

Prevalence of *Escherichia coli* O157:H7 in water and meat and meat products and vegetables sold in the Eastern Cape Province of South Africa and its impact on the diarrhoeic conditions of HIV/AIDS patients

by

Benard Omondi Abong'o

A thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

(MICROBIOLOGY)

In the Faculty of Science and Agriculture at the University of Fort Hare.

2008

Supervisor: Prof MNB Momba

Co-Supervisor: Prof AI Okoh

DECLARATION

I, the undersigned, declare that this thesis submitted to the University of Fort Hare for the degree of Doctor of Philosophy in Microbiology in the Faculty of Science and Agriculture, School of Science, and the work contained herein is my original work with exemption to the citations and that this work has not been submitted at any other university in partial or entirety for the award of any degree.

Name: _____

Signature: _____

Date: _____

Acknowledgements

With gratitude to the Almighty God, I wish to express my profound gratitude to all persons and institutions that have made immense contributions towards the successful completion of this study. In particular, I thank my supervisor, Prof MNB Momba, currently of Tshwane University of Technology, Environmental, Water and Earth Sciences, for her mentorship, sustained interest, and encouragement as well as material and financial support. I lack the words to express my heartfelt gratitude to her. Thanks are due to my Co-Supervisor Prof Anthony I. Okoh, University of Fort Hare, Department of Biochemistry and Microbiology, to whom I owe much of my success in the study. His coming to Fort Hare is exquisite and I may not have the exact and proper words to thank him enough. I will never forget his comfort, encouragement, moral and material contribution as well as his zeal until my successful completion of the study. To Mr. LV Mabinya and Prof. G. Bradley, my gratitude knows no bounds. I also thank Ms Veronica Khathutshelo Malakate for her tireless assistance during the molecular aspect of this study. My thanks also goes to Mrs Gwentshe, Prof. Pironcheva, Mr. Ngwenya, Mr. Mazomba and the other staff members as well as all my fellow student colleagues and friends for their kindness, support and love.

The Doctors and Sisters at Frere Hospital in East London, in particular to Dr. Chris Horsfall, Dr. Pandey, Dr. Macingwane, Dr. Makhomba and Sr. A. Pagel are appreciated for allowing me to sample from their hospital complex. My gratitude is equally extended to Prof. P. Becker of Biostatistics Unit Pretoria, Medical Research Council of South Africa and also to Ms Jeanine Mwambakana, Department of Mathematical Sciences, Tshwane University of Technology for her statistical input in the analysis of data. Dr. Nicola Rodda is highly appreciated for Risk analysis.

Many thanks goes to the staff of National Research Foundation (NRF), staff of Govan Mbeki Research and Development Centre and staff of Fort Hare Foundation for their co-operation and co-ordination in all the financial issues pertaining to this study. Also thanks are due to the staff of the Department of Health Bisho, especially Dr. Thomas Dlamini for the ethical approvals.

I would like to thank my family especially my brother Mr. G. W. Abong'o for his support and my wife Teresa and our children Victor, Felis, Collins, Roselyn, and Pauline Fleria Auma for their love, perseverance, understanding, kindness, patience, and support throughout the period of this study.

COPYRIGHT

© Copyright by Benard Omondi Abong'o. 2007. All rights reserved.

Dedications

This Thesis is

Dedicated to my beloved soul mate and wife

Teresa Atieno Omondi; Our Children: Melvic, Felis, Collins,

Roselyn and Pauline Fleria Auma.

LIST OF ABBREVIATIONS

A	–	Adenine
AE	–	Adhesion-effacing
AFLP	–	Amplified Fragment Length Polymorphism
AGA	–	American Gastroenterological Association
AIDS	–	Acquired immune deficiency syndrome
APHIS	–	Animal and Plant Health Inspection Service
ASSA	–	Actuarial Society of South Africa
ATCC	–	American Type Culture Collection,
AZT	–	azidothymidine
BCIG-SMAC	–	bromo-4-chloro-3-indoxyl-b-D-glucuronider SMAC
BCM	–	Biosynth Culture Media
bp	–	base pair
BPW	–	Buffered peptone water
C	–	Cytosine
cAMP	–	cyclic adenosine monophosphate
CCDR	–	Canadian Communicable Disease Report
CCPs	–	Critical Control Points
CD4+ T	–	T cells expressing CD4
CDC	–	Communicable Disease Control
CDC-MMWR	–	Communicable Disease Control - Morbidity and Mortality Weekly Report
cDNA	–	Complementary Deoxyribonucleic acid

CFSP	–	Certified Food Safety Professional
CFSPH	–	Center for Food Security and Public Health
CFU	–	Colony forming units
CFUg ⁻¹	–	Colony forming units per one gram
CFUmL ⁻¹	–	Colony forming units per one millilitre
CSIR	–	Council of Scientific and Industrial Research
CT-SMAC	–	Cefixime Tellurite-Sorbitol MacConkey Agar
DALYS	–	Disability adjusted life years ATCC
DIC	–	Disseminated intravascular coagulation
DNA	–	Deoxyribonucleic acid
dNTPs	–	deoxynucleoside triphosphates
DWAF	–	Department of Water and Forestry
e.g.	–	<i>exempli gratia</i> , for example
<i>eae</i>	–	attaching and effacing gene
EAF	–	adherence factor
EAggEC	–	Enterotoxigenic <i>Escherichia coli</i>
EC	–	Eastern Cape
EDTA	–	Ethylenediaminetetraacetic acid
EHEC	–	Enterohaemorrhagic <i>Escherichia coli</i>
EIEC	–	Enteroinvasive <i>Escherichia coli</i>
EMBA	–	Eosin Methylene Blue Agar
EPA	–	Environmental Program Agency
EPEC	–	Enteropathogenic <i>E. coli</i>

<i>et al</i>	–	(<i>et alii</i>) and others
EtBr	–	Ethidium bromide
ETEC	–	Enterotoxigenic <i>E. coli</i>
FAO	–	Food and Agricultural Organization
FDA	–	Food and Drug Administration
<i>fliC</i>	–	flagellin gene
FSIS	–	Food Safety and Inspection Service
G	–	Guanine
g	–	gram
GB3	–	Globotriaosyl ceramide
GDP	–	Gross Domestic Product
h	–	Hour
H ⁺	–	Flagella antigen present
H [–]	–	Flagella antigen absent
H7	–	Flagella antigen
HACCP	–	Hazard Analysis and Critical Control Points
HIV	–	Human Immune Virus
HIV/AIDS	–	Human Immune Virus/Acquire Immune Deficiency Syndrome
<i>hlyA</i>	–	haemolysin gene
HSRC	–	Human Sciences Research Council
HUS	–	hemolytic-uremic syndrome
ILSI	–	International Life Sciences Institute

IMS	–	Immunomagnetic Separation
IMViC	–	Indole, Methyl red, Voges-Proskauer, Citrate
LCDC	–	Laboratory Center of Disease Control
LD ₅₀	–	Lethal Dose, 50%
L-EMBA	–	Levine's Eosin Methylene Blue
LPS	–	Lipopolysaccharide
LT	–	Heat-labile enterotoxin
MAC -	–	MacConkey Agar
MDa	–	Mega Dalton
MDL	–	Molecular Diagnostic Laboratory
mEC- Broth	–	Modified <i>E. coli</i> broth
mg	–	Milligram
Mg ²⁺	–	magnesium ions
MgCl ₂	–	Magnesium Chloride
MHA	–	Mueller-Hinton Agar
min	–	Minute
ml	–	Millilitre
mM	–	Millimole
Mn ²⁺	–	manganese ions
MPC-S	–	magnetic particle concentrator
MPN	–	most probable number
MRC	–	Medical Research Council
MUG	–	4-Methyl-umbelliferyl-β-D-glucuronide

NA	—	Nutrient Agar
NaCl	—	Sodium Chloride
NAS	—	National Academy of Sciences
NCCLS	—	National Committee for Clinical Laboratory Standards
Nº	—	Number
O	—	Somatic antigen
°C	—	Degrees Celsius
OIs	—	Oral Infections
PCA	—	Plate Count Agar
PCR	—	Polymerase Chain Reaction
PFGE	—	Pulsed-field gel electrophoresis
QMRA	—	Quantitative Microbiological Risk Assessment
QRT-PCR	—	Quantitative Real Time PCR
RAPD	—	Random amplification of polymorphic
<i>rfb</i>	—	gene cluster that encodes for the biosynthesis of the O-antigen (pO157)
RFLP	—	Restriction fragment length polymorphisms
RNA	—	ribonucleic acid
Rnase	—	Ribonuclease
rRNA	—	Ribosomal Ribonucleic acid
RT	—	Reverse transcriptase
RT-PCR	—	Real-Time PCR
SA	—	South Africa

SAAFost	–	South African Association for Food Science and Technology
SANHS	–	South African National HIV Survey
SAS	–	Statistical Analysis System
SDS-PAGE	–	Sodium Dodecyl Sulphate Poly-Acrylamide Gel Electrophoresis
SLT	–	Shiga -like toxin
SMAC	–	Sorbitol MacConkey Agar
SSA	–	Statistics South Africa
ST	–	Heat-stable enterotoxin
STD	–	Sexually Transmitted Diseases
STEC	–	Shiga Toxigenic <i>Escherichia coli</i>
Stx	–	Shigatoxin
T	–	Thymine
TAE	–	Tris-acetate
<i>Taq</i>	–	<i>Thermus aquaticus</i>
TB	–	Tuberculosis
Tris-HCl	–	Trishydroxymethylaminomethane-Hydrochloric acid
TSA	–	Tryptic Soy Agar
U	–	Uracil
UF/IFAS	–	University of Florida Institute of Food and Agriculture
UK	–	United Kingdom
UNAIDS	–	United Nations Programme on HIV and AIDS
UNDP	–	United Nations Development Programme
US	–	United States

USA	–	United States of America
USDA	–	United States Department of Agriculture
USEPA	–	Environmental Protection Agency
V	–	Voltage
VCT	–	Voluntary Counseling and Testing
<i>vt1</i>	–	Verotoxin 1 gene
<i>vt2</i>	–	Verotoxin 2 gene
VTEC	–	Erocytotoxin-producing <i>Escherichia coli</i>
W/V	–	Weight per volume
WHO	–	World Health Organization
µg	–	Microgram
µℓ	–	microlitre
β	–	<i>Beta</i>
1 U	–	One Unit

TABLE OF CONTENTS

Page №

Declaration.....	i
Acknowledgements.....	ii
Dedications.	iv
List of Abbreviations.....	v
Table of contents.....	xii
General Abstract.....	xiv

CHAPTER

1. General introduction.....	1
2. Background of the study.....	36
3. Prevalence of enterohemorrhagic <i>Escherichia coli</i> O157:h7 in drinking water in the Amathole District, Eastern Cape Province – South Africa.....	214
4. Prevalence of <i>Escherichia coli</i> O157:H7 in meat and meat products sold in the Amathole district, Eastern Cape Province – South Africa.....	244
5. The prevalence of <i>Escherichia coli</i> O157:H7 in vegetables sold in the Amathole District, Eastern Cape Province – South Africa.....	274
6. Prevalence of <i>Escherichia coli</i> O157:H7 among confirmed and non-confirmed HIV/AIDS patients with diarrhoea in the amathole district, Eastern Cape Province – South Africa.....	304
7. Molecular relatedness of <i>Escherichia coli</i> O157:H7 isolated from drinking water, meat and meat products, and vegetables, and stools of diarrhoeic confirmed and non-confirmed HIV/AIDS patients in the Amathole District – South Africa.....	338

8.	Health risk of <i>Escherichia coli</i> O157:H7 in drinking water and meat and meat products and vegetables to diarrhoeic confirmed and non-confirmed HIV/AIDS patients.....	370
9.	General Discussion.....	401
	Appendices.....	438

GENERAL ABSTRACT

General Abstract

Water and food borne *Escherichia coli* O157:H7 could be one of the pathogens posing high health risk to patients suffering from Acquired Immunodeficiency Syndrome (AIDS) because of its incrimination in diarrhoea cases in AIDS patients. The present study, which was conducted between March 2005 and August 2006, investigated the prevalence of *E. coli* O157:H7 in water, meat and meat products and vegetables and its impact on diarrhoeic conditions of confirmed and non-confirmed HIV/AIDS patients in the Amathole District in the Eastern Cape Province of South Africa. The water samples used in the study were obtained from stand pipes supplying treated drinking water to communities residing in Fort Beaufort, Alice, Dimbaza and Mdantsane whereas borehole waters were sampled from Ngwenya and Kwasaki. The meat and meat products and vegetable samples were purchased from shops, butcheries, supermarkets and open air markets in Fort Beaufort, Alice and Mdantsane. The stool swabs used in the study were obtained from HIV/AIDS and outpatient clinics at Frere Hospital in East London. A total of 180 each of water, meat and meat products and vegetable samples and another 360 stool samples were analyzed for *E. coli* O157:H7.

Presumptive *E. coli* O157 was isolated from the samples by culture-based methods and confirmed using Polymerase Chain Reaction techniques. Anti-biogram as well as risk assessment were also carried out using standard methods. The viable counts of presumptive *E. coli* O157 for water samples ranged between 3.3×10^4 and 1.71×10^5 CFU/ml, and between 1.8×10^4 and 5.04×10^6 CFU/g for meat and meat products, whereas those for vegetables ranged between 1.3×10^3 and 1.6×10^6 CFU/g. The counts of presumptive *E. coli* O157 for the water and vegetable samples were not significantly different whereas those for meat and meat products were found to be significantly different ($P \leq 0.05$).

The prevalence rates of presumptive *E. coli* O157 in meat and meat products was 35.55% (64/180), and 25.55% (46/180) and 21.66% (39/180) for water and vegetables respectively. Prevalence of presumptive *E. coli* O157 in the stool samples of HIV/AIDS patients was 36.39% (131/360), of which 56.5% (74/131) and 43.5% (57/131) were from stools of confirmed and non-confirmed HIV/AIDS patients, respectively. Molecular analysis of representative presumptive *E. coli* O157 indicated that 10.29% (4/39) of vegetables; 14.81% (4/27) of water and 38.46% (5/13) of meat and meat products carried *E. coli* O157:H7. Also 36% (9/25) and 17.24% (5/29) of the stool samples were positive for *E. coli* O157:H7.

Antimicrobial susceptibility profile revealed that all of the *E. coli* O157:H7 isolated from water, meat and meat products and vegetables as well as those isolated from stools of confirmed and non-confirmed HIV/AIDS patients were resistant (R) to gentamycin and erythromycin. However, 75% (20/27) of these isolates were resistant (R) to ampicillin and tetracycline whereas approximately 25% (6/27) were resistant (R) to nalidixic acid, ceftriaxone, and chloramphenicol. All the isolates (27/27) were susceptible (S) to amikacin.

Probability of risk of *E. coli* O157:H7 infection was high for confirmed HIV/AIDS patients than for the non-confirmed HIV/AIDS patients. Estimated probability of risk of *E. coli* O157:H7 due to ingestion of water was 1.00 for 100 confirmed and non-confirmed HIV/AIDS patients. Risk due to meat and meat products was estimated at 0.27 and 0.20 and for vegetables at 0.21 and 0.15 per 100 confirmed and non-confirmed HIV/AIDS patients.

The findings of this study predicted a possible link between *E. coli* O157:H7 isolated from drinking water, meat and meat products and vegetables and diarrhoeic conditions in both confirmed and non-confirmed HIV/AIDS patients, and concludes that confirmed HIV/AIDS

patients can be at higher risk of contracting water and food borne *E. coli* O157:H7 than non-confirmed HIV/AIDS patients. It is thus recommended that proper water treatment and food handling, maximum food and water safety and sanitation as well as personal body hygiene should be maintained, in order to prevent *E. coli* O157:H7 infections. Education initiatives and active surveillance of *E. coli* O157:H7 should be taken by all the stake-holders working directly or indirectly towards ensuring enduring sound public health.

CHAPTER 1

INTRODUCTION

TABLE OF CONTENTS	Page №
Table of contents.....	1
General introduction.....	2
References.....	15

GENERAL INTRODUCTION

Human illnesses associated with water and food-borne pathogens are as old as the universe. Globally, waves of water and food borne disease outbreaks have been reported (Harris, 2001; Dontorou *et al.*, 2003; Ihekweazu *et al.*, 2006; Mashood *et al.*, 2006). The pathogens often implicated in water and food borne disease outbreaks are but not limited to *Campylobacter*, *Salmonella*, *Shigella*, *Yersinia* and toxigenic *Escherichia coli*, *Giardia* and *Cryptosporidium* (Svenungsson *et al.*, 2000; Hayes *et al.*, 2005). Some enteric viruses have also been reported to cause food and water borne diseases (Monroe *et al.*, 2000; Kapikian, 2000; Svenungsson *et al.*, 2000; Hayes *et al.*, 2003).

Escherichia coli was first described by a German paediatrician Dr. Theodor Escherich as *Bacterium coli commune* that had been isolated from the faeces of an infant patient (Escherich, 1885). It is one of the several types of micro flora of the intestines of humans and animals. However, to date several strains of pathogenic *E. coli* have been reported universally, most of which have caused water and food borne disease outbreaks (Cunin 1999; Jackson *et al.* 2000; Effler *et al.*, 2001; Faiella-Tommasino and Reigert, 2002; Hansen and Knochel, 2003; Jay *et al.*, 2004).

These pathogenic *E. coli* strains include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enterohemorrhagic *E. coli* (EHEC) of which *E. coli* O157:H7 is a member (Kaper *et al.*, 2004; Torres *et al.*, 2005).

Escherichia coli O157:H7 is the predominant serotype of enterohemorrhagic *E. coli*. It was first associated with severe gastroenteritis in 1982 when it caused two major outbreaks of haemolytic uremic syndrome (HUS) (Riley *et al.*, 1983). Since then, more infections caused by

this bacterium have been reported in both developed and developing countries (Harris, 2001; Dontorou *et al.*, 2003; Ihekweazu *et al.*, 2006; Mashood *et al.*, 2006). Outbreaks have been documented from countries in America, Europe, Asia and Africa (WHO, 1997a; Besser *et al.*, 1999, Mashood *et al.*, 2006).

In 1996, *E. coli* O157:H7 outbreak occurred in British Columbia, California, Colorado and Washington, involving 70 illnesses, mostly children, in which more than one third of patients were hospitalized (Cody *et al.*, 1999). A separate outbreak of *E. coli* O157:H7 in the United States was reported in 2002. It was associated with consumption of ground beef (Vogt and Dippold, 2005). In this outbreak, only one person was diagnosed with the bacterium. Between November and December 2006, 71 persons were infected with *E. coli* O157:H7 in about five states in the US. This was reported in the states of New Jersey, New York, Pennsylvania, Delaware and South Carolina. Among these 71 victims, 53 (75%) were hospitalized and 8 (11%) developed HUS. Cases in 52 of the 71 patients were confirmed positive for *E. coli* O157:H7 using DNA fingerprinting (CDC, 2006).

In the UK, most outbreaks of *E. coli* O157:H7 have been sporadic (Parry *et al.*, 1998). In 2002, a young schoolchild was diagnosed with *E. coli* O157:H7 after returning to UK from a holiday trip in France. The infection was suspected on cucumber and hot-dog pastry that the students fed on during the trip (Duffell *et al.*, 2003). In August 2004, 7 cases of *E. coli* O157 infection were identified in children on a holiday in Cornwall, Southwest England. The outbreak was suspected on a contaminated freshwater stream flowing across a seaside beach. Reportedly a heavy rainfall in the days preceding the outbreak had led to faeces from cattle potentially contaminated by *E. coli* O157 contaminating the stream through surface run-offs, thereby leading to the outbreak (Ihekweazu *et al.*, 2006).

Another European country that has suffered from *E. coli* O157:H7 outbreak is Sweden (Söderström *et al.*, 2005). In 1996, outbreaks of *E. coli* O157:H7 linked to contaminated radish sprouts in school lunches in Japan led to 9 451 cases (Mermin and Griffin, 1999). In the same year, there was another infection of *E. coli* O157:H7 among factory workers in Kyoto, Japan. The food suspected in this outbreak was radish sprout salad that was served to the factory workers during lunch (Watanabe *et al.*, 1999).

Epidemiological data on *E. coli* O157: H7 in developing countries are limited, as surveillance for this pathogen is not done routinely. In South Africa the first case of *E. coli* O157:H7 infection was reported in 1990. This bacterium was isolated from a man in the city of Johannesburg (Browning *et al.*, 1990). Since then, many sporadic cases of bloody diarrhoea have been reported in other areas of South Africa. An outbreak of diarrhoeal infections in Gauteng region of South Africa reported a detection rate of 7.7% for *E. coli* O157:H7 out of 151 *E. coli* isolates from patients with diarrhoea and 30 isolates from healthy individuals (Galane and Le Roux, 2000).

Even though no cases of *E. coli* O157:H7 outbreaks in South Africa have been reported since 2001, studies on environmental samples and drinking water have yielded some suspicious findings (Müller *et al.*, 2001, 2003; Momba *et al.*, 2006a, b). In their study, Müller *et al.* (2001) reported that some water samples contained *E. coli* strains that carried virulence properties, such as Shiga toxin 1 and 2 (*Stx1* and 2), and enterohaemolysin, which they suggested could pose a health risk if the community ingested such contaminated waters. Another study conducted by Müller *et al.* (2003) in a bid to assess the prevalence of *E. coli* O157:H7 in selected sewage and environmental waters in South Africa reported a 20% isolation rate for *E. coli* O157: H7.

Isaacson *et al.* (1993) reported an outbreak of the bacterium in Swaziland, which occurred in 1992. During this outbreak, 40 912 patients of less than five years of age were infected. This was the largest ever-recorded *E. coli* O157:H7 outbreak in the African continent. Out of the 40, 912 patients, only 778 were screened for the bacteria of which 327 were found positive. Beef and untreated water were the prime suspected vehicles of transmission. In the same year, there was an outbreak of *E. coli* O157:H7 in refugee camps in central and southern regions of Malawi where more than 20 000 cases occurred (Paquet *et al.*, 1993). Other *E. coli* O157:H7 isolates from stools of patients with diarrhoeal illness have also been reported from other African countries such as Kenya (Sang *et al.*, 1996; Arimi *et al.*, 2000), Cote d'Ivoire (Dadie *et al.*, 2000), Uganda (Kaddu-Mulindwa *et al.*, 2001) and Nigeria (Olorunshola *et al.*, 2003).

In the Central African Republic, 290 cases and 2 fatalities were reported in 1997 (Germani *et al.*, 1997). The outbreak was associated with the consumption of smoked zebu meat and contaminated drinking water. In Kenya, Arimi *et al.* (2000) reported *E. coli* O157:H7 serotype in two samples of milk collected in Nairobi and one of the two isolates was confirmed positive for verocytotoxins. Another African country that has witnessed an *E. coli* O157:H7 outbreak is Cameroon. In this country, *E. coli* O157:H7 infection was reported in November 1997 and April 1998. Approximately 298 people were diagnosed with diarrhoea suspected on *E. coli* O157:H7 (Germani *et al.*, 1998).

In Cote d'Ivoire (Ivory Coast) the first reported case of enterohemorrhagic producing *E. coli* was by the works of Dadie *et al.* (2000). They reported O157:H7 strains from children with bloody diarrhoea. The *E. coli* O157 strains were found to carry *Stx 2*, *eae*, and glucoronidase gene (*UidA*). In Kampala, Uganda, faecal samples from 237 diarrhoeic infants and 59 healthy

cattle from a ranch in the central region of Uganda were screened for *E. coli* O157:H7 and other types of enterohaemorrhagic producing *E. coli* (Kaddu-Mulindwa *et al.*, 2001).

Since 2001, cases of acute bloody diarrhoea have been increasing in various parts of Africa. For instance, in Kinshasa Democratic Republic of Congo (DRC), rainy periods are often characterized by outbreaks of *E. coli* O157:H7 infections. Koyange *et al.* (2004) reported *E. coli* O157:H7 outbreak that occurred in 2003. During this outbreak 463 children aged 15 and below were diagnosed with severe diarrhoea at a Paediatric Hospital of Kalembelembe in Kinshasa, (DRC). The routes of transmission were suspected on person-to-person contact, unhygienic conditions and contaminated food and water (Koyange *et al.*, 2004).

Elsewhere in Lagos, Nigeria, Olorunshola *et al.* (2003) examined the prevalence of *E. coli* O157:H7 in 100 patients with diarrhoea. The detection rate of the bacterium was noted at 6%. In a separate study, Smith *et al.* (2003) also reported to have isolated the bacterium from healthy animals in the same city.

The above studies clearly show that people happen to acquire *E. coli* O157:H7 infections through various routes namely contaminated water, meat and meat products and vegetables (Todar, 2002; Kassenborg *et al.*, 2004). Most illnesses have been associated with the eating of undercooked contaminated ground beef such as hamburger (Su, *et al.*, 1995; Prier *et al.*, 2000; Gansheroff and O'Brien, 2000; Donnenberg and Whittam, 2001). Waterborne *E. coli* O157:H7 transmissions have included contaminated drinking, river and recreational waters (Isaacson *et al.*, 1993; Keene *et al.*, 1994; Armstrong *et al.*, 1996; Friedman *et al.*, 1999). Water can be contaminated at the source, during its distribution, collection from the distribution points or in the homes due to contaminated water storage vessels (Muyima and Ngcakani, 1998; Momba and Notshe, 2003). Established routes that can lead to water being contaminated with *E. coli*

O157:H7 while at the source include various human activities such as uncontrolled grazing of cattle (Isaacson *et al.*, 1993; Hubbard *et al.*, 2004; Zamxaka *et al.*, 2004), answering calls of nature in the velds (Phaswana-Mafuya, 2006) and use of manures in the farms (Solomon *et al.*, 2002).

Surface waters may be heavily contaminated after heavy rainfall, as was the case with the Walkerton *E. coli* O157:H7 outbreak in 2000 as well as the Swaziland outbreak (Bruce-Grey-Owen Sound Health Unit, 2000; Effler *et al.*, 2001). Surface water sources that are used by communities may act as disposal points of human and animal wastes (Fatoki *et al.*, 2001).

Dumping of human and animal wastes particularly excreta into drinking water sources plays an important role in the contamination of such water sources and acts as reservoirs of disease causing bacteria such as *E. coli* O157:H7. Pit latrines may also act as potential sources of microbial contaminants to underground water through contaminated aquifers by water seepage to such natural sources (WHO, 2006).

Foods such as meat and meat products can acquire *E. coli* O157:H7 contamination during slaughter process, distribution or even in the shops. Contamination of meat and meat products by this bacterium can also occur at home during cooking from contaminated cooking vessels or by the persons preparing the foods or cross-contamination due to contact with foods, mostly those of animal origin. Other bovine products that have as well been implicated in *E. coli* O157:H7 outbreaks are raw milk, and cheese (Feng, 2000; Kassenborg *et al.*, 2004).

Most fresh produce like vegetables are grown using bovine manure and untreated irrigation waters (Solomon *et al.*, 2002; Mukherjee *et al.*, 2004). This kind of practice leads to contamination of vegetables with enteric pathogens (Mukherjee *et al.*, 2004; Johannessen *et al.*, 2005). An increasing number of diarrhoeal diseases due to *E. coli* O157:H7 outbreaks have been

associated with the consumption of such contaminated vegetables (Mukherjee *et al.*, 2004; Johannessen *et al.*, 2005). People have developed diarrhoea suspected on *E. coli* O157:H7 after eating contaminated bean sprouts, lettuce, spinach and vegetable salads (Abdul-Raouf *et al.*, 1993; Bender *et al.*, 1999; Mukherjee *et al.*, 2004; Johannessen *et al.*, 2005). Person-to-person contact in families, childcare centres and nursing homes is also a known mode of transmission (Griffin, 1995; Armstrong *et al.*, 1996). Person-to-person transmissions mostly occur if infected people do not observe proper personal hygiene.

As noted above, the presence of water and food borne pathogens in drinking waters and foods causes a number of diseases one of which is diarrhoea. Diarrhoeal cases have been reported in both immune-competent and immune-suppressed persons such as those suffering from Human Immunodeficiency Virus/Acquired Immunodeficiency Syndromes, otherwise known as HIV/AIDS (Hayes *et al.*, 2003). Human Immunodeficiency Virus (HIV) is the virus that causes Acquired Immunodeficiency Syndrome (AIDS), an immune condition that renders the human immune system incapacitated to protect itself against infectious agents such as those found in water and contaminated foods (MMWR, 1981a, b). Water and food borne pathogens normally manifest themselves through diarrhoea in immunocompetent persons but could be worse in immunocompromised persons such as HIV/AIDS patients (Hayes *et al.*, 2003). Reports indicate that diarrhoea occur in 30 to 60% of HIV/AIDS patients in developed countries and in about 90% of such patients in developing countries (Sapkota *et al.*, 2004). Contaminated waters and foods are the likely culprits inducing diarrhoea in HIV/AIDS patients (Prier *et al.*, 2000; Hayes *et al.*, 2003). Due to their compromised immune system HIV/AIDS patients are perceived most vulnerable to water and food borne diarrhoeal disease causing pathogens.

Bacterial pathogens namely *Salmonella* species, *Campylobacter jejuni*, *Vibrio* species and *Listeria monocytogenes* (Morris and Potter, 1997; Prier, *et al.*, 2000; Hoffman, 2004, Obi *et al.*, 2007b), have been reported to account for significant number of diarrhoeal episodes in HIV/AIDS patients. Just as it would be for persons with competent immunity, accidental ingestion of contaminated waters and foods would consequently lead to unprecedented infections in persons with incompetent immune system (Law and Kelly, 1995; Prier *et al.*, 2000; Hayes *et al.*, 2003).

A study in South Africa by Obi *et al.* (2007b) attempted to show a link that exists between bacterial pathogens namely *Shigella dysenteriae*, *Salmonella sp.* and *E. coli* that were isolated from stools of HIV/AIDS positive patients and the water samples from the households of these patients. Another study by Obi and Bessong (2002) isolated diarrhoeagenic bacterial pathogens from the stools of HIV positive patients with diarrhea in rural communities of the Limpopo Province of South Africa. However, to date there is no study in South Africa that is known to our knowledge, which has linked *E. coli* O157:H7 isolated from water, meat and meat products and vegetables with diarrhoeic conditions in HIV/AIDS patients.

Highly polluted waters and foods constitute a health risk to the population who are using such waters and eating these foods (Friedman, 1999; Keene *et al.*, 1999; Teunis *et al.*, 2004). Microbial risk assessment of water and foods consumed by confirmed and non-confirmed HIV/AIDS patients is therefore very vital. This is due to the changing epidemiology of food and water borne diseases because of complex interactions and changes in pathogens, foods, food distribution and consumption as well as the rising loss in population immunity (Helderberg *et al.*, 1994; Smith and Fratamico, 1995; Altekruse *et al.*, 1997). The essence of microbial risk

assessment is the description of a system in which a microbial hazard reaches its host and causes infection.

Risk assessment consists of four main steps namely hazard identification, exposure assessment, dose-response assessment and risk characterization (Potter, 1996; Cassin *et al.*, 1998; Haas *et al.*, 1999). The knowledge in each step is combined to represent a cause-and-effect chain from the prevalence and population of a given microbial agent to the probability and magnitude of health effects.

In risk assessment, both the probability and impact of disease are considered. Risk assessment model is therefore developed, based on the pathogen population in the water or food product throughout the distribution chain. The variability and uncertainty in the model are accommodated using the probabilistic representations of many of the parameters. To generate a representative distribution of risk, the model is simulated with values from the probability distributions (Thompson *et al.*, 1992; Vose, 1996; Cassin *et al.*, 1998). The direct output of the model is a distribution of health risk from ingesting water or eating a given food product by a particular group of population (in this case confirmed and non-confirmed HIV/AIDS patients with diarrhoea).

To characterize and analyse health risk of a disease-causing organism such as *E. coli* O157:H7, the parameters like presumptive counts in the waters or food, the susceptibility variation between the population of study must be understood. The information is then used to estimate the probability of infection of the pathogen i.e. *E. coli* O157:H7 to the patients. The quantities of daily intake of the items under study, for instance water, meat and meat products and vegetables by the patients are estimated based on a published data (Helena and Steyn, 2002).

Antibiotics are often used therapeutically and prophylactically to treat human and animal infections and in addition used as growth promoters in animal production (Bogaard, 1996, 1999). Before, *E. coli* O157:H7 were susceptible to many antibiotics used for treating most human and animal infections (Tsuboi *et al.*, 1998; Johnson *et al.*, 2000; Oie *et al.*, 2004). However, some researchers have reported on the marked increase in antimicrobial resistance in *E. coli* O157:H7 strains of clinical, environmental and food origins (Magwira *et al.*, 2005). Resistance to cephalothin, sulphatriad, colistin sulphate, sulfamethoxazole and tetracycline has been reported in a number of studies (Magwira *et al.*, 2005).

The link between use of antibiotics and development of bacterial resistance is well documented (Threlfall *et al.*, 2000; Teal, 2002). Use of antibiotics in animals plays an important role in accelerating the development and dissemination of antibiotic-resistant bacteria (Barza and Travers, 2002). It has been reported that application of antibiotics in animals meant for food may facilitate the development of resistant genes in pathogens infecting these animals, which may eventually be transferred to human through the foods (Bogaard and Stobberingh, 1999; Teal, 2002). Different antibiotic resistance profiles have been detected in *E. coli* O157:H7 isolates from different sources, including humans, animals and foods (Magwira *et al.*, 2005; Ju-Yeon *et al.*, 2006).

Antibiotics are not recommended for use therapeutically for human *E. coli* O157: H7 infections as there are evidence indicating that antibiotic resistance in *E. coli* O157: H7 of human isolates is increasing (Zhao *et al.*, 2001). Because many human *E. coli* O157:H7 infections are acquired through ingestion of contaminated water, meat and meat products and vegetables, it is important to determine if *E. coli* O157: H7 isolated from water, meat and meat products and

vegetables as well as from the stools of confirmed and non-confirmed HIV/AIDS patients have antimicrobial resistance properties.

The Eastern Cape Province is one of the nine provinces of South Africa and is located along the South-East coast covering an estimated 170 000 km². The province represents about 14% of South Africa's land mass (Phaswana-Mafuya and Shukla, 2005). It has a population size of approximately seven million people faced with high levels of poverty due to unemployment. The area is also characterized with underdeveloped infrastructure (Phaswana-Mafuya and Shukla, 2005). This vast population is also faced with a myriad of diseases like HIV/AIDS, diarrhoea and other socio-economic problems (Sowetan News, 2003; Sooka *et al.*, 2004). Looking at the socio-economic development levels in the Eastern Cape Province, its high levels of poverty is a major factor that co-exists with other diseases (Department of Health, 2001; Sooka *et al.*, 2004).

The small villages in the Amathole District of Eastern Cape Province where this study was conducted still lack safe water supplies (Momba *et al.*, 2006a). Human practices such as irrigation and waste disposal in this area act as potential sources of contamination to dams and other water bodies that serve as sources of municipal water supplies (Fatoki *et al.*, 2001; Phaswana-Mafuya and Shukla, 2005). Effluents from sewage treatment plants also contaminate water sources such as rivers (Mathuthu *et al.*, 1995). Generally, there is lack of information on the role that water and food borne *E. coli* O157:H7 infections play in determining the diarrhoeic conditions of HIV/AIDS patients in the African countries one of which is South Africa. Such inadequacy in information was our motivation for the study.

The study firstly aimed at assessing the prevalence of *E. coli* O157:H7 in water, meat and meat products and vegetables as well as in the stools of confirmed and non-confirmed diarrhoeic

HIV/AIDS patients. The molecular linkage of *E. coli* O157:H7 isolated from water, meat and meat products and vegetables and from stools of confirmed and non-confirmed diarrhoeic HIV/AIDS patient was determined. Health risk associated with *E. coli* O157:H7 to confirmed and non-confirmed diarrhoeic HIV/AIDS patients as a result of drinking the waters and consuming meat and meat products and vegetables sold in the Amathole District was evaluated. The other aim was to assess the antimicrobial susceptibility of the *E. coli* O157:H7 isolates.

To achieve the above aims, the following objectives were pursued:

- (i) To determine the prevalence of *E. coli* O157:H7 in drinking water sources, meat and meat products and vegetables sold in the Amathole District in the Eastern Cape Province of South Africa.
- (ii) To determine the prevalence of *E. coli* O157:H7 in stools of confirmed and non-confirmed HIV/AIDS patients with diarrhoea in the Amathole District in the Eastern Cape Province of South Africa.
- (iii) To establish the molecular relationship between *E. coli* O157:H7 isolated from water, meat and meat products and vegetables to those isolated from stools of confirmed and non-confirmed HIV/AIDS patients using Polymerase Chain Reaction (PCR).
- (iv) To assess the health risk associated with *E. coli* O157:H7 in water and meat and meat products and vegetables to diarrhoeic conditions in confirmed and non-confirmed HIV/AIDS patients.
- (v) To evaluate the antibiotic susceptibilities of *E. coli* O157:H7 isolated from water, meat and meat products and vegetable as well as stools of confirmed and non-confirmed HIV/AIDS patients with diarrhoea.

This study was expected to generate valuable information that could be used by Water Engineers' Wastewater Plant Managers, Local Water Authority, and Microbiologists. Others include Plant-bioprospectors, Ethno-botanists, Traditional Healers, and Medical Doctors. Finally, Epidemiologists, Food Industries, Local Markets and especially the Department of Health, Water Affairs and Forestry would benefit in their respective obligation to develop appropriate strategies for the control of water and food borne diseases in the Eastern Cape Province. This would enable them have a focused decision in the provision of public health protection through didactic fora, better water treatment and monitoring of water and food safety, hygiene and sanitation.

References

- Abdul-Raouf, U. M., L. R. Beuchat, and M. S. Ammar.** 1993. Survival and growth of *Escherichia coli* O157:H7 in ground, roasted beef as affected by pH, acidulants, and temperature. *Appli. Environ. Microbiol.*; **59(8)**: 2364–2368.
- Altekruse, S. F., D. A. Street, S. B. Fein, and A. S. Levy.** 1996. Consumer knowledge of foodborne microbial hazards and food-handling practices. *J. Food Prot.*; **59(3)**: 287–294.
- Arimi, S. M., E. Koroti, E. K. Kang'ethe, A. O. Omore, J. J. McDermott, K. Macharia K, et al.** 2000. Risk of infection from *E. coli* O157:H7 through informally marketed raw milk in Kenya 3rd All Africa Conference on Animal Agriculture.
- Armstrong, G. L., J. Hollingsworth, and J. G. Jr. Morris.** 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.*; **18**: 29–51.
- Barza, M. and K. Travers.** 2002 Excess infections due to antimicrobial resistance: the "attributable fraction". *Clin. Infect. Dis.*; **34(3)**: S126–S130.
- Bender, J. B., E. S. Kirk, H. Craig, and T. M. Osterholm.** 1999. Food-borne disease in the 21st century: what challenges await us? *Postgrad. Med.*; **106(2)**: 109–119
- Bogaard, V, D. A. E., and E. E. Stobberingh.** 1996. Is it time to ban all antibiotics as animal growth-promoting agents? *Lancet*; **348**: 1454–1456.
- Browning, N. G., J. Botha, H. Sacho, and P. J. Moore.** 1990. *Escherichia coli* O157:H7 haemorrhagic colitis. Report of the first South African case. *SA Surg. J.*; **28**: 28–29.

- Bruce-Grey-Owen Sound Health Unit.** 2000. Waterborne outbreak of gastroenteritis associated with a contaminated municipal water supply, Walkerton, Ontario, May–June 2000. *Canadian Comm. Dis. Rep.*; **26(2)**:170–173.
- Cassin, M. H., M. L. Anna, C. D. Ewen, R. William, and R. S. McColl.** 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. *Int. J. Food Microbiol.* **41(1)**: 21–44.
- CDC.** 2006. Multistate Outbreak of *E. coli* O157 Infections Linked to Taco Bell. Available from <http://www.cdc.gov/ecoli/>. (Accessed 2007 June 12).
- Cody, S. H., M. K. Glynn, J. A. Farrar, K. L. Cairns, P. M. Griffin, J. Kobayashi, M. Fyfe, R. Hoffman, A. S. King, J. H. Lewis, B. Swaminathan, R. G. Bryant, and D. J. Vugia.** 1999. An Outbreak of *Escherichia coli* O157:H7 Infection from Unpasteurized Commercial Apple Juice. *Intern. Med.* **130**: 202–209.
- Cunin, P., T. Etienne, G. Yves, N. Chouaïbou, B. Raymond, M. Jacques, and M. V. M. Paul.** 1999. An Epidemic of Bloody Diarrhea: *Escherichia coli* O157 Emerging in Cameroon? *Emerg. Infect. Dis.*; **5(2)**: 285–290.
- Dadie, A., T. Karou, N. Adom, A. Kette, and M. Dosso.** 2000. Isolation of enteric pathogenic agents in Cote d' Ivoire: *Escherichia coli* O157:H7 and enteroaggregative *Escherichia coli*. *Bull Soc of Pathol Exot.*; **93**: 95 – 96.
- Department of Health, EC.** 2001. HIV and Syphilis survey Provincial Report. Epidemiological Notes. Available from: <http://www.ecdoh.gov.za/uploads/files/>. Accessed May, 17th 2007.

- Donnenberg, M. S. and T. S. Whitam.** 2001. Pathogenesis and evolution of virulence in enteropathogenic and enterohemorrhagic *Escherichia coli*. *The J. of Clin. Invest.*; **107(5)**: 539–548.
- Dontorou, C., C. Papadopoulou, G. Filioussis et al.** 2003. Isolation of *Escherichia coli* O157:H7 from foods in Greece. *Int. J. Food Microbiol.*; **82**: 273–279.
- Duffell, E., E. Espiè, T. Nichols, G. Adak, H. De Valk, and J. Stuart.** 2003. Investigation of an outbreak of *E. coli* O157 infections associated with a trip to France of schoolchildren from Somerset, England. *Eur. Surv.*; **8(4)**: 81–86.
- Effler, E., M. Isaacson, L. Arntzen, R. Heenan, P. Canter, and T. Barrett.** 2001. Factors contributing to the emergence of *Escherichia coli* O157:H7 in Africa. *Emerg. Infect. Dis.* **7**: 812–819.
- Escherich, T.** 1885. Die Darmbakterien des Neugeborenen und des Säuglings. *Fortschritte der Med.*; **3**: 515–522, 547–554.
- Faiella-Tommasino, J., and H. Reigert.** 2002. *Escherichia coli* O157:H7—An Emerging bacterial threat. *Clin. Rev.*; **12**: 47–53.
- Fatoki, O. S., N. Y. O. Muyima, and N. Lujima.** 2001. Situation analysis of water quality in the Umtata river catchment. *Water, SA.*; **27(4)**: 467–473.
- Feng, P.** 2000. *Escherichia coli* serotype O157:H7: novel vehicles of infection and emergence of phenotypic variants. *Emerg. Infect. Dis.* **1**: 9.
- Friedman, M. S., T. Roels, J. E. Koehler, L. Feldman, L., Bibb, W. F. and P. Blake.** 1999. *Escherichia coli* O157:H7 outbreak associated with an improperly chlorinated swimming pool. *Clin. Infect. Dis.*; **29**: 298–303.

- Galane, P. M and M. Le Roux.** 2001. Molecular Epidemiology of *Escherichia coli* isolated from young South Africa children with diarrheal diseases. *J. Health Popl. Nutr.* **19(1):** 311–318
- Gale.** 2001. Developments in microbiological risk assessment for drinking water. *J. Appl. Microbiol.* **91:** 191–205.
- Gansheroff, L. J. and A. D. O'Brien.** 2000. *Escherichia coli* O157:H7 in beef cattle presented for slaughter in the U.S.: Higher prevalence rates than previously estimated. Proceedings of the National Academy of Sciences USA **97:** 2959 –2961.
- Germani, Y., B. Soro, M. Vohito, O. Morel, and J. Morvan.** 1997. Enterohaemorrhagic *Escherichia coli* in Central African Republic. *Lancet*; **349:** 1670.
- Germani, Y., Cunin, P., Tedjouka, E., Bou-Neharre, C., Morvan, J. and P. Martin.** 1998. Enterohaemorrhagic *Escherichia coli* in Ngoila (Cameroon) during an outbreak of bloody diarrhoea. *Lancet*; **352:** 625–626.
- Griffin, P. M.** 1995. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*. In “Infections of the Gastrointestinal Tract. M.J. Blaser, P.D. Smith, J. I. Ravdin, H. B. Greenberg, and R. L. Guerrant, eds. Raven Press Ltd., New York. Pg. 739–761.
- Haas, C. N., J. Rose, and C. Gerba.** 1999. *Quantitative microbial risk assessment*, John Wiley & Sons
- Hansen, T. B. and S. Knochel.** 2003 Image analysis method for evaluation of specific and non-specific hand contamination. *J. Appl. Microbiol.*; **94:** 483–494.
- Harris, L. J.** 2001. *Escherichia coli*. Available from: <http://www.vetmed.ucdavis.edu/>
Accessed 05th June 2007.

- Hayes, C. E. Elliot, E. Krales, D. Goulda.** 2003. Food and Water Safety for Persons Infected with Human Immunodeficiency Virus. *Clin. Infect. Dis.*; **36(S2)**: S106–S109.
- Helderberg, C. W., K. L. MacDonald, and M. T. Osterholm.** 1994. Changing epidemiology of food-borne disease: a Minnesota perspective. *Clin. Infect. Dis.*; **18**: 671– 682.
- Helena J. N. and P. Steyn.** 2002. Report on South African food consumption studies undertaken amongst different population groups (1983-2000): Average intakes of foods most commonly consumed. Available from: <http://www.doh.gov.za/docs/reports/2002/>. Accessed April 16th 2007.
- Hoffman, E. W.** 2004. Evaluation of food safety education materials for persons with HIV/AIDS. Thesis p (1 - 165). Washington State University, 2004. Department of Food Science and Human Nutrition, Washington State University.
- Hubbard, R. K., G. L. Newton, and G. M. Hill.** 2004. Water quality and the grazing animal. *J. Animal Sci.*; **82**: E255–E263
- Ihekweazu, C., M. Barlow, S. Roberts, H. Christensen, B. Guttridge, D. Lewis.** 2006. Outbreak of *E. coli* O157 infection in the south west of the UK: risks from streams crossing seaside beaches. *Euro. Surveill.*; **11(4)**: 128–130
Institute of Food and Agricultural Sciences, University of Florida.
- Isaacson, M., P. H. Canter, P. E. Effler, L. Arntzen, P. Bomans, and R. Heenon.** 1993. Haemorrhagic colitis epidemic in Africa. *Lancet*; **341**: 961.
- Jackson, L. A., W. E. Keene, J. M. McAnulty, E. R. Alexander, M. Diermayer, M. A. Davis, K. Wedberg, J. Boase, T. J. Barrett, M. Samadpour, and D. W. Fleming.** 2000. Where's the beef? The role of cross-contamination in 4 chain restaurant-

- associated outbreaks of *Escherichia coli* O157:H7 in the Pacific Northwest. *Arch. Intern. Med.*; **160**: 2380–2385
- Jay, L. S., D. Comar and L.D. Govenlock.** 1999. A video study of Australian domestic food-handling practices, *J. Food Prot.*; **62**: 1285–1296.
- Johannessen, G. S., G. B. Bengtsson, B. H. Heier, S. Bredholt, Wasteson, Y. and L. M. Rørvik.** 2005. Potential uptake of *Escherichia coli* O157:H7 from organic manure into crisphead lettuce. *Appl. Environ. Microbiol.*; **71**: 2221–2225.
- Johnson, K, N. Marano, R. Howard, T. Root et al.** 2000. NARMS Working Group. The antimicrobial resistance patterns of *Escherichia coli* O157:H7 - NARMS, 1996- 1999. 2nd International Conference on Emerging Infectious Diseases. Atlanta, GA, July 2000
- Ju-Yeon Y., M. Bo-Mi O. In-Gyeong, B. Byeong-Kirl, L. Li-Guang, K. Byeong-Su, D. S. Barry, and H. L. John.** 2006. Antimicrobial resistance of *Escherichia coli* O157 from cattle in Korea. *Int. J. Food Microbiol.*; **106(1)**: 74–78
- Kaddu-Mulindwa, D. H., T. Aisu, K. Gleier, S. Zimmermann, and L. Beutin.** 2001. Occurrence of shiga -toxin-producing *Escherichia coli* in faecal samples from children with diarrhoea and from healthy Zebu cattle in Uganda. *Int. J. Food Microbiol.*; **66**: 95–101.
- Kaper, J. B., J. P. Nataro, and H. L. T. Mobley.** 2004. Pathogenic *Escherichia coli*. *Nature Rev.*; **2**: 123 – 140.
- Kapikian, A. Z.** 2000. The discovery of the 27-nm Norwalk virus: an historic perspective. *J. Infect. Dis.*; **181**: S295–302.
- Kassenborg, H. D. W. H. Craig, H. Michael, C. E. Mary, E. C. Arthur, M. Ruthanne, J. V. Duc, S. Kirk, D. A. Shama, S. Laurence, and M. G. Patricia.** 2004. Farm Visits

- and Undercooked Hamburgers as Major Risk Factors for Sporadic *Escherichia coli* O157:H7 Infection: Data from a Case - Control Study in 5 FoodNet Sites. *Clin. Infect. Dis.*; **38**: 271–278.
- Keene, M. E., J. M. McAnulty, F. C. Hoesly, L. P. Jr. Williams, K Hedberg, G. L. Oxman, T. J. Barrett, M. A. Pfaller, and D. W. Fleming.** 1994. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. *New Eng. J. Med.*; **331(9)**: 579–584
- Keene, W. E.** 1999. Lessons from investigations of food borne disease outbreaks. *JAMA*; **281**: 1845–1847
- Koyange, L., G. Ollivier, J. J. Muyembe, B. Kebela, M. Gouali, and Y. Germani.** 2004. Enterohemorrhagic *Escherichia coli* O157, Kinshasa. *Emerg. Infect. Dis.* Available from: <http://www.cdc.gov/ncidod/EID>. Accessed 17th February 2007
- Law, D. and J. Kelly.** 1995. Use of heme and hemoglobin by *Escherichia coli* O157 and other shiga-like-toxin-producing *Escherichia coli* serogroups. *J. Infect. Immun.*; **63**: 700–702.
- Magwira C.A., B. A. Gashe, and E. K. Collison.** 2005. Prevalence and antibiotic resistance profiles of *Escherichia coli* O157:H7 in beef products from retail outlets in Gaborone, Botswana. *J. Food Prot.*; **68 (2)**: 403–406.
- Mashood, A. R., U. Minga, and R. Machangu.** 2006. Current epidemiological status of enterohaemorrhagic *Escherichia coli* O157:H7 in Africa. *J. Chin. Med.*; **119 (3)**: 217–222.

- Mathuthu, A. S., M. Zaranyika, S. Tafirenyika, and B. Chibanda.** 1995. Impact assessment of sewage effluent discharges on the quality of the receiving lower Mukuvisi River in Harare, Zimbabwe. *J. Environ. Sci. Health.*; **30**: 281–297.
- Mermin J. H. and P. M. Griffin.** 1999. Public health in crisis: outbreaks of *Escherichia coli* O157:H7 infections in Japan. *Am. J. Epidemiol.*; **150(8)**: 797–803
- MMWR.** 1981a. Kaposi's sarcoma and pneumocystis pneumonia among homosexual men—New York City and California. *MMWR.*; **30**: 305–308
- MMWR.** 1981b. Pneumocystis pneumonia in Los Angeles. *MMWR.*; **30**: 250–252
- Momba, M. N. B., and T. L. Notshe.** 2003. The effect of long storage and household containers on the microbiological quality of drinking water in rural communities of South Africa. *J. Water Supp. Res. Techno.-AQUA.*; **52 (1)**: 67–76
- Momba, M. N. B., V. K. Malakate, and J. Theron.** 2006a. Abundance of pathogenic *Escherichia coli*, *Salmonella typhimurium* and *Vibrio cholerae* in Nkonkobe drinking water sources. *J. Water Health*; **04**: 289–296.
- Momba, M. N. B., Z. Tyafa, N. Makala, B. M. Brouckaert, and C. L. Obi.** 2006b. Safe drinking water still a dream in rural areas of South Africa. Case study: The Eastern Cape Province. *Water, SA*; **32**: 715–720.
- Monroe, S. S., T. Ando, and R. I. Glass.** 2000. Introduction: human enteric calicivirus—an emerging pathogen whose time has come. *J. Infect. Dis.*; **181**: S249–251.
- Morris, J. G. and M. Potter.** 1997. Emergence of new pathogens as a function of changes in host susceptibility. *Emerg. Infect. Dis.*; **3**: 435–441.

- Mukherjee, A., D. Speh, E. Dyck, and F. Diez-Gonzalez.** 2004. Pre-harvest evaluation of coliforms, *Escherichia coli*, *Salmonella* and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. *J. Food Prot.*; **67(5)**: 894–900
- Müller, E. E., Ehlers, M. M. and W. O. Grabow.** 2001. The occurrence of *Escherichia coli* O157:H7 in South African water sources intended for direct and indirect human consumption. *Water Res.*; **35**: 3085–3088.
- Müller, E. E., Grabow, W. and M. M. Ehlers.** 2003. Immunomagnetic separation of *Escherichia coli* O157:H7 from environmental and wastewater in South Africa. *Water, SA*; **29**: 427–432.
- Muyima, N. Y. O. and F. Ngcakani.** 1998. Indicator bacteria and regrowth potential of the drinking water in Alice, Eastern Cape. *Water, SA*; **24 (1)**: 29–34.
- Obi, C. L. and P. O. Bessong.** 2002. Diarrhoeagenic bacterial pathogens in HIV positive patients with diarrhea in rural communities of Limpopo Province, South Africa. *J. Health Pop. Nut.*; **20(3)**: 230–234.
- Obi, C. L. J. Ramalivhana, M. N. B Momba and J. Igumbor.** 2007b. Scope and frequency of enteric bacterial pathogens isolated from HIV/AIDS patients and their household drinking water in Limpopo Province. *Water, SA*; **33(4)**: 539–538
- Oie, S., I. Junko, S. Akihiro, T. Masaaki, and K. Akir.** 2004. *In vitro* bactericidal activity of antimicrobial agents against enterohaemorrhagic *Escherichia coli*. *J. Antimicrobial Chemo.*; **54(5)**: 897-903
- Olorunshola, I. D., S. I. Smith, and A. O. Cker, A. O.** 2000. Prevalence of EHEC O157:H7 in patients with diarrhoea in Lagos, Nigeria. *APMIS.*; **108**: 761–763.

- Paquet, C., W. Perea, P. Grimont, M. Collin, and M. Guillod.** 1993. Aetiology of haemorrhagic colitis epidemic in Africa. *Lancet*; **342**: 175
- Parry, S. M., R. L. Salmon, G. A. Willshaw, and T. Cheasty.** 1998. Risk factors for and prevention of sporadic infections with vero cytotoxin (shiga toxin) producing *Escherichia coli* O157. *Lancet*; **351**: 1019–1022.
- Phaswana-Mafunya N. and N. Shukla.** 2005. Factors that could motivate people to adopt safe hygienic practices in the Eastern Cape Province, South Africa. *SA Health Sci.*; **5(1)**: 21–28.
- Phaswana-Mafunya, N.** 2006. Hygiene status of rural communities in the Eastern Cape of South Africa. *Int. J. Environ. Health Res.*; **16(4)**: 289–03.
- Potter, M. E.** 1996. Risk assessment terms and definitions. *J. Food Prot. (Supplement)* S6–S9.
- Prior, R. and V. S. Jay.** 2000. Foodborne and waterborne infectious diseases: Contributing factors and solutions to new and reemerging pathogens. *The Pract. peer-Rev. J. Primary Care Phy.*; **107(4)**.
- Sang WK, Saidi SM, Yamamoto H, Ezaki T, Iida T, Yoh M, et al.** 1996. Haemorrhagic colitis due to *Escherichia coli* O157:H7 in Kenya. *J. Trop. Pediatr.*; **42**:118-119.
- Sapkota, D., P. Ghimire, and S. Manandhar.** 2004. Enteric parasitosis in patients with Human Immunodeficiency Virus (HIV) infections and Acquired Immunodeficiency Syndrome (AIDS) in Nepal. *J. Nepal Health Res. Council*; **2(1)**: 1 – 6.
- Smith, J. L. and P. M. Fratamico.** 1995. Factors involved in the emergence and persistence of food-borne diseases. *J. Food Prot.*; **58**:696–708.

- Smith, S. I., O. O. Aboaba, P. Odeigha et al.** 2003. Plasmid profile of *Escherichia coli* O157:H7 from apparently healthy animals. *African J. Biotech.*; **2**: 317–322.
- Söderström, A., A. Lindberg, and A. Yvonne.** 2005. EHEC O157 outbreak in Sweden from locally produced lettuce, August-September 2005. *Eurosurveillance weekly Releases*; 10(9). Accessed 12 June 2007. Available from <http://www.eurosurveillance.org>
- Solomon, E. B., S. Yaron, and K. R. Matthews.** 2002. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Appl. Environ. Microbiol.*; **68**: 397–400.
- Sooka, A., M. Du Plessis, and K. Keddy.** 2004. Enterovirulent *Escherichia coli*. *The Southern African J. Epidemiol. Infect.*; **19(1)**: 23 – 33.
- Sowetan News.** 2003. Waters for some. In the Sowetan newspaper of 21 January, 2003. Available at: http://queensu.ca/msp/pages/In_The_News/2003/January/3.htm. Accessed 5th September 2007.
- Su, C. and L. J. Brandt.** 1995. *Escherichia coli* O157:H7 infection in humans. *Ann. Int. Med.*; **123**: 698 – 714.
- Svenungsson, B. L. Åsa, E. Erik, E. Birgitta, O. H. Kjell, K. Anders, L. Sven, S. Lennart, and W. Andrej.** 2000. Enteropathogens in adult patients with diarrhea and healthy control subjects: A 1-year prospective study in a Swedish clinic for infectious diseases. *Clin. Infect. Dis.*; **30**: 770–778.
- Teal, C. J.** 2002. Antimicrobial resistance and the food chain. *J. Appl. Microbiol. Symp. Sup.*; **92**: S85–S89
- Teunis, P. T. Katsuhisa, and S. Kunihiro.** 2004. Dose response for infection by *Escherichia coli* O157:H7 from outbreak data. *Risk Anal.*; **24(2)**: 401–407.

- Thompson, K. M., D. E. Burmaster, and E. A. C. Crouch.** 1992. Monte Carlo techniques for quantitative uncertainty analysis in public health risk assessments. *Risk Analysis*; **12**: 53 – 63.
- Threlfall, E.J., L. R. Ward, J. A. Frost, and G. A. Willshaw.** 2000. Spread of resistance from food animals to man – the UK experience. *Acta Vet. Scandinavica Supp.*; **93**: 63–69.
- Todar, K.** 2002. Online Textbook of Bacteriology: available on line at www.textbookofbacteriology.net
- Torres, A. G., X. Zhou, and J. B. Kaper.** 2005. Adherence of diarrheagenic *Escherichia coli* strains to epithelial cells. *Infect. Imm.*; **73**: 18 – 29.
- Tsuboi, I., H. Ida, E. Yoshikawa et al.** 1998. Antibiotic susceptibility of enterohemorrhagic *Escherichia coli* O157:H7 isolated from an outbreak in Japan in 1996. *Ant. Agents Chem.*; **42**, 431–432.
- UNDP (United Nations Development Programme).** 2006. Human Development Report. Beyond scarcity: Power, poverty and the global water crisis. Pp 1 – 440. Palgrave Macmillan, New York. ISBN 0-230-50058-7.
- Vogt, R. L, and L. Dippold.** 2005. *Escherichia coli* O157:H7 outbreak associated with consumption of ground beef, June-July 2002. *Public Health Rep.*; **120(2)**: 174–178
- Vose, D.** 1996. Quantitative risk analysis: a guide to Monte Carlo simulation modelling. John Wiley & Sons, Inc. New York: USA.
- Watanabe, Y., O. Kotaro, H. Jonathan et al.** 1999. Factory Outbreak of *Escherichia coli* O157:H7 Infection in Japan. *Emerg. Infect. Dis.*; **5(3)**: 424–428.

- WHO.** 1997a. Foodborne diseases possibly 350 times more frequent than reported. Geneva, Switzerland.
- Zamxaka, M., G. Pironcheva, and N. Y. O. Muyima.** 2004. Microbiological and physicochemical assessment of the quality of domestic water resources in selected rural communities of the eastern Cape Province. *Water, SA*; **30(3)**: 333–340.
- Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D. G. White, D. Wagner, and J. Meng.** 2001. Prevalence of *Campylobacter*, spp, *Escherichia coli* and *Salmonella serovars* in retail chicken, turkey, pork, and beef from the Greater Washington, DC area. *Appl. Environ. Microbiol.*; **67(12)**: 5431–5436.

CHAPTER 2

TABLE OF CONTENTS	Page №
Table of contents.....	28
List of Tables.....	34
List of Figures	35
Background of the study.....	36
2.1 Introduction.....	36
2.2 Pathogenic <i>E. coli</i>: A Disease Causing Agent.....	38
2.2.1 Classification of <i>E. coli</i>.....	38
2.2.1.1 <i>Enterotoxigenic E. coli (ETEC)</i>	38
2.2.1.2 <i>Enteroinvasive E. coli (EIEC)</i>	39
2.2.1.3 <i>Enteropathogenic E. coli (EPEC)</i>	40
2.2.1.4 <i>Enterobaggative E. coli (EAggEC)</i>	41
2.2.1.5 <i>Enterohemorrhagic E. coli (EHEC)</i>	42
2.2.2 Microbiological characteristics of <i>E. coli</i> O157:H7 and other	
<i>E. coli</i> strains	44
2.2.2.1 <i>Biochemical characteristics of <i>E. coli</i></i>	44
2.2.2.2 <i>Acid tolerance of <i>E. coli</i> O157:H7</i>	49
2.2.2.3 <i>Resistance of <i>E. coli</i> O157:H7 to antimicrobial</i>	
<i>Agents</i>	51
2.2.2.4 <i>Thermal inactivation of <i>E. coli</i> O157:H7</i>	51

2.2.3	Pathogenecity of <i>E. coli</i> O157:H7.....	52
2.2.3.1	<i>Shigatoxins of <i>E. coli</i> O157:H7.....</i>	52
2.2.3.2	<i>Attaching and effacing by <i>E. coli</i> O157:H7.....</i>	52
2.2.3.3	<i>Infectious dose of <i>E. coli</i> O157:H7.....</i>	53
2.2.4	Factors affecting growth of <i>E. coli</i> O157:H7.....	53
2.2.4.1	<i>Temperature.....</i>	53
2.2.4.2	<i>pH.....</i>	54
2.2.4.3	<i>Water activity (a_w).....</i>	55
2.2.4.4	<i>Nutrients.....</i>	55
2.2.4.5	<i>Background microflora.....</i>	56
2.2.4.6	<i>Salinity.....</i>	57
2.2.4.7	<i>Irradiation.....</i>	57
2.3	Sources and routes of <i>E. coli</i> O157:H7 transmission to Human	58
2.3.1	Water.....	58
2.3.1.1	<i>Drinking and recreational waters.....</i>	58
2.3.1.2	<i>Sewage and irrigation water</i>	60
2.3.2	Meat and meat products.....	61
2.3.2.1	<i>Bovine and Bovine products.....</i>	61
2.3.2.2	<i>Undercooked ground meat and meat products.....</i>	62
2.3.2.3	<i>Milk and milk products.....</i>	62
2.3.2.4	<i>Other farm animals.....</i>	62
2.3.3	Fruits and vegetables and their products.....	64
2.3.4	Other sources of transmission.....	65

2.3.4.1	<i>Wild animals.....</i>	65
2.3.4.2	<i>Humans.....</i>	66
2.4	Direct Impact of water and food contaminated with <i>E. coli</i> O157:H7	
	on public health.....	67
2.4.1	Haemolytic uremic syndrome (HUS) and Haemolytic colitis	
	(HC).....	67
2.4.2	Gastrointestinal illness caused by <i>E. coli</i> O157:H7.....	67
2.4.3	Disease burdens of <i>E. coli</i> O157:H7.....	68
2.4.3.1	<i>Hospitalizations and deaths due to <i>E. coli</i></i>	
	<i>O157:H7 in other parts of the world</i>	68
2.4.3.2	<i>Hospitalizations and deaths due to <i>E. coli</i></i>	
	<i>O157:H7 in Sub-Saharan Africa.....</i>	69
2.5	Link between contaminated water/food and HIV/AIDS Diarrheic	
	Conditions.....	71
2.5.1	HIV as the etiological agent of AIDS.....	72
2.5.2	General considerations of HIV and AIDS worldwide.....	73
2.5.3	HIV/AIDS epidemiology in South Africa.....	78
2.5.3.1	<i>HIV/AIDS related deaths in South Africa.....</i>	82
2.5.4	HIV/AIDS prevalence in the Eastern Cape Province between	
	1997 and 2000.....	83
2.5.4.1	<i>Prevalence by region.....</i>	83
2.5.4.2	<i>A cross age groups among antenatal clients.....</i>	84
2.5.4.3	<i>Among men and women.....</i>	85

2.5.4.4	<i>By level of education.....</i>	86
2.5.5	Incidence of HIV/AIDS in the Eastern Cape in 2006.....	87
2.5.6	Susceptibility of HIV/AIDS patients to water and food borne illnesses of <i>E. coli</i> O157: H7 and of other pathogenic organisms.....	88
2.5.7	Direct and indirect impact of water, and food contaminated with <i>Escherichia coli</i> O157:H7 and other bacteria causing diarrhoeal diseases on HIV/AIDS patients.....	89
2.5.7.1	<i>Escherichia coli</i> O157:H7 infections and diarrhoea in HIV/AIDS patients.....	89
2.5.7.2	Economic Implications of water/food borne infections on HIV/AIDS patients and the public	91
2.5.7.3	Hotel and catering Industry.....	92
2.6	Control of the spread of <i>E. coli</i> O157:H7 and protection of public Health.....	92
2.6.1	Protection of water sources from <i>E. coli</i> O157:H7 Contamination.....	93
2.6.1.1	<i>Protection of surface water sources.....</i>	93
2.6.1.2	<i>Ground water protection.....</i>	99
2.6.1.3	<i>Protection of water in the distribution system.....</i>	101
2.6.1.4	<i>Protection of water at household level.....</i>	104
2.6.2	Control of <i>E. coli</i> O157:H7 food borne outbreak.....	108
2.6.2.1	<i>On-farm control.....</i>	109
2.6.2.2	<i>Use of antibiotics.....</i>	109

2.6.2.3	<i>Vaccination.....</i>	110
2.6.2.4	<i>Use of bacteriophages.....</i>	111
2.6.2.5	<i>Slaughtering and dressing.....</i>	112
2.6.2.6	<i>Control of E. coli O157:H7 during food Transportation.....</i>	114
2.6.2.7	<i>Protection at retail levels.....</i>	115
2.6.2.8	<i>Personal Hygiene.....</i>	116
2.6.2.9	<i>Food preparation, cooking and serving.....</i>	117
2.6.2.10	<i>Handling of leftover foods.....</i>	118
2.6.2.11	<i>Ionizing radiation.....</i>	118
2.6.2.12	<i>Handling of refuse.....</i>	118
2.6.2.13	<i>Sanitation facilities.....</i>	119
2.6.2.14	<i>Exclusion.....</i>	119
2.6.3	Education and hygiene.....	120
2.6.4	Hazard analysis and critical control point	122
2.7	Techniques used for the Study of E. coli O157:H7 in Clinical, Food and Environmental Samples.....	125
2.7.1	Conventional methods for detection and isolation of E. coli O157:H7.....	125
2.7.1.1	<i>Culture-based techniques.....</i>	126
2.7.1.2	<i>Immunoassays.....</i>	128
2.7.1.3	<i>Resuscitation and identification of E. coli O157:H7.....</i>	130
2.7.1.4	<i>Serological tests.....</i>	130

2.7.2	Molecular diagnostic methods for <i>E. coli</i> O157:H7.....	131
2.7.2.1	<i>Nucleic acid sequence based analysis.....</i>	132
2.7.2.2	<i>Polymerase Chain Reaction (PCR).....</i>	139
2.7.3	Amplified Fragment Length Polymorphism (AFLP).....	143
2.7.4	Pulse Field Gel Electrophoresis.....	144
2.8	Techniques of Assessing Risk Associated with <i>E. coli</i> O157:H7 in	
	Drinking Water and Foods.....	145
2.8.1	Quantitative risk assessment steps.....	146
2.8.1.1	<i>Hazard identification.....</i>	147
2.8.1.2	<i>Exposure assessment.....</i>	147
2.8.1.3	<i>Dose-response analysis.....</i>	148
2.8.1.4	<i>Risk characterization.....</i>	149
	References.....	152

LIST OF TABLES

Table 2.1:	Food vehicles implicated in outbreaks of <i>E. coli</i> O157:H7 in the United States.....	65
Table 2.2:	Largest <i>E. coli</i> O157:H7 outbreaks in some of the developed countries.....	69
Table 2.3:	Summary of <i>E. coli</i> O157:H7 outbreak cases in the African continent Since 1990.....	70
Table 2.4:	World epidemic of HIV/AIDS as at end of 2006.....	76
Table 2.5:	Regional statistics for HIV & AIDS, end of 2006.....	77
Table 2.6:	Estimated HIV prevalence among South Africans by age and gender.....	79
Table 2.7:	Estimated HIV prevalence among antenatal clinic attendees, by age.....	80
Table 2.8:	Estimated HIV prevalence among South Africans aged 2 years and older, by sex and race.....	81
Table 2.9:	Estimated HIV prevalence among South Africans aged 2 years and older by province.....	82
Table 2.10:	Effects of plating media on the percentages of <i>E. coli</i> O157:H7 positive samples detected and the 50% detection limit of each media formulation.....	131

LIST OF FIGURES

Figure 2.1:	Global trends of HIV/AIDS.....	74
Figure 2.2:	HIV prevalence among antenatal clients per region in Eastern Cape Province.....	84
Figure 2.3:	HIV prevalence in different age groups among antenatal clients in the Eastern Cape Province since 1997 – 2000.....	85
Figure 2.4:	Estimated level of HIV infection among men and women in different age groups in the Eastern Cape Province by 2001.....	86
Figure 2.5:	HIV prevalence by level of education by the year 2000.....	87
Figure 2.6:	Generic HACCP plan for beef slaughter and fabrication.....	125

CHAPTER 2

BACKGROUND OF THE STUDY

2.1 Introduction

Microbial contaminants of water sources and food products are the largest and the most immediate health hazard (Hayes *et al.*, 2003; Kivaria *et al.*, 2006, Momba *et al.*, 2006a, b). The presence of microorganisms in water and food causes a heavy burden of disease worldwide, especially in developing countries (Prüss *et al.*, 2002). One of such water and food contaminating microorganisms are *E. coli*.

Escherichia coli are the most predominant species of facultative anaerobes in the human gut and are usually considered harmless to the host; however, a group of pathogenic *E. coli* has emerged that causes diseases in humans (Riley *et al.*, 1983; Levine, 1987; Pupo, 1997; Nataro and Kaper, 1998). Pathogenic *E. coli* are classified based on their unique virulence and antigenic factors and can only be identified by these traits. The present review focuses on water and food borne pathogenic *E. coli* with a special interest on *E. coli* O157:H7, which is the strain of *E. coli* that is mostly implicated in water and food borne diseases outbreaks.

The review takes into account the classification, microbiological as well as biochemical characteristics of this group of *E. coli*. The pathogenicity and factors influencing the growth of *E. coli* O157:H7 are also discussed. Sources of its transmission such as water, meat and meat products, and vegetables as well as other possible routes of transmission are outlined in section 2.3. Section 2.5 of the review gives a brief account on the link between contaminated water and foods and diarrhoeic conditions in HIV/AIDS patients. The section is introduced by an outline of

the history of HIV/AIDS since its discovery in 1981 and an overview of HIV as an etiological agent of AIDS. The global picture of HIV/AIDS and the current situation of the pandemic in South Africa, particularly in the Eastern Cape Province are discussed in sub sections 2.5.2. The section further explores the susceptibility of HIV/AIDS patients to water and food borne illnesses. It discusses the possible direct and indirect impact of water and foods contaminated with *E. coli* O157:H7 on HIV/AIDS patients in subsection 2.5.6.

To protect the public from water and food borne *E. coli* O157:H7, there must be interventions at strategic points in the distribution of waters and foods to the consumer. Several studies have proposed the multiple barrier approach system to protect water contamination from the source up to the household level (Momba and Brouckaert, 2005; Nath *et al.*, 2006). Hazard analysis and critical control points is the baseline in protecting water and foods from contamination (NACMCF, 1998, 1999; Hulebak and Sclosser, 2002; Quinn and Marriott, 2002).

Control measures aimed at curbing the spread of *E. coli* O157:H7 and protection of the public health are discussed in section 2.6. The section is divided into two subsection 2.6.1 and 2.6.2. Sub section 2.6.1 discusses the protection of water sources starting with surface waters from the catchment up to the household level. Subsection 2.6.2 tackles the strategies employed in protecting foods from the field to the table and briefly discusses personal hygiene and food handling practices in the kitchen.

Different cultural and molecular techniques used in the isolation and identification of *E. coli* O157: H7 from clinical, environmental and food samples are described in section 2.7 of the chapter. The chapter concludes by reviewing the risk assessment techniques in section 2.8.

2.2 Pathogenic *E. coli*: A Disease Causing Agent

Pathogenic *E. coli* that cause diseases in humans are either water or food borne (Riley *et al.*, 1983; Su and Brandt, 1995; Nataro and Kaper, 1998). These bacteria are classified based on the virulence properties exhibited by each sub group. The six sub groups currently known include: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Levine, 1987; Nataro and Kaper, 1998). In this section, emphasis will be on each of the groups, especially on enterohemorrhagic *E. coli* O157:H7.

2.2.1 Classification of *E. coli*

2.2.1.1 Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic *E. coli* have adhesins in the form of fimbriae, which are species-specific (Karen, 1992). The fimbrial adhesins help them to adhere to specific receptors on enterocytes of the proximal small intestine (Karen, 1992; Todar, 2002). They produce enterotoxins referred to as LT (heat-labile) toxin and/or the ST (heat-stable) toxin (Levine, 1987; Nataro and Kaper, 1998). These are genes, which are responsible for the encoding of the toxins, which may occur on the same or separate plasmids (Nataro and Kaper, 1998). The LT enterotoxin is very similar to cholera toxin in both structure and mode of action (Slutsker *et al.*, 1997). The LT (heat-labile) enterotoxin binds to the same identical ganglioside receptors that are also recognized by the cholera toxin (i.e. GM1). The enzymatic activity is also said to be identical to that of the cholera toxin (Nataro and Kaper, 1998).

The ST enterotoxin is actually a family of toxins, which are peptides and are of molecular weight of about 2000 daltons (Nataro and Kaper, 1998). These toxins cause an increase in cyclic guanosine monophosphate (cGMP) in host cell cytoplasm causing an increase in cyclic adenosine monophosphate (cAMP) (Levine, 1987). The toxins act by binding to a guanylate cyclase that is located on the apical membranes of host cells making the enzyme to be activated. This kind of reaction leads to secretion of fluid and electrolytes, which eventually leads to diarrhoea in the infected patients (Nataro and Kaper, 1998). The well-known symptom of ETEC infections is diarrhoea without fever. The bacteria colonize the gastrointestinal tract (GI) by means of a fimbrial adhesion, however, they are non-invasive, but produce either the LT or ST toxin.

Enterotoxigenic *E. coli* are an important cause of diarrhoea in infants and travellers in underdeveloped countries (Michael *et al.*, 1997). The disease varies from minor discomfort to a severe cholera-like syndrome. This group of *E. coli* is acquired by ingestion of contaminated foods and water. Adults in endemic areas have been reported to develop immunity (Nataro and Kaper, 1998). The disease requires colonization and elaboration of one or more enterotoxins, which are plasmid-encoded (Levine, 1987; Nataro and Kaper, 1998; Todar, 2002).

2.2.1.2 *Enteroinvasive E. coli (EIEC)*

This group of *E. coli* apparently lack fimbrial adhesins; however, they do possess specific adhesins that is thought to be an outer membrane protein as in *Shigella*. Similarly, like *Shigella*, they are invasive bacterium. However, they neither produce LT nor ST toxin as well as shiga toxins (Nataro and Kaper, 1998). Nevertheless, enteroinvasive *E. coli* (EIEC) closely resembles *Shigella* in their pathogenic mechanisms and the kind of clinical illness they cause (Michael *et*

al., 1997). They penetrate and multiply within the epithelial cells of the host's colon causing widespread cell destruction (Todar, 2002). The clinical syndrome is identical to that of *Shigella dysenteriae* and includes dysentery-like diarrhoea accompanied with fever (Michael *et al.*, 1997; Donnenberg and Whittam, 2001; Todar, 2002).

2.2.1.3 Enteropathogenic *E. coli* (EPEC)

Enteropathogenic *E. coli* (EPEC) was the first group of *E. coli* strains recognized as pathogens following serological studies comparing strains cultured from outbreaks of neonatal diarrhoea with other strains isolated from healthy infants (Nataro and Kaper, 1998; Donnenberg and Whittam, 2001). Although such outbreaks are now rare in developed countries, this strain continues to be the leading cause of diarrhoea among infants from developing countries (Nataro and Kaper, 1998; Donnenberg and Whittam, 2001).

The pathogenesis of this group of *E. coli* has proved to be agreeable to genetic analysis, and several themes have emerged, reports Nataro and Kaper (1998). Enteropathogenic *E. coli* have an exacting pattern of adherence to tissue culture cells, which is allied with the presence of a large adherence factor (EAF) plasmid (Jerse *et al.*, 1990; Nataro and Kaper, 1998).

Enteropathogenic *E. coli* induces watery diarrhoea similar to that of enterotoxigenic *E. coli* only that they do not possess the same colonization factors and do not produce ST or LT toxins (Michael *et al.*, 1997). However, they produce a non-fimbrial adhesin designated intimin that mediates the final stages of adherence (Nataro and Kaper, 1998). Even though, EPEC do not produce LT or ST toxins, it has been reported that EPEC produce an enterotoxin similar to that of *Shigella*. This suggests that other virulence factors may be associated to those found in *Shigella* (Michael *et al.*, 1997; Nataro and Kaper, 1998).

In other studies, it has been established that adherence of EPEC to the intestinal mucosa is a complex process and may produce dramatic effects in the ultra-structure of the cells resulting in reorganization of actins in the vicinity of adherent bacteria (Donnenberg and Whittam, 2001). This process is called "attaching and effacing" of cells (Nataro and Kaper, 1998; Donnenberg and Whittam, 2001). Enteropathogenic *E. coli* are said to be "moderately-invasive" meaning they are not as invasive as *Shigella*, and unlike ETEC or Enteroaggregative *E. coli* (EAggEC), they cause an inflammatory response (Nataro and Kaper, 1998).

Diarrhoea and other symptoms of infections of these strains of *E. coli* probably are caused by bacterial invasion of host cells and interference with normal cellular signal transductions rather than by production of toxins (Donnenberg and Whittam, 2001). Some types of EPEC are referred to as Enteroadherent *E. coli* (EAEC) or diffuse adherent *E. coli* (DAEC) based on specific patterns of adherence (Michael *et al.*, 1997, Donnenberg and Whittam, 2001). Enteropathogenic *E. coli* have been documented as important causes of traveller's diarrhoea in Mexico and in North Africa (Michael *et al.*, 1997; Nataro and Kaper, 1998). This group of *E. coli* have been reported in HIV/AIDS patients with chronic diarrhoea in Bangui Central Republic of Africa (Mossoro *et al.*, 2002).

2.2.1.4 *Enteroaggregative E. coli (EAggEC)*

The distinguishing features of these strains are their ability to attach to tissue culture cells in an aggregative manner (Levine, 1987; Michael *et al.*, 1997). These strains are associated with persistent diarrhoea in young children (Levine, 1987; Michael *et al.*, 1997, Nataro and Kaper, 1998). They resemble enterotoxigenic *E. coli* (ETEC) strains in that they adhere to the intestinal mucosa and cause non-bloody diarrhoea without invading or causing inflammation (Levine,

1987; Michael *et al.*, 1997). This in itself suggests that the organisms produce a toxin of some sort.

A distinctive heat-labile plasmid-encoded toxin called the EnteroAggregative ST (EAST) toxin has been isolated from these strains (Levine, 1987). Enteroaggregative *E. coli* also produce a haemolysin related to the haemolysin produced by *E. coli* strains involved in urinary tract infections. However, the roles of the EnteroAggregative ST (EAST) toxin and the haemolysin gene in virulence have not been established (Levine, 1987; Nataro and Kaper, 1998). The significance of EAaggEC strains in human disease is controversial though they are also armed with a thin fimbriae, which is approximately 2 to 4 nm that they use to bind tissue matrix proteins (Levine, 1987).

2.2.1.5 *Enterohemorrhagic E. coli (EHEC)*

Enterohemorrhagic *E. coli* are considered “moderately invasive” though nothing is known concerning their colonization antigens, however, fimbriae are presumed to be involved. The bacteria do not invade mucosal cells as readily as *Shigella*, *Yersinia* or *Aeromonas*, which are armed with fimbriae (Levine, 1987; Nataro and Kaper, 1998). Nevertheless, infection of human intestinal epithelial cells by these bacteria is said to activate the enzyme mitogen activated protein (MAP) kinase (p38 and ERK 1/2) and NF κ B signaling pathways, both of which are required for the induction of an intestinal epithelial cell pro-inflammatory response (Berine *et al.*, 2002).

Enterohemorrhagic *E. coli* (EHEC) are represented by a single serotype of *E. coli* called *E. coli* O157:H7, which causes diarrhoeal set of symptoms discrete from that of enteroinvasive *E. coli* (EIEC) and *Shigella* in that there is copious bloody discharge without fever (Michael *et*

al., 1997). *Escherichia coli* O157:H7 is a gram-negative rod-shaped bacterium. The letter "O" in the name refers to the somatic antigen number, whereas the "H" refers to the flagella antigen (Nataro and Kaper, 1998). *Escherichia coli* O157:H7 serotypes apparently arose because of horizontal gene transfer of virulence factors (Whitam, 1988)

According to Kassenborg *et al.* (2000) and other authors (Prier *et al.*, 2000; Gansheroff and O'Brien, 2000; Donnenberg *et al.*, 2001; Todar, 2002) these *E. coli* strains have been recognized as a cause of serious diseases often linked to eating of inadequately cooked hamburger meat. Pediatric diarrhoea caused by these strains can be fatal due to acute kidney failure (Galane and Le Roux, 2000).

The permutation of letters and numbers in the name of *E. coli* O157:H7 refers to the specific markers found on these bacteria's cell surface; these letters and numbers tell between the dangerous O157:H7 variety from other types of *E. coli*. The virulence of *E. coli* O157:H7 is due to its ability to produce Shiga-like toxins, or verotoxins (Wells *et al.*, 1991). Shiga-like toxins hinder protein synthesis in eukaryotic cells and play a role in hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS). The toxin is also said to accelerate this by causing damage to endothelial cells in the kidneys, pancreas, brain, and other organs, thus inhibiting the ability of these organs to function (Nataro and Kaper, 1998; Paton and Paton, 1998; Donnenberg and Whittam, 2001).

The pathogenicity of *E. coli* O157:H7 is discussed in section 2.2.3 whereas section 2.7 discusses the conventional and molecular diagnostic techniques used for the isolation and detection of *E. coli* O157:H7 in clinical, environmental and food samples.

2.2.2 Microbiological characteristics of *E. coli* O157:H7 and other *E. coli* strains

Characterization of *E. coli* O157:H7 involves the application of conventional indole-methyl red-Voges-Proskauer-citrate and lysine decarboxylation tests (Radu *et al.*, 1998). Triple Sugar Iron reactions and identification by use of API 20E test kits are also among the reactions used in the characterization of *E. coli* O157:H7 (Radu *et al.*, 1998; Guyon *et al.*, 2001). The clonal nature of serotype *E. coli* O157:H7 has facilitated its phenotypic identification using specific O157 and H7 anti-sera (Feng, 1995; Guyon *et al.*, 2001).

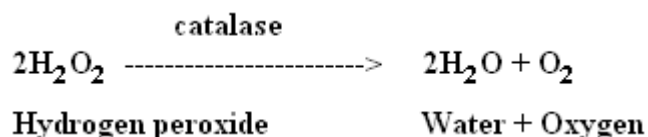
2.2.2.1 Biochemical characteristics of *E. coli*

Gram stain – Gram staining is an empirical method of differentiating bacterial species into two large groups: Gram-positive and Gram-negative based on the chemical and physical properties of their cell walls (Holt *et al.*, 1994).

The method is named after its inventor, the Danish scientist Hans Christian Gram who developed the technique in 1884 to discriminate between pneumococci and *Klebsiella pneumoniae* bacteria (Gram, 1884). All strains of *E. coli* are gram-negative short rods (bacilli) however; cultures of *E. coli* that are more than 24 h old may appear as cocci when viewed under the microscope (Bettelheim, 1994, Prescott *et al.*, 1996).

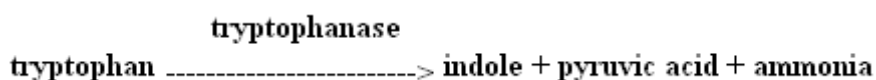
Catalase reaction for E. coli – Some bacteria can reduce diatomic oxygen to hydrogen peroxide or superoxide (Alberts *et al.*, 2002). Both of these molecules are toxic to bacterial cells. *Escherichia coli* are examples of bacteria, which uses two enzymes namely catalase and hydrogen peroxidase to catalyze the conversion of hydrogen peroxide and superoxide back into diatomic oxygen and water (Soomro *et al.*, 2002). The catalase test involves the addition of hydrogen peroxide to a culture sample or agar slant. The evolution of gas causes formation of

bubbles and is indicative of a positive test (Soomro *et al.*, 2002). A chemical equation of a catalase reaction is shown below.



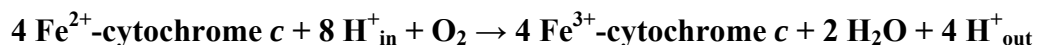
Breakdown of tryptophan by E. coli – The ability to degrade amino acids to identifiable products is often used to differentiate among bacteria (Ikeda, 2002). *Escherichia coli* hydrolyse tryptophan to indole, pyruvic acid and ammonia by use of tryptophan synthase. The pyruvic acid can be further metabolized to produce large amounts of energy. The ammonia is then available for use in the synthesis of new amino acids.

To identify strains of *E. coli* such as *E. coli* O157:H7, an indole test is always performed (Alexandre *et al.*, 2000; Soomro *et al.*, 2002). The test involves the use of a tube of tryptone broth that is inoculated with a 24 h old presumptive *E. coli* O157 culture and incubated for 48 h at 37 °C. The production of a distinct red coloured upper layer of the broth-culture on addition of 0.2 - 0.3 ml of Kovacs' reagent indicates a positive *E. coli* O157:H7 (Soomro *et al.*, 2002). This particular test has been used by most researchers to confirm for *E. coli* O157:H7 (Heizmann *et al.*, 1988). A chemical equation showing the production of indole is shown below.



Oxidase production by E. coli – Oxidase test is used to determine if a bacterium produces cytochrome c oxidases. It involves the use of disks impregnated with a reagent such as

N,N,N',N'-Tetramethyl-*p*-phenylenediamine (Prescott, 1996). Cytochrome oxidase, in the presence of oxygen, oxidizes the para-amino dimethylalanine oxidase reagent to form a rose-colored indophenol. Cytochrome oxidase helps in the transfer of electrons to oxygen in some electron transport chains.



Thus, the enzyme oxidizes reduced cytochrome c to make this transfer of energy possible. *E. coli* are oxidase negative bacteria and so do not oxidize the para-amino dimethylalanine oxidase. In this case, no colour change would be detected, hence confirming that the test is negative for *E. coli* (Health Protection Agency, 2004).

Fermentation of simple sugars – Escherichia coli are known to ferment simple sugars like glucose and lactose resulting in the production of an acid and liberation of gaseous products. *Escherichia coli* O157:H7 rapidly ferments lactose and is indistinguishable from most other *E. coli* on traditional lactose-containing media. However, unlike approximately 80% of other *E. coli*, *E. coli* O157:H7 do not ferment D-sorbitol in 24 h at 37 °C (March and Ratnam, 1986; Feng, 1995).

The failure to ferment D-sorbitol is relatively rare among other strains of *E. coli* and so is extremely useful in differentiating *E. coli* O157:H7 from other *E. coli* strains (Feng, 1995). Strains of *E. coli* of non-H7 serotype that are not pathogenic and do not ferment sorbitol, have occasionally been isolated from foods (Alexandre *et al.*, 2000). Due to the presence of phenotypically similar species, sorbitol negative isolates are therefore serologically confirmed

with O157 and H7 antisera (Feng, 1995). Most strains of *E. coli* are not able to ferment complex carbohydrate compounds such as starch. This is evidenced by the lack of zone of clearance upon addition of mercuric chloride on colonies of *E. coli* grown in media containing starch as the only source of carbon. This is because most *E. coli* strains are amylase negative (Holt *et al.*, 1994).

Voges-Proskauer-Methyl Red (VP-MR) test for E. coli – MR-VP media is primarily used for the identification of enteric bacteria like *E. coli*, *Shigella* and *Salmonella spp.* and *Enterobacter aerogenes* (Prescott *et al.*, 1996). MR-VP test is used to determine the pathway a given organism uses to ferment glucose; to this end, reagents such as methyl red (a pH indicator) and alpha-naphthol and potassium hydroxide are added to detect end products or intermediates produced by a given fermentation pathway (Holt *et al.*, 1994; Prescott *et al.*, 1996). The MR-VP broth contains dextrose as the carbohydrate source. Some coliforms ferment simple sugars like glucose and lactose to acidic end products that eventually causes the pH to drop below 5. After incubation the addition of methyl red, a dye, which turns red below pH 4.4, will indicate a positive MR reaction. Other coliforms will convert the sugars to less acidic products such as ethanol or butanediol. These bacteria are negative for the methyl red test (Holt *et al.*, 1994).

Butanediol fermentation is demonstrated by the Voges-Proskauer test, which measures the presence of acetoin (acetyl methyl carbinol), a precursor to butanediol. This test uses the same medium as the methyl red test and both tests are usually performed in parallel. Barritt's reagents, alpha-naphthol and potassium hydroxide, are added to a 48 h old culture and the tube is shaken to aerate the solution. The development of a pink or red colour after agitation is a positive reaction for the production of acetoin. *Escherichia coli* (including *E. coli* O157:H7) have been shown to be MR positive but VP negative (Holt *et al.*, 1994; Prescott *et al.*, 1996).

Citrate reduction – The ability to metabolize citrate is also useful for differentiating among enterobacteriaceae. Simmons Citrate Agar is a medium containing citrate as the sole carbon source and ammonium salts as the sole nitrogen sources. Organisms that metabolize citrate utilize the ammonium salts, releases ammonia and resulting in an increase in the pH of the medium (Holt *et al.*, 1994; Prescott *et al.*, 1996). Bromo thymol blue is present in the medium as the indicator dye. *Escherichia coli* are citrate negative and when grown in Simmons Citrate Agar does not change the agar from green to blue (Holt *et al.*, 1994).

Motility test for E. coli – Bacterial motility can be observed directly from motility agar tubes following incubation (Shelton *et al.*, 2002, Prescott *et al.*, 1996). Growth spreads out from the line of inoculation if the organism is motile. Highly motile organisms show growth throughout the tube (Farmer, 1999, Ware *et al.*, 2000). Growth of non-motile organisms only occurs along the stab line. *Escherichia coli* and more specifically serotype O157:H7 are highly motile and will show turbidity throughout the tube. This is due to the possession of motility antigens such as the flagella antigen by these organisms (Holt *et al.*, 1994).

Serological tests – Serological technique is often employed to identify the antigenic properties such as the flagella H and the somatic O-antigens that are possessed by some strains of *E. coli*. For instance, to identify *E. coli* O157:H7, their ability to agglutinate in O157 antiserum is always tested using slide, tube or latex agglutination test (Rice *et al.*, 1992). During this test, it is important to perform the appropriate control for auto agglutination. There have been reports of false positive agglutination test results for *E. coli* O157:H7 due to other sorbitol-negative species such as *E. hermannii* (Chart *et al.*, 1989; Rice *et al.*, 1992).

MUG test for E. coli – MUG means methylumbelliferyl-beta-D-glucuronide. There are certain strains of *E. coli* that produce beta-glucuronidase and are thus MUG positive, however, most *E. coli* O157 strains are MUG negative (Doyle and Schoeni, 1984; Thompson *et al.*, 1990). In a study to identify verocytotoxin-positive strains of *E. coli* serotype O157, Thompson *et al.* (1990) established that 156 *E. coli* O157:H7 and 10 *E. coli* O157: H– isolates were MUG negative. According to these authors, MUG test was much dependent on verotoxin production by the *E. coli* O157 isolates. They concluded that *E. coli* strains that do not produce verotoxins are MUG positive whereas those that produce verotoxins like *E. coli* O157:H7 are MUG negative.

2.2.2.2 *Acid tolerance of E. coli O157:H7*

The hydrogen ion concentration (H^+) is an important qualitative phenomenon that governs the growth of many microorganisms (Conner and Kotrola, 1995; Presser *et al.*, 1997). It has been reported that the pH responses of microorganisms consists of a plateau of constant growth rates over a range near the optimal and a more rapid decline in the growth rate as the pH is reduced until no growth can be observed (Conner and Kotrola, 1995; Cheville *et al.*, 1996).

The above observation leads to a conclusion that the growth rate of microorganisms is directly proportional to hydrogen ion concentration (H^+). For instance, *E. coli* O157:H7 has been found to grow on foods with pH of greater than 4 (Buchanan and Michael, 1997). Nevertheless, *E. coli* O157:H7 shows poor growth at pH of less than 4. For example, it exhibits low growth in mayonnaise, apple and cider juice as was reported by Buchanan and Michael (1997); Presser *et al.* (1997) and Garcia-Graells *et al.* (1998).

A high pH trisodium phosphate has been used to control *E. coli* O157:H7 in meat (Buchanan and Michael, 1997). This preservative agent has been reported to show a reduction on

the growth of *E. coli* O157:H7 in a similar manner to other organic acids (Buchanan and Michael, 1997).

2.2.2.3 *Resistance of E. coli O157:H7 to antimicrobial agents*

Escherichia coli O157:H7 does not appear to have any increased resistance to antimicrobial food additives (Buchanan and Michael, 1997). Previous studies established that this bacterium has higher susceptibility to most antibiotics (Tsuboi *et al.*, 1998; Johnson *et al.*, 2000). Cattle treated with neomycin sulfate showed reduced shedding of *E. coli* O157:H7 (Callaway *et al.*, 2003). However, antibiotics are not recommended for control of *E. coli* O157:H7 due to fear of release of toxins into the systems of patients (Wong *et al.*, 2000). It is also thought that treatment with some antibiotics may lead to kidney complications (Walterspiel *et al.*, 2003). Nonetheless, these bacteria have high sensitivity to disinfectants. For instance, the use of 2% sodium hypochlorite, 70% ethanol, phenolic or iodine-based disinfectants, glutaraldehyde and formaldehyde has demonstrated lethal effect on these bacteria (CFSPH, 2004).

2.2.2.4 *Thermal inactivation of E. coli O157:H7*

Escherichia coli have proteins that are often synthesized to counteract any increase in temperature (Cheville *et al.*, 1996). This phenomenon is regulated by *rpoS* genes, which are a sigma subunit of the RNA polymerase enzyme (Missiakas *et al.*, 1993; Cheville *et al.*, 1996). However, the expression of *rpoS* by *E. coli* O157:H7 have not been reported; consequently these bacteria are easily inactivated when foods are heated sufficiently enough at high temperatures (Cheville *et al.*, 1996; Buchanan and Michael, 1997). Moist heat at 121°C for at least 15 min or dry heat at 160 – 170 °C for at least 1 h is far much enough for inactivation of *E. coli* O157:H7

and therefore foods are always safe when cooked to a minimum temperature of 71°C (CFSPH, 2004).

2.2.3 Pathogenicity of *E. coli* O157:H7

The pathogenicity of *E. coli* O157:H7 is a function of its virulence factors (Nataro and Kaper, 1998). Among these virulence factors are a periplasmic catalase and shiga-like toxins. Shiga-like toxins are iron-regulated toxins that catalytically inactivate 28S ribosomal RNA subunits of eukaryotic cells blocking mRNA translation and causing cell death (Reisberg *et al.*, 1981).

Shiga-like toxins are functionally identical to toxins produced by virulent *Shigella* species (Reisberg *et al.*, 1981). Strains of *E. coli* that express shiga-like toxins gained this ability due to infection with a prophage containing the structural coding for the toxin. Non-shigatoxin producing strains may also become infected and produce shiga-like toxins after incubation with shiga toxin positive strains (O'Brien *et al.*, 1984; Strockbine *et al.*, 1986). The periplasmic catalase is encoded on the pO157 plasmid and is believed to be involved in virulence by providing additional oxidative protection when infecting the host (Brunner *et al.*, 1996).

2.2.3.1 Shigatoxins of *E. coli* O157:H7

Among the most important virulence characteristics of *E. coli* O157:H7 is its ability to produce one or more Shiga-like toxins called verocytotoxins (Law and Kelly, 1995; Nataro and Kaper, 1998; Donnenberg and Whittam, 2001). The term “verocytotoxin” refers to *E. coli* - derived toxins that are cytotoxic to vero cells (Donnenberg and Whittam, 2001). These Shiga like toxins (*Stx1* and *Stx2*), are believed to cause HUS and HC due to their systemic action on vascular epithelial cells (Donnenberg and Whittam, 2001; Björn-Arne *et al.*, 2003). The enterotoxigenic

effect of Stx on ileal loops is thought to be due to selective damage and loss of villus absorptive epithelial cells (Nataro and Kaper, 1998). Each toxin is composed of a single A subunit non-covalently associated with a pentamer composed of identical B subunits (Perna *et al.*, 2001). The B subunits bind specifically to globotriaacyl ceramide and related glycolipids on host cells (Perna *et al.*, 2001). The A subunit is taken up by endocytosis and transported to the endoplasmic reticulum of the host's cell (Donnenberg and Whittam, 2001; Perna *et al.*, 2001). It is believed that the toxin binds at a specific target on the 28S rRNA, which is A depurinated adenine residue, causing protein synthesis to cease and infected cells die by apoptosis (Reisberg *et al.*, 1981; Yoshida *et al.*, 1999; Donnenberg and Whittam, 2001).

It has been established that receptors for Stx are found on endothelial cells. Renal microvascular endothelial cells are particularly sensitive to the toxin (Donnenberg and Whittam, 2001; Perna *et al.*, 2001). According to Nataro and Kaper (1998), the toxin then enters the systemic circulation after being translocated across the intestinal epithelium. This eventually causes damage to the endothelial cells, which leads to activation of coagulation cascades, formation of microthrombic, intravascular haemolysis and ischemia (Law and Kelly, 1995; Donnenberg and Whittam, 2001).

2.2.3.2 *Attaching and effacing by E. coli O157:H7*

Escherichia coli O157:H7 can colonize the ceca and colon of infected hosts by an adhesion-effacing (AE) mechanism (Nataro and Kaper, 1998; Donnenberg and Whittam, 2001). The bacteria are believed to adhere closely to the mucosal cells of the large bowel, causing disruption of the brush border (Nataro and Kaper, 1998). The AE lesion is characterized by intimate

attachment of the bacteria to the intestinal cells with effacement of the underlying microvilli and accumulation of filamentous actins in the subjacent cytoplasm (Nataro and Kaper, 1998).

2.2.3.3 *Infectious dose of E. coli O157:H7*

Very low cell numbers of *E. coli* O157: H7 have been detected in foods and waters associated with disease outbreaks (Mead and Griffin, 1998, Müller *et al.*, 2003). *Escherichia coli* O157: H7 cell numbers isolated from foods and waters implicated in disease outbreaks have been reported to be as low as 10–100 CFU/g or CFU/ml of the food or water analyzed (Feng, 2000). This low numbers of cells are actually sufficient to cause clinical manifestations in a host (Mead and Griffin, 1998; Hawker *et al.*, 2001). Such very low infectious doses necessitate the need for more sensitive methods for the detection of these bacteria.

2.2.4 **Factors affecting growth of *E. coli* O157:H7**

Like all other bacteria the survival and growth of *E. coli* O157:H7 depend on the interaction between intrinsic and extrinsic factors (Buchanan and Michael, 1997). These factors include temperature, pH, water activity, salinity, nutrients and background micro flora (Buchanan and Michael, 1997). These factors are discussed in this section.

2.2.4.1 *Temperature*

Many *E. coli* O157:H7 isolates do not grow well, if at all, at temperatures above 51 °C (Doyle and Schoeni, 1984; Szabo *et al.*, 1986). These bacteria are differentiated from other *Enterobacteriaceae* based on their ability to grow and produce gas in *E. coli* broth at 44 °C (Buchanan and Michael, 1997). The optimal temperature for growth of *E. coli* O157:H7 is

approximately 37 °C (Doyle and Schoeni, 1984; Szabo *et al.*, 1986; Buchanan and Michael, 1997). However, the upper temperature for *E. coli* O157:H7 growth is culture medium – dependent (Buchanan and Michael, 1997).

Most strains of *E. coli* O157:H7 grow in Brain Heart Infusion (BHI) broth at 45 °C, as opposed to *E. coli* broth where it optimally grows at 44 °C (Buchanan and Michael, 1997). The minimum growth temperature for *E. coli* O157:H7 under optimal conditions is approximately 8–10°C. However, the organism can survive refrigeration temperature but show a decline in concentration (Buchanan and Michael, 1997).

2.2.4.2 *pH*

The pH of water and food products influences the survival of microorganisms (Abdul-Raouf, 1993; Presser *et al.*, 1997). The pKa of an acid and the pH of water or food determine the amount of dissociated and undissociated acid that will occur in the water or food product (Abdul-raouf, 1993; Presser *et al.*, 1997).

High levels of undissociated acid in water or food; leads to the acid being able to permeate the cell wall of the bacteria and become dissociated as a result of higher intracellular pH (Barbut and Griffiths, 2003). *Escherichia coli* O157:H7 has been reported by various researchers to grow at low pH values (Leyer *et al.*, 1995; Cheville *et al.*, 1996; Yuk and Marshall, 2004; Samelis *et al.*, 2005). This property is believed to be under the control of *rpoS*–regulated proteins, which are activated when, *E. coli* O157:H7 cells are subjected to low pH (Leyer *et al.*, 1995; Cheville *et al.*, 1996).

Low pH is also believed to induce an increase in saturated fatty acids like palmitic acid and reduces the concentration of unsaturated fatty acids such as *cis*–vaccenic acid in the bacterial

cell membranes (Brown *et al.*, 1997; Yuk and Marshall, 2004; Samelis *et al.*, 2005). However, this property is said to be dependent with other growth factors such as temperature and water activity (a_w) and that *E. coli* O157:H7 cells grown at temperatures equal to or less than 4 °C tend to be more acid resistant than cells grown at temperatures above 15 °C (Cheville *et al.*, 1996; Samelis *et al.*, 2005). Acid tolerance in *E. coli* is a complex phenomenon, both growth phase-dependent and inducible (Buchanan and Michael, 1997). *Escherichia coli* cells in the stationary phase of growth are substantially more acid tolerant than cells in the exponential phase. This increased tolerance is associated with expression of genes regulated by the *rpoS* sigma factor operon (Buchanan and Michael, 1997).

2.2.4.3 *Water activity (a_w)*

The intrinsic factors of a food product like water activity are very important in determining the product's shelf life and safety with respect to microbial quality. A reduced a_w affects bacterial cells by increasing the lag phase and decreasing the growth rate, resulting in a reduction in bacterial population (Barbut and Griffiths, 2003). The effect of water activity on the survival and growth of *E. coli* O157:H7 focuses primarily on the effect of humectants (Buchana and Michael, 1997). *Escherichia coli* O157:H7 can survive for many weeks when desiccated, particularly at refrigeration temperatures. Under ideal conditions, the minimum water activity for its growth is approximated at 0.98 (Buchana and Michael, 1997).

2.2.4.4 *Nutrients*

New selective media have been developed to increase the effectiveness of *E. coli* O157:H7 isolation (Jennifer *et al.*, 1998; Malihe and Kadir, 2000). The agar medium mostly used for the

isolation of *E. coli* O157:H7 is Sorbitol MacConkey Agar (SMAC agar) (Jennifer *et al.*, 1998). This medium contains 1% sorbitol in place of lactose in the standard MacConkey medium. Sorbitol-MacConkey agar plates are inoculated with the suspected *E. coli* O157:H7 positive specimen and examined after 18 to 24 h of incubation at 37°C for the presence of colourless, sorbitol-negative colonies (Jennifer *et al.*, 1998). A few sorbitol non-fermenting colonies may be selected for testing as potential *E. coli* O157:H7 (Malihe and Kadir, 2000). This aspect is further discussed in section 2.7.

2.2.4.5 *Background micro flora*

High levels of competing background micro flora always compound the difficulty of isolating *E. coli* O157:H7 (Ogden *et al.*, 2000). In a report by Stewart (1999), the survival of verocytotoxic *E. coli* serotype O157 in cattle dosed with commensal *E. coli* and *Proteus mirabilis* strains recovered from ruminant faeces was highly reduced. Bell *et al.* (1999) also reported that *Pseudomonas aeruginosa* from the rumen of sheep inhibited the growth of *E. coli* O157 *in vitro*. In their study, these authors argued that *P. aeruginosa* strains from different animals produced different bacteriocins (pyocins), which they suspected had an ecological role in determining the dominant biotype (Bell *et al.*, 1999; Stewart, 1999).

Two pigments from *P. aeruginosa* (pyocyanin and fluorescin) have also been shown to inhibit both commensal and *E. coli* serotype O157- strains (Stewart, 1999). It seems that some of the bacteria interacting directly with *E. coli* O157:H7 in the ruminant gut are relatively minor members of the gut micro biota (Stewart, 1999). Such bacteria may also influence the survival of *E. coli* in shed faeces and in the soil.

Bell (1999) and Stewart (1999) suggested that pyocyanin is presumably active if present in media exposed to air. In a separate experimental study on the effect of competitor micro flora on the growth kinetics of *E. coli* O157:H7; Duffy *et al.* (1999) noted that growing *E. coli* O157:H7 together with *Hafnia alvei* in the same growth media; lengthened lag phase for *E. coli* O157:H7. However, *H. alvei* had a much shorter lag phase; suggesting that *H. alvei* inhibited *E. coli* O157:H7 under similar growth conditions.

2.2.4.6 *Salinity*

Various researchers have reported the use of salts in foods as food preservative agents (Buchanan and Michael, 1997; Buchanan and Klawitter, 1992). Sodium benzoate used together with fumaric acid has served well in the preservation of apple cider against spoilage by *E. coli* O157:H7 (Buchanan and Michael, 1997). When this preservative mixture was added to apple cider, Buchana and Michael (1997) observed a 5 log₁₀ reduction in *E. coli* O157:H7 population.

The above treatment was also found to be very efficient by Chikthimmah *et al.* (2003) when the holding temperature of cider juice was increased from 25 °C to 35 °C. At 35 °C, no *E. coli* O157:H7 cells were recovered from the juice. Callaway *et al.* (2003) equally established that water supplemented with Sodium chlorate reduced *E. coli* O157:H7 population in the faecal shedding of test cattle.

2.2.4.7 *Irradiation*

Food safety can also be improved by food irradiation, a process whereby energy is applied to the food from a radioactive source (cobalt or cesium), electron beams, or x-rays (Lagunas-Solar, 1995). The chemical changes thus produced in the food impair the ability of microorganisms to

grow. The use of ionizing irradiation has been realized to eliminate or reduce contamination of foods by pathogens, such as *E. coli* O157:H7, *Salmonella* and *Campylobacter* spp. (Kim and Thayer, 1996).

Foods that are treated with low-level gamma radiation shows reduced levels of pathogenic *E. coli* O157:H7 (Buchanan and Michael, 1997; Zafer *et al.*, 2007). This technique works better when used in combination with refrigeration to inactivate *E. coli* O157:H7 in foods (Buchanan *et al.*, 1998). Buchanan *et al.* (1998) equally demonstrated that low dose irradiation could readily eliminate *E. coli* O157:H7 from fresh apple juice while maintaining the product at refrigeration temperatures (Buchanan *et al.*, 1998). Regrettably, Buchanan *et al.* (1998) reported that mutant strains of *E. coli* O157:H7 could occur in foods and this could jeopardize the efficacy of radiation. However, Thayer and Boyd (1993) concluded in their study that application of irradiation energy at doses ranging between 1.5 and 3.0 kGy at low temperatures was sufficient to inactivate *E. coli* O157:H7 cells.

2.3 Sources and routes of *Escherichia coli* O157:H7 transmission to human

2.3.1 Water

2.3.1.1 Drinking and recreational waters

There is a complex interaction of environmental and behavioural factors of both animals and humans, which facilitate the spread of *E. coli* O157:H7 infections. Poor sanitation and hygiene conditions as well as lack of or little environmental awareness among people is considered as a major cause of source water contamination (Zamxaka *et al.*, 2004; Phaswana-Mafuya, 2006). Example of such ways is agricultural practices that involve usage of sewage waters and/or cattle

manure on farms. Another practice is uncontrolled waste disposal, bathing and swimming in water sources such as rivers and dams, which serve as sources to municipal water supplies, answering calls of nature in velds and grazing of cattle next to catchment areas (Phaswana-Mafuya, 2006; WHO, 2006a).

Studies have reported that many rural communities in the Eastern Cape Province still lack access to treated drinking water and a sizeable number do not have any proper water storage facilities (Momba and Kaleni, 2003; Momba and Notshe, 2003; Phaswana-Mafuya, 2006). Some people dispose of wastewater and solid waste in very unsafe manners. A recent survey in the Eastern Cape Province established that most of the families have no toilet facilities; hence, relieve themselves in the veld (Phaswana-Mafuya, 2006). This kind of human behaviour is indeed a primary source of water contamination mostly to communities using surface water sources. This is in agreement with findings of Zamxaka *et al.* (2004), who reported that surface waters, which serve as sources to municipal water supplies for Nkonkobe municipality (within the Amathole District), was not suitable for human consumption due to high levels of microbial contaminants.

Other studies on potable water in the Eastern Cape Province of South Africa have demonstrated that, despite the efforts by the South African Government to provide potable water to the communities, mostly to those in the informal settlements, the region is still faced with poor drinking water quality (Momba *et al.*, 2004a, b; Momba *et al.*, 2006a, b). Life threatening microorganisms such as *Salmonella typhimurium*, *E. coli*, *Vibrio cholera* and *Shigella flexneri* have been isolated from the waters used for domestic purposes (Momba *et al.*, 2004a, b; Momba *et al.*, 2006b; Obi *et al.*, 2007a). A previous study by Momba *et al.* (2003a) in the Eastern Cape reported that household containers used for the storage of drinking waters also support re-growth

and survival of pathogenic microorganisms. This in a way sheds light on how drinking water can be contaminated not only at the point of treatment and distribution systems but also at household levels.

Swimming in pool waters is such a refreshing experience. However, such recreational waters have also caused *E. coli* O157:H7 outbreaks. An outbreak of *E. coli* O157:H7 due to contact with contaminated recreational waters was reported by Armstrong *et al.* (1996). Another outbreak of *E. coli* O157:H7 associated with swimming was in 1999 in Battle Ground Lake in Clark County, Washington. *Escherichia coli* O157:H7 was isolated from duck faeces, as well as from water samples from Battle Ground Lake. The isolates were confirmed to be *E. coli* O157:H7 using pulsed-field gel electrophoresis, which revealed that the isolates from the water samples and those of the patient had genetic homology (Washington State Department of Health, 2001). Swimming pool waters are contaminated with *E. coli* O157:H7 when persons who are carriers of the bacteria swim in the waters.

2.3.1.2 *Sewage and irrigation water*

Ingress of sewage-contaminated water through cracked pipes may result in high pathogen concentrations. The use of sewage water on the field can also lead to contamination of water catchment areas with disease causing pathogens. The origin and type of water used for irrigation are therefore risk factors (Easton *et al.*, 2005). In less developed areas like the Amathole District in the Eastern Cape Province, pollution from human settlements lacking appropriate sanitary infrastructure, partially treated or untreated wastewater or water from land fertilized with manure are the major pollution sources of source waters (Fatoki *et al.*, 2001; Momba and Mfenyana, 2005).

2.3.2 Meat and meat products

Escherichia coli O157:H7 have been isolated from cattle, their carcasses, hides and faeces (Brichta-Harhay *et al.*, 2007). A study in the UK showed that *E. coli* O157:H7 was isolated from 752 (15.7%) of 4,800 cattle (Chapman *et al.*, 1997a; FSAI, 1999). However, there is very limited information on the prevalence of *E. coli* O157:H7 in the animal population in Africa in general and South Africa in particular.

2.3.2.1 Bovine and bovine products

Studies on *E. coli* O157:H7 in bovine and bovine products have reported prevalence rates of 8% in adult cows (Wells *et al.*, 1991) and 15.7% (Chapman *et al.*, 1997a) and 1.8% in cattle herds (Hancock *et al.*, 1997). A study by Blanco *et al.* (1996) reported that the proportion of animals infected with *E. coli* O157:H7 varied from 0 - 60%. Elder *et al.* (2000) pointed out that the overall prevalence of *E. coli* O157 in cattle and their carcasses during processing was much higher. However, they predicted that reduction in carcass prevalence from pre-evisceration to post processing could be achieved if sanitary procedures are strictly followed (Elder *et al.*, 2000).

There is seasonal variation in *E. coli* O157:H7 shedding in cattle. Peak *E. coli* O157:H7 faecal shedding are reported to occur during summer and early fall, and vary from low to high, depending on the season (Edrington *et al.*, 2006). The number, frequency and timing of sampling, handling, transport and storage of samples, the type and age of cattle, the type of sample, the season of sampling and the unit of analysis may affect *E. coli* O157:H7 prevalence estimation (Van Donkersgoed *et al.*, 1999). However, no factors have been identified, other than season that consistently affects the *E. coli* O157:H7 shedding rates of cattle (Elder *et al.*, 2000).

2.3.2.2 *Undercooked ground meat and meat products*

Undercooked ground beef have been a kingpin in disease outbreaks of *E. coli* O157:H7 (Riley *et al.*, 1983; Armstrong *et al.*, 1996; Kassenborg *et al.*, 2004). Epidemiological data indicate that the prime risk foods of bovine origin are undercooked hamburgers (Reilly, 1998; Pennington, 1997). Such contaminated hamburgers or ground beef tend to possess a characteristic pink colour at the middle (Riley *et al.*, 1983). Different foods that act as vehicles in *E. coli* O157:H7 outbreaks are shown in Table 2.1. Processed foods such as yoghurt, cheese and fermented sausage have as well been involved in food-borne outbreaks caused by *E. coli* O157:H7 (Prier, 2000; Gansheroff and O'Brien, 2000).

2.3.2.3 *Milk and milk products*

The contamination of milk by *E. coli* O157:H7 is often suspected to occur during the milking process. Due to contamination of milk during its collection and/or processing, milk and milk products has been implicated in *E. coli* O157:H7 disease outbreaks (Karmali *et al.*, 1987; LCDC, 1987). The bacteria have been isolated from cheese sandwiches (Karmali *et al.*, 1987; Armstrong *et al.*, 1996; Kassenborg *et al.*, 2004). This has been possible most probably because the organism is acid tolerant and can grow under low acidic conditions (Conner and Kotrola, 1995).

2.3.2.4 *Other farm animals*

Escherichia coli O157:H7 have been detected in other farm animals. Horse have been reported to be carriers of *E. coli* O157:H7 (Chalmers *et al.*, 1997; Hancock *et al.*, 1998; Bauwens *et al.*, 2000; Pichner *et al.*, 2005). Other animals that have been reported to carry the bacteria include

rats (Cizek *et al.*, 1999), dogs (Beutin *et al.*, 1995; Kaufmann *et al.*, 2006), an opossum (Renter *et al.*, 2003) and cats and also a few zoo animals like an orangutan (Beutin *et al.*, 1996), monkeys and lemurs (Bauwens *et al.*, 2000).

Sheep are also significant source of *E. coli* O157:H7 and these bacteria have been detected in mutton (Chapman *et al.*, 2001; Barlow *et al.*, 2006). Monitoring of a flock of sheep revealed that the animals shed *E. coli* O157:H7 mostly during the summer seasons (Kudva *et al.*, 1997).

Goats have also been reported as reservoirs of *E. coli* O157:H7 (Beutin *et al.*, 1995; Dontorou *et al.*, 2004; Keen *et al.*, 2006). Crampin *et al.* (1999) reported the development of attaching and effacing lesions in the colon and recto-anal junction of goats following an oral dose of *E. coli* O157:H7 to the goats. Both wild and domestic rabbits have equally been reported to harbor *E. coli* O157:H7 (Pritchard *et al.*, 2001; Garcia and Fox, 2003; LeClercq and Mahillon, 2003).

Poultry meat sometimes carry *E. coli* O157:H7 on the skin surface and these bacteria have also been demonstrated to persist in the ceca of experimentally infected chicks (Schoeni and Doyle, 1994; Caprioli *et al.*, 2005). However, there are few reports on the isolation of *E. coli* O157:H7 in chicken and turkey feces (Heuvelink *et al.*, 1999; Pilipcinec *et al.*, 1999; Schouten *et al.*, 2005). Recently it was reported that 26 of 720 cloacae swab samples from living layer hens in Italy tested positive for *E. coli* O157:H7 (Dipineto *et al.*, 2006).

Escherichia coli O157:H7 have also been isolated from Swine (Johnsen *et al.*, 2001; Osek, 2004; Kijima-Tanaka *et al.*, 2005; Schouten *et al.*, 2005; Zweifel *et al.*, 2006). Experimentally infected pigs have been reported to shed these bacteria in their faeces (Cornick and Helgersson, 2004).

2.3.3 Fruits and vegetables and their products

The common type of fruits and vegetables that have always been implicated in *E. coli* O157:H7 outbreaks are fresh and ready to eat cut fruits and vegetables like tomatoes, lettuce, cucumber, onions and carrots, especially those used in salads (Abdul-Raouf *et al.*, 1993; Brooks *et al.*, 2004; Mukherjee *et al.*, 2004). There have also been numerous outbreaks of *E. coli* O157:H7 implicating apple juice (Cody *et al.*, 1999).

Most researchers have established that the commonest cause of *E. coli* O157:H7 on vegetables is the use of cattle manure or irrigation waters that are potentially contaminated with *E. coli* O157:H7 in the growing of fruits and vegetables (Patriquin, 2000; Solomon *et al.*, 2002; Johannessen *et al.*, 2005). Outbreaks of diseases caused by shiga toxigenic *Escherichia coli* have been associated with the consumption of leafy lettuce (Ackers *et al.*, 1998), potatoes (Chapman *et al.*, 1997b), radish sprouts (Michino *et al.*, 1999; Watanabe *et al.*, 1999) and alfalfa sprouts (Barrett *et al.*, 2001).

The rapid spread of *E. coli* O157:H7 infections because of consuming contaminated vegetables are because the microbial numbers increase tremendously during the process of cutting and slicing of the vegetables (Cassin *et al.*, 1998). The process of cutting and slicing, somehow, blow up the bacterial numbers to the tune of six and seven fold. The cases of *E. coli* O157:H7 on vegetables have been common mostly in salad bars (Brooks *et al.*, 2004). Various food vehicles implicated in outbreaks of *E. coli* O157:H7 in the United States is shown in Figure 2.1.

Table 2.1: Food vehicles implicated in outbreaks of *E. coli* O157:H7 in the United States
(FAO/WHO, 2004)

Vehicle	1998	1999	2000	2001	Total
Ground beef/hamburger	10	9	4	4	27
Roasted beef	0	2	0	1	3
Green leafy vegetables	4	7	1	1	13
Salad	1	1	1	1	4
Milk	2	0	0	0	2
Other	5	5	6	2	18
Total No of outbreaks	21	21	11	8	63

2.3.4 Other sources of transmission

2.3.4.1 Wild animals

Deers often graze from some environments that are used by cattle, sheep, and goats and so may be infected with *E. coli* O157:H7 from such environments (Cody *et al.*, 1999; Fischer *et al.*, 2001). Numerous studies in the U.S. and other countries have documented the presence of *E. coli* O157:H7 and other verotoxin-producing *E. coli* in wild deer (Asakura *et al.*, 1998; Sargeant *et al.*, 1999; Wasteson *et al.*, 1999; Fischer *et al.*, 2001; Lahti *et al.*, 2001; Rice *et al.*, 2003; Dunn *et al.*, 2004).

Birds are also thought to be possible transport hosts for *E. coli* O157:H7. For example, pigeons might spread these bacteria through their faecal droppings (Schmidt *et al.*, 2000; Morabito *et al.*, 2001; Grossmann *et al.*, 2005). Others birds that have been reported to harbour *E. coli* O157:H7 include gulls (Wallace *et al.*, 1997) and a rook (Ejidokun *et al.*, 2006). Experimentally infected pigeons have shed *E. coli* O157:H7 with their faecal droppings (Cizek *et al.*, 2000).

2.3.4.2 *Humans*

Individuals can acquire *E. coli* O157:H7 infections in many ways (Feng, 1995; CDC–MMWR, 2001). Person-to-person transmission have been reported in day care centres, nursing homes and in cases of close contact between family members (Griffin, 1995; Armstrong *et al.*, 1996; Koyange *et al.*, 2004).

Social gatherings in South Africa have not received adequate thoughts as possible occasions that can make possible the spread of *E. coli* O157:H7. In some of the occasions, people have contracted *E. coli* O157:H7 infections after consuming fast foods in social functions (Welinder-olsson *et al.*, 2004). People also involve themselves in close interactions in social gatherings, which may lead to transmission of the bacteria from one person to another. The structure of settlement in South Africa is another compounding factor to the transmission of water and food borne diseases.

2.4 Direct impact of water and food contaminated with *Escherichia coli* O157:H7 on public health

2.4.1 Haemolytic uremic syndrome (HUS) and Haemolytic colitis (HC)

The most serious complication of *E. coli* O157:H7 infection is haemolytic-uremic syndrome (HUS). This is a potentially life-threatening condition characterized by haemolytic anaemia, thrombotic thrombocytopenia purpura and renal failure (Cray and Moon, 1995). Thrombotic thrombocytopenia purpura and renal failure, conditions associated with *E. coli* O157:H7 infections, most commonly occurs in children and immune compromised persons.

The kidneys are the end organs most severely affected, but ischemic necrosis of the intestines, central nervous system (stroke), and to any other organ may occur (Nataro and Kaper, 1998; Paton and Paton, 1998). Typically, the illness begins with abdominal cramps and then diarrhoea, which is initially watery but becomes grossly bloody within 2 – 3 days (Mead *et al.*, 1999; CFSPH, 2004).

On approximation, 15% of persons with HUS die or develop chronic renal failures (Donnenberg and Whittam, 2001). The illness is usually self-limiting and lasts for an average of 8 days (CFSPH, 2004).

2.4.2 Gastrointestinal illness caused by *Escherichia coli* O157:H7

As a pathogen, *E. coli* O157:H7 is known for its ability to cause intestinal diseases (Michael *et al.*, 1997; Todar, 2002). *Escherichia coli* O157:H7 infection presents as mild non-bloody or severe bloody diarrhoea (hemorrhagic colitis) (Brady *et al.*, 1992; Nataro and Kaper, 1998; Donnenberg and Whittam, 2001).

Patients infected with *E. coli* O157:H7 also experience severe abdominal pain, with little or no fever (Brady *et al.*, 1992; Nataro and Kaper, 1998; Donnenberg and Whittam, 2001; CFSPH, 2004). Because of *E. coli* O157:H7 infections, paediatric patients are particularly at risk of developing strokes, pancreatitis, colonic perforations, hypertension and coma may be noticed (CFSPH, 2004). Toxins produced by *E. coli* O157:H7 mediate symptoms that have been shown to have cytotoxic effects on Vero cells (Brady *et al.*, 1992; Nataro and Kaper, 1998; Donnenberg and Whittam, 2001).

2.4.3 Disease burdens of *E. coli* O157:H7

2.4.3.1 Hospitalizations and deaths due to E. coli O157:H7 in other parts of the world

From 1982 to 2002, 350 outbreaks were reported from 49 states, accounting for 8,598 cases of *E. coli* O157 infection in the United States alone (Rangel *et al.*, 2005). Among these cases, there were 1,493 (17.4%) hospitalizations, 354 (4.1%) cases of HUS, and 40 (0.5%) deaths. An estimated 73,480 illnesses due to *E. coli* O157 infection cases are reported to occur each year in the United States, leading to an estimated 2,168 hospitalizations and 61 deaths annually (Mead *et al.*, 1999; Rangel *et al.*, 2005). A total of 38 cases of *E. coli* O157:H7 were reported in Oklahoma in 2005, resulting in an incidence rate of 1.10 cases per 100,000 populations (Anonymous, 2007a). Of the reported cases in Oklahoma, 12 (32%) were hospitalized, however, no deaths were reported (Anonymous, 2007a).

An outbreak of *E. coli* O157 infections was reported in the east of Scotland in 2006 whereby 13 cases were confirmed (NHS, 2006). Table 2.2 shows some of the largest *E. coli* O157:H7 outbreaks in some developed countries.

Table 2.2: Largest *E. coli* O157:H7 outbreaks in some of the developed countries
(Petridis *et al.*, 2002).

Year	Place	Nº of people sick	Source
1989	Montana, US	243	undercooked ground beef
1996	Sakai, Japan	5, 727	white radish sprout
1996	Scotland, UK	496	undercooked ground beef
2000	Walkerton, Canada	over 2000	contaminated drinking water
2002	Pennsylvania, US	51	infected dairy animals

2.4.3.2 Hospitalizations and deaths due to *E. coli* O157:H7 in sub-Saharan Africa

Surveillance of *E. coli* O157:H7 infection is well established in many developed countries and it may be apparent that there are geographical differences in the incidences of infection. However, many African countries do not routinely test for *E. coli* O157:H7, hence many infections may go unrecognized. This has created substantial gaps in knowledge about epidemiology of *E. coli* O157:H7 in developing countries.

Although reported outbreaks of *E. coli* O157: H7 in sub-Saharan Africa have been few, available information indicates the presence the pathogen within communities. *Escherichia coli* O157: H7 cases have been isolated in South Africa (Browning *et al.*, 1990; Galane and Le Roux,

2001); Swaziland (Isaacson *et al.*, 1993), Malawi and Mozambique (Paquet *et al.*, 1993); Central African Republic (Germani *et al.*, 1997) and Kenya (Sang *et al.*, 1996; Arimi, 2000); Nigeria (Olorunshola *et al.*, 2000) and Ivory Coast (Dadie *et al.*, 2000). In Zemio, a small village located on the Democratic Republic of Congo border witnessed outbreaks of bloody diarrhoea in 1996, which was attributed to *E. coli* O157. In December 1997, an epidemic of bloody diarrhoea due to *E. coli* O157:H7 was reported in Ngoïla. The outbreak resulted in the deaths of 11 patients (Germani *et al.*, 1998). Table 2.3 summarizes *E. coli* O157:H7 outbreaks in the African continent since 1990.

Table 2.3: Summary of *E. coli* O157:H7 outbreak cases in African continent since 1990

Year	Place	Nº of people sick	Source
1990	South Africa	1	unknown
1992	Swaziland	40912	beef and water
1992	Kenya	1	unknown
1997	South Africa	151	water
1998	Cameroon	298	water
2003	DRC	463	water
2003	Central African	463	zebu meat
2003	Nigeria	187	water

Information on the incidence, epidemiological risk factors, interaction of HIV/AIDS and *E. coli* O157:H7, seasonal variation, current state of resistance to antimicrobial agents by *E. coli* O157:H7 is profoundly lacking. Currently, there is no literature on *E. coli* O157:H7 infection in HIV/AIDS patients. It is clear that there will be increasing incidences of severe diseases, water and foodborne being some of the diseases, among HIV/AIDS patients due to their compromised immune systems. The following section discusses the possible link between the quality of water and food and the diarrhoeic conditions of HIV/AIDS individuals.

2.5 Link between contaminated water/food and HIV/AIDS diarrhoeic conditions

In 1981, Clinicians in the City of New York and California first observed an immunodeficiency syndrome among young, previously healthy homosexual men who had rare diseases such as Kaposi sarcoma, and opportunistic infections like *Pneumocystis carinii* pneumonia and unexplained persistent lymphadenopathy (MMWR, 1981a, b, Fauci, 2003; Hoffman, 2004). The term Acquired Immunodeficiency Syndrome (AIDS) was used to describe the condition. After several unclear diagnoses, investigators concluded that AIDS cases and their occurrence in diverse risk groups could be explained only if an infectious microorganism was responsible for causing the condition and that this organism was transmittable through sexual contact and/or through blood transfusion (MMWR, 1981a, b, Fauci, 2003; Hoffman, 2004).

In 1983, a French research team published experimental data that linked retrovirus and AIDS (Barre-Sinoussi *et al.*, 1983). A year later, the virus was isolated from some individuals with AIDS symptoms and from asymptomatic individuals at high risk for AIDS in the state of California (Levy *et al.*, 1984). In the same year (1984) some French and US researchers at the

U.S. National Institute of Health published virological and epidemiological evidence that AIDS was being caused by the virus now known as Human Immunodeficiency Virus (HIV), hence the acronym HIV/AIDS (Gallo, 2002; Montagnier, 2002). These series of scientific discoveries show the birth of the acronym HIV/AIDS, which means Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome.

2.5.1 HIV as the etiologic agent of AIDS

The pathogenic mechanisms of HIV infection are extremely complicated and multi-factorial (Fauci, 2003, Hoffman, 2004). HIV enters the body and binds to Langerhans or dendritic cells, which then carries the virus to CD4⁺ T cells (Fauci, 2003). The HIV virus has an envelope that contains two copies of RNA (Hoffman, 2004; Washington State Health Department, 2003). Through a surface glycoprotein, the virus binds to cellular receptors, most commonly CD4⁺ T cells in association with a chemokine receptor (Fauci, 2003; Hoffman, 2004).

Once the virus is inside the cytoplasm of the hosts' cell, the viral reverse transcriptase (RT) enzyme, along with other viral proteins converts the viral RNA into a double-stranded DNA molecule (Mandell *et al.*, 2000; Hoffman, 2004). To do this, the virus uses the viral RNA as a template to polymerize DNA and form RNA: DNA hybrid (Mandell *et al.*, 2000; Hoffman, 2004). Then the virus degrades the RNA with the RNase H activity associated with the enzyme (Mandell *et al.*, 2000; Hoffman, 2004).

The resulting single-stranded DNA is then used as a template to polymerize the second DNA strand (Mandell *et al.*, 2000; Hoffman, 2004). Once double-stranded viral DNA is formed, it is transported to the nucleus of the CD4⁺ T cell, where it is integrated into the cellular genome with the aid of other enzymes (Mandell *et al.*, 2000; Hoffman, 2004). After integration; HIV

DNA is then transcribed and translated using cellular transcription machinery and protein synthesis (Mandell *et al.*, 2000; Hoffman, 2004). HIV efficiency of transmission includes higher viremia in the infecting partner. Risk practices, which facilitate the spread of the virus include but are not limited to receptive anal intercourse, sex during menses, and the presence of other Sexually Transmitted Diseases (STD) (Mandell *et al.*, 2000; Hoffman, 2004).

AIDS is the manifestation of a clinical spectrum of illnesses caused by HIV (Mandell *et al.*, 2000; Hoffman, 2004). It is defined by the development of serious opportunistic infections or other life-threatening manifestations resulting from progressive HIV-induced immunosuppression (Mandell *et al.*, 2000; Lashley and Durham, 2002; Hoffman, 2004). The progression of an untreated HIV infection to AIDS takes a window period of on average 10 years (Lashley and Durham, 2002; Hoffman, 2004). However, in untreated HIV infection, ongoing viral replication generally leads to a rapid damage of the immune system, ultimately resulting in AIDS and eventual death (Mandell *et al.*, 2000; Hoffman, 2004). Use of antiretroviral therapy can prolong the lives of HIV positive individuals but may not completely stop the progression to AIDS (Hoffman, 2004).

2.5.2 General considerations of HIV and AIDS worldwide

Globally, HIV/AIDS is one of the most serious health problems of the 21st century generation. More than 25 million people have died of AIDS since 1981 (UNAIDS/WHO, 2006). The number of people living with HIV rose from around 8 million in 1990 to nearly 40 million by the end of 2005. Figure 2.1 shows the global trend of HIV/AIDS.

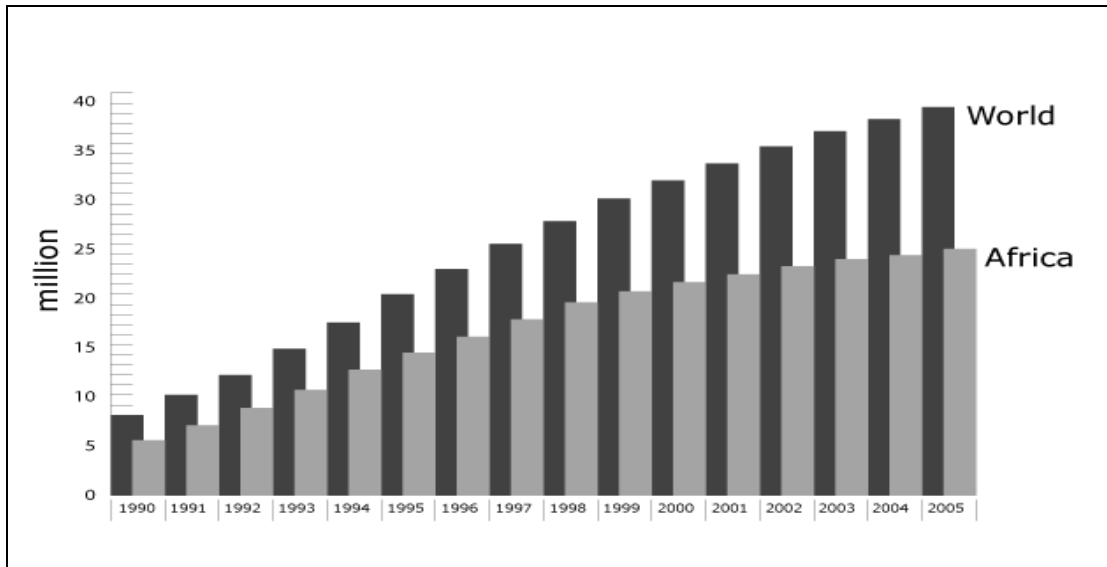


Figure 2.1: Global trends of HIV/AIDS

At the end of 2006, women accounted for 48% of all adults living with HIV worldwide with sub-Saharan Africa housing a staggering 59%. Young people under 25 years old have been reported as the most affected worldwide. Around 6,000 of young people become infected with HIV every day (UNAIDS/WHO, 2006). The situation is said to be even worse in developing countries where almost 7.1 million people are in immediate need of life-saving AIDS drugs. Of these, it has been reported that only 2.0 million (28%) are receiving the drugs (UNAIDS/WHO, 2006). Table 2.4 shows world epidemic of HIV/AIDS as at the end of 2006. Around 63% of people living with HIV are in sub-Saharan Africa. The estimated number of adults and children living with HIV/AIDS in Sub Saharan Africa is about 24.7 million with the number of deaths due to AIDS totalling to 2.1 million.

The total number of children orphaned due to AIDS was estimated at 12 million at the end of 2005 (UNAIDS/WHO, 2006). Table 2.5 shows the global regional statistics for HIV/AIDS as at the end of 2006. During 2006 around four million adults and children became infected with HIV and by the end of 2006, an estimated 39.5 million people worldwide were living with HIV/AIDS. The year also witnessed the death of around three million people due to HIV/AIDS despite recent improvements in access to antiretroviral treatment (UNAIDS/WHO, 2006).

Table 2.4: World epidemic of HIV/AIDS as at end of 2006 (UNAIDS/WHO, 2006).

Cases	Living with HIV/AIDS		New Infections	
	Estimate*	Range*	Estimate*	Range*
People (Adults + Children)	39.5	34.1–47.1	4.3	3.6–6.6
Adults (Male & Female)	37.2	32.1–44.5	3.8	3.2–5.7
Women	17.7	15.1–20.9	–	–
Children	2.3	1.7–3.5	0.53	0.41–0.66
<u>Reported Deaths</u>				
AIDS deaths			2.9	2.5–3.5
Adult AIDS			2.6	2.2–3.0
Child AIDS			0.38	0.29–0.50

*The figures are in millions

Table 2.5: Regional statistics for HIV & AIDS, end of 2006 (UNAIDS/WHO, 2006).

Region	Adults & Children living with HIV/AIDS	Adults & Children Newly infected	% Adult Prevalence	Death of Adults and Children
Sub-Saharan Africa	24.7	2.8	5.9%	2.1
South and South-East Asia	7.8	0.86	0.6%	0.59
North Africa & Middle East	0.46	0.068	0.2%	0.036
East Asia	0.75	0.1	0.1%	0.043
Oceania	0.081	0.007	0.4%	0.004
Latin America	1.7	0.14	0.5%	0.065
Caribbean	0.25	0.027	1.2%	0.019
Eastern Europe & Central Asia	1.7	0.27	0.9%	0.084
Western & Central Europe	0.74	0.022	0.3%	0.012
North America	1.4	0.043	0.8%	0.018
Global Total	39.5	4.3	1.0%	2.9

*Proportion of adults aged 15–49 who were living with HIV/AIDS. The figures are in millions

2.5.3 HIV/AIDS epidemiology in South Africa

Information regarding the prevalence of HIV in South Africa is characterised by contradictory reports from one study to another. However, an estimated 5.3 million South Africans were HIV-positive in 2003 (UNAIDS, 2004). In 2005, Human Sciences Research Council (HSRC) released updated information on HIV prevalence and HIV-related risk behaviours (HSRC, 2005). The study was based on a representative sample of more than 15,000 South Africans (HSRC, 2005). Of the study population, 10.8% were revealed to be HIV-positive (HSRC, 2005).

A separate study by the Department of Health (2005) reported that 30.2% of antenatal clinic attendees in South Africa were HIV positive. In the same year, the National HIV Survey in South Africa reported that 10.8% of South African population were HIV positive with 16.2% of those aged between 15 and 49 years suffering from the disease (Department of Health, 2005; SANHS, 2005).

As per the 2005 study by South African National HIV Survey, HIV prevalence was recorded the highest among females between 25 and 29 years old whereas among males, the peak was in the group aged 30 and 39 years. The estimated percentage of HIV prevalence among South Africans classified by age and gender is shown in Table 2.6 (SANHS, 2005).

Table 2.6: Estimated HIV prevalence among South Africans by age and gender
(SANHS, 2005).

<u>Age</u> (yrs)	<u>Prevalence in (%) by gender</u>	
	Male	Female
2-4	4.9	5.3
5-9	4.2	4.8
10-14	1.6	1.8
15-19	3.2	9.4
20-24	6.0	23.9
25-29	12.1	33.3
30-34	23.3	26.0
35-39	23.3	19.3
40-44	17.5	12.4
45-49	10.3	8.7
50-54	14.2	7.5
55-59	6.4	3.0
60+	4.0	3.7
Total	8.2	13.3

The study showed that males aged 15 to 49 years old had 58% chance of being infected as their female counterparts in the same age group (SANHS, 2005). Estimated HIV prevalence

among antenatal clinic attendees by age is shown in Table 2.7. Tables 2.8 and 2.9 show the estimated HIV prevalence among South Africans aged 2 years and older as at 2005 classified by sex and race (SANHS, 2005; Department of Health, 2005).

Table 2.7: Estimated HIV prevalence among antenatal clinic attendees, by age from the year 2000 to 2005 (Department of Health, 2005).

Age group	(%) prevalence					
	2000	2001	2002	2003	2004	2005
<20	16.1	15.4	14.8	15.8	16.1	15.9
20-24	29.1	28.4	29.1	30.3	30.8	30.6
25-29	30.6	31.4	34.5	35.4	38.5	39.5
30-34	23.3	25.6	29.5	30.9	34.4	36.4
35-39	15.8	19.3	19.8	23.4	24.5	28.0
40+	11.0	9.8	17.2	15.8	17.5	19.8

Table 2.8: Estimated HIV prevalence among South Africans aged 2 yrs and older, by sex and race (SANHS, 2005).

Sex and Race	Number of people surveyed	Prevalence %
Female	9,509	13.3
Male	6,342	8.2
African	9,950	13.3
Colored	3,382	1.9
Indian	1,319	1.6
White	1,173	0.6
National	15,851	10.8

By province, the study revealed that five out of the nine South African provinces had HIV/AIDS prevalence above 10%. The Kwa-Zulu Natal province had the highest prevalence (16.5%) followed closely by Mpumalanga (15.1%) whereas Free State North West and Gauteng had a prevalence of 12.6%, 10.9% and 10.8% respectively. The province that had the least prevalence of HIV/AIDS according to the survey was Western Cape (1.9%). Table 2.9 show the estimated HIV prevalence among South Africans aged 2 years and older by province (Department of Health, 2005).

Table 2.9: Estimated HIV prevalence among South Africans aged 2 years and older by province (SANHS, 2005).

Province	Number of people surveyed	Prevalence (%)
Kwa-Zulu Natal	2, 729	16.5
Mpumalanga	1, 224	15.2
Free State	1, 066	12.6
North West	1, 056	10.9
Gauteng	2, 430	10.8
Eastern Cape	2, 428	8.9
Limpopo	1, 570	8.0
Northern Cape	1, 144	5.4
Western Cape	2, 204	1.9
Total	15, 851	10.8

2.5.3.1 HIV/AIDS related deaths in South Africa

According to a study by the Medical Research Council (MRC) of South Africa in 2005, HIV related deaths were found to be 53,185 for men aged 15 to 59 years and 59,445 for women aged 15 to 59 years. Children under the age of 5 years old were 40,727 in the year 2000-2001 (Groenewald *et al.*, 2005). The recent death estimates in South Africa linked to HIV/AIDS is said to be 336,000 for the period between mid-2005 and 2006 (Washington Post, 2006).

However, a report by the Centre for Actuarial Research, South African Medical Research Council and Actuarial Society of South Africa, by use of a computer based model estimated that 345,640 people in South Africa died because of AIDS in 2006. Among adults aged 15 to 49 years, the Centre for Actuarial Research estimated that 71% of all the deaths were due to AIDS (Dorrington *et al.*, 2006) whereas UNAIDS/WHO estimated that AIDS claimed 320,000 lives in South Africa in 2005 (UNAIDS/WHO, 2006).

2.5.4 HIV/AIDS prevalence in the Eastern Cape Province between 1997 and 2000

2.5.4.1 *Prevalence by region*

Following antenatal HIV survey in 2000, Eastern Cape Province was estimated to be home to more than a half a million people living with HIV and that about 10, 500 HIV positive children were born in the Province in that 2000 (Department of Health, EC, 2001). The HIV prevalence rates were reported based on regions. The province was divided into five regions A: Port Elizabeth, B: Queenstown, C: East London, D: Umtata and E: Kokstad. Kokstad had the highest HIV prevalence (24%) whereas Queenstown had the lowest prevalence of 15.1%. Port Elizabeth was divided into two sub-regions. The urban-based prevalence for Port Elizabeth was 26% whereas the rural based prevalence was 7%. East London and Umtata had a prevalence of 17.8% and 22.2% respectively. Figure 2.2 shows HIV prevalence among antenatal clients per region in the Eastern Cape Province (Department of Health, EC, 2001).

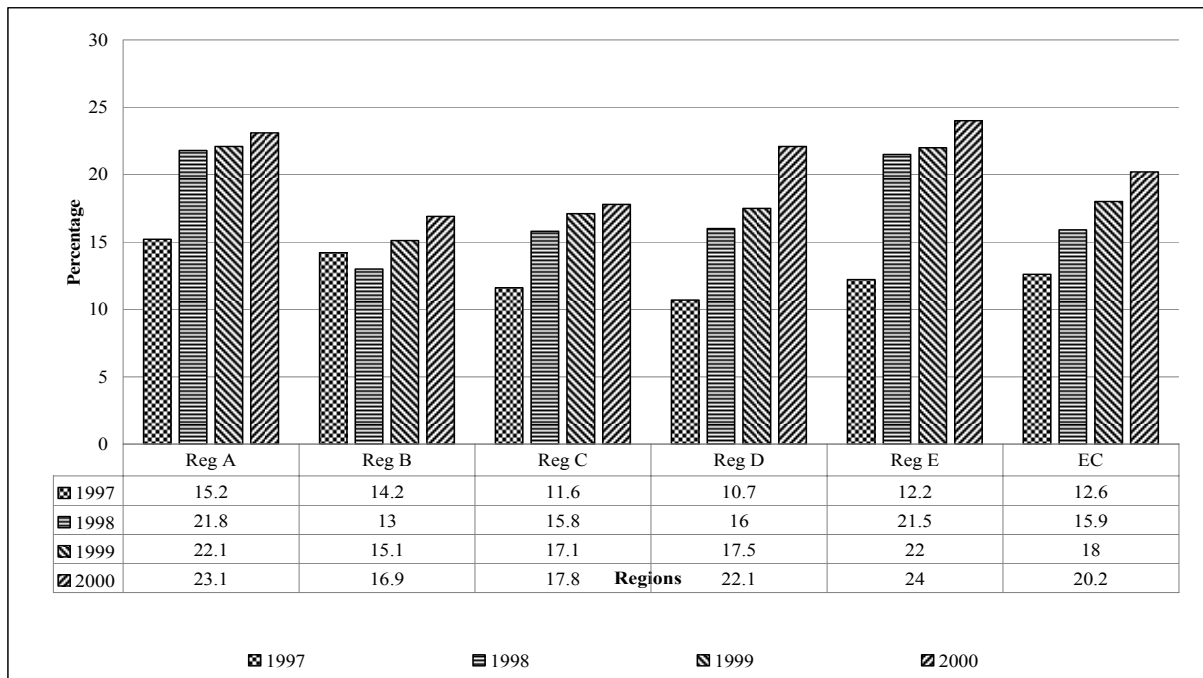


Figure 2.2: HIV prevalence among antenatal clients per region in Eastern Cape Province. Region A: Port Elizabeth, B: Queenstown, C: East London, D: Umtata, E: Kokstad and EC: Eastern Cape (Department of Health, EC, 2001).

2.5.4.2 *A cross age groups among antenatal clients*

The 2000 HIV prevalence survey in the Eastern Cape Province among different age groups of antenatal clinic attendees revealed highest prevalence of HIV among pregnant women in the age group of 20 and 24 at 25.9% followed by the age group of 25 and 29 at 23.1% (Department of Health, EC, 2001). Also observed was a consistent increase in HIV infection rate in almost all the age groups except for those who were under twenties. Unlike an earlier report by the national HIV Antenatal Survey in 1998 that noted a constant increase in HIV prevalence amongst those aged below twenty from 0% in 1990 to 15.1% in 1998. There was a marked decrease in the prevalence of HIV in the under 20 yrs old from 15.1% in 1990 to 14.2% in 1999 and a further decrease to 13.6% in 2000. This trend somehow was a positive

gesture that there was some level of combating of HIV/AIDS in Eastern Cape Province. Histograms shown in Figure 2.3, represents HIV prevalence in different age groups among antenatal clients in the Eastern Cape Province (Department of Health, EC, 2001).

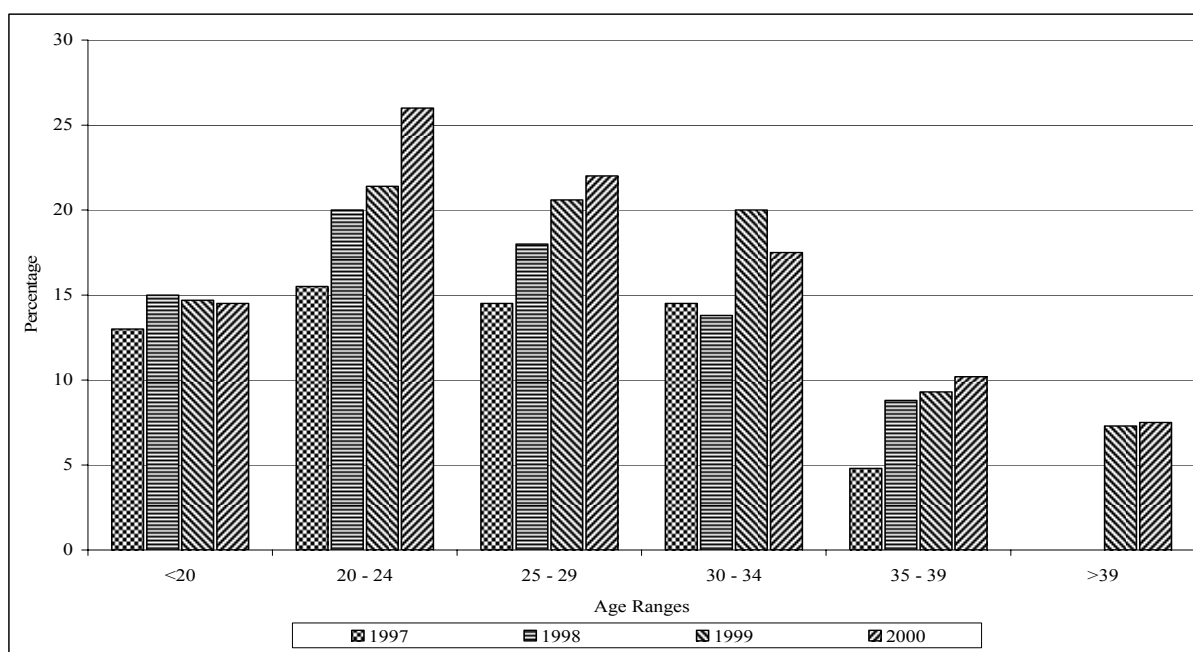


Figure 2.3: HIV prevalence in different age groups among antenatal clients in the Eastern Cape Province since 1997 – 2000 (Department of Health, EC, 2001).

2.5.4.3 *Among men and women*

HIV prevalence for the sexually active men was estimated using ages of fathers to children born of HIV positive mothers. The assumption was that these fathers were also HIV positive since their offsprings were positive. The survey established that the prevalence of HIV was 18% for the sexually active men who were fathers to the HIV positive children (Department of Health, EC, 2001). Concurrently observed in this particular survey was a higher HIV prevalence rate among older men in comparison to the women. Figure 2.4 highlights estimated

level of HIV infection among men and women in different age groups in the Eastern Cape Province as at the year 2001 (Department of Health EC, 2001).

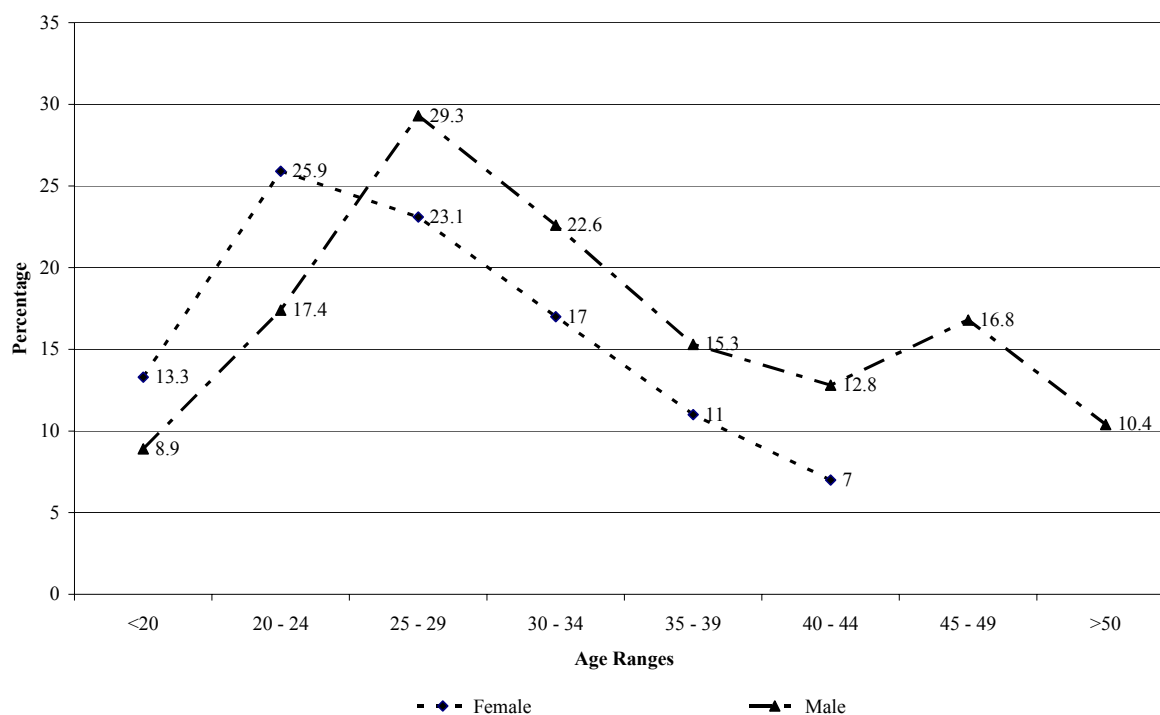


Figure 2.4: Estimated level of HIV infection among men and women in different age groups in the Eastern Cape Province by 2001(Department of Health EC, 2001).

2.5.4.4 *By level of education*

When the level of education was included in the 2000 survey for HIV prevalence in the Eastern Cape Province, it was established that there was an increase in HIV prevalence across all the levels of education, however, post matric and university going antenatal clinic attendees recorded low prevalence level. Figure 2.5 summarizes the trends of HIV prevalence by level of education in the Eastern Cape Province as from 1997 up to 2000 (Department of Health, EC, 2001).

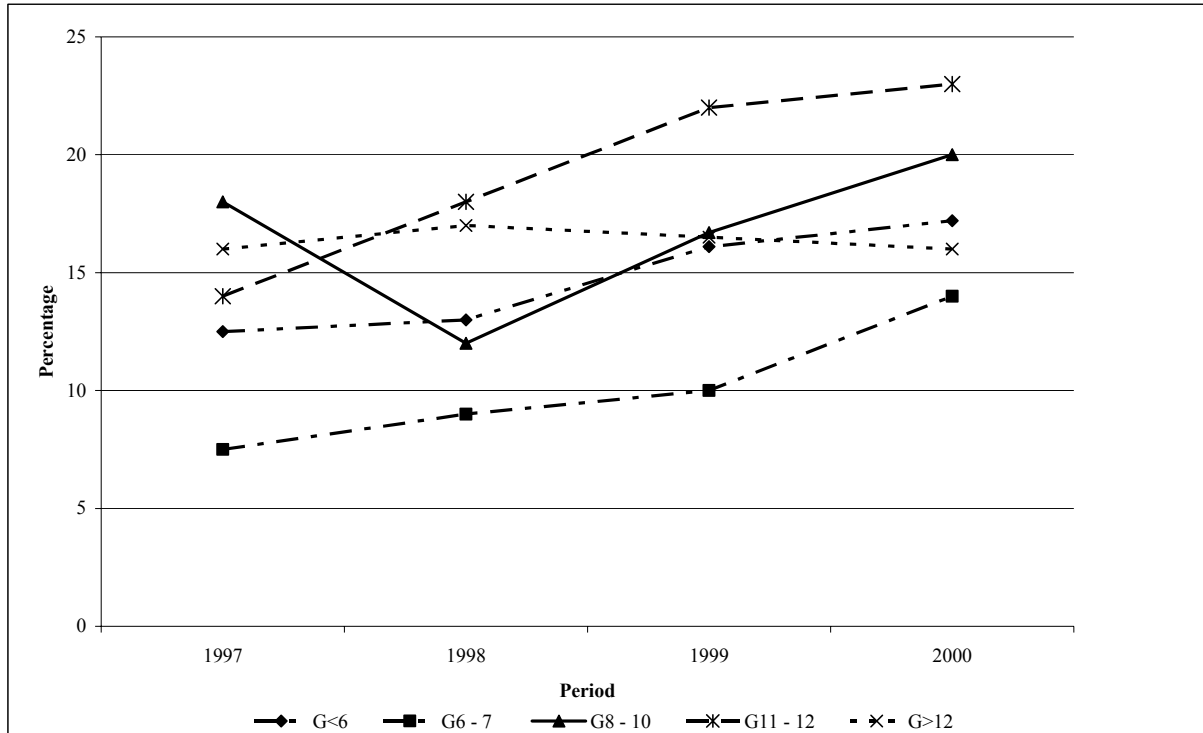


Figure 2.5: HIV prevalence by level of education by the year 2000

2.5.5 Incidence of HIV/AIDS in the Eastern Cape in 2006

Currently the Eastern Cape Province is a home to 10% of HIV positive persons in South Africa (Dorrington *et al.*, 2006). Dorrington *et al.* (2006) estimated that by June 2006, the majority of persons who were positive of HIV in the Eastern Cape Province were youths. The predicted incidence of HIV infections in the province was at 1.3% and as at June 2006, 194, 443 people had died of HIV/AIDS and another 64, 095 were already sick with the diseases (Dorrington *et al.*, 2006). Not only HIV/AIDS has ravaged the Eastern Cape Province; but other diseases have also been reported as well. These include stroke, hypertensive heart disease, diarrhoea, diabetes mellitus, tuberculosis and cholera (Bradshaw *et al.*, 2000).

2.5.6 Susceptibility of HIV/AIDS patients to water and food borne illnesses of *E. coli*

O157: H7 and of other pathogenic organisms

Provision of safe water and foods is very important not only to HIV/AIDS individuals but to all and sundry. *Escherichia coli* O157:H7 (a common water and food borne pathogen), infections pose the greatest risk to immune-suppressed individuals because it can easily invade cells of such patients. Studies on water and food borne illness in HIV/AIDS patients have found that these patients are more susceptible to pathogens such as *Toxoplasma gondii*, *Salmonella* species, *Campylobacter jejuni*, *Vibrio* species and *Listeria monocytogenes*, *Cryptosporidium*, *Giardia* as well as viral pathogens (Morris and Potter, 1997; Prier *et al.*, 2000; Hayes *et al.*, 2003; Hoffman, 2004; Obi *et al.*, 2007a).

Data on salmonellosis suggest that risk for nontyphi *Salmonella* infections is increased 20 to 100 folds among HIV/AIDS patients compared to the general population (Morris and Potter, 1997; Tauxe, 1997; Prier *et al.*, 2000). This is because persons infected with HIV/AIDS and salmonellosis has several fold of risk for septicaemia. HIV/AIDS also increases risk of infection at extra intestinal sites, compatible with an overall increase in risk for dissemination of the infecting organism (Morris and Potter, 1997; Tauxe, 1997; Prier, *et al.*, 2000).

An increase in the *Campylobacter jejuni* cases among persons with HIV/AIDS has also been noted (Prier *et al.*, 2000; Obi *et al.*, 2007a, b). Other data indicate that HIV/AIDS positive patients can contract persistent *C. jejuni* infections, with chronic diarrhoea, fever, and faecal leukocytes (Prier, *et al.*, 2000; Obi *et al.*, 2007a, b). Before, *T. gondii* was of concern principally because of the risk for congenital infection in mothers who had acute toxoplasmosis illness during pregnancy (Prier *et al.*, 2000; Hoffman, 2004). Nonetheless, this

pathogen has been reported as one of the leading cause of cranial lesions in persons with HIV/AIDS (Prier, *et al.*, 2000). Similar works by Morris and Potter, (1997) and Hoffman, (2004) claimed that 5 to 10% of HIV/AIDS patients develop toxoplasmic encephalitis. This condition in most of the cases has been linked to food-borne infection (Morris and Potter, 1997; Hoffman, 2004).

The AIDS epidemic has also drawn attention onto microorganisms not previously recognized as pathogens (Morris and Potter, 1997; Hayes *et al.*, 2003; Hoffman, 2004). In earlier investigations of AIDS-associated diarrhoea, it became apparent that most patients were not infected with traditional enteric pathogens, though; many of these patients were infected with *Cryptosporidium parvum* (Morris and Potter, 1997; Hoffman, 2004; Prier *et al.*, 2000). According to Morris and Potter, (1997), approximately 10 to 20% of cases of AIDS-associated diarrhoea are due to *Cryptosporidium parvum* (Morris and Potter, 1997). This observation has equally been supported by Hayes *et al.* (2003).

2.5.7 Direct and indirect impact of water, and food contaminated with *Escherichia coli*

O157:H7 and other bacteria causing diarrhoeal diseases on HIV/AIDS patients

2.5.7.1 Escherichia coli O157:H7 infections and diarrhoea in HIV/AIDS patients

Escherichia coli O157:H7 is emerging to be the most common cause of bloody diarrhoea (Prier *et al.*, 2000; Brooks *et al.*, 2003; Ritchie *et al.*, 2003). The risk of susceptibility to water and food borne pathogens such as *E. coli* O157:H7 are dreaded to be on the increase (Fauci, 2003; Hoffman, 2004).

Human Immune Virus /Acquired Immunodeficiency Syndrome (HIV/AIDS) cases over the years have been associated with other ill-health conditions such as tuberculosis (TB)

and diarrhoea. As for the cases of tuberculosis, numerous *mycobacteria* strains known to be causing TB have been isolated from HIV/AIDS patients (Sharma *et al.*, 2005). As much as *E. coli* O157:H7 is always a suspected causative agent for diarrhoea in persons with competent immune system, not much has been documented on *E. coli* O157:H7 with regard to diarrhoea in HIV/AIDS patients.

In a survey in Western Kenya, Brooks *et al.* (2003) only isolated *Shigella spp.* from patients experiencing bloody diarrhoea. In the study, non-HIV patients with bloody diarrhoea were used as subjects but there was no investigation of *E. coli* O157:H7 as a causative agent of bloody diarrhoea (Brooks *et al.*, 2003).

A study by Obi *et al.* (2007b) established a link between *Shigella dysenteriae*, *Salmonella sp.* and *E. coli* that were isolated from water samples and from stool specimens of HIV/AIDS patients with and without diarrhoea. This molecular linkage revealed Obi *et al.* (2007b) study was a clear indication that water consumed by HIV/AIDS individual is a potential route of acquiring pathogenic infections. *Shigella dysenteriae*, *Salmonella sp.* and *E. coli* isolated from the stool specimens as well as from drinking waters obtained from the households of these patients indicated identical electrophoretic profiles. Obi *et al.* (2007b) concluded that these bacteria could easily infect HIV/AIDS patients owing to their compromised immune systems.

A survey in the US by Prier *et al.* (2000), revealed an increase in susceptibility of HIV/AIDS persons to bacterial pathogens causing water and food borne infections. Because most *E. coli* O157:H7 outbreaks are always suspected on either a food product or water, there is a possibility that HIV/AIDS patients can acquire these pathogens through the foods and water they consume. This possibility is further compounded by the fact that HIV/AIDS

patients are immunocompromised, hence, consumption of water and/or foods contaminated with *E. coli* O157:H7 will ultimately lead to infections in these patients.

2.5.7.2 *Economic implications of water and food borne infections on HIV/AIDS patients and the public*

Serious financial losses accrue due to water and food borne disease outbreaks suspected on *E. coli* O157:H7 and other bacteria causing diarrhoeal diseases. Treating HIV/AIDS patients who are suffering from diarrhoea is an uphill task that must be approached carefully. In addition, during this period of diarrhoea, there is a lot of dehydration and most HIV/AIDS patients require the replacement of the lost electrolytes. At the same time, blood transfusion is vital and the cost of maintaining safe blood for transfusion has increased (McFarland *et al.*, 1995).

Training of laboratory personnel to prevent cross contamination in the laboratory during blood donation and subsequent transfusion is an issue that must be undertaken with extreme sterility (Jacobs and Mercer, 1999). The emerging of single use of syringes and needles is just one of the factors that poor people cannot cope with in countries where medical bills are footed by patients. The fear of mother to child transmission has contributed to the use of formula-feeding interventions (Soderlund, 1999).

The cost of voluntary counselling and testing is a burden that most families in developing African and Far East countries cannot afford (Sangiwa *et al.*, 2000). Interventions, such as prevention of mother-to-child transmission, tuberculosis treatments, and home based care, are few options considered as cost effective in caring for HIV/AIDS patients (Msobi and Msumi, 2002). The fear of passing *E. coli* O157:H7 infections to home based caretakers cannot be ignored and so such persons may come at a high cost due to the risks involved.

2.5.7.3 *Hotel and catering industry*

Hotels, restaurants and fast food shops have at times served foods, which have subsequently caused *E. coli* O157:H7 outbreaks (Riley *et al.*, 1983; Pebody *et al.*, 1999). There have been numerous reports of travellers' diarrhoea associated with *E. coli* O157:H7 infections due to consumption of fast foods (Pebody *et al.*, 1999). This condition is now common in both developed and developing countries due to tremendous increase in the numbers of people with HIV/AIDS over the past years (Pebody *et al.*, 1999).

Food and water borne diarrhoea are common in the public in developing countries and people with HIV/AIDS are more likely to become ill. Diarrhoea caused by *E. coli* O157:H7 infections may occasionally disappear in non-HIV/AIDS patients, however, this may not be the case in HIV/AIDS positive persons and the condition may require thorough regimen (Prier *et al.*, 2000; Hoffman, 2004).

2.6 **Control of the spread of *E. coli* O157:H7 and protection of public health**

Key approaches in preventing water and food borne *E. coli* O157:H7 outbreak is both individual and collective responsibility. Protection of water and foods from contamination is a crucial initiative in ensuring that water and foods reach consumers in their safe forms. This section discusses the steps taken in ensuring that the public are protected from water and food borne pathogens from the source to the table. The section is divided into two sub-sections. Sub-section 2.6.1 gives a discussion on protection of water sources starting from surface waters from the catchment areas up to the household level. Sub-section 2.6.2 tackles the

strategies employed in protecting foods from the field to the table. The section briefly discusses personal hygiene and food handling practices in the kitchen.

2.6.1 Protection of water sources from *E. coli* O157:H7 contamination

In most cases contamination of water sources resulting from poor sanitation has been linked to endemic outbreaks of *E. coli* O157:H7. In developing countries, the use of poorly protected water sources has been linked to acute diarrhoeal diseases (Grabow *et al.*, 2000; Nasinyama *et al.*, 2000). Control measures are therefore necessary to protect water from microbial contamination. Protection of water sources is categorized into protection of surface and ground water sources. The combined use of multiple treatment processes or "barriers" is a widely embraced principle in drinking water science and is widely applied in drinking water supplies (NHMRC, 2004; Nath *et al.*, 2006).

2.6.1.1 Protection of surface water sources

Protection of water abstraction facilities against potential inundation by contaminated surface waters due to overland flows caused by heavy rainfall or damage of drainage systems by animals is very crucial (WHO, 2006b). After heavy rainfall, cattle and human faeces may be swept to streams and dams, which serve as reservoirs for municipal water supplies (Effler *et al.*, 2001). Restricting access to water source by both humans and animals is important in reducing risks of contamination and thus, where possible, water sources should be enclosed by a fence (Pedley and Howard, 1997; Robertson and Edberg, 1997).

Surface waters are more prone to microbial contamination. Therefore, control of microbial contaminants present in surface waters are usually achieved by water treatment

approaches, which aim at removing the microbial contaminants. Multiple barrier approach system is normally adopted in water treatment, which involves coagulation, flocculation, sedimentation/floatation, filtration and disinfection with long contact times in the supply and storage reservoirs (Momba and Brouckaert, 2005).

Coagulation and flocculation –Typically coagulation and flocculation involve adding a coagulant to a vessel of water, stirring rapidly to disperse the coagulant, followed by a period of standing with slow stirring to encourage the formation of large flocs. The flocs are polycationic and attract negatively charged colloidal particles and microorganisms (Momba and Brouckaert, 2005; Nath *et al.*, 2006). Thus, the process achieves a significant improvement in turbidity, and under optimum conditions can remove 90-99% of pathogenic bacteria and viruses (NHMRC, 2004; Nath *et al.*, 2006). However, the pathogens remain viable in the floc and separation is essentially by settlement or filtration to prevent re-contamination of the clean water (Momba and Brouckaert, 2005). Alternatively, the treated water can further be dosed with a chemical disinfectant such as hypochlorite (Momba and Brouckaert, 2005). Field studies have confirmed the validity of this approach especially in areas where water has a high turbidity (Momba and Brouckaert, 2005; Nath *et al.*, 2006). The most common chemical flocculants are alums, aluminium chlorohydrate, aluminum sulphate, calcium oxide, iron (III) chloride, iron (II) sulphate, sodium aluminate and sodium silicate. These chemicals are effective, but moderately expensive (Nath *et al.*, 2006).

Alternative low costing materials such as clays and natural plant extracts can be used. Natural plant extracts that have been used include proteins extracted from the seeds of the moringa tree. These plant extracts have received considerable attention and field trials have

shown that if used properly, they can be as effective as alum (Sutherland *et al.*, 1990; Nath *et al.*, 2006; Gupta and Chaudhuri, 1992). Other plant materials such as polysaccharides, for example *Strychnos potatorum*, have been used (Gupta and Chaudhuri, 1992).

Optimum coagulation to achieve optimum level of turbidity and maximum removal of microorganisms requires careful control of coagulant dose, correct pH balance (normally between 6.0 and 7.0) and consideration of the quality of the water being treated, as well as appropriate mixing conditions for optimum flocculation. A coagulant dose required to achieve such optimum level of turbidity is known as coagulant demand (Momba and Brouckaert, 2005).

Under optimum conditions, coagulation-flocculation and sedimentation with alum or ferric chloride can achieve microbial reductions of >90 to >99% for all classes of waterborne pathogens (Ahammed *et al.*, 1999; Nath *et al.*, 2006). However, poor microbial reductions (<90%) may occur when coagulation-flocculation or precipitation conditions are sub-optimal (Ongerth, 1990). Even greater microbial reductions (>99.99%) can be achieved with lime coagulation-flocculation or precipitation if high pH levels are achieved in the process (pH >11) to cause microbial inactivation (Nath *et al.*, 2006).

Sedimentation – Sedimentation is the process in which most of the original contaminants that were in the raw water are removed (Momba and Brouckaert, 2005). This process normally proceeds by holding or storing water undisturbed and without mixing long enough for larger particles to settle out or sediment by gravity as the settled water flows into the settled water launders (Momba and Brouckaert, 2005). Sedimentation is done using small water storage vessels or larger settling basins, reservoirs and storage tanks (Momba and

Brouckaert, 2005). Vessels used for sedimentation need to be regularly cleaned and accumulated sediments removed by desludging (Momba and Brouckaert, 2005). Microbial films will also tend to grow on the vessel walls and these too need to be removed by scrubbing and or chemical disinfection. As a pre-treatment process, settlement is very cost-effective requiring only a suitable vessel, labour and time (Nath *et al.*, 2006).

Use of wrong coagulation dose during coagulation can affect sedimentation. Flocculation process should achieve larger flocs, which often settle easily during sedimentation. Water should not be pumped from the flocculation channels to clarifiers as this process breaks up flocs and eventually interferes with sedimentation. The flow rate through the clarifier should be slow for flocs to settle out easily (Momba and Brouckaert, 2005).

Filtration – Filtration covers a wide range of technologies from simple straining out of large particulates to sophisticated membrane systems operating under high pressure that is capable of removal of particles down to nanometer size (Nath *et al.*, 2006). Some membrane filtrations are recommended for emergency water treatment (Sobsey, 2002). The practicality, ease of use, availability, accessibility and affordability of these filtration media and methods vary widely and often depend on local factors. The effectiveness of these filtration methods in reducing microbes also varies widely, depending on the type of microbe and the type and quality of the filtration medium or system (Sobsey, 2002; Nath *et al.*, 2006). Filtration through porous granular media, typically sand or successive layers of anthracite coal and sand, is the most widely used physical method for water treatment at the community level, and it has been used extensively for on-site treatment of both community and household water filtration since ancient times (Logsdon, 1990; LeChevallier and Au, 2000).

Filtration can be a cheap and effective way to improve water quality provided care is taken in both setting up the filter device and regular maintenance (Momba and Brouckaert, 2005). Potential risks occurs when there is leakage especially around seals such that some water may not be filtered; channelling of water through poorly packed filtration media or through cracks in solid filters; growth of bacteria within the filter if a bacteriostatic agent (e.g. silver) is not employed and eventual breakthrough of pathogens (Nath *et al.*, 2006).

Disinfection – The preferred and most widely used chemical disinfectants for disinfecting drinking water are chlorine, ozone, chlorine dioxide, chloramines (mostly monochloramine), and oxidants generated by electrolysis of sodium chloride solution (Camel and Bermond, 1998; Ainsworth, 2004; NHMRC, 2004; DWAF, 2005; Momba and Brouckaert, 2005).

Historically, chlorine and its compounds (e.g. calcium hypochloride, sodium hypochloride and chlorine dioxide) are the most popular chemical disinfection agents. Free chlorine is an excellent biocide and provides a persistent residual to maintain the microbiological safety of the finished water as it passes through the distribution system (Ainsworth, 2004; DWAF, 2005 Momba and Brouckaert, 2005). The Advantages of chlorine are that it provides residual disinfectant. Residual chlorine can easily be measured (Momba *et al.*, 2003a, b, 2004a, b). It is readily available at reasonable cost; it requires low electrical supply to apply and it can be used for multiple water problems such as removal of bacteria, iron etc. It is also appropriate as both primary and secondary disinfectant (DWAF, 2005; Nath *et al.*, 2006).

The main disadvantages of chlorine are that it requires contact time of 30 min for simple chlorination and it is not very effective in treating very turbid waters. There is an after-taste of chlorine in chlorine treated waters; it does not kill *Giardia* cysts at the low concentrations that it is normally used for water treatment. Its reactivity with almost any organic material also means its activity can be easily quenched if the correct process is not used. Careful storage and handling of gaseous chlorine is difficult (DWAF, 2005; Nath *et al.*, 2006). For the above reasons, chloramines have become better alternatives in the place of chlorine. Chloramines are chlorine compounds formed by the addition of ammonia and chlorine and have the advantage of having a long half-life (DWAF, 2005).

Iodine, silver, copper, quaternary ammonium compounds have also been proposed for use in water treatment (NHMRC, 2004, Sobsey, 2002). Iodine is very effective at destroying water-borne pathogens and has been widely used for drinking water treatment in emergencies like during outdoor recreations such as camping and hiking; field military training as well as during natural disasters and human conflicts like wars, tsunami and other societal disruptions (Sobsey, 2002).

Iodine is available in the form of tablets or as ion exchange resins (Sobsey, 2002). However, use of iodine in water treatment comes with some disadvantages. High levels of iodine impart an unpleasant taste to the water. Long-term consumption may damage one's health, whereas too low a level may also fail to inactivate all the target pathogens. Water-borne enteric viruses have been reported to be more resistant to be inactivated by iodine compared to bacteria (Nath *et al.*, 2006). It is expensive and there is a risk of soluble iodine leaching into water; requires replacement of spent resin as well as special disposal practices (Sobsey, 2002).

Silver is used as a bacteriostatic agent for point-of-use or household water treatment by storing water in vessels composed of silver or passing water through porous or granular filter media impregnated with silver (Sobsey, 2002; Clasen *et al.*, 2004). Domestic water filters are often impregnated with a silver compound to prevent growth of bacteria within the filter body, but this is not sufficient to destroy pathogens in the water being filtered. However, the use of silver in treating drinking water is not supported by WHO since bacteria may develop silver resistance and many microbes such as viruses, protozoan cysts and oocysts as well as bacterial spores are not inactivated at silver concentrations employed for point-of-use drinking water treatment (WHO, 2006a).

Other disinfectants that have been used include ozonation, ultraviolet radiation and chlorine dioxide (Nath *et al.*, 2006). These methods have been reported to have lethal effects on bacteria in waters and have achieved maximum reduction in viral loads as well as protozoans such as *Giardia* and *Cryptosporidium*.

2.6.1.2 *Ground water protection*

Ground waters are often regarded as microbiologically safe; however, shallow or unconfined aquifers can be contaminated because of human activities such as agricultural land-use and waste disposal. Other avenues of groundwater contamination are pit latrines, septic tanks, burial sites, landfill stockpiles, industrial effluents and manufacturing wastes, sewage and wastewater lagoons and urban storm waters. It is believed that for contamination of ground water to occur there must be a transfer pathway from point and diffuse sources to an underlying aquifer (Obi *et al.*, 2007a).

Control of spread of pathogenic microorganisms in ground water includes protection of aquifers and the local area around the ground water source from contamination to ensure the integrity of the waters. This section focuses on the protection of ground water from microbial contaminants.

Protection of boreholes – Boreholes should be constructed in a manner that minimizes direct ingress of contaminated surface water. This can be achieved by providing a bentonite grout seal of 1-3 m at the top, which should be continuous with a concrete apron surrounding the top of the borehole (WHO, 2006a).

Animals can be watered by use of water troughs located a few distance from dams and boreholes. Once a water source is identified as a source of *E. coli* O157:H7, alternative sources of drinking water should be used, or a "boil water notice" issued to consumers until the source of the infection is resolved (FSAI, 1999).

Protection of dug-wells – Dug-wells can be designed with an apron surrounding the top of the well (usually of 1-3 m radius) with lining extended 30 – 50 cm above the top of the apron to provide protection against direct entrance of surface water. Collins, (2000) recommended that a cover should always be put on the well to prevent direct contamination of the water.

The means of abstraction should also minimize the potential for introducing contaminants from dirty containers. Collins (2000) also suggested that inclusion of linings is effective in preventing shallow waters from entering the well during wet seasons. Such linings include but are not limited to pre-cast concrete, concrete cast in-situ and brick linings.

Protection of springs – A spring is a natural groundwater source, which can be protected by providing a concrete wall or spring box around the eye of the spring. This prevents direct contamination of the spring due to animal or human activities (Howard *et al.*, 2001; Meuli and Wehrle, 2001). The eye of the spring can also be excavated and the area backfilled with loose material. Such materials should be sufficiently fine to provide reasonable filtration of the groundwater from the spring eye and any surface water percolating through the immediate area (Howard *et al.*, 2001). Meuli and Wehrle, (2001) noted that overlaying the eye of the spring with either a clay or concrete cover minimizes the possibility of direct infiltration of surface water into the spring.

2.6.1.3 *Protection of water in the distribution system*

Water treatment should be optimized to prevent microbial re-growth, corrosion of pipe materials and the formation of deposits. This can be achieved through measures such as continuous and reliable elimination of particles, and the production of water of low turbidity (DWAF, 1996; Ainsworth, 2004). Precipitation and removal of dissolved iron and manganese; minimizing the carry-over of residual coagulant, which may precipitate in reservoirs and pipe work. Reducing as far as possible the dissolved organic matter especially easily biodegradable organic carbon, which provides nutrients for microorganisms and maintaining corrosion within limits that avoid damage to the structural materials and consumption of disinfectant (DWAF, 1996; Ainsworth, 2004; Momba and Brouckaert, 2005). Maintaining good water quality in the distribution system entirely depend on the design and operation of the system and on maintenance and survey procedures to prevent contamination and reduce accumulation of internal deposits (Ainsworth, 2004; Momba and Brouckaert, 2005).

Water entering the distribution system must be microbiologically safe and ideally should be chemically and biologically stable (Ainsworth, 2004; Momba and Brouckaert, 2005). The distribution system itself must provide a secure barrier to contamination as the water is transported to users. Maintaining a disinfectant residual throughout the distribution system can provide some protection against contamination and limit microbial re-growth problems (Ainsworth, 2004; Momba and Brouckaert, 2005).

Water distribution systems should always be enclosed and storage facilities such as reservoir tanks covered to minimize microbial contaminants (Ainsworth, 2004; Momba and Brouckaert, 2005). Backflow into reservoir tanks should not occur as this normally allows for re-introduction of contaminants into the water (Ainsworth, 2004). Faulty and burst pipes should be replaced in ways that do not re-introduce microbial contaminants into the water in the distribution system. Adequate training of workers including contractors responsible for repairing water distribution system is very crucial to minimize the potential of introducing contaminants into the system during construction and repair of pipe networks (Ainsworth, 2004; Momba and Brouckaert, 2005).

It is also important that appropriate security measures be put in place to prevent unauthorized access to or interference with the drinking-water system infrastructure. Control measures may include using stable secondary disinfecting chemical (e.g. chloramines instead of free chlorine), flushing, relining and maintaining positive pressure in the distribution system (Ainsworth, 2004; Momba and Brouckaert, 2005). Reducing the time that water is in the system by avoiding stagnation in storage tanks, loops and dead-end sections is essential (Ainsworth, 2004; Momba and Brouckaert, 2005).

Biofilm development on the inside surfaces of distribution pipes and reservoirs should always be controlled. This is suspected on high levels of turbidity and dissolved organic and biodegradable materials in the waters, if disinfectants e.g. chlorine is not consistently supplied and pipes are corroded (Momba and Brouckaert, 2005). The best strategy to reduce the formation of biofilms on surfaces of distribution pipes and water supply reservoirs is to ensure that water meant for distribution achieves recommended turbidity levels of between 0.5 and 1 NTU, which is regarded as having no significant risks of being associated with transmission of infectious micro-organisms (DWAF, 1996).

Maintaining chlorine residual levels of between 0.2 and 0.6 mg/l even though this chlorine residual level normally gives a chlorine odour and taste to water, the risk of microbial infection is minimal (Momba and Brouckaert, 2005). Certain disinfectants may have properties more conducive to controlling biofilm populations. Less reactive, more persistent compounds, such as chloramine, maintain a higher disinfectant residual throughout the distribution system and penetrate the biofilm more effectively, thus controlling biofilm-forming organisms (Van der Wende and Characklis, 1990; Momba *et al.*, 2000).

Stabilization of finished water also helps in controlling biofilm formation, as there are reduced risks of corrosion of the pipes and storage reservoirs. Corroded pipes and reservoirs are characterized with rough surfaces, which encourage biofilm deposits. Untreated water should not seep back into the reticulation system. Heterotrophic microorganisms should be monitored frequently to check for their growth (Momba and Brouckaert, 2005). Biofilms once established within treated water distribution pipes and reservoirs normally requires shock dosing of disinfectants and/or high velocity flushing or swabbing to remove them (Ainsworth, 2004).

2.6.1.4 *Protection of water at household level*

Water can become contaminated by unsafe consumer storage and handling practices at the household level. This was confirmed by the works of Muyima and Ngcakani, (1998), Momba and Kaleni, (2003). Contamination of water at household level can happen when the water has to be collected from a communal source for domestic use. Many of the world's people continue to obtain their water on a daily basis from any available source and either carry it or have it delivered to their dwellings for personal use. In many rural areas, the municipal water supply is intermittent and water has to be stored for significant periods in the home (Muyima and Ngcakani, 1998; Quick *et al.*, 2002; Momba and Kaleni, 2003). Typically, water is stored in containers of various designs, materials and sizes ranging from small earthenware or other vessels to relatively large underground or overhead tanks (Quick *et al.*, 1996). Often, the water is not protected from subsequent contamination during use (Nath *et al.*, 2006).

Factors contributing to microbial contamination of waters at household levels are inadequate protection (open, uncovered or poorly covered) of water collection and storage containers (Wright *et al.*, 2004). Use of unhygienic methods to dispense water from household storage containers, including faecally contaminated hands and dippers (Nath *et al.*, 2006). Inadequate protection of water against contaminants introduced by vectors such as flies, cockroaches and rodents has also been reported (Sobsey, 2002; Wright *et al.*, 2004).

Inadequate cleaning of storage tanks to prevent biofilm formation and accumulation of sediments increases chances bacterial re-growth (Nath *et al.*, 2006). However, various water treatment strategies at household levels have demonstrated significant benefits to families adopting such strategies (Quick *et al.*, 2002; Crump *et al.*, 2005; Lule *et al.*, 2005). The

following discussions highlights household water treatment methods that have been used in both developed and developing countries to reduce episodes of water related diarrhoea.

Solar radiation disinfection – A study by Conroy *et al.* (1996) demonstrated that solar disinfected water had reduced cases of diarrhoea among some Massai children who were drinking solar disinfected than their counterparts who were drinking non-solar treated water. In a further study, Conroy *et al.* (2001) demonstrated the benefits of solar disinfected water, which resulted in reduced incidence of cholera in 131 Maasai households. However, the study revealed insignificant difference in the risk of cholera in adults or older children in households who used solar disinfection to those who did not. Nonetheless, there were some cases of cholera (3 cases of cholera) out of 155 children aged <6 yrs who were drinking solar disinfected water compared with 20 out of 144 controls (Conroy *et al.*, 2001).

Water boiling – Water boiling prior to drinking has also been reported to be effective in controlling water borne diarrhoea. A study on the effects of boiling of water in some rural villages in Kenya where the main source of water were wells, ponds or rivers demonstrated a reduction in the numbers of coliforms in the waters after boiling (Lijima *et al.*, 2001). Incidences of diarrhoea also dropped amongst families who were drinking boiled water compared to families drinking unboiled waters (Lijima *et al.*, 2001).

Water filtration using clothes – Clean pieces of clothes have also been used in the rural areas to filter water (Colwell *et al.*, 2003). Water filtration using clothes like sari or nylon have been found to be effective in the removal of *Vibrio cholera* from untreated surface

waters meant for household use (Colwell *et al.*, 2003). This water purification system was reported to have led to a reduction in the incidence of cholera in the families who had adopted it as opposed to those who never filtered their waters (Huo *et al.*, 1996). However, this practice would not be adequate in the removal of viruses.

Covering water containers – When water containers are covered, there is minimal chances of flies, cockroaches and rodents having access to water inside the containers (Sobsey, 2002; Wright *et al.*, 2004). This kind of practice was experimented in a refugee camp in Malawi and was found to have an impact on the reduction of water borne diarrhoea. However, it was established that most of the waters were contaminated either by the hands of the people collecting the water or by the water buckets (Roberts *et al.*, 2001). However, rinsing the inside of the water buckets before filling with waters was found to lead to a reduction in the number of coliforms (Roberts *et al.*, 2001).

In-house water chlorination – Research have also investigated the impact of in-home water chlorination on the protection of household water from microbial contamination (Roberts *et al.*, 2001). However, this approach would require some level of knowledge on the correct dosage of chlorine and improved water storage containers and good handling practice (Quick *et al.*, 1996). It has been reported that some households have used this form of water treatment to their advantage (Kirchoff *et al.*, 1985; Roberts *et al.*, 2001). A study in Brazil revealed that there was a significant reduction in faecal coliform count in chlorinated household water than in the unchlorinated water (70 compared with 1500 organisms/dl) (Kirchoff *et al.*, 1985; Nath *et al.*, 2006).

Quick *et al.* (2002) carried out a study on water treatment, safe storage and education in Zambia. The water sources were shallow wells. The participating families were instructed on chlorine disinfection of water and use of safe storage vessel as well as the importance of clean drinking water, and its relationship to health. Analysis of water from households of families who practiced chlorinated and stored water using narrow-mouthed vessels exhibited less *E. coli* contamination and had fewer cases of diarrhoea than families who did not practice the instructions (Quick *et al.*, 2002).

Benefits of in-house chlorination of water have been reported in other countries like Calcutta (Deb *et al.*, 1986), Saudi Arabia (Mahfouz *et al.*, 1995), Bolivia and Bangladesh (Quick *et al.*, 1999; Sobsey *et al.*, 2003). Water chlorination can also be used in combination with other water treatment approaches like flocculation. Reller *et al.* (2003) demonstrated that chlorination combined with flocculation of water stored in containers with a narrow mouths meant to prevented ingress of contaminants into the water was very effective in reducing cases of water borne diarrhoea among families living in some rural villages in Guatemala.

Elsewhere, the use of chlorination in combination with flocculation to treat drinking water has been reported. Luby *et al.* (2004) reported reduction in cases of water borne diarrhoea among some 800 households in Karachi following treatment of their drinking water using chlorination and flocculation. In a separate study, Crump *et al.* (2005) reported reduced incidences in diarrhoea among some families in Kenya who were using flocculation-chlorination to treat their waters as opposed to their counterparts who only used chlorination.

2.6.2 Control of *E. coli* O157:H7 food borne outbreak

Control of food borne diseases outbreaks can only be achieved by the involvement of all the role players involved in the production, processing, regulation and preparation of the foods. Factors that contribute to outbreaks of bacterial food borne diseases include obtaining foods from unsafe sources, contaminated raw food items, improper food storage, and poor personal hygiene during food preparation; inadequate cleaning of kitchen equipments and utensils; inadequate cooking, cooling and reheating of food leftover and prolonged time lapse between cooking and eating of the foods.

Due to the high frequency of *E. coli* O157:H7 cases implicating foods both in developed and developing countries (Kassenborg *et al.*, 2004; Rangel *et al.*, 2005); it is of paramount importance to ensure practices that would hamper the survival and growth of these bacteria in foods. This section discusses how the above-mentioned factors lead to food contamination and the control of transmission of food borne *E. coli* O157:H7.

For effective control of *E. coli* O157:H7; control measures must start from production step to the consumers. This step begins with controlling critical steps that carry the potential of contaminating the food with *E. coli* O157:H7. Hazard Analysis and Critical Control (HACCP) system has been recommended as the preferred approach in controlling the spread of *E. coli* O157:H7 from the farm to the consumers (NACMCF, 1998, 1999). In the context of combating the risk from *E. coli* O157:H7, food safety management systems based on the principles of HACCP should be adopted by all sectors in the food chain. To ensure that food producers, retailers and caterers in South Africa comply with the relevant food legislation, an array of Government agencies including the Department of Health, Agriculture, Trade and Industry should be involved. The government departments are only overseers, but the

involved stakeholders should adopt a consistent approach to compliance and that no gaps exist in the continuum from farm to fork.

2.6.2.1 *On-farm control*

Since it is evident that cattle are the primary source of *E. coli* O157:H7; control measures should start with farmers controlling the occurrence of these bacteria in cattle and their environments. Cattle troughs can be reservoirs that disseminate *E. coli* O157:H7 (Callaway *et al.*, 2003; LeJeune *et al.*, 2004). However, the significance of this transmission has not been proven in cattle (LeJeune *et al.*, 2004).

Interventions at the water trough level offer potential to decrease *E. coli* O157:H7 contamination and cross-contamination of other animals (LeJeune *et al.*, 2004). Strategies for making this option workable are through chlorination, ozonation, frequent cleaning and screens that would decrease organic solids accumulating in the troughs (Callaway *et al.*, 2003). Diet management has also been reported to be helpful in controlling the prevalence of *E. coli* O157:H7 in cattle. This may involve dietary change and use of growth promoting antibiotics that are widely used in cattle production to increase production efficiency (Callaway *et al.*, 2003). For instance, barley feeds have been reported to reduce shedding of *E. coli* O157:H7 by cattle (Dargatz *et al.*, 1997).

2.6.2.2 *Use of antibiotics*

The use of antibiotics in animals as growth promotants and human as therapeutic agent to control *E. coli* O157:H7 has received fierce criticism, which is yet to continue in the near future (Callaway *et al.*, 2003; Panos *et al.*, 2006). This could be due to all the disadvantages

this particular option comes with. For instance, the use of antibiotics in human medicine and animal agriculture has led to the wide spread dissemination of antibiotic resistance genes in the target pathogens (Bogaard and Stobberingh, 1999; Barza and Travers, 2002; Teal, 2002). Although, some antibiotics (neomycin) have shown positive effects in reducing the population of intestinal *E. coli* O157:H7, there is need for evaluation. The use of antibiotics is not highly recommended in treating diarrhoeic conditions in HIV/AIDS patients since it is suspected that this would aggravate the situation (Panos *et al.*, 2006).

2.6.2.3 *Vaccination*

Immunization of an animal (or humans) can help in controlling infections by bacteria and other pathogens. Vaccination is the exposure of an animal (or humans) to an attenuated pathogen or antigen of a virulent microorganism to produce immunity (Buchanan and Michael, 1997). When the animals (and/or humans) are immunized against pathogenic bacteria such as *E. coli* O157:H7, the probability of pathogen shedding and colonization would be greatly reduced.

A study in Canada in 1998 evaluated the effect of vaccinating dairy calves against *E. coli* O157:H7 and revealed that vaccinated calves did not shed significantly fewer *E. coli* O157:H7 cells compared to the control calves that were not vaccinated (Rena, 2000). In a separate study, Dean-Nystrom *et al.* (2002) reported that vaccination of pregnant pigs with intimin from *E. coli* O157:H7 gives rise to high intimin-specific antibody titres in the serum and colostrum. Neonatal piglets allowed to suckle such vaccinated pigs exhibited increased resistance to colonization. They were also no intestinal damage following experimental

inoculation of such pigs with *E. coli* O157:H7 compared to piglets allowed to suckle mock-vaccinated pigs (Dean-Nystrom *et al.*, 2002).

A vaccine has also been developed for use in feedlot cattle that significantly decreases faecal *E. coli* O157:H7 shedding (Finlay, 2003). Further experimental investigations on the vaccine indicated that it could decrease *E. coli* O157:H7 shedding in feedlot cattle from 23% to less than 9% (Moxley *et al.*, 2003)

2.6.2.4 *Use of bacteriophages*

Isolation of *E. coli* O157-specific phages and testing their use in controlling *E. coli* O157:H7 in vivo and in vitro have been done by various researchers (Morita, *et al.*, 2002; Fischer *et al.*, 2004; O'Flynn, *et al.*, 2004; Tanji *et al.*, 2004; Haiqing *et al.*, 2006). However, control of *E. coli* O157:H7 in cattle with phage treatment in natural farm settings is not well documented. Use of bacteriophage to control *E. coli* O157:H7 in sheep has shown some promising results (Bach *et al.*, 2003). Bach *et al.* (2003) revealed that sheep lambs orally dosed with *E. coli* O157:H7 and then treated with a single dose of O157-specific phage DC22 did not clear the pathogen, even though this phage was effective in controlling *E. coli* O157:H7 in an artificial rumen system. However, recently Haiqing *et al.* (2006) demonstrated the use of bacteriophages in cattle to control *E. coli* O157:H7 with some excellent results. They observed that oral treatment of cattle with a bacteriophage SH1 or a mixture of SH1 and KH1 at phage/bacterium ratios greater than 10^2 terminated the presence of faecal *E. coli* O157:H7 within 2 to 6 days after phage treatment (Haiqing *et al.*, 2006). Even though there has been promising scientific revelation on the use of bacteriophages in the control of *E. coli* O157:H7, further work is needed to expound on this interesting innovation.

2.6.2.5 *Slaughtering and dressing*

Escherichia coli O157:H7 has been isolated from the hides and skins of animals (Pennington, 1997; FSAI, 1999). Hides and skins serve as reservoirs of *E. coli* O157:H7 contaminating the carcasses during slaughter (Heuvelink *et al.*, 2001; Gun *et al.*, 2003). For this reason, cattle and other animals should be washed before being taken for slaughter. There should be a veterinary inspector to ascertain the level of cleanliness of slaughter animals and to declare the animals as fit or unfit for slaughter for human consumption based on the hygienic condition. This kind of practice has been adopted successfully in Ireland (FSAI, 1999).

Commercial hauliers and livestock transporters must also ensure that animals do not become dirty or wet while in transit to the abattoir (Barham *et al.*, 2002; Arthur *et al.*, 2007). Trailer design, the loading and off-loading of animals must not make the animals dirty during their transportation to the abattoir. Farmers should insist on good practice in the transport of their animals. The transport company used by the buyer should be obliged not to load dirty animals for carriage to abattoirs as this may facilitate cross-contamination of other animals with *E. coli* O157:H7 (Barham *et al.*, 2002; Arthur *et al.*, 2007).

Transfer of *E. coli* O157:H7 from the hide or skin of the animal to the carcass surface can be prevented during slaughter. Chemical washing of carcass have been reported to have a reduction on the population of *E. coli* O157:H7. For instance, King *et al.* (2005) reported that washing the carcass with 2% L-lactic acid helped to reduce *E. coli* O157:H7 counts to $2.7 \log_{10}$ CFU/cm². King *et al.* (2005) also established that Peroxyacetic acid concentrations of 1000 ppm reduced *E. coli* O157:H7 by up to $1.7 \log_{10}$ CFU/cm² of carcass surface. However, Good Manufacturing Practice (GMP) in slaughtering could be very beneficial in minimising carcass contamination with *E. coli* O157:H7 (FSAI, 1999).

The most feasible approach that has been adopted by most commercial slaughterers is referred to as the “Multiple hurdles” carcass interventions, which aim at preventing carcass contamination at every level of slaughter (Bacon *et al.*, 2000; Arthur *et al.*, 2004). This involves steam vacuuming while targeting areas contacted by knives or machines during the skinning process (Dorsa *et al.*, 1997a, b; Nutsch *et al.*, 1997). It is then followed with a pre-evisceration wash using hot water or organic acid (Bosilevac *et al.*, 2004). After evisceration and splitting, carcasses are passed through a thermal pasteurization chamber, where heated water (74 °C) or steam is applied (Dorsa *et al.*, 1997a, b; Nutsch *et al.*, 1997). This treatment is lethal to bacteria on the carcass surface and further cleanses the carcass. Finally, a heated organic acid or acidified chlorine rinse is applied before carcasses are packed into hotbox (Castillo *et al.*, 1999).

For the above multiple hurdles to successfully control carcass contamination; personal hygiene of persons involved in the slaughter process are equally important. It is imperative that personnel handling carcass wash their hands and put on apron at all times (Republic of South Africa, 1999). Workers with illness that can lead to contamination of carcass should be given sick leave or if they have open wounds then the wounds should be covered with waterproof bandages (Nel *et al.*, 2004). Cleaning and disinfection as well as inspection of slaughter facilities and equipment before and after slaughter should be a priority (Nel *et al.*, 2004). Hot running water and detergent has been reported to have a significant effect in reducing levels of contaminants on slaughter equipment and facilities (Griffith *et al.*, 2000; Redmond *et al.*, 2003).

Animal slurry and manure should be managed in a manner, which prevents contamination of water supplies or ready-to-eat fruit and vegetables (Lung *et al.*, 2001; Jiang

et al., 2002; Nicholson *et al.*, 2005). An increasing number of *E. coli* O157:H7 outbreaks have been associated with consumption of fresh fruit and vegetables (CFIA, 2006; FDA News. 2006a, b). This is attributed to changes in production, harvesting, processing, and consumption patterns thus fruits and vegetables to be eaten raw must first be washed thoroughly with potable water (Abdul-Raouf *et al.*, 1993; Mukherjee *et al.*, 2004).

Research has also shown that *E. coli* O157:H7 strains can penetrate damaged lettuce leaves at cut edges (Seo and Frank, 1999). It is thought that once embedded within the vegetable tissues, these bacteria are protected from sanitising chemicals that have little tissue penetration power. The safety of vegetables like lettuce and other leafy produce may be enhanced by discarding damaged leaves and exposing undamaged leaves to effective levels of sanitizer before packing.

2.6.2.6 *Control of E. coli O157:H7 during food transportation*

Foods may become contaminated with *E. coli* O157:H7 or other microorganisms during transportation. Transportation vessels must be clean and under refrigeration conditions of less than 5°C. Unprotected foods should not touch the sides or floors of transport vehicles. Vehicles should be inspected before loading to ensure their suitability in transporting foods (FSAI, 1999). Such transport vehicles can be inspected by Environmental Health and Food Safety inspectors to ensure that they comply with the required hygiene standards and that they have HACCP systems in operation. Vehicles that have been used for transporting refuse must not be used to transport food, until they are thoroughly cleaned.

2.6.2.7 *Protection at retail levels*

Escherichia coli O157:H7 can grow in foods during retailing process. The first approach to controlling the spread of these bacteria in foods is by avoiding contamination and/or cross-contamination of the foods. This can be achieved by ensuring that raw meat and cooked meat/meat products and other ready-to-eat foods and vegetables are physically separated from each other during production, storage, display, sale, and cooking. This is achievable by using separate refrigerators and production equipment, utensils and, wherever possible, separate staff. Fruit and vegetables should be separated from other ready-to-eat foods (FSAI, 1999).

There should be safe storage of foods both at retail outlets and at homes as this may also control contamination of food and/or growth of *E. coli* O157:H7 (Du Toit and Irma, 2005). Results from studies conducted in the UK (Spriegel, 1991), Australia (Jay *et al.*, 1999), US (Li-Cohen and Bruhn, 2002) and South Africa (Du Toit and Irma, 2005) indicate that many consumers do not follow bacterial food borne disease prevention guidelines, such as keeping high-risk food products at or below 4°C. Safe food storage can be achieved by the following strategies:

Hot holding – Cooked or pre-cooked, cooled and reheated foods should be held at a temperature greater than 63°C. This is especially important if the food is to be stored hot for longer than 2 h. When reheating foods, a temperature of 70°C for 2 min or equivalent should be achieved.

Chill holding – Storage temperatures of less than 5°C should be used when chill storing food (Brown, 2000; Medeiros *et al.*, 2001). Chilling of foods does not kill *E. coli* O157:H7 and it is important that food is not returned to chill storage after being exposed to

environments above 5°C. Storing raw meat, poultry or fish on the top shelf in the refrigerator increases the risk of *E. coli* O157:H7 cross-contamination and eventual infection especially if the foods stored on the underneath shelves are ready-to-eat items that do not necessarily require heating before consumption (Chicken safety tips, 2001; Du Toit and Irma, 2005).

Frozen meat and poultry should be thawed by putting them in the refrigerator while placed in sealed packages or they can be placed in cold water or in a microwave oven. Defrosting frozen food items at room temperature or in warm water is a hazardous practice as temperatures between 5 and 60°C can lead to the growth of pathogens in the food (Du Toit and Irma, 2005).

Cooling food – Rapid cooling of food to chill temperatures for storage is essential. Cooked food should be placed under refrigerated conditions within 90 min after cooking and should reach a temperature of about 5°C within 150 min after cooling has commenced (Hertzman and Barrash, 2007).

2.6.2.8 *Personal hygiene*

When hands are not washed correctly and at appropriate times, pathogens such as *E. coli* O157: H7 can be transmitted to prepared or ready-to-eat food items. The safe practice of washing hands with soap and water before handling of food makes food borne disease outbreaks less likely to occur (Altekruse *et al.*, 1996; Hertzman and Barrash, 2007).

It is important to maintain a high degree of personal cleanliness starting with the body to the garments (Hertzman and Barrash, 2007). Food handlers and any other persons; should wash their hands and exposed portions of their arms thoroughly in a warm soapy water. The

hands should be rinsed and dried properly before handling foods, and as often as necessary, especially after handling raw meat or poultry as these products have been reported to carry *E. coli* O157:H7 (Griffith *et al.*, 2000; Ralston *et al.*, 2000; Zhao *et al.*, 2001; Borch and Arinder, 2003; Redmond *et al.*, 2003; Hertzman and Barrash, 2007).

2.6.2.9 *Food preparation, cooking and serving*

During food preparation, pathogenic organisms may be transferred to food items by the handlers both directly or indirectly by cross-contamination through hands, surfaces, utensils and equipment that have been inadequately cleaned and disinfected between the preparations of different types of food (Roberts, 1990; Scott and Bloomfield, 1990).

Utensils and food contact surfaces should be thoroughly cleaned and where necessary, sanitized before being used. Studies by Gorman *et al.* (2002), Mattick *et al.* (2003) and Haysom and Sharp (2004), noted that pathogenic microorganisms may spread from raw chickens to hands and other contact surfaces in kitchens during domestic meal preparations. It is thus recommended that cutting boards should be sanitised by using 5 ml of chlorine bleach in a 250 ml of water (Du Toit and Irma, 2005).

The consumption of undercooked food products, such as hamburger can contribute to outbreaks of *E. coli* O157:H7 (Riley *et al.*, 1983). It is thus recommended that all high-risk food items be cooked to temperature slightly above 74°C (Brown, 2000; Bennion and Scheule, 2004; Du Toit and Irma, 2005). Food items should be served as soon as possible after preparation. If food items are kept for extended periods, they must be kept either above 60 °C or below 5 °C (Brown, 2000).

2.6.2.10 *Handling of leftover foods*

The handling of leftover foods is a high-risk action that in some cases promotes proliferation of microbial contaminants in the foods. In most cases, leftover foods have been reported to carry high levels of bacterial populations (Brinkman *et al.*, 1999). Leftover foods should be handled hygienically, kept in clean containers and cooled as quickly as possible (Beumer and Kusumaningrum, 2003). Leaving foods to cool at room temperature before refrigeration allows for an uncontrolled growth of bacteria in the foods due to favourable temperatures between 5 °C and 60 °C (Knabel, 1995; Brown, 2000).

2.6.2.11 *Ionizing radiation*

Radiation effectively controls the population of *E. coli* O157:H7 on most foods (Buchanan and Michael, 1997). *Escherichia coli* O157:H7 is radiation sensitive and radiation pasteurization dose of 1.5 – 3.0 kGy is sufficient to reduce its population to desired levels (Buchanan and Michael, 1997). Radiation meant to reduce *E. coli* O157:H7 in meat achieves best results when done at frozen temperatures (Buchanan and Michael, 1997).

2.6.2.12 *Handling of refuse*

All refuse should be kept in leak-proof, non-absorbent containers, which should always be covered with tight-fitting lids when stored or not in continuous use. Each container room or storage area should be thoroughly cleaned after emptying or removal of refuse. All refuse should be disposed of often enough to prevent contamination of the salvaged food products and surrounding processing areas.

2.6.2.13 *Sanitation facilities*

Toilet facilities that are user friendly should be made available and separate areas for changing napkins should be provided with surfaces that are easy to clean. Cleaning procedures for bathroom and napkin changing areas should be written and complied with (FDA, 1999; IFAS, 2005). Cleaning and food preparation tasks should be completely separated and preferably performed by different people. Basic items such as disposable gloves, paper towels and soap should be provided at public eating areas (FDA, 1999; IFAS, 2005).

2.6.2.14 *Exclusion*

The potential for spread of *E. coli* O157:H7 from one person to another has been reported (FSAI, 1999). The workers at food service outlets or even nurses at nursing homes, attendees at crèches or day-care centres who tests positive for *E. coli* O157:H7 should remain at home until they are confirmed free of the bacteria. They can only resume duty after they have 2 consecutive *E. coli* O157:H7 negative stool samples (FDA, 1999; FSAI, 1999). Such samples should be taken at least 24 h apart and should not be taken within 48 h of the patient taking antibiotics. Children under the age of 5 who suffering from *E. coli* O157:H7 should be identified and exclusion from school devised with the guidance of public health medical adviser (FSAI, 1999).

2.6.3 Education and hygiene

An essential pre-requisite of any water and food safety system is staff training. It is of great benefit when owners of water and food businesses train workers employed by them as well as ensuring that they are supervised and instructed in water and food safety and hygiene matters commensurate with their work activities.

It is of fundamental importance that results and recommendation of water and food sanitation research be channelled to the public for a timeous consumption (Phaswana-Mafunya and Shukla, 2005). In most countries, journalists work in collaboration with research institutions to pass information to the public on foods that are suspected to be potential sources of *E. coli* O157:H7. Even though South Africa has its Local Government under the supervision of Department of Health to monitor safe provision of water and ensure that food stores and street vended foods comply with the laid down hygiene standards, there are still cases of water and food borne disease outbreaks (Mosupye and von Holy, 1999).

Information regarding the prevalence of *E. coli* O157:H7 in water and foods should be made available to the public to enable their understanding of the disease, foods most associated with it and its prevention and control strategies (Vicki *et al.*, 2000). In most cases, governments have failed to provide the necessary guidance and structure for the implementation of recommendations by researchers to contain the outbreak of *E. coli* O157:H7. A survey in the Eastern Cape Province of South Africa revealed that lack of adequate training in water and food hygiene to persons involved in the distribution chain is one drawback that has contributed to rampant uncontrolled outbreak of water and food borne outbreaks (Phaswana-Mafunya and Shukla, 2005).

Hygiene training of food workers is very fundamental and can be organized by the respective food industries by contracting the services of specialists (Adams and Moss, 1997). In South Africa, information on the registration of food safety specialist and trainers can be sought from South African Association for Food Science and Technology (SAAFost www.saafofst.org.za). Continual education on the need to ensure good personal hygiene and water as well as food handling practices is very important. The strategy should be tailored to reach the maximum number of persons, particularly vulnerable groups like the immunocompromised persons such as the elderly, HIV/AIDS and cancer patients as well as their caretakers.

The emergence of infectious diseases such as bloody diarrhoea, HIV/AIDS and microbial resistance to therapeutic drugs and emphasis on disease prevention have necessitated the role of communication as an important component of public health practice (Vicki, 2000). However, the dissemination of information regarding the possible causes, control and prevention of *E. coli* O157:H7 water and food borne infections to the public has received very little attention, unlike other HIV/AIDS related illnesses such as tuberculosis (Heersink, 1996). This trend of events has been very common in developing nations where diarrhoeal diseases are taken so lightly, yet social functions are possible venues where people can contract *E. coli* O157:H7 infections. Gatherings such as weddings, funerals, church and political rallies as well as seminars create exceptionally good grounds that various water and food hygiene and sanitation activists can inform the public of potential health hazard.

2.6.4 Hazard analysis and critical control point (HACCP)

Hazard analysis and critical control point-type (HACCP) prevention programmes using scientifically based critical management points is perceived as a viable approach to controlling the spread of *E. coli* O157:H7 and other water and food borne pathogens (Rena, 2000; Guyon *et al.*, 2001). *Escherichia coli* O157:H7, however, pose some unique problems when developing and implementing HACCP plans (Guyon *et al.*, 2001). The low prevalence of these bacteria in water and foods makes direct microbiological testing for the pathogen as a means of verifying the effectiveness of a HACCP program of limited benefit (Guyon *et al.*, 2001). Therefore a method that is lethal like thermal inactivation of *E. coli* O157:H7 would be recommended for use together with microbiological testing for verification of HACCP program efficiency (Buchanan and Michael, 1997). Other processes that do not involve thermal inactivation such as high-pressure inactivation in combination with HACCP to achieve the desired level of inactivation have been used (Rena, 2000). There is HACCP plan for beef slaughter and fabrication (Buchanan and Michael, 1997). Other factors associated with animal production practices and distribution, marketing and consumption of the final product, needs critical control (Rena, 2000; Guyon *et al.*, 2001).

HACCP system in the context of a food supply chain is basically a management system, which identifies critical points at which a food product can become contaminated. In the meat processing industry, HACCP plan is developed to avoid adulteration of meat either by physical or microbiological contaminants. Hazard analysis and critical control point is developed based on seven principles (NACMCF, 1998; 1999). These include Hazard analysis, critical control points (CCP's), critical limits, monitoring procedures, corrective actions, verification procedures and record keeping (NACMCF, 1998, 1999; Hulebak and Sclosser,

2002; Quinn and Marriott, 2002). Hazard analysis from a food perspective involves looking at how the food product is made from start to finish and identifying the steps that carry potential risk of contamination to the food. The effectiveness of a HACCP program depends on correctly identifying what hazards may be present and assessing their risk and severity. The hazard must be such that its prevention, elimination, or reduction to acceptable levels is essential to the production of a safe food (Hulebak and Schlosser, 2002).

The second and very important step is the critical control points (CCP's) (Hulebak and Schlosser, 2002). A critical control point (CCP) is defined as a point, step, or procedure at which control can be applied and a food-safety hazard can be prevented, eliminated, or reduced to an acceptable level. All significant hazards identified during the hazard analysis must be addressed. CCPs include cooking, chilling, specific sanitation procedures, prevention of cross-contamination, product-formulation controls, and employee and environmental hygiene. All CCPs must be carefully developed and documented (Hulebak and Schlosser, 2002).

Once the CCP's are determined, the critical limit(s) for each identified CCP step is defined. The critical limit(s) explores the entire criterion that must be met for each preventive measure associated with a particular CCP in the food continuum. CCPs are most often based on process parameters, such as temperature, time, physical dimensions, humidity, moisture level, water activity, pH, salt concentration, etc (Hulebak and Schlosser, 2002; Quinn and Marriott, 2002). After the identification of the CCP's and their control limits set, the HACCP team is then expected to monitor if the CCP's are under control. Monitoring consists of observations or measurements taken to assess the control of a given CCP (Quinn and Marriott, 2002). It helps in determining any deviation from a CCP and suggests the remedies to be

adopted for bringing the production process back on track (FSIS, 1996; Sohrab, 1996). Should the HACCP team notice a deviation from any CCP, then a corrective action must be taken to remedy the deviation (Quinn and Marriott, 2002). From a water and food perspective, any product that a corrective measure cannot remedy are usually discarded or processed for other uses but not for human consumption (FSIS, 2000). Before any process continues, a control is re-established for the CCP that was affected (FSIS, 2000).

The next principle is the verification (Hulebak and Schlosser, 2002; Quinn and Marriott, 2002). This principle describes the procedures taken to verify that the HACCP system is being followed as planned and is effective at controlling the food safety hazard(s) (Lupin, 2000; Quinn and Marriott, 2002). Verification procedures include a review of the HACCP system and records any deviations and product dispositions, confirmation that the CCPs are kept under control and even interviewing employees (Mayes, 1999). The seventh principle of HACCP is to establish adequate record keeping procedures. Record keeping serves as a proof of evidence as to whether the HACCP plan is being followed and is compliant with the documented system (NACMCF, 1998; 1999). Records are useful in providing a basis for trends and for systematic improvement of the process over time. Records on monitoring, correction actions and audits of HACCP plan should always be filed and made available should it be required by any inspection agency. A schematic diagram illustrating CCP in meat slaughter system is summarized in Figure 2.6.

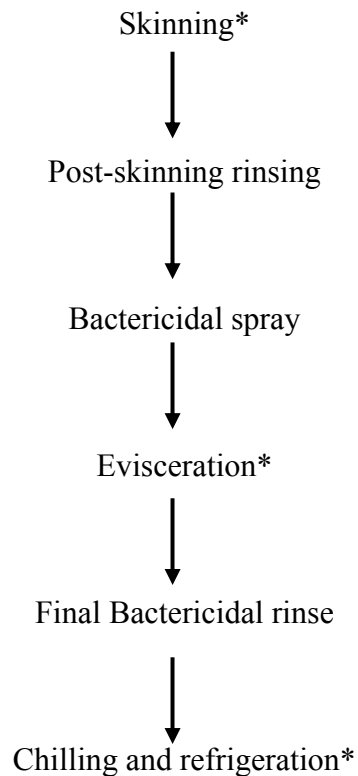


Figure 2.6: Generic HACCP plan for beef slaughter and fabrication (Buchanan and Michael, 1997), (*) critical points that need control to minimize contamination of the carcass.

2.7 Techniques used for the study of *E. coli* O157:H7 in clinical, food and environmental samples

2.7.1 Conventional methods for detection and isolation of *E. coli* O157:H7

The need to detect *E. coli* O157: H7 in clinical, food and environmental samples requires enrichment using selective media, which are discriminately sensitive and only select *E. coli* O157:H7 strains instead of other *E. coli* (Malihe and Kadir, 2000). Several methods have been used to isolate *E. coli* O157:H7 from clinical, food and environmental samples (Wang *et al.*,

2002; Cagney *et al.*, 2004; Müller, 2005). This section discusses some of the techniques used in the isolation of *E. coli* O157:H7 from clinical, food and environmental samples.

2.7.1.1 Culture-based techniques

The very first step in the isolation of *E. coli* O157:H7 is sample enrichment. Modified *E. coli* (mEC) broth supplemented with novobiocin has been reported to increase the selection for *E. coli* O157:H7 (Oberst *et al.*, 1998). Modified *E. coli* (mEC) broth and a single-tube screening medium for *E. coli* O157: H7, which contains sorbitol and flagella antigen H7 antisera, is also an alternative media for the isolation of *E. coli* O157:H7. GN Broth Hajna and trypticase soy broth supplemented with cefixime and vancomycin have both been used with success in the isolation of these bacteria (Karch *et al.*, 1996).

Tryptose soya broth (TSB) supplemented with phosphate buffered saline (PBS) in combination with bile salt (pH 7.5) and novobiocin have shown positive results (Doyle and Schoeni, 1984). Buffered peptone water made into a selective enrichment broth by the addition of vancomycin, cefixime, and cefsulodin have equally been used in the enrichment step for *E. coli* O157:H7 isolation (Malihe and Kadir, 2000). After the enrichment step, the enriched samples are then plated onto selective agar media. The agar media commonly used for the isolation of *E. coli* O157:H7 include Sorbitol MacConkey (SMAC) Agar, (March and Ratnam, 1986); Sorbitol MacConkey Agar supplemented with cefixime and tellurite (CT-SMAC) (Elder *et al.*, 2000; Bopp *et al.*, 2003) and Enterohaemolysin Agar (Beutin *et al.*, 1989). Rainbow Agar O157 (Manafi and Kremsmaier, 2001; Ingrid *et al.*, 2003) and Fluorocult *E. coli* O157 Agar (Szabo *et al.*, 1986) have been used.

The selectivity of SMAC Agar has also been improved by the addition of cefixime-rhamnose resulting in the formulation of Cefixime-Rhamnose-Sorbitol MacConkey (CR-SMAC) Agar (Chapman *et al.*, 1991) and 4-methylumbelliferyl-b-D-glucuronide (MSA-MUG) Agar (Szabo *et al.*, 1986). The above media contain sorbitol replacing the lactose of the standard MacConkey medium.

Unlike other *E. coli* strains, isolates of serotype O157:H7 do not ferment D-sorbitol within 24 h since they lack glucuronidase activity and do not grow at 45.5 °C. Sorbitol non-fermenting colonies, indicative of the typical *E. coli* O157:H7 are normally colourless on this medium. However, many organisms other than *E. coli* O157:H7, especially other serogroups of *E. coli* and *Proteus spp.*, may not ferment sorbitol, and thus may be confused with *E. coli* O157:H7. Rhamnose is not fermented by *E. coli* O157:H7 but is fermented by most sorbitol non-fermenting *E. coli* of other serogroups.

The inclusion of novobiocin in 5-bromo-4-chloro-3-indoxyl-b-D-glucuronide SMAC (BCIG-SMAC) (Okrend *et al.*, 1990) has also improved the selectivity of SMAC for *E. coli* O157:H7 strains. The inability of *E. coli* O157:H7 to produce β -glucuronidase has been utilized by including MUG into some media as non-O157:H7 *E. coli* strains produce a fluorescent compound on it (Doyle and Schoeni, 1984; Slutsker *et al.*, 1997).

Rainbow Agar is enriched with chromogenic substrates that are specific for both β -galactosidase and β -glucuronidase and yields a spectrum of coloured colonies ranging from black to gray, to red, to blue and to violet that purportedly differentiates *E. coli* O157:H7 among other *E. coli* strains (Manafi and Kremsmaier, 2001). On Rainbow Agar O157, *E. coli* strains grow, yielding colonies ranging in colour through various shades of red, magenta,

purple, violet, blue, and black. The typical *E. coli* O157 strains form distinctive charcoal grey or black colonies (Manafi and Kremsmaier, 2001).

Another differential medium used in the isolation of *E. coli* O157:H7 is Enterohaemolysin Agar (Beutin *et al.*, 1989; Beutin *et al.*, 1994), which detects the enterohaemolysin expressed by Stx-producing *E. coli* isolates. The disadvantage with this medium is the need to read and record haemolytic colonies 3 to 4 h after plating when only α -haemolysin is evident and again after overnight incubation when both α -haemolysin and enterohaemolysins are evident (Beutin *et al.*, 1994; Nataro and Kaper, 1998).

Hemorrhagic Colitis Agar is another medium that has been used. It is a differential media and has been used in direct plating to isolate O157:H7 from foods (Murat *et al.*, 2004). It includes an enrichment step and is a new method developed sequel to food borne outbreaks (Sanderson *et al.*, 1995). Other media have also been developed for the isolation of *E. coli* O157:H7. These include Biosynth Culture Media O157:H7, (BCM O157:H7) and CHROMagar O157 (Manafi and Kremsmaier, 2001). *Escherichia coli* O157:H7 grow with blue-black colonies and *E. coli* non-O157 with green-yellow colonies on Biosynth Agar (Restaino *et al.*, 1996; 1999).

2.7.1.2 Immunoassays

In immunoassay, antibody-coated super paramagnetic beads/particles are used in the isolation of *E. coli* O157:H7. One such type of technique, which uses immunoassay, is the Dynabead anti- *E. coli* O157 system. The Dynabeads anti- *E. coli* O157 test offers a rapid culture technique based on the selective enrichment for *E. coli* O157:H7 directly from pre-enriched

samples using the technology of Immunomagnetic Separation (IMS) (Barkocy-Gallagher *et al.*, 2002; Sun *et al.*, 2002; Fu *et al.*, 2004).

Dynabeads are coated with adsorbed and affinity purified anti – *E. coli* O157 antibodies (DynaL Product Brochure, 2006). The beads are incubated with 1 ml of pre-enriched sample (Müller *et al.*, 2003). During this incubation, *E. coli* O157:H7 present in the sample will bind to the surface of the bead (Müller *et al.*, 2003). The bacterial-bead complexes are then separated from the sample using a magnetic particle concentrator (DynaL MPC-S) (Müller *et al.*, 2003; DynaL Product Brochure, 2006). After Immunomagnetic separation, the bacterial-bead complexes are re-suspended in phosphate buffered saline and appropriate volume (usually 20 – 50 µl) of the re-suspended beads is plated onto a selective culture media and incubated at 37 °C for 24 h (DynaL Product Brochure, 2006). After incubation, the plates are examined for colonies that exhibit characteristics typical for the growth of *E. coli* O157:H7 (Müller *et al.*, 2003; Fu *et al.*, 2004).

Another system that uses immunoassay technology is the Envisio system. The Envisio™ *E. coli* O157 assay is a rapid detection system comprising a proprietary enrichment medium, a magnetic nanoparticle immunoassay and an automated reader for detection (Centrus International, 2006). Assay detection threshold is improved relative to traditional immunoassays by using a magnetic nanoparticle label and a highly sensitive magnetic particle detector. Assay specificity is improved through use of a selective medium that reduces the growth of non-target organisms that are immunologically related to the targeted one. This system has only been tested for the detection of *E. coli* O157:H7 in beef products (Centrus International, 2006).

2.7.1.3 *Resuscitation and identification of E. coli O157:H7*

Due to adverse conditions that *E. coli* O157:H7 cells are often subjected to during growth in their experimental environments, the cells experience sub lethal cellular injuries (Garcia-Graells, 1998; Hara-Kudo *et al.*, 2000). The only viable media mostly used for resuscitation and presumptive identification of *E. coli* O157:H7 is Eosin Methylene Blue (EMB) Agar (Radu *et al.*, 1998). This differential media gives *E. coli* colonies with a characteristic blue-black metallic sheen appearance (Radu *et al.*, 1998). This only applies to colonies that appear pink or colourless on Sorbitol MacConkey Agar (sorbitol fermentors) (Feng, 1995; Cagney *et al.*, 2004). Sorbitol non-fermentors produce colourless colonies on Sorbitol MacConkey Agar (Radu *et al.*, 1998).

2.7.1.4 *Serological test*

After the isolation of presumptive *E. coli* O157:H7 on any of the above mentioned media, the isolates are normally subjected to serological tests for confirmation. Confirmation of *E. coli* O157:H7 is done using latex agglutination kits or O157 anti-sera. Latex test has been found to be a simple, highly efficient and reliable in detecting *E. coli* O157:H7 with 100% sensitivity and specificity (March and Ratnam, 1986).

Escherichia coli O157 latex test includes two reagents: test latex, consisting of latex particles sensitized with specific rabbit antibody reactive with the *E. coli* O157 antigen, and control latex, consisting of latex particles sensitized with pre-immune rabbit globulin. The test is designed to demonstrate, by slide agglutination, *E. coli* strains possessing the O157 antigen. The control latex is used only if agglutination occurs with the test latex, and it is meant to rule

out autoagglutination (March and Ratnam, 1986). Table 2.10 illustrates the effects of some plating media on the isolation of *E. coli* O157:H7.

Table 2.10: Effects of plating media on the percentages of *E. coli* O157:H7 positive samples detected and the 50% detection limit of each media formulation (Sanderson *et al.*, 1995).

Plating medium	% of positive samples	50% detection limit (CFU/g)
Sorbitol MacConkey Agar	3	2, 511
Sorbitol MacConkey Agar Cefixime	25	562
Sorbitol MacConkey Agar + Cefixime + Tellurite	70	25

2.7.2 Molecular-based diagnostic methods for *E. coli* O157:H7

Molecular microbiologists have used a number of molecular based techniques to diagnose pathogenic microorganisms (Wong *et al.*, 2000; Wang *et al.*, 2002; Obi *et al.*, 2004). Modern molecular biology is a technique, which is providing a number of solutions to diagnostic problems (Savelkoul *et al.*, 1999; Wang *et al.*, 2002). These are based on the assumption that each species of microorganism has some unique genetic makeup. In this section, molecular diagnostic methods for the detection of *E. coli* O157:H7 are discussed. These methods have

been categorized into nucleic acid sequence based approach as well as polymerase chain reaction (PCR) methods.

2.7.2.1 *Nucleic acid sequence based analysis (NASBA).*

Nucleic acid sequence based diagnostic techniques have been employed for the detection of microorganisms. These techniques explore the nucleic acid structures of the microorganism to establish their presence in the environments, foods and in clinical specimens. Nucleic acids are complex, high-molecular-weight biochemical macromolecules composed of nucleotide chains that convey genetic information. They are either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) (Clausen-Schaumann *et al.*, 2000). Nucleic acids are made up of four nucleotide monomers referred to as deoxynucleotide bases made up of Guanine (G), Cytosine (C), Adenine (A) and Thymine (T) in the case of DNA. However, when the Thymine in the DNA is replaced with Uracil (U), then RNA is formed instead of DNA (Maier *et al.*, 2000; Green, 2003).

Nucleotides are usually written in the abbreviated form and have a backbone made of sugars and phosphate atoms joined by ester bonds. Attached to each sugar is one of four types of molecules called bases. Each type of base on one strand forms a bond with just one type of base on the other strand (Clausen-Schaumann *et al.*, 2000). This arrangement of two nucleotides binding together across the double helix is called a base pair (Ponnuswamy and Gromiha, 1994).

The backbone of the DNA strand is made from alternating phosphate and sugar residues. The sugar in DNA is 2-deoxyribose, which is a pentose sugar (Ghosh and Bansal, 2003). The sugars are joined together by phosphate groups that form phosphodiester bonds

between the third and fifth carbon atoms of adjacent sugar rings. These asymmetric bonds means a strand of DNA has a direction. In a double helix, the direction of the nucleotides in one strand is opposite to their direction in the other strand. This arrangement of DNA strands is called anti-parallel. The asymmetric ends of a strand of DNA bases are referred to as the 5' and 3' ends. One of the major differences between DNA and RNA is the sugar, with 2-deoxyribose being replaced by the alternative pentose sugar ribose in RNA (Berg *et al.*, 2002).

Two techniques such as gene probing and polymerase chain reaction (PCR) are well recognized for the elucidation of nucleic acid structures of microorganisms (Kudva *et al.*, 1996; Garcia-Aljaro *et al.*, 2005). In this section, techniques that are often used in the elucidation of nucleic acid structures present in *E. coli* O157:H7 are discussed.

Gene Probes and Probing – Gene probe is a single-stranded DNA or RNA sequence that is specifically labelled (e.g. radioactively) and that binds to complementary DNA sequences. Because of this labelling, the bound sequences can be identified (Maier *et al.*, 2000). Probing is the process whereby a fragment of DNA of variable length (usually 100-1000 bases long), is used to detect in DNA or RNA samples the presence of nucleotide sequences that are complementary to the sequence in the probe (Kudva *et al.*, 1996; Garcia-Aljaro *et al.*, 2005).

Hybridization probe is labelled radioactively (commonly with ^{32}P) or with immunological markers such digoxigenin (Garcia-Aljaro *et al.*, 2005). The labelled probe is then denatured by heating into single DNA strands and hybridized to target DNA or RNA immobilized on a membrane or in situ (Garcia-Aljaro *et al.*, 2005). DNA sequences or RNA transcripts that have moderate to high sequence similarity to the probe are detected by

visualizing the hybridized probe via autoradiography or other imaging techniques (Garcia-Aljaro *et al.*, 2005).

Hybridization probes used in DNA microarrays refer to DNA covalently attached to an inert surface, such as coated glass slides or gene chips, and to which a mobile DNA target is hybridized (Garcia-Aljaro *et al.*, 2005). Several researchers have used hybridization to detect DNA or RNA samples of *E. coli* O157:H7 (Southern, 1975; Maier *et al.*, 2000; Gerrish *et al.*, 2007). Hybridization is divided into southern, northern, western and southwestern hybridization.

Southern blotting – Southern blotting is a method routinely used to check for the presence of a DNA sequence in a sample of specimen. Southern blotting in combination with agarose gel electrophoresis helps in the separation of DNA and the transfer of the separated DNA to a filter membrane for probe hybridization. This technique was developed by a British biologist Edwin Southern and named after him (Southern, 1975).

The process of detecting *E. coli* O157:H7 DNA can be by filtering the water or food homogenate or by using DNA extracted from suspected colonies of these bacteria. After DNA extraction; using a DNA extraction kit (Gerrish *et al.*, 2007) or any other published methods (Torres *et al.*, 2003); the DNA is then subjected to PCR procedure using target primers specific to *E. coli* O157:H7. Such primers include but are not limited to *eae*, *stx1* and 2, *hlyA*, *katP*, and *tox B* of pO157 in order to get the amplicons (Blanco *et al.*, 1996; Brunder *et al.*, 1996; Burland *et al.*, 1998; Makino *et al.*, 1998; Tatsuno *et al.*, 2001). The amplicons are then cut out and a suitable solution containing an alkaline like sodium hydroxide is used as a

transfer medium (Gerrish *et al.*, 2007). This involves placing the gel into the solution to denature the double-stranded DNA.

A sheet of nitrocellulose or nylon membrane is placed on top or below of the gel (depending on the direction of the transfer) (Garcia-Aljaro *et al.*, 2005). Pressure is applied evenly to the gel either using suction, or by placing a stack of paper towels and a weight on top of the membrane and gel to ensure good and even contact between gel and membrane.

Buffer transfer by capillary action (usually filter paper and paper tissues) is then used to move the DNA from the gel on to the membrane; ion exchange interactions bind the DNA to the membrane due to the negative charge of the DNA and positive charge of the membrane (Garcia-Aljaro *et al.*, 2005; Gerrish *et al.*, 2007).

The membrane is exposed to high temperature (60 to 100 °C) (Garcia-Aljaro *et al.*, 2005) or exposed to ultraviolet radiation to permanently and covalently crosslink the DNA to the membrane and the membrane exposed to a hybridization probe (Gerrish *et al.*, 2007). The probe DNA is labelled so that it can be detected, usually by incorporating radioactivity or tagging the molecule with a fluorescent or chromogenic dye. In some cases, the hybridization probe may be made from RNA, rather than DNA (Gerrish *et al.*, 2007). After hybridization, excess probe is washed from the membrane and the pattern of hybridization is visualized on X-ray film by autoradiography in the case of a radioactive or fluorescent probe, or by development of colour on the membrane if a chromogenic detection method is used (Gerrish *et al.*, 2007).

Northern blotting – Northern blot is a technique used to study gene expression with a key difference from southern blotting that RNA, rather than DNA, is the substance being analyzed by electrophoresis and detection with a hybridization probe (Maier *et al.*, 2000;

Gerrish *et al.*, 2007). A notable difference in the procedure (as compared with the Southern blot) is the addition of formaldehyde in the agarose gel, which acts as a denaturant. As in the Southern blot, the hybridization probe may be made from DNA or RNA (Gerrish *et al.*, 2007).

Western hybridization – Western blotting or immunoblotting was dubbed because it is similar to Southern blotting (Burnette, 1981). It allows investigators to determine, with a specific primary antibody, the relative amounts of the protein present in different samples. Samples are prepared from tissues or cells that are homogenized in a buffer that protects the protein of interest from degradation (Burnette, 1981).

The sample is separated using Sodium Dodecyl Sulphate Poly-Acrylamide Gel Electrophoresis (SDS-PAGE) and then transferred to a membrane for detection. The membrane is incubated with a generic protein to bind to any remaining sticky places on the membrane. A primary antibody is then added to the solution, which is able to bind to its specific protein. A secondary antibody-enzyme conjugate, which recognizes the primary antibody, is then added to find locations where the primary antibody bound (Burnette, 1981). Using western blot technology, Son *et al.* (2002); demonstrated the presence of *E. coli* O157:H7 monoclonal antibody Intγ1 in some humans and animal strains. This technique has also been used to detect *E. coli* O157:H7 in sewage water and foods (Maite and Juan, 1998).

Southwestern hybridization – Southwestern blotting, first described by Bowen *et al.* (1980), is a powerful technique for identifying and characterizing DNA-binding proteins by their ability to bind to specific oligonucleotide probes. Southwestern blot mapping" is performed for rapid characterization of both DNA binding proteins and their specific sites on

genomic DNA. Proteins are separated on a Sodium Dodecyl Sulfate (SDS), polyacrylamide gel (PAGE), renatured by removing SDS in the presence of urea, and blotted onto nitrocellulose by diffusion (Jeffrey and Helene, 1997).

The genomic DNA region of interest is digested by restriction enzymes selected to produce fragments of appropriate but different sizes, which are subsequently end-labelled and allowed to bind to the separated proteins (Jeffrey and Helene, 1997). The specifically bound DNA is eluted from each individual protein-DNA complex and analyzed by acrylamide gel electrophoresis. The usefulness of this technique is hampered by its requirement for relatively large amounts of nuclear proteins (typically 50-100 mg), problems with protein degradation during isolation, and the difficulties in achieving efficient electrophoresis separation and transfer of a wide molecular size range of proteins (Jeffrey and Helene, 1997).

Dot blotting – DNA/RNA or protein samples are spotted onto a membrane and hybridized with a labelled probe that hybridizes to a specific DNA/RNA or protein molecule. This technique is similar to the Western blot but differing in that the samples are not separated electrophoretically (Tenover and Unger, 1993). Antigens may be applied directly to nitrocellulose membrane as a discrete spot (dot) to give a simple and reliable assay. Dot blotting has been reported to be effective in detecting *E. coli* O157:H7 in foods such as ground beef, goat milk, blueberries, and surimi-based delicatessen salads (Samadpour *et al.*, 1990).

DNA Microarrays – DNA microarrays are used to measure the relative abundance of DNA or RNA in order to compare genomes or gene expression profiles. DNA microarrays are typically composed of DNA probes that are bound to a solid substrate such as glass (Beattie *et*

al., 1995; Beier *et al.*, 1999; Call, 2001a). In genomic profiling experiments, DNA extracted from a sample is fluorescently labelled (or amplified by PCR and labelled) and hybridized to the microarray. For RNA expression profiles fluorescently labelled cDNA copies of the total RNA pool from each sample are prepared by reverse transcriptase reaction and hybridized to the microarray (Derisi *et al.*, 1996). The microarray is scanned to measure the fluorescence intensity of every spot. The ratio of the fluorescent intensities reflects the relative abundance of the samples.

During hybridisation, DNA targets diffuse passively across the glass surface, when sequences complementary to a probe finally anneal and form a DNA duplex (Derisi *et al.*, 1996; Call, 2001). Hybridised targets can then be detected using one of the many reporter molecule systems. In essence, a microarray is a reverse dot-blot that employs the same principles of hybridisation and detection with membrane-bound nucleic acids (e.g. Southern and Northern blots) (Call, 2001).

DNA microarrays can be used for determining the presence or absence of specific pathogens in food systems. Chandler *et al.* (2001) applied automated immunomagnetic separation coupled with PCR amplification and microarray hybridization to detect *E. coli* O157:H7 from poultry carcass rinse. A process-level detection limit was noted at $< 10^3$ CFU/ml. Bekal *et al.* (2003) developed a DNA microarray for rapid identification of *E. coli* pathotypes by virulence gene detection. However, pathogen detection using DNA microarray has been reported to be less sensitive (Call *et al.*, 2001).

2.7.2.2 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is a technique for isolating and exponentially amplifying a fragment of DNA via enzymatic replication (Cheng *et al.*, 1994; Sambrook and Russel, 2001; Pavlov *et al.*, 2004). Polymerase chain reaction is used to amplify specific regions of a DNA strand. This can be a single gene, part of a gene, or a non-coding sequence.

Most PCR methods typically amplify DNA fragments of up to 10 kilo-base pairs (kb), although some techniques allow for amplification of fragments up to 40 kb in size (Cheng *et al.*, 1994). PCR requires several basic components. These components are i) DNA template that contains the region of the DNA fragment to be amplified. ii) One or more primers, which are complementary to the DNA regions at the 5' and 3' ends of the DNA that is to be amplified (Sambrook and Russel, 2001). iii) DNA polymerase (e.g. *Taq* polymerase or another DNA polymerase with a temperature optimum at around 70°C) is used to synthesize a DNA copy of the region to be amplified. iv) Deoxynucleotide triphosphates (dNTPs) from which the DNA polymerase builds the new DNA, v) Buffer solution, which provides a suitable chemical environment for optimum activity and stability of the DNA polymerase (Sambrook and Russel, 2001). vi) Divalent cation, magnesium or manganese ions; generally Mg^{2+} is used, but Mn^{2+} can be utilized for PCR-mediated DNA mutagenesis, as higher Mn^{2+} concentration increases the error rate during DNA synthesis, vii) monovalent cation potassium ions (Sambrook and Russel, 2001; Pavlov *et al.*, 2004).

Polymerase chain reaction is carried out in small reaction tubes (0.2-0.5 ml volumes), containing a reaction volume typically of 15 to 100 μ l, that are inserted into a thermal cycler (Wang *et al.*, 2002; Cagney *et al.*, 2004). This machine heats and cools the reaction tubes

within it to the precise temperature required for each step of the reaction. Most thermal cyclers have heated lids to prevent condensation on the inside of the reaction tube caps.

Polymerase chain reaction procedure is usually carried out in three steps, often preceded by one temperature hold at the start and followed by one hold at the end. These steps include initialization, annealing and finally extension/elongation step (Sharkey *et al.*, 1994). During the initialization step prior to the first cycle, the PCR reaction is often heated to temperatures of 94 – 96°C after which the temperature is then held for 1-9 min. This first hold is employed to ensure that the DNA is melted by disrupting the hydrogen bonds between complementary bases of the DNA strands. The second step, which is the annealing, is where the reaction temperature is lowered so that the primers can anneal to the single-stranded DNA template. The temperature at this step depends on the melting temperature of the primers and is usually between 50-64°C for 20-40 seconds (Sharkey *et al.*, 1994).

The final step is the extension/elongation step during which the DNA polymerase synthesizes new DNA strands complementary to the DNA template strands. The temperature at this step depends on the DNA polymerase used. Taq polymerase has a temperature optimum of 70 to 74°C; thus, in most cases a temperature of 72°C is used (Wang *et al.*, 2002; Obi *et al.*, 2004). Several PCR based techniques have been employed for the diagnosis of *E. coli* O157:H7. These include Reverse transcriptase PCR (RT-PCR), simplex PCR, and doublex PCR, multiplex PCR, nested PCR.

Reverse transcriptase PCR (RT-PCR) – RT-PCR is a two-step process for converting RNA to DNA and the subsequent amplification of the reversely transcribed DNA. In the first step of RT-PCR, called the “first strand reaction,” complementary DNA (cDNA) is made from

mRNA template using dNTP's and a reverse transcriptase. The above components are combined with a DNA primer in a reverse transcriptase buffer for 1 h at 37 °C (Marone *et al.*, 2001; McIngvale *et al.*, 2002).

After the reverse transcriptase reaction is complete and cDNA has been generated from the original single-stranded mRNA, standard PCR called the “second strand reaction” is initiated. In the two-step RT-PCR, a thermo stable DNA polymerase and the upstream and downstream DNA primers are added. Heating the reaction to temperatures above 37°C facilitates binding of DNA primers to the cDNA, and subsequent higher temperatures allow the DNA polymerase to make double-stranded DNA from the cDNA. Heating the reaction to ~95°C melts the two DNA strands apart, enabling the primers to bind again at lower temperatures and begin the chain reaction again. After ~30 cycles, millions of copies of the sequence of interest are generated (Marone *et al.*, 2001). Diagnosis of *E. coli* O157:H7 can also be done using conventional, doublex, multiplex or Real-Time PCR. These different forms of PCR are improvements of the conventional PCR. Multiplex-PCR is a widely used, fast and accurate method for detecting *E. coli* having virulence-associated genes such as *stx1*, *stx2*, *eaeA* and *hlyA* (Paton and Paton, 1998; Radu *et al.*, 2001; Hahm *et al.*, 2003). Hahm *et al.* (2003) successfully used multiplex-PCR to amplify the *stx1*, *stx2*, *eaeA* and *hlyA* genes of *E. coli* O157:H7. In their study, they were able to establish the presence of the three-targeted genes in 26 out of 30 *E. coli* O157:H7 that were tested. Recently, the use of Real-Time PCR (RT-PCR) is very much on the increase due to the advantages attached to this form of PCR.

Real-time PCR, also known as quantitative real time PCR (QRT-PCR) is used to simultaneously quantify and amplify specific parts of a given DNA molecule. It is used to determine whether or not a specific sequence is present in the sample; and if so, the number of

copies in the sample (Nolan *et al.*, 2006). This method requires extensive optimisation of the number of PCR cycles to obtain results during logarithmic DNA amplification (Nolan *et al.*, 2006). This form of PCR is increasingly being used for clinical diagnostic purposes as well as to identify microorganisms in food and environmental samples.

Ibekwe and Grieve (2003) used real-time TaqMan PCR to detect as few as 10 CFU of *E. coli* O157:H7 per gram of soil without enrichment. Spano *et al.* (2005) developed a real-time polymerase chain reaction (RT-PCR) that enabled a rapid identification of *E. coli* O157:H7 in cattle and dairy wastewater samples produced from mozzarella cheese factories in Italy. In their study, Spano *et al.* (2005) noted that RT-PCR was very sensitive and managed to detect *eae*, *stx1*, *stx2* and 16SrRNA in the *E. coli* O157:H7 that were isolated from the cattle and wastewater samples that were tested.

nested PCR – Nested polymerase chain reaction is a modification of polymerase chain reaction intended to reduce the contaminations in products due to the amplification of unexpected primer binding sites. Conventional PCR requires primers complementary to the termini of the target DNA. A commonly occurring problem is primers binding to incorrect regions of the DNA, giving unexpected products.

Nested polymerase chain reaction involves two sets of primers, used in two successive runs of polymerase chain reaction, the second set intended to amplify a secondary target within the first run product. Nested PCR was used to detect as low as 24 CFU of *E. coli* O157:H7 in 10 g of ground beef in the US (Guan and Levine, 2002). Garcia-Aljaro *et al.* (2005) used nested PCR together with most probable number (MPN) method to quantify *stx2*

gene carrying bacteria colonizing an aquatic environment and detected the gene in all the 67 water samples that were tested for the bacteria.

2.7.3 Amplified Fragment Length Polymorphism (AFLP)

Amplified-fragment length polymorphism analysis is a selective restriction-fragment-amplification technique, which is based on the ligation of adapters (i.e. linkers and indexers) to genomic restriction fragments followed by a PCR-based amplification with adapter-specific primers. In amplified-fragment length polymorphism analysis, only a small amount of purified genomic DNA is needed (Guan *et al.*, 2002). This is digested with two restriction enzymes, one with an average cutting frequency (like *EcoRI*) and a second one with a higher cutting frequency (like *MseI* or *TaqI*) (Savelkoul *et al.*, 1999; Guan *et al.*, 2002).

Double-stranded oligonucleotide adapters are designed in such a way that the initial restriction site is not restored after ligation. This allows simultaneous restriction and ligation, while religated fragments are cleaved again (Kuiper *et al.*, 1995; Guan *et al.*, 2002). An aliquot is then subjected to two subsequent PCR amplifications under highly stringent conditions with adapter-specific primers that have at their 3' ends an extension of one to three nucleotides running into the unknown chromosomal restriction fragment (Kuiper *et al.*, 1995; Guan *et al.*, 2002).

An extension of one selective nucleotide amplifies 1 of 4 of the ligated fragments, whereas three selective nucleotides in both primers amplify 1 of the many fragments (Savelkoul *et al.*, 1999). The PCR primer, which spans the average-frequency restriction site, is labelled (Jin, 2005). After polyacrylamide gel electrophoresis, a highly informative pattern of bands can be obtained (Nataro and Kaper, 1998; Guan *et al.*, 2002). The patterns obtained

from different strains are polymorphic due to i) mutations in the restriction sites, ii) mutations in the sequences adjacent to the restriction sites and complementary to the selective primer extensions, and iii) insertions or deletions within the amplified fragments (Nataro and Kaper, 1998; Guan *et al.*, 2002). Iyoda *et al.* (1999) and Zhao *et al.* (2000) have used AFLP as a sub-typing method for *E. coli* O157:H7.

2.7.4 Pulse Field Gel Electrophoresis

Pulsed Field Gel Electrophoresis (PFGE) is a method for separating large DNA molecules, which unlike gel electrophoresis uses alternating electrical field (Chu, 1990). In PFGE, by changing the direction of the electrical field frequently, much greater size resolution is obtained. During PFGE, the smaller nucleic acid pieces are able to re-orient themselves to the new field more quickly than the larger ones. This delay in re-orientation means larger nucleic acid pieces end up migrating down the gel slower than smaller pieces.

Frequent changing of the nucleic acid pieces migration direction means that over the entire run, the movement sums to one direction (Chu, 1990). PFGE sub-typing of *E. coli* O157:H7 isolates have been successful in determining the link of these bacteria with some water and food borne disease outbreaks as well as none food related cases (Barrett *et al.*, 2001; Richards *et al.*, 2006). Bopp *et al.* (2003) successfully used PFGE to compare patients and environmental isolates of *E. coli* O157:H7 during a waterborne outbreak in the United States that occurred in upstate New York following a county fair in 1999. The findings of the study demonstrated that different types of *E. coli* O157:H7 were responsible for the outbreak.

2.8 Techniques of Assessing Risk Associated with *E. coli* O157:H7 in Drinking Water and Foods

Risk assessment is a methodology used to organize and analyze scientific information to estimate the probability and severity of an adverse event. Applied to water and food safety, the methodology can help to identify the stages in the operation, distribution, handling, and consumption of water and foods with potential risk of water and food-borne illnesses. It focuses on resources and efforts to reduce the risk of water and food-borne pathogens (Cassin *et al.*, 1998). The probable reasons why risk assessments related to pathogens present a key problem in most countries is due to lack of data on which to base estimates of disease impacts.

A risk assessment study may be done by use of different approaches. This may be done either through quantitative or epidemiological risk assessment approach (Cassin *et al.*, 1998; Haas *et al.*, 1999). In epidemiological studies, investigator attempt to establish the level of disease that can be attributed to a particular targeted pathogenic organism. Such approaches have been used in a number of cases (Walls and Scott, 1997).

Quantitative risk assessment approaches uses data on quality of a product or adverse event with respect to a target pathogenic microorganism or a chemical and derive a disease burden from the obtained data based on the likely risks associated with the levels of the target hazard that presents the adverse event. This disease burden is thus defined as a risk rather than proven level of diseases. Other researchers (Haas *et al.*, 1999; Havelaar and Melse, 2003) have also described the basis for such approaches.

To specify a risk assessment, one needs to identify a pathogen, a product like water or food, a food pathway and a population (Cassin *et al.*, 1998). For the present study, the health

risk of *E. coli* O157:H7 in drinking water, vegetable and bovine products presumably consumed by HIV/AIDS patients with diarrhoea in the Amathole District, in the Eastern Cape Province of South Africa will be evaluated. The reference pathogen in this study (*E. coli* O157:H7), was chosen because of its prevalence in most water sources in the Eastern Cape Province as evidenced by the works of Momba and Mfenyana, (2005) and also its implication in most water and food borne disease outbreaks (Riley *et al.*, 1983; Bruce-Grey-Owen Sound Health Unit, 2000).

Bovine products are the most important reservoirs of *E. coli* O157:H7 (Wells *et al.*, 1991; Zhao *et al.*, 1995). Therefore, bovine products were chosen as one of the foods for which the risk could be evaluated as numerous work has been done to establish the prevalence of this organism on bovine products (Mark and Roberts, 1993; Walls and Scott, 1997). The most important reason for conducting a risk assessment for pathogenic *E. coli* O157:H7 is due to its potential impact on public health (AGA, 1994, USDA: APHIS: VS, 1994). There is a considerable evidence of persistent diarrhoea among adults, children, and persons who are HIV positive or suffering from AIDS (Sathaporn *et al.*, 1996). However, the risk that this organism pose on HIV/AIDS patients and the possible link of water, meat and meat products and vegetables that may be containing this bacterium to the diarrhoeic conditions of HIV/AIDS patients has not been pursued. The discussions in this section will be restricted to quantitative risk assessment.

2.8.1 Quantitative risk assessment steps

Quantitative risk assessment is a mathematical modeling approach, which was developed to assess human health risks associated with exposure to chemical hazards (NAS, 1983;

Australian Government – Department of Health and Aging, 2006). It mainly consists of four steps namely: hazard assessment, exposure assessment, dose-response analysis, and risk characterization (NAS, 1983). For microorganisms, hazard assessment refers to the identification of a pathogen as an agent of potential significance and it is generally a straightforward task (Australian Government – Department of Health and Aging, 2006).

The major steps of Quantitative Microbiological Risk Assessment (QMRA) include: i) Hazard identification; ii) Exposure assessment; iii) Dose-response; iv) Risk characterization (Potter, 1996; Australian Government – Department of Health and Aging, 2006).

2.8.1.1 Hazard identification

A hazard is a biological, chemical or physical agent or property in food that has a potential to cause an adverse effect (Potter, 1996). This involves the identification of a known or potential health effect associated with a particular agent. In this study, *E. coli* O157:H7 was identified as the hazard (Potter, 1996). In principle, outbreaks are associated with ingestion of water and food that in the course of distribution were contaminated with *E. coli* O157:H7 (Potter, 1996; Cassin *et al.*, 1998).

2.8.1.2 Exposure assessment

The exposure assessment explores the avenues that can lead to a person or a population acquiring *E. coli* O157:H7 infection (FSIS, 2001). It estimates the occurrence of the bacterium in the study population, in the present study; it is the presence of *E. coli* O157:H7 in confirmed and non-confirmed HIV/AIDS patients with diarrhoea. Risk exposure also

estimates the occurrence of the pathogen in the water or a food product presumably consumed by the study population.

A study by Food Safety and Inspection Service (FSIS) (2001) in the US developed a model to assess the risk of exposure to *E. coli* O157:H7 from ground beef. Exposure assessment of *E. coli* O157:H7 in water, meat, and meat products and vegetables presumed eaten by HIV/AIDS depends on the population of the bacterium in such waters, meat and meat products and the vegetables.

In exposure assessment, with regard to microbiological risk assessment on water and foods, parameters such as daily quantities of water and/or foods consumed by the patients under investigation must be known or derived based on a previously published work (Helena and Steyn, 2002). In some cases, factors that may affect the growth of the bacterium in water and foods are also taken into consideration. Such factors include but are not limited to storage temperature of the water and/or food, cooking of the food and the season during which the study was done. For instance, some risk assessment studies in the US on *E. coli* O157:H7 in the grinder loads for raw beef patties varied with seasons, with summer season registering high risk of the bacterium than winter seasons (Haas *et al.*, 1999; Elmali *et al.*, 2005).

2.8.1.3 *Dose-response analysis*

Dose response analysis is the determination of the relationship between the magnitude and frequency of exposure of adverse effects (Cassin *et al.*, 1998). The possibility of illness is determined by the probability of illness caused by a single *E. coli* O157:H7 cell multiplied by the numbers of *E. coli* O157:H7 cells presumed ingested (Cassin *et al.*, 1998).

2.8.1.4 Risk characterization

Under risk characterization, the adverse effects likely to occur in a given population and a summary of assumptions and sources of uncertainty are considered. Both the probability of infection and illness depends on the amount of viable *E. coli* O157:H7 cells ingested (Cassin *et al.*, 1998). In other words, it looks at the dose-response relationship (FSIS, 2001). Due to lack of effective treatment for *E. coli* O157:H7 infection and the perceived outcome on human, experimental studies exposing humans to the bacterium have not been done and may not be possible in the near future (FSIS, 2001).

To derive a dose response function for *E. coli* O157:H7, a number of steps are always considered. These include:

- i. The estimation of the number of *E. coli* O157:H7 related illnesses to the study population attributable, in this present case to the consumption of water and/or meat and meat products and vegetables by confirmed and non-confirmed HIV/AIDS patients with diarrhoea.
- ii. The estimation of the level of *E. coli* O157:H7 in the waters and the meat and meat products and vegetables based on daily intake of the water and foods investigated as revealed by a previous study (Helena and Steyn, 2002)
- iii. The derivation of the lower (5th) and upper (95th) bounds of *E. coli* O157:H7 dose response function, based on previous clinical studies of the bacterium (Teunis *et al.*, 2004).

The lower and upper bound dose response functions help in the estimation of the number of cases due to the intake of water and/or a food product. In a dose response, the susceptibility of the study population is also taken into consideration based on factors like age and immunity. For instance, children would be highly susceptible to *E. coli* O157:H7 infection than adults upon consumption of a particular food product contaminated with the bacterium (Teunis *et al.*, 2004). In addition, it is perceived that immune-suppressed individuals are always at greater risk, especially the elderly and those suffering from diseases such as HIV/AIDS and therefore would be more vulnerable to water and food borne pathogens such as *E. coli* O157:H7 (Cassin *et al.*, 1998). In the Eastern Cape Province, *E. coli* has been reported to be predominant in treated water sources (Momba and Kaleni, 2002; Momba and Notshe, 2003; Momba *et al.*, 2004a, b 2005a, b, 2006a, b). However, not a specific study has elucidated on the prevalence of *E. coli* O157:H7 in water, meat and meat products and vegetables consumed by residents of the Eastern Cape Province as well as in the stools of individuals in the province suffering from diarrhoea. The most important fact is that diarrhoeal diseases have been endemic in the region and no link has been established between the presence of these bacteria in the water and foods as well as in the stools and the potential health risks associated with this organism.

Case studies of the prevalence of *E. coli* O157:H7 in drinking water, meat and meat products and vegetables consumed by the communities of the Amathole District in the Eastern Cape Province of South Africa are described in chapters 3, 4 and 5 respectively. Chapter 6 focuses on the prevalence of *E. coli* O157:H7 in the stools of confirmed and non-confirmed HIV/AIDS patients with diarrhoea who were visiting Frere Hospital in the Amathole District for treatment during the study period. Molecular link between the prevalence of this organism

in water, meat and meat products and vegetables and in the stools of confirmed and non-confirmed HIV/AIDS patients has been discussed in chapter 7. Finally, chapter 8 dwells on the health risks of *E. coli* O157:H7 in drinking water, meat and meat products and vegetables to diarrhoeic HIV/AIDS patients.

As stated in chapter 1, the information gathered from this study will assist Water Engineers, Wastewater Plant Managers, Local Water Authority and Microbiologists. Medical Doctors, Epidemiologists, Food Industries and Local Markets and especially the Department of Health and the Department of Water Affairs and Forestry in their respective obligation to develop appropriate strategies for the control of water and food borne diseases in the Eastern Cape Province and South Africa in general to provide public health protection through didactic fora, better water treatment and monitoring of water and food safety, hygiene and sanitation.

References

- Abdul-Raouf, U. M., L. R. Beuchat, and M. S. Ammar.** 1993. Survival and growth of *Escherichia coli* O157:H7 in ground, roasted beef as affected by pH, acidulants, and temperature. *Appli. Environ. Microbiol.*; **59(8)**: 2364–2368.
- Ackers, M. L., B. E. Mahon, E. Leahy, B. Goode, T. Damrow, P. S. Hayes, W. F. Bibb, D. H. Rice, T. J. Barret, L. Hutwagner, P. M. Griffin, and L. Slutsker.** 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. *J. Infect. Dis.*; **177**: 1588–1593.
- Adams, M. R. and M. O. Moss.** 1997. Food microbiology. , The Royal Society of Chemistry, Cambridge.
- AGA, (American Gastroenterological Association).** 1995. Consensus conference statement: *Escherichia coli* O157:H7 infections. An emerging national health crisis, July 11–13, 1994. *Gastro.*; **108**: 1923–1934.
- Ahammed, M. M., and M. Chaudhuri.** 1999. A low cost home water filter. *J. Water Serv. Res Technol.*; **48**: 263–267.
- Ainsworth, R.** 2004. *Safe Piped Water: Managing Microbial Water Quality in Piped Distribution Systems*. IWA Publishing, Alliance House, UK. ISBN 1 84339 0396.
- Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter.** 2002. *Peroxisomes, in Molecular Biology of the Cell*, 4th ed., Garland. ISBN 0815332181
- Alexandre, L., L. Bernard, P. Denis, and M. Jacques.** 2000. Particular biochemical profiles for enterohemorrhagic *Escherichia coli* O157:H7 isolates on the ID 32E system. *J. of Clin. Microbiol.*; **39**: 1161–1164.

- Altekruse, S. F., D. A. Street, S. B. Fein, and A. S. Levy.** 1996. Consumer knowledge of foodborne microbial hazards and food-handling practices. *J. Food Prot.*; **59(3)**: 287–294
- Anonymous.** 2007a. Enterohemorrhagic *E. coli*, Including *E. coli* O157:H7. Available at <http://www.health.state.ok.us/program/cdd>. Accessed May 07th 2007.
- Arimi, S. M., E. Koroti, E. K. Kang'ethe, A. O. Omore, J. J. McDermott, K. Macharia K, et al.** 2000. Risk of infection from *E. coli* O157:H7 through informally marketed raw milk in Kenya 3rd All Africa Conference on Animal Agriculture.
- Armstrong, G. L., J. Hollingsworth, and J. G. Jr. Morris.** 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.*; **18**: 29–51.
- Arthur, M., J.M. Bosilevac, X. Nou, S.D. Shackelford, T.L. Wheeler and M.P. Kent et al.** 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants, *J. Food Prot.*; **67**: 658–665.
- Arthur, T. M., J. M. Bosilevac, D. M. Brichta-harhay, M. N. Guerini, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie.** 2007. Transportation and lairage environment effects on prevalence, numbers, and diversity of *Escherichia coli* O157:h7 on hides and carcasses of beef cattle at processing. *J. Food Prot.*; **70(2)**: 280–286.
- Asakura, H., S. Makino, T. Shirahata, T. Tsukamoto, H. Kurazono, T. Ikeda, and K. Takeshi.** 1998. Detection and genetical characterization of shiga toxin-producing *Escherichia coli* from wild deer. *Microbiol. Imm.*; **42**: 815–822.

- Australian Government – Department of Health and Aging.** 2006. Environmental Health Risk Assessment Guidelines for assessing human health risks from environmental hazards. Available at: <http://www.health.gov.au>. Accessed 24 July 2007.
- Bach, S. J., T. A. McAllister, D. M. Veira, V. P. J. Gannon, and R. A. Holley.** 2003. Effect of bacteriophage DC22 on *Escherichia coli* O157:H7 in an artificial rumen system (Rusitec) and inoculated sheep. *Anim. Res.*; **52**: 89–101.
- Bacon, R.T., K. E. Belk, J. N. Sofos, R. P. Clayton, J. O. Reagan, and G. C. Smith.** 2000. Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination, *J. Food Prot.*; **63**: 1080–1086.
- Barbut, S. and M. W. Griffiths.** 2003. Developing Validation Models for *Escherichia coli* O157 Inactivation in Dry Fermented Sausages, Department of Food Science, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.
- Barham, A. R., B. L. Barham, A. K. Johnson, D. M. Allen, J. R. Blanton, and M. F. Miller.** 2002. Effects of the transportation of beef cattle from the feed yard to the packing plant on prevalence levels of *Escherichia coli* O157 and *Salmonella* spp. *J. Food Prot.*; **65**: 280–283.
- Barkocy-Gallagher, G. A., E. D. Berry, M. Rivera-Bentacourt, T. M. Arther, X. Nou, and M. Koohmaraie.** 2002. Development of a method for the recovery of *Escherichia coli* O157:H7 and *Salmonella* from beef carcass sponge samples and bovine faecal and hide samples. *J. food Prot.*; **65**: 1527–1534.

- Barlow, R. S., K. S. Gobius, and P. M. Desmarchelier.** 2006. Shiga toxin-producing *Escherichia coli* in ground beef and lamb cuts: Results of a one year study. *Int. J. Food Microbiol.*; **111(1)**: 1–5.
- Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, et al.** 1983 Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS) *Sci.*; **220**: 868–871
- Barrett, T., J. Benkel, H. Denise, T. Breuer et al.** 2001. A multistate outbreak of *Escherichia coli* O157:H7 infections linked to alfalfa sprouts grown from contaminated seeds. Available from: <http://www.encyclopedia.com/doc/1G1-80951231.html>. Accessed 24 July 2007.
- Barza, M. and K. Travers.** 2002 Excess infections due to antimicrobial resistance: the "attributable fraction". *Clin. Infect. Dis.*; **34(3)**: S126–S130.
- Bauwens, L, W. De Meurichy, and F. Vercammen.** 2000. Isolation of *Escherichia coli* O157 from zoo animals. *Vlaams Diergeneeskundig Tijdschr* **69**: 76–79.
- Beattie, K. L., W. G. Beattie, L. Meng, S. L. Turner, R. Coral-Vazquez, D. D. Smith, P. M. McIntyre, and D. D. Dao.** 1995. Advances in genosensor research. *Clin. Chem.* **41**: 700–706.
- Beier, M., and D. H. Jörg.** 1999. Versatile derivatisation of solid support media for covalent bonding on DNA-microchips. *Nucleic Acids Res.*; **27**: 1970–1977.
- Bekal, S., R. Brousseau, L. Masson, G. Prefontaine, J. Fairbrother, and J. Harel.** 2003. Rapid identification of *Escherichia coli* pathotypes by virulence gene detection with DNA microarrays. *J. Clin. Microbiol.*; **41(5)**: 2113–2125.

- Bell, B. P., M. Goldoft, P. M. Griffin, M. A. Davis, D. C. Gordon, P. I. Tarr, C. A. Bartleson, J. H. Lewis, T. J. Barrett, and J. G. Wells.** 1999. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhoea and hemolytic uremic syndrome from hamburgers: The Washington experience. *JAMA*. **272(17)**: 1349–1353.
- Bennion, M and B. Scheule.** 2004. *Introductory foods*. 12th ed. Upper Saddled River, NJ. Pearson Education International.
- Berg, J., J. Tymoczko, and L. Stryer.** 2002 *Biochemistry*. W. H. Freeman and Company ISBN 0-7167-4955-6
- Berine, C. M., A. Darfeuille-Michaud, J. E. Laurence, M. Yukiko, and F. K. Martin.** 2002. Role of EHEC O157:H7 virulence factors in the activation of intestinal epithelial cell NFκ B and MAP kinase pathways and the up regulated expression of interleukin 8. *Cellular Microbiol.*; **4(10)**: 635–647.
- Bettelheim, K. A.** 1994. Biochemical characteristics of *Escherichia coli*, p. 3-30. In C. L. Gyles (ed.), *Escherichia coli* in domestic animals and humans. CAB International, Wallingford, United Kingdom
- Beumer, R. R. and H. Kusumaningrum.** 2003. Kitchen hygiene in daily life. *International Biodeterior. Biodegrad.*; **51(4)**: 299–302.
- Beutin, L., D. Geier, S. Zimmermann, and H. Karch.** 1995. Virulence markers of shiga-like toxin-producing *Escherichia coli* strains from healthy domestic animals of different species. *J. Clin. Microbiol.*; **33**: 631–635.
- Beutin, L., G. Knollmann-Schanbacher, W. Rietschel, and H. Seeger.** 1996. Animal reservoirs of *Escherichia coli* O157:H7. *Vet. Res.*; **139**: 70–71.

- Beutin, L., M. A. Montenegro, I. Ørskov, F. Ørskov, J. Prada, S. Zimmermann, and R. Stephan.** 1989. Close association of verotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. *J. Clin Microbiol.* **27**: 2559–2564.
- Beutin, L., S. Aleksic, S. Zimmermann, and K. Gleier.** 1994. Virulence factors and phenotypical traits of verotoxigenic strains of *Escherichia coli* isolated from human patients in Germany. *Med. Microbiol. Immunol.*; **183**: 13–21.
- Björn-Arne, L., H. Even, G. Elizabet, V. Traute, and K. Georg.** 2003. DNA fingerprinting of Shiga-toxin producing *Escherichia coli* O157 based on multiple-locus variable-number tandem-repeats analysis (MLVA). *Ann. Clin. Microbiol. Antimicrob.*; **2**: 12.
- Blanco, M., J. E. Blanco, J. Blanco, E. A. Gonzalez, A. Mora, C. Prado, L. Fernandez, M. Rio, J. Ramos, and M. P. Alonso.** 1996. Prevalence and characteristics of epidemiology and infection of *Escherichia coli* Serotype O157:H7 and other verotoxin-producing *E. coli* in healthy cattle. *Exp. Biol. Med.*; **117**: 251–257.
- Bogaard, V, D, A. E., and E. E. Stobberingh.** 1996. Is it time to ban all antibiotics as animal growth-promoting agents? *Lancet*; **348**: 1454–1456.
- Bopp, D. J., B. D. Sauders, A. L. Waring, J. Ackelsberg, N. Dumas, E. Braun-Howland, D. Dziewulski, B. J. Wallace, M. Kelly, T. Halse, K. A. Musser, P. F. Smith, D. L. Morse, and R. J. Limberger.** 2003. Detection, Isolation, and Molecular Subtyping of *Escherichia coli* O157:H7 and *Campylobacter jejuni* Associated with a Large Waterborne Outbreak. *J. Clin. Microbiol.*; **41**: 174–180.
- Borch, E. and P. Arinder.** 2003. Bacteriological safety issues in red meat and ready-to-eat meat products, as well as control measures. *Meat Sci.*; **62(3)**: 381–390.

- Bosilevac, J.M., T. M. Arthur, T. L. Wheeler, S. D. Shackelford, M. Rossman, and J. O. Reagan *et al.***, 2004. Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and *Enterobacteriaceae* are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant, *J. Food Prot.*; **67**: 646–650.
- Bowen, B., J. Steinberg, U. K. Laemmli, and H. Weintraub.** 1980. The detection of DNA-binding proteins by protein blotting. *Nucleic Acids Res.*; **3**: 1–20
- Bradshaw, D., N. Nadine, L. Ria, G. Pam, J. Jané, N. Beatrice, N. Rosana, P. Desirée, P. and S. Michelle.** 2000. South African National Burden of Disease Study: Mortality Estimates for Eastern Cape Province. A publication OF Medical Research Council of South Africa: Burden of Disease Research Unit; 1–16. Available from: <http://www.mrc.ac.za>. Accessed 06th February, 2007.
- Brady, R. C, D. L. Swerdlow, and B. A. Woodruff.** 1992. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. *Ann. Internal Med.* **117**: 812–819.
- Brichta-Harhay, D. M., T. M. Arthur, J. M. Bosilevac, M. N. Guerini, N. Kalchayanand, and M. Koohmaraie.** 2007. Enumeration of *Salmonella* and *Escherichia coli* O157:H7 in ground beef, cattle carcass, hide and faecal samples using direct plating methods. *J. Appl. Microbiol.*; doi:10.1111/j.1365-2672.2007.03405.x
- Brinkman, E., R. Dijk, Van Nieuwland, and R. R. Beumer.** 1999. Microbiological quality leftovers of foods from domestic environments and effect of chilled storage. In Tuijtelaars, ACJ, Samson, RA, Rombouts, FM & Notermans, S. (Eds). 1999. *Food microbiology and food safety into the next millennium: proceedings of the 17th*

- International Conference on Food Microbiology and Hygiene (ICFMH)*, Veldhoven, The Netherlands, 13-17 September 1999. TNO. Zeist, the Netherlands.
- Brooks, J. T., B. O. John, K. Lata, O. George, L. S. Roger, G. W. Joy, B. Michele, B. Cheryl, C. Wairimu, E. B. Mark, C. Tom, M. V. John, M. Eric, and S. Laurence.** 2003. Surveillance for bacterial diarrhea and antimicrobial resistance in rural western Kenya, 1997–2003. *J. Clin. Infect. Dis.*; **43**: 393–401.
- Brown, A.** 2000. *Understanding food: principles and preparation*. Belmont, CA. Wadsworth-Thompson Learning.
- Brown, J. L., T. Ross, T. A. McMeekin, and P. D. Nichols.** 1997. Acid habituation of *Escherichia coli* and the potential role of cyclopropane fatty acids in low pH tolerance. *Internat. J. Food Microbiol.*; **37**: 163–173.
- Browning, N. G., J. Botha, H. Sacho, and P. J. Moore.** 1990. *Escherichia coli* O157:H7 haemorrhagic colitis. Report of the first South African case. *SA Surg. J.*; **28**: 28–29.
- Bruce-Grey-Owen Sound Health Unit.** 2000. Waterborne outbreak of gastroenteritis associated with a contaminated municipal water supply, Walkerton, Ontario, May–June 2000. *Canadian Comm. Dis. Rep.*; **26(2)**:170–173.
- Brunder, W., H. Schmidt, and H. Karch.** 1996. KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. *J. Microbiol.*; **142**: 3305–3315.
- Buchanan, R. L. and L. A. Klawitter.** 1992. The effect of incubation, initial pH, and sodium chloride on the growth kinetics of *Escherichia coli* O157:H7. *J. Food Microbiol.*; **9**: 185–196.

- Buchanan, R. L. and P. D. Michael.** 1997. Foodborne Disease Significance of *Escherichia coli* O157:H7 and Other Enterohemorrhagic *E. coli*. *Sci. Status Summ.*; **51**:69–76.
- Buchanan, R. L., S. G. Edelson, K. Snipes, and G. Boyd.** 1998. Inactivation of *Escherichia coli* O157:H7 in apple juice by irradiation. *Appl. Environ. Microbiol.*; **64**: 4533–4535.
- Burland, V., Y. Shao, N. T. Perna, G. Plunkett, H. J. Sofia, and F. R. Blattner.** 1998. The complete DNA sequence and analysis of the large virulence plasmid of *Escherichia coli* O157:H7. *Nucleic Acids Res.*; **26**: 4196–4204.
- Burnette, W. N.** 1981. “Western blotting”: electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.*; **112**: 195–203.
- Cagney, C., H. Crowley, G. Duffy, J-J. Sheridan, S. O’Brien, E. Carney, W. M. Anderson, D. A. Dowell, I. S. Blair, and R. H. Bisho.** 2004. Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. *J. Food Microbiol.* **21**: 203-212.
- Call, D.** 2001. DNA microarrays - principles and applications in molecular diagnostics. An online current awareness journal. ISSN 1569 0530. Available at: <http://www.vetscite.org/publish/articles/000022/index.html>. Accessed 25 July 2007.
- Call, D. R., F. J. Brockman, and D. P. Chandler.** 2001. Detecting and genotyping *Escherichia coli* O157:H7 using multiplexed PCR and nucleic acid microarrays. *Int. J. Food Microbiol.*; **67**: 71–80.

- Callaway, T. R. R. C. Anderson, T. S. Edrington, K. J. Genovese, K. M. Bischoff, T. L. Poole, Y. S. Jung, R. B. Harvey, and D. J. Nisbet.** 2003. What are we doing about *Escherichia coli* O157:H7 in cattle? *J. Anim. Sci.*; **82**: 93–99
- Camel, V. and A. Bermond.** 1998. The use of ozone and associated oxidation processes in drinking water treatment. *Water Res.*; **31(11)**: 3208–3222.
- Caprioli, A., Morabito, S., Brugere, H. and E. Oswald.** 2005. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Vet. Res.*; **36**: 289–311.
- Cassin, M. H., M. L. Anna, C. D. Ewen, R. William, and R. S. McColl.** 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. *Int. J. Food Microbiol.* **41(1)**: 21–44.
- Castillo, A., L. M. Lucia, G. K. Kemp and G. R. Acuff.** 1999. Reduction of *Escherichia coli* O157:H7 and *Salmonella typhimurium* on beef carcass surfaces using acidified sodium chlorite, *J. Food Prot.*; **62**: 580–584.
- CDC - MMWR.** 2001. Outbreaks of *Escherichia coli* O157:H7 infections among children associated with farm visits – Pennsylvania and Washington, 2000. *MMWR.*; **50(15)**: 293–297.
- CDC.** 2001. Dignosis and management of foodborne illness, a primer for physicians. *MMWR.*; **50**:2.
- Centrus International.** 2006. The Envisio System: The detection of *E. coli* O157:H7 in ground beef. Available at: <http://www.qdusa.com>. Accessed 25 July 2007.
- CFIA (The Canadian Food Inspection Agency).** 2006. *E. coli* O157:H7 Outbreak in the United States Associated with Bagged Fresh Spinach. *CCDR.*; **32(22)**: 272–274

- CFSPH.** 2004. *Escherichia coli* O157:H7 infections. Institute for international cooperation in animal biologics, Iowa State University.
- Chalmers, R. M., R. L. Salmon, G. A. Willshaw, T. Cheasty, N. Looker, I. Davies, and C. Wray.** 1997. Vero-cytotoxin-producing *Escherichia coli* O157 in a farmer handling horses. *Lancet*; **349**: 1816.
- Chandler, D.P., J. Brown, D. R. Call, S. Wunschel, J. W. Grate, D. A. Holman, L. Olson, M. S. Stottlemire, and C. J. Bruckner-Lea.** 2001. Automated immunomagnetic separation and microarray detection of *E. coli* O157:H7 from poultry carcass rinse. *Int. J. Food Microbiol.*; **70**: 143–154.
- Chapman, P. A., A. T. Cerdan Malo, M. Ellin, R. ASHTON, and M. A. Harkin.** 2001. *Escherichia coli* O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products in South Yorkshire, UK. *Int. J. Food Microbiol.*; **64**: 139–150.
- Chapman, P. A., C. A. Siddons, A. T. Cerdan Malo, and M. A. Harkin.** 1997a. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. *Epidemiol. Infect.*; **119**: 245–250
- Chapman, P. A., C. A. Siddons, P. M. Zadik, and L. Jewes.** 1991. An improved selective medium for the isolation of *Escherichia coli* O157. *J. Med. Microbiol.*; **35**:107–110.
- Chapman, P. A., C. A., Siddons, J. Manning, and C. Cheetham.** 1997b. An outbreak of infection due to verocytotoxin-producing *Escherichia coli* O157 in four families: the influence of laboratory methods on the outcome of the investigation. *Epid. Infect.*; **119**:113–119.

- Chart, H., S. M. Scotland, and B. Rowe.** 1989. Serum antibodies to *Escherichia coli* serotype O157: H7 in patients with hemolytic uremic syndrome. *J. Clin. Microbiol.*; **27**: 285–290.
- Cheng S, C. Fockler, W. M. Barnes, and R. Higuchi.** 1994. "Effective amplification of long targets from cloned inserts and human genomic DNA". *Proc Natl Acad Sci.* **91**: 5695–5699.
- Cheville, A. M., K. W. Arnold, C. Buchrieser, C.-M. Cheng, and C. W. Kaspar.** 1996. *rpoS* regulation of acid, heat and salt tolerance in *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.*; **62**: 1822–1824.
- Chicken Safety Tips.** 2001. *Consumer Institute of New Zealand*. Available from: <http://www.consumer.org.nz>. Accessed 17 July 2007.
- Chikthimmah, N. C., L. F. Laborde, and R. B. Beelman.** 2003. Critical factors affecting the Destruction of *Escherichia coli* O157:H7 in Apple Cider Treated with Fumaric Acid and Sodium Benzoate. *J. Food Sci.*; **68**: 1438–1442.
- Chu, G.** 1990. Pulsed-field electrophoresis: theory and practice. In *Methods: A Companion to Methods of Enzymology. Pulsed-Field Electrophoresis* (B. Birren and E. Lai, eds.), Academic Press, San Diego. pg. 129-142.
- Cizek, A., I. Literak, and P. Scheer.** 2000. Survival of *Escherichia coli* O157 in faeces of experimentally infected rats and domestic pigeons. *Lett. Appl. Microbiol.*; **31**: 349–352.
- Cizek, A., P. Alexa, I. Literak, J. Hamrik, P. Novak, and J. Smola.** 1999. Shiga toxin-producing *Escherichia coli* O157 in feedlot cattle and Norwegian rats from a large-scale farm. *Lett. Appl. Microbiol.*; **28**: 435–439.

- Clasen, T. J. Brown, S. Collins, O. Suntura, and S. Cairncross,** 2004. Reducing diarrhoea through the use of household-based water filters: a randomised controlled trial in rural Bolivia. *Amer. J. Trop. Med. and Hyg.*; **70**: 651–657.
- Clausen-Schaumann H, M. Rief, C. Tolksdorf, and H. Gaub.** 2000. "Mechanical stability of single DNA molecules. *Biophys.*; **78(4)**: 1997–2007
- Cody, S. H., M. K. Glynn, J. A. Farrar, K. L. Cairns, P. M. Griffin, J. Kobayashi, M. Fyfe, R. Hoffman, A. S. King, J. H. Lewis, B. Swaminathan, R. G. Bryant, and D. J. Vugia.** 1999. An Outbreak of *Escherichia coli* O157:H7 Infection from Unpasteurized Commercial Apple Juice. *Intern. Med.*; **130**: 202–209.
- Collins, S.** 2000. *Hand-dug Shallow Wells*. SKAT Publications, St. Gallen.
- Colwell, R. R., A. Huq, M. S. Islam et al.** 2003. Reduction of cholera in Bangladeshi villages by simple filtration. *Proc. Natl. Acad. Sci. USA*; **100**: 1051–1055.
- Conner, D. E. and J. S. Kotorola.** 1995. Growth and survival of *Escherichia coli* O157:H7 under acidic conditions. *J. Appl. Environ. Microbiol.*; **61**: 382–385.
- Conroy, R. M., M. E. Meegan, T. Joyce, K. McGuigan, J. Barnes.** 2001. Solar disinfection of drinking water protects against cholera in children under 6 years of age. *Arch. Dis. Child.* **85(4)**: 293–295.
- Conroy, R. M., M. Elmore-Meegan, T. Joyce, K. G. McGuigan, and J. Barnes.** 1996. Solar disinfection of drinking water and diarrhoea in Maasai children: a controlled field trial. *Lancet*; **348**: 1695–97.
- Cornick, N. A and A. F. Helgersson.** 2004. Transmission and infectious dose of *Escherichia coli* O157:H7 in swine. *Appl. Environ. Microbiol.*; **70**: 5331–5335.

- Crampin M, G. Willshaw, R. Hancock, T. Djuretic, C. Elstob, A. Rouse, T. Cheasty, and J. Stuart.** 1999. Outbreak of *Escherichia coli* O157 infection associated with a music festival. *European J. Clin. Microbiol. Infect. Dis.*; **18**: 286–288.
- Cray, W. C. and H. W. Moon.** 1995. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.*; **61(4)**: 1586–1590.
- Crump, J. A., P. Otieno, L. Slutsker, B. Keswick, D. Rosen, and S. P. Luby.** 2005. Household based treatment of drinking water with flocculant – disinfectant for preventing diarrhoea in areas with turbid source water in rural western Kenya: cluster randomised controlled trial. *BMJ.*; **331**: 478.
- Dadie, A., T. Karou, N. Adom, A. Kette, and M. Dosso.** 2000. Isolation of enteric pathogenic agents in Cote d' Ivoire: *Escherichia coli* O157:H7 and enteroaggregative *Escherichia coli*. *Bull Soc of Pathol Exot.*; **93**: 95 – 96.
- Dargatz, D. A., S. J. Wells, L. A. Thomas, D. D. Hancock, and L. P. Garber.** 1997. Factors associated with the presence of *Escherichia coli* O157 in feces of feedlot cattle. *J. Food Prot.*; **60**: 466–470
- Dean-Nystrom, E. A., L. J. Gansheroff, M. Mills, H. W. Moon, and A. D. O'Brien.** 2002. Vaccination of pregnant dams with intimin(O157) protects suckling piglets from *Escherichia coli* O157:H7 infection. *Infect Immun.*; **70**: 2414–2418.
- Deb, B. C., P. G. Sircar, S. T. Sengupta, S. K. Mondal, D. N. Gupta, N. G. Saha, S. Gosh, U. Mitra, S. C. Pal.** 1986. Studies on interventions to prevent eltor cholera transmission in urban slums. *Bull. WHO.*; **64**: 127–131.

- Department of Health, EC.** 2001. HIV and Syphilis survey Provincial Report. Epidemiological Notes. Available from: <http://www.ecdoh.gov.za/uploads/files/>. Accessed May, 17th 2007.
- Department of Health, South Africa.** 2005. *National HIV and syphilis antenatal sero-prevalence survey in South Africa 2004*. Pretoria, Department of Health.
- Derisi, J., L. Penland, P. O. Brown, M. L. Bittner, P. S. Meltzer, M. Ray, Y. Chen, Y. A. Su, and J. M. Trent.** 1996. Use of a cDNA microarray to analyze gene expression patterns in human cancer. *Natl Gen.*; **14**: 457–460.
- Dipineto, L., A. Santaniello, M. Fontanella, K. Lagos, A. Fioretti, and L. F. Menna.** 2006. Presence of Shiga toxin-producing *Escherichia coli* O157:H7 in living layer hens. *Lett. Appl. Microbiol.*; **43**: 293–295.
- Donnenberg, M. S. and T. S. Whitam.** 2001. Pathogenesis and evolution of virulence in enteropathogenic and enterohemorrhagic *Escherichia coli*. *The J. of Clin. Invest.*; **107(5)**: 539–548.
- Dontorou, C., C. Papadopoulou, G. Filioussis et al.** 2003. Isolation of *Escherichia coli* O157:H7 from foods in Greece. *Int. J. Food Microbiol.*; **82**: 273–279.
- Dorrington, R. E., L. F. Johnson, D. Bradshaw, and T. Daniel.** 2006. The Demographic Impact of HIV/AIDS in South Africa. National and Provincial indicators for 2006. Cape Town: Centre for Actuarial Research, South African Medical Research Council and Actuarial Society of South Africa. ISBNB 0-7992-2322-0
- Dorsa, W. J., C. N. Cutter, and G.R. Siragusa,** 1997. Effects of steam-vacuuming and hot water spray wash on the microflora of refrigerated beef carcass surface tissue

- inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes*, *J. Food Prot.*; **60**: 114–119
- Doyle, M. P. and J. L. Schoeni.** 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. *Appl. Environ. Microbiol.*; **48(4)**: 855–856.
- Du Toit L. and V. Irma.** 2005. Food practices associated with increased risk of bacterial food-borne disease of female students in self-catering residences at the Cape Peninsula University of Technology. *J. Fam. Ecol. Cons. Sci.*; **33**: 73–88
- Duffy, G. R. C. Whiting, and J. J. Sheridan.** 1999. The effect of a competitive microflora, pH and temperature on the growth kinetics of *Escherichia coli* O157:H7. *J. Food Microbiol.*; **16**: 299–307.
- Dunn, J. R., J. E. Keen, D. Moreland, and R. A. Thompson.** 2004. Prevalence of *Escherichia coli* O157:H7 in white-tailed deer from Louisiana. *J. Wildlife Dis.*; **40**: 361–365.
- DWAF (Department of Water Affairs and Forestry).** 1996. *South African Water Quality Guidelines for Domestic Use* (2nd edn.) Pretoria. pg 1–197.
- DWAF (Department of Water affairs and Forestry).** 2005. A drinking water quality framework for South Africa; pp 1 – 69. Available from: <http://www.dwaf.gov.za>. Accessed April 09th 2007.
- Dynal Product Brochure.** 2006. *Dynabeads® anti-E coli*. For rapid selective enrichment of *Escherichia coli* O157:H7. Dynal A.S. Oslo, Norway.
- Easton, J. H., J. J. Gauthier, M. M. Lalor, and R. E. Pitt.** 2005. Die-Off of Pathogenic *E coli* O157:H7 in Sewage Contaminated Waters. *JAWRA.*; **41(5)**: 1187–1193.

- Edrington, T. S. R. C. Todd, E. I. Sam, J. E. Mike, L. L. Mike, C. A. Robin, and J. N. David.** 2006. Seasonal shedding of *Escherichia coli* O157:H7 in ruminants: A new hypothesis. *Food borne patho. Dis.*; **3(4)**: 413–421.
- Effler, E., M. Isaacson, L. Arntzen, R. Heenan, P. Canter, and T. Barrett.** 2001. Factors contributing to the emergence of *Escherichia coli* O157:H7 in Africa. *Emerg. Infect. Dis.* **7**: 812–819.
- Ejidokun, O. O., A. Walsh, J. Barnett, Y. Hope, S. Ellis, M. W. Sharp, G.A. Paiba, M. Logan, G. A. Willshaw, and T. Cheasty.** 2006. Human Vero cytotoxigenic *Escherichia coli* (VTEC) O157 infection linked to birds. *Epidemiol. and Infect.* **134**: 421–423.
- Elder, R. O., E. K. James, R. S. Gregory, A. B. Genevieve, K. Mohammad, and W. L. William.** 2000. Correlation of enterohemorrhagic *Escherichia coli* O157:H7 prevalence in feces, hides and carcasses of beef cattle during processing. *PNAS*; **97(7)**: 2999–3003.
- Elmali, M., U. Zeynep, Y. Hilmi, T. Mehmet, G. Kenan, and C. Perihan.** 2005. A seven-month survey for the detection of *e. coli* o157:h7 from ground beef samples in the markets of turkey. *Pakistan J. Nut.*; **4(3)**: 158–161.
- FAO/WHO.** 2004. Discussion paper on the risk profile for enterohemorrhagic *Escherichia coli* including the identification of the compounds of concern, including sprouts, ground beef and pork.
- Farmer.** 1999. In: Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

- Fatoki, O. S., N. Y. O. Muyima, and N. Lujima.** 2001. Situation analysis of water quality in the Umtata river catchment. *Water, SA.*; **27(4)**: 467–473.
- Fauci, A. S.** 2003. HIV and AIDS: 20 years of science. *Nature Med.*; **9**: 839–843.
- FDA News.** 2006a. Pacific Coast Fruit Company Voluntarily Recalls Spinach Salad Products and Pizza Because of Possible Health Risk. Available at: <http://www.fda.gov/oc/po/firmrecalls>. Accessed 1 September 2007.
- FDA News.** 2006b. Investigations of *E. coli* O157 Infections associated with taco bell restaurants in Northeast. Available at: <http://www.fda.gov/oc/opacom/hottopics>. Accessed 1 September 2007
- FDA.** 1999. White paper: Evaluation of risks related to microbial contamination of ready-to-eat- food by food preparation workers and the effectiveness of interventions to minimize those risks. Available from: <http://www.cfsan.fda.gov/~ear/rterisk.html>. Accessed 16 August, 2007.
- Feng, P.** 1995. *Escherichia coli* Serotype O157:H7: Novel Vehicles of Infection and Emergence of Phenotypic Variants. Ph.D. U.S. Food and Drug Administration, Washington, D.C., USA. *Emerg. Infect. Dis.* **1**: 46–52.
- Feng, P.** 2000. *Escherichia coli* serotype O157:H7: novel vehicles of infection and emergence of phenotypic variants. *Emerg. Infect. Dis.* **1**: 9.
- Finlay, B.** 2003. Pathogenic *Escherichia coli*: From molecules to vaccine. Page 23 in Proc. 5th Int. Symp. on Shiga Toxin-Producing *Escherichia coli* Infections, Edinburgh, U.K.

- Fischer, C. R., M. Yoichi, H. Unno, and Y. Tanji.** 2004. The coexistence of *Escherichia coli* serotype O157:H7 and its specific bacteriophage in continuous culture. *Lett. Appl. Microbiol.*; **241**: 171–177.
- Fischer, J. R, T. Zhao, M. P. Doyle, M. R. Goldberg, C. A. Brown, C. T. Sewell, D. M. Kavanaugh, and C. D. Bauman.** 2001. Experimental and field studies of *Escherichia coli* O157:H7 in white-tailed deer. *Appl. Environ. Microbiol.*; **67**: 1218–1224.
- FSAI (Food Safety Authority of Ireland).** 1999. The prevention of *E. coli* O157:H7 infection A shared responsibility. Food Safety Authority of Ireland Abbey Court Lower Abbey Street Dublin 1. pp 1 – 70. ISBN 0-9533624-1-8. Available from: Website: www.fsai.ie. Accessed 12 May 2007.
- FSIS, (Food Safety and Inspection Service).** 1996. Pathogen reduction; Hazard Analysis and Critical Control Point (HACCP) systems; Final rule. *Register*; **61**: 38806–38944
- Fu, Z., R. Snezna, and L. K. Thomas.** 2004. Rapid detection of *Escherichia coli* O157:H7 by Immunomagnetic Separation and real-time PCR. *Int.. J. Food Microbiol.* **99**: 47–57.
- Galane, P. M and M. Le Roux.** 2001. Molecular Epidemiology of *Escherichia coli* isolated from young South Africa children with diarrheal diseases. *J. Health Popl. Nutr.* **19(1)**: 311–318
- Gallo, R. C.** 2002. Historical essay. The early years of HIV/AIDS. *Science*; **298**: 1728–1730.
- Gansheroff, L. J. and A. D. O'Brien.** 2000. *Escherichia coli* O157:H7 in beef cattle presented for slaughter in the U.S.: Higher prevalence rates than previously estimated. *Proceedings of the National Academy of Sciences USA* **97**: 2959 –2961.

- Garcia, A. and J. G. Fox.** 2003. The rabbit as a new reservoir host of enterohemorrhagic *Escherichia coli*. *Emerg. Infect. Dis.*; **9**: 1592–1597.
- García-Aljaro, C., M. Muniesa, J.E. Blanco, M. Blanco, J. Blanco, J. Jofre, and A.R. Blanch.** 2005. Characterization of Shiga toxin-producing *Escherichia coli* isolated from aquatic environments. *Lett. Appl. Microbiol.*; **246(1)**: 55–65.
- Garcia-Graells, C., J. A. H., Kristel. and W. M. Chris.** 1998. High pressure inactivation and sublethal injury of pressure-resistant *Escherichia coli* mutants in fruit juices. *App. Environ. Microbiol.*, **64**: 1566–1568.
- Germani, Y., B. Soro, M, Vohito, O. Morel, and J. Morvan.** 1997. Enterohaemorrhagic *Escherichia coli* in Central African Republic. *Lancet*; **349**: 1670.
- Germani, Y., Cunin, P., Tedjouka, E., Bou-Neharre, C., Morvan, J. and P. Martin.** 1998. Enterohaemorrhagic *Escherichia coli* in Ngoila (Cameroon) during an outbreak of bloody diarrhoea. *Lancet*; **352**: 625–626.
- Gerrish, R. S., J. E. Lee, J. Reed, J. Williams, L. D. Farrell, K. M. Spiegel et al.** 2007. PCR versus hybridization for detecting virulence genes of enterohemorrhagic *Escherichia coli*. *Emerg. Infect. Dis.* ISSN: 1080-6059. Available from <http://www.cdc.gov/EID/content/13/8/1253.htm>. Accessed 19 August 2007.
- Ghosh, A. aand M. Bansal.** 2003. A glossary of DNA structures from A to Z *Acta Crystallogr D. Biol. Crystallogr.*; **59(4)**: 620–626.
- Gorman, R., S. Bloomfield, and C.A. Adley.** 2002. A study of cross-contamination of food-borne pathogens in the domestic kitchen in the Republic of Ireland. *Int. J. Food Microbiol.*; **76(1–2)**: 143–150.

- Grabow W. O. K, M. B. Taylor, C. G. CLAY, and J. C. Develliers.** 2000. Molecular detection of viruses in drinking water: implications for safe and disinfection. *Proc. 2nd Conf. of the Int. Life Science Inst.: The Safety of Water Disinfection: Balancing Chemical and Microbiology Risks*. Radisson Denville Resort, Miami Beach, Florida, USA, 15-17 November.
- Gram, H. C.** 1884. "Über die isolierte Färbung der Schizomyceten in Schnitt-und Trockenpräparaten". *Fortschritte der Med.*; **2**: 185–189.
- Green, Z.** 2003. The use of molecular methods to determine virulence encoding genes in *Escherichia coli* isolates from stool samples and river water sources in rural venda communities, South Africa. Masters Thesis. pp 88. University of Venda. South Africa.
- Griffin, P. M.** 1995. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*. In "Infections of the Gastrointestinal Tract. M.J. Blaser, P.D. Smith, J. I. Ravdin, H. B. Greenberg, and R. L. Guerrant, eds. Raven Press Ltd., New York. Pg. 739–761.
- Griffith, C. J.** 2000. Good hygiene practices for food handlers and consumers. In Blackburn, C.W. and McClure, P.J. (Eds), *Foodborne Pathogens: Hazards, Risk and Control*, Woodhead Publishing Ltd, London.
- Groenewald, P., N. Nannan, D. Bourne, R. Laubscher, and D. Bradshaw.** 2005. Identifying deaths from AIDS in South Africa. South African National HIV Prevalence Incidence Behavior and Communication Survey, 2005.
- Grossmann, K., B. Weniger, G. Baljer, B. Brenig, and L. H. Wieler.** 2005. Racing, ornamental and city pigeons carry Shiga toxin producing *Escherichia coli* (STEC)

- with different Shiga toxin subtypes, urging further analysis of their epidemiological role in the spread of STEC. *Berlin Munch Tierarzt Wochenschr*; **118**: 456–463.
- Guan, J. and R. E. Levin.** 2002. Sensitive and rapid detection of *Escherichia coli* O157:H7 in ground beef by nested PCR incorporating immunomagnetic separation. *Food Biotech.*; **16(2)**: 145–154
- Guan, S., R. Xu, S. Chen, J. Odumeru, and C. Gyles.** 2002. Development of a procedure for discriminating among *Escherichia coli* isolates from animal and human sources. *Appl. Environ. Microbiol.*; **68**: 2690–2698.
- Gun, H. A. Yilmaz, S. Turker, A. Tanlasi and H. Yilmaz.** 2003. Contamination of bovine carcasses and abattoir environment by *Escherichia coli* O157:H7 in Istanbul, *Int. J. Food Microbiol.*; **84**: 339–344.
- Gupta, A. and M. Chaudhuri.** 1992. "Domestic water purification for developing countries." Aqua (Oxford). 45(5):290-298.
- Guyon, R., F. Dorey, J. P. Malas, and A. LeClercq.** 2001. Hazard analysis of *Escherichia coli* O157:H7 contamination during beef slaughtering in Calvados, France. *J. Food Prot.*; **64**: 1341–1345.
- Guyon, R., F. Dorey, J. P. Malas, and A. LeClercq.** 2001. Hazard analysis of *Escherichia coli* O157:H7 contamination during beef slaughtering in Calvados, France. *J. Food Prot.*; **64**: 1341–1345.
- Haas, C. N., J. Rose, and C. Gerba.** 1999. *Quantitative microbial risk assessment*, John Wiley & Sons
- Hahm, B.K., Y. Maldonado, E. Schreiber, A. K. Bhunia and C. H. Nakatsu.** 2003. Subtyping of foodborne and environmental isolates of *Escherichia coli* by

- multiplex-PCR, rep-PCR, PFGE, ribotyping and AFLP, *J. Microbiol. Methods*; **53**: 387–399.
- Haiqing, S., H. J. Knecht, I. T. Kudva, and C. J. Hovde.** 2006. Application of bacteriophages to control intestinal *Escherichia coli* O157:H7 levels in ruminants. *Appl. Environ. Microbiol.*; 72(8): 5359–5366
- Hancock, D. D., T. E. Besser, and D. H. Rice.** 1998a. Ecology of *Escherichia coli* O157:H7 in cattle and impact of management practices. In: Kaper, J. B., and A. D. O'Brien, eds. *Escherichia coli O157:H7 and other shiga toxin-producing E. coli strains*. Washington, DC: ASM Press, pg 85–91.
- Hancock, D.D., D. H. Rice, D. E. Herriot, T. E. Besser, and E. D. Ebel.** 1997. Effects of farm manure handling practices on *Escherichia coli* O157 prevalence in cattle. *J. Food Prot.*; **60**: 363–366.
- Hara-Kudo, Y., M. Ikeda, H. Kodaka, H. Nakagawa, K. Goto, T. Masuda, H. Konuma, T. Kojima, and S. Kumagai.** 2000. Selective enrichment with a resuscitation step for isolation of freeze-injured *Escherichiacoli*O157:H7from foods. *Appl.Environ. Microbiol.*; **66**: 2866–2872.
- Havelaar, A. H. and J. M. Melse.** 2003, Quantifying public health risks in the WHO Guidelines for Drinking-Water Quality: a burden of disease approach, Report 734301022/2003, RIVM, Bilthoven, Netherlands.
- Hawker, J., N. B. Begg, B. Blair, R. Reintjes, and J. Weinberg.** 2001. Communicable Disease Control Handbook. Oxford: Blackwell, 2001.
- Hayes, C. E. Elliot, E. Kroles, D. Goulda.** 2003. Food and Water Safety for Persons Infected with Human Immunodeficiency Virus. *Clin. Infect. Dis.*; **36(S2)**: S106–S109.

- Haysom, I and Sharp, K.** 2004. Cross contamination from raw chicken during meal preparation. *J. British Food* **106(1)**: 38–50
- Health Protection Agency.** 2004. *Oxidase test*. National Standard Method BSOP TP 26 Issue 1. http://www.hpa-standardmethods.org.uk/pdf_sops.asp
- Heersink, M.** 1996. *Escherichia coli* O157: the true story of a mother's battle with a killer microbe. New Jersey: New Horizon; 1996.
- Heizmann, W., P. C. Doôler, B. Gutbrod, and H. Werner.** 1988. Rapid identification of *Escherichia coli* by Fluorocult media and positive indole reaction. *J. Clin. Microbiol.*; **26**: 2682–2684.
- Helena J. N. and P. Steyn.** 2002. Report on South African food consumption studies undertaken amongst different population groups (1983-2000): Average intakes of foods most commonly consumed. Available from: <http://www.doh.gov.za/docs/reports/2002/>. Accessed April 16th 2007.
- Hertzman, J. and D. Barrash.** 2007. An assessment of food safety knowledge and practices of catering employees. *J. British Food*; **109(7)**: 562–576
- Heuvelink, A. E., J. T. M. Zwartkruis-nahuis, F. I. A. M Van Den Biggelaar, W. J. Van Leeuwen, and E. de Boer.** 1999. Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 from slaughter pigs and poultry. *Int. J. Food Microbiol.*; **52**: 67–75.
- Heuvelink, A.E., G. L. Roessink, K. Bosboom, and E. de Boer.** 2001. Zero-tolerance for faecal contamination of carcasses as a tool in the control of O157 VTEC infections. *Int. J. Food Microbiol.*; **66**: 13–20.

- Hoffman, E. W.** 2004. Evaluation of food safety education materials for persons with HIV/AIDS. Thesis p (1 - 165). Washington State University, 2004. Department of Food Science and Human Nutrition, Washington State University.
- Holt, J. G., J. T. Staley, M. P. Bryant, and N. Phennig.** (eds). 1994. Bergey's Manual of Systematic Bacteriology, 9th ed, vol. 3. Williams & Wilkins, Baltimore.
- Howard, G., R. Mutabazi, and M. Nalubega.** 2001. Rehabilitation of protected springs in Kampala, Uganda. In Water, Sanitation and Hygiene: challenges of the millennium, *Proc. of the 26th WEDC Conference*, Dhaka, Bangladesh, pg. 20–23.
- HSRC, (Human Sciences Research Council).** 2005. South Africa National HIV Prevalence, HIV Incidence, Behaviour and Communications Survey 2005.
- Hulebak, K.L., and W. Schlosser.** 2002. "Hazard Analysis and Critical Control Point (HACCP) History and Overview." *Risk Analysis* **22**: 547–52.
- Huo, A., B. Xu, M. A. Chowdhury, M. S. Islam, R. Montilla, and R. R. Colwell.** 1996. A simple filtration method to remove plankton-associated *Vibrio cholerae* in raw water supplies in developing countries. *Appl. Environ. Microbiol.*; **62**: 2508–2512
- Ibekwe, A. M. and C. M. Grieve.** 2003. Detection and quantification of *Escherichia coli* O157:H7 in environmental samples by real-time PCR, *J. Appl. Microbiol.*; **94(3)**: 421– 431.
- IFAS (Institute of Food and Agricultural Sciences).** 2005. Good Worker Health and Hygiene Practices: Training Manual for Produce Handlers. Available at:<http://edis.ifas.ufl.edu>. Accessed 16 August 2007.

- Ikeda, M.** 2002. Amino acid production processes. In *Advances in Biochemical Engineering and Biotechnology. Microbial Production of L-Amino Acids*. Faurie R, Thommel J. Berlin, Eds. Springer. pp. 1-35
- Ingrid, F., W. Chandi, G. C. Paoli, and T. Shu-i.** 2003. Evaluation of enrichment media for detection of *Escherichia coli* O157:h7 in ground beef using immunomagnetic-electrochemiluminescence. *J. Rapid Methods and Automation in Microbiol.*; **15(1)**: 92–106. doi:10.1111/j.1745-4581.2007.00080.x
- Isaacson, M., P. H. Canter, P. E. Effler, L. Arntzen, P. Bomans, and R. Heenon.** 1993. Haemorrhagic colitis epidemic in Africa. *Lancet*; **341**: 961.
- Iyoda, S., A. Wada, J. Weller, S. J. A. Flood, E. Schreiber, B. Tucker, and H. Watanabe.** 1999. Evaluation of AFLP, a high-resolution DNA fingerprinting method, as a tool for molecular subtyping of enterohemorrhagic *Escherichia coli* O157:H7 isolates. *Microbiol. Immunol.*; **34**: 803–806.
- Jacobs, B. and A. Mercer.** 1999. Feasibility of hospital-based blood banking: a Tanzanian case study, *Health Policy Plan.*; **14**: 354–362.
- Jay, L. S., D. Comar and L.D. Govenlock.** 1999. A video study of Australian domestic food-handling practices, *J. Food Prot.*; **62**: 1285–1296.
- Jeffrey, S. H. and F. R. Helene.** 1997. *An improved method for southwestern blotting*. Frontiers in Bioscience, 2, c9 -11, June 15. *The Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland*
- Jennifer, L. J., L. B. Cherryl, and J. F. Scott.** 1998. Comparison of the BAX for screening/*Escherichia coli* O157:H7 method with conventional methods for detection of

- extremely low levels of *Escherichia coli* O157:H7 in ground beef. *Appl. Environ. Microbiol.*; **64**: 4390–4395.
- Jerse, A. E., J. Yu, B. D. Tall, and J. B. Kaper.** 1990. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proc. Natl. Acad. Sci. USA*; **87**: 7839–7843.
- Jiang, X., J. Morgan, and M. P. Doyle.** 2002. Fate of *Escherichia coli* O157:H7 in manure-amended soil. *Appl. Environ. Microbiol.*; **68**:2605–2609
- Jin, H.Y., Tao, K. H., Li, Y. X., Li, F. Q. and S. Q. Li.** 2005. Microarray analysis of *Escherichia coli* O157:H7. *World J. Gastro.*; **11**(37): 5811–5815.
- Johannessen, G. S., G. B. Bengtsson, B. H. Heier, S. Bredholt, Wasteson, Y. and L. M. Rørvik.** 2005. Potential uptake of *Escherichia coli* O157:H7 from organic manure into crisphead lettuce. *Appl. Environ. Microbiol.*; **71**: 2221–2225.
- Johnsen, G., Y. Wasteson, E. Heir, O. I. Berget, and H. Herikstad.** 2001. *Escherichia coli* O157 H7 in faeces from cattle, sheep and pigs in the southwest part of Norway during 1998 and 1999. *Int. J. Food Microbiol.*; **65**: 193–200.
- Johnson, K, N. Marano, R. Howard, T. Root et al.** 2000. NARMS Working Group. The antimicrobial resistance patterns of *Escherichia coli* O157:H7 - NARMS, 1996-1999. 2nd International Conference on Emerging Infectious Diseases. Atlanta, GA, July 2000
- Karch, H., J-M. Claudia, A. Stojanka, and D. Martina.** 1996. Isolation of Enterohemorrhagic *Escherichia coli* O157 Strains from Patients with Hemolytic-Uremic Syndrome by Using Immunomagnetic Separation, DNA-Based Methods, and Direct Culture. *J. Clin. Microbiol.*; **34**(3): 516–519.

- Karen, S. C., E. Levente, J. T. Trevor, and W. K. William.** 1992. Thin aggregative fimbriae from diarrheagenic *Escherichia coli*. *J. Bacteriol.*; **174**: 4490 – 4495.
- Karmali, M. A., A. O. Carter, A. A. Borczyk, J. A. Carlson, B. Harvey, and J. C. Hockin.** 1987. A severe outbreak of *Escherichia coli* O157:H7-associated hemorrhagic colitis in a nursing home. *New Eng. J. Med.*; **316**: 1496–500.
- Kassenborg, H. D. W. H. Craig, H. Michael, C. E. Mary, E. C. Arthur, M. Ruthanne, J. V. Duc, S. Kirk, D. A. Shama, S. Laurence, and M. G. Patricia.** 2004. Farm Visits and Undercooked Hamburgers as Major Risk Factors for Sporadic *Escherichia coli* O157:H7 Infection: Data from a Case - Control Study in 5 FoodNet Sites. *Clin. Infect. Dis.*; **38**: 271–278.
- Kaufmann, M., C. Zweifel, M. Blanco, J. E. Blanco, J. Blanco, L. Beutin, and R. Stephan.** 2006. *Escherichia coli* O157 and non-O157 Shiga toxin-producing *Escherichia coli* in fecal samples of finished pigs at slaughter in Switzerland. *J. Food Prot.*; **69**: 260–266
- Keen, J. E., J. E. Wittum, J. R. Dunn et al.** 2006. Shiga-toxigenic *Escherichia coli* O157 in agricultural fair livestock, United States. *Emerg. Infect. Dis.*; **12**: 780–786.
- Kijima-Tanaka, M., K. Ishihara, A. Kojima, A. Morioka, R. Nagata, M. Kawanishi, M. Nakazawa, Y. Tamura, and T. Takahashi.** 2005. A national surveillance of Shiga toxin-producing *Escherichia coli* in food-producing animals in Japan. *J. Vet. Med. Ser.* **52**: 230–237.
- Kim, A. Y. and D. W. Thayer.** 1996. Mechanism by which gamma irradiation increases the sensitivity of *Salmonella typhimurium* ATCC 14028 to heat. *Appl. Environ. Microbiol.*; **62**: 1759–1763.

- King, D.A., L.M. Lucia, A. Castillo, G.R. Acuff, K.B. Harris and J.W. Savell.** 2005. Evaluation of peroxyacetic acid as a post-chilling intervention for control of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on beef carcass surfaces, *Meat Sci.*; **69**: 401–407
- Kirchhoff, L. V, K. E. McClelland, M. Do Carmo Pinho, J. G. Araujo, M. A. De Sousa, and R. L. Guerrant.** 1985. Feasibility and efficacy of in-home water chlorination in rural North eastern Brazil. *J. Hyg.*; **94**: 173–180.
- Kivaria, F., J. Noordhuizen, and A. Kapaga.** 2006. Evaluation of the hygienic quality and associated public health hazards of raw milk marketed in Dar es Salaam region, Tanzania. *Trop. Animal Health and Prod.*; **38(3)**: 185–194.
- Knabel, S. J.** 1995. Foodborne illness: role of home food handling practices. *Food Techno.*; **49**: 119–131.
- Koyange, L., G. Ollivier, J. J. Muyembe, B. Kebela, M. Gouali, and Y. Germani.** 2004. Enterohemorrhagic *Escherichia coli* O157, Kinshasa. *Emerg. Infect. Dis.* Available from: <http://www.cdc.gov/ncidod/EID>. Accessed 17th February 2007
- Kudva, I. T, P. G. Hatfield, and C. J. Hovde.** 1996. *Escherichia coli* O157:H7 in microbial flora of sheep. *J. Clin. Microbiol.*; **34**: 431–433.
- Kudva, I. T, P. G. Hatfield, and C. J. Hovde.** 1997. Characterization of *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli* serotypes isolated from sheep. *J. Clin. Microbiol.*; **35**: 892–899.
- Kuiper, M., P. Vos, R. Hogers, M. Bleeker, M. Reijans, T. Van De Lee, M. Hornes, A. Frijters, J. Pot, and J. Peleman.** 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.*; **23**: 4407–4414.

- Lagunas-Solar, M. C.** 1995. Radiation processing of foods: an overview of scientific principles and current status. *J. Food Prot.*; **58(2)**: 186–192.
- Lahti, E., V. Hirvela-Koski, and T. Honkanen-buzalski.** 2001. Occurrence of *Escherichia coli* O157 in reindeer (*Rangifer tarandus*). *Vet. Res.*; **148**: 633–634.
- Lashley, F. R., and J. D. Durham.** 2002. *Emerging Infectious Diseases: Trends and Issues*. New York: Springer Publishing Company.
- Law, D. and J. Kelly.** 1995. Use of heme and hemoglobin by *Escherichia coli* O157 and other shiga-like-toxin-producing *Escherichia coli* serogroups. *J. Infect. Immun.*; **63**: 700–702.
- LCDC, (Laboratory Center of Disease Control).** 1987. Outbreak of gastrointestinal disease - Ontario. *Canada Dis. Weekly Rep.*; **13**: 5–8.
- LeChevallier, M. W. and K. K. Au.** 2000. Impact of Treatment on Microbial Water Quality: A Review Document on Treatment Efficiency to Remove Pathogens, (Draft). World Health Organization, Geneva
- LeClercq, A. and J. Mahillon.** 2003. Farmed rabbits and ducks as vectors for VTEC O157:H7. *Vet. Res.*; **152**: 723–724.
- LeJeune, J. T., T. E. Besser, D. H. Rice, J. L. Berg, R. P. Stilborn, and D. D. Hancock.** 2004. Longitudinal study of fecal shedding of *Escherichia Coli* O157:H7 in feedlot cattle: Predominance and persistence of specific clonal types despite massive cattle population turnover. *Appl. Environ. Microbiol.*; **70**: 377–384.
- Levine, M. M.** 1987. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. *J. Infect. Dis.*; **55(3)**: 377–389

- Levy, J.A., A. D. Hoffman, S. M. Kramer, J. A. Landis, J. M. Shimabukuro, and L. S. Oshiro, L.S.** 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS *Sci.*; **225**: 840–842.
- Leyer, G. J., L. L. Wang, and E. A. Johnson.** 1995. Acid adaptation of *Escherichia coli* O157:H7 increases survival in acidic foods. *Appl. Environ. Microbiol.*; **61**: 3752–3755.
- Li-Cohen, A. E. and C. M. Bruhn.** 2002. Safety of consumer handling of fresh produce from the time of purchase to the plate: a comprehensive consumer survey. *J. Food Prot.*; **65(8)**: 1287–1296.
- Lijima, Y., M. Karama, J. O. Oundo, T. Honda.** 2001. Prevention of bacterial diarrhoea by pasteurization of drinking water in Kenya. *Microbiol. Immunol.*; **45**: 413–416.
- Logsdon, G. S.** 1990. Microbiology and Drinking Water Filtration. In: Drinking Water Microbiology: Progress and Recent Developments. G. A. McFeters (ed.). New York, Springer-Verlag: pg. 120–146.
- Luby, S., M. Agboatwalla, R. Hoekstra, M. Rahbar, W. Billhimer, and B. Keswick.** 2004. Delayed effectiveness of homebased interventions in reducing childhood diarrhoea, Karachi, Pakistan. *Am. J. Trop. Med. Hyg.*; **71(4)**: 420–427.
- Lule, J. R., J. Mermin, J. P. Ekwaru, S. Malamba, R. Downing, R. Ransom, D. Nakanjako, W. Wafula, P. Hughes, R. Bunnell, F. Kaharuza, A. Coutinho, A. Kigozi, and R. Quick.** 2005. Effect of home-based water chlorination and safe storage on diarrhea among persons with Human Immunodeficiency Virus in Uganda. *Am. J. Trop. Med. Hyg.*; **73**: 926–933.

- Lung, A. J., C. M. Lin, J. M. Kim, M. R. Marshall, R. Nordstedt, N. P. Thompson, and C. I. Wei.** 2001. Destruction of *Escherichia coli* O157:H7 and *Salmonella enteritidis* in cow manure composting. *J. Food Prot.*; **64**: 1309–1314.
- Lupin, H.M.** 2000. Internal auditing of HACCP-based systems in the fishery industry. *Infofish Intern.*; **4**: 56–64.
- Mahfouz, A. A., M. Abdel-Moneim, R. A. Al-Erian, and O. M. Al-Amari.** 1995. Impact of chlorination of water in domestic storage tanks on childhood diarrhoea: a community trial in the rural areas of Saudi Arabia. *J. Trop. Med. Hyg.*; **98**: 126–130.
- Maier, R. M., I. L. Pepper, and C. P. Gerba.** 2000. Nucleic Acid-Based Methods of Analysis. In: “Environmental Microbiology” (E. M., Marlowe, K. L., Josephson, I. L. Pepper. Eds) Academic Press. California. USA. Pg 287–315.
- Maite M. and J. Juan.** 1998. Abundance in Sewage of Bacteriophages That Infect *Escherichia coli* O157:H7 and That Carry the Shiga Toxin 2 Gene. *Appl. Environ. Microbiol.*; **64(7)**: 2443–2448.
- Makino, K., K. Ishii, T. Yasunaga, M. Hattori, K. Yokoyama, C. H. Yutsudo et al.** 1998. Complete nucleotide sequences of 93-kb and 3.3-kb plasmids of an enterohemorrhagic *Escherichia coli* O157:H7 derived from Sakai outbreak. *DNA Res.*; **5**:1–9.
- Malihe, R. N. and H. Kadir.** 2000. A study on selective broths and agar media for the isolation of *Escherichia coli* O157:H7 serotype. *J. Vet. Ani. Sci.*; **24**: 459–464.

- Manafi, M. and B. Kremsmaier.** 2001. Comparative evaluation of different chromogenic/fluorogenic media for detecting *Escherichia coli* O157:H7 in food. *Int. J. Food Microbiol.*; **71(2-3)**: 257–262.
- Mandell, G. L., F. Douglas, and J. E. Bennett.** 2000. *Principles and practice of infectious diseases: volume 2*. Philadelphia: Churchill Livingstone.
- March, S. B. and S. Ratnam.** 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J. Clin. Microbiol.*; **23**: 869–872.
- Mark, S. and T. Roberts.** 1993. *Escherichia coli* O157:H7 ranks as the fourth most costly food borne disease. *Food Rev.*; **16**: 51–59.
- Marone, M., S. D. Mozzetti, L. De Ritis, L. Pierelli, and G. Scambia.** 2001. Semiquantitative RT-PCR analysis to assess the expression levels of multiple transcripts from the same sample. *Biol. Proced.*3(1): 19-25. Available at: <http://www.biologicalprocedures.com>. Accessed 25 July 2007.
- Mattick, K. L, K. Durham, M. Hendrix, J. Slader, C. Griffith, M. Sen, and T. Humphrey.** 2003. The microbiological quality of washing-up water and the environment in domestic and commercial kitchens. *J. Appl. Microbiol.*; **94(5)**: 842–847.
- Mayes, T.** 1999. How can the principles of validation and verification be applied to hazard analysis? *Food Cont.*; **10(4/5)**: 277–279.
- McFarland, W., J. G. Kahn, D. A. Katzenstein, D. Mvere, and R. Shamu.** 1995. Deferral of blood donors with risk factors for HIV infection saves lives and money in Zimbabwe, *J. AIDS Human Retro. Virol.*; **9**: 183–92.

- McIngvale, S. C., D. Elhanafi, and M. A. Drake.** 2002. Optimization of reverse transcriptase PCR to detect viable Shiga toxin-producing *Escherichia coli*. *Appl. Environ. Microbiol.*; **68**:799-806.
- Mead, P. S, and P. M. Griffin.** 1998. *Escherichia coli* O157:H7. *Lancet*; **352(9135)**: 1207–1212.
- Medeiros, L. C., V. N., Hillers, P. A. Kendall, and A. Mason.** 2001. Food safety education: what should we be teaching consumers? *J. Nutri. Edu.*; **33(2)**: 108–114.
- Meuli, C. and K. Wehrle.** 2001. *Spring Catchment*, SKAT, St Gallen.
- Michael, D. P., T. Zao, J. Meng, and S. Zao.** 1997. *Escherichia coli* O157:H7. In: Food Microbiology: Fundamentals of frontiers (M. P. Doyle, L. R. Beuchat, T. J. Montville. (Eds) pp 171 – 191. ASM Press, Washington D. C.
- Michino, H., K. Andaraki, and S. Minami.** 1999. Massive outbreak of *Escherichia coli* O157:H7 infection in schoolchildren in Sakai City, Japan associated with consumption of white radish sprouts. *Am. J. Epidemiol.*; **150**:787–796.
- Missiakas, D., G. Costa, and R. Satish.** 1993. The *Escherichia coli* heat shock gene *htpY*: Mutational analysis, cloning sequencing and transcriptional regulation. *J. Bacteriol.*; **175**: 2613–2624.
- MMWR.** 1981a. Karposi’s sarcoma and pneumocystis pneumonia among homosexual men- New York City and California. *MMWR.*; **30**: 305–308
- MMWR.** 1981b. Pneumocystis pneumonia in Los Angeles. *MMWR.*; **30**: 250–252
- Momba M. N. B. and C. Mfenyana.** 2005. Inadequate treatment of wastewater: A source of coliform bacteria in receiving surface water bodies in developing countries – Case study: Eastern Cape Province of South Africa. *In Water Encyclopedia –Domestic*,

Municipal, and Industrial water supply and waste disposal (Edt J.H. Lehr and J. Keeley). John Wiley & Son, Inc. pg 661–667.

Momba M. N. B., Z. Tyafa, and N. Makala. 2004a. Rural water treatment plants fail to provide potable water to their consumers: Alice water treatment plant in the Eastern Cape Province of South Africa. *SA J. Sci.*; **100**: 307–310.

Momba, M. N. B. and B. M. Brouckaert. 2005. Guidelines for ensuring sustainable effective disinfection in small water supply systems. Report Number: TT 249/05.

Momba, M. N. B., and P. Kaleni. 2003. Re-growth and survival of indicator microorganisms on the surfaces of household containers used for the storage of drinking water in rural communities of South Africa. *Water Res.*; **36**: 3023–3028.

Momba, M. N. B., and T. L. Notshe. 2003. The effect of long storage and household containers on the microbiological quality of drinking water in rural communities of South Africa. *J. Water Supp.; Res. Techno.-AQUA.*; **52 (1)**: 67–76

Momba, M. N. B., N. Makala, B. Brouckaert, Z. Tyafa, P. Thompson and A. C. Buckley. 2004b. Improving the efficiency and sustainability of disinfection at a small rural water treatment plant. *Water, SA.* **30(5)**: 617–622

Momba, M. N. B., R. Kfir, S. N. Venter, and T. E. Cloete. 2000. An overview of biofilm formation in potable water distribution systems and its impact on the deterioration of water quality. *Water, SA*; **26(1)**: 59–66

Momba, M. N. B., N Makala, B. Zani, and B. M. Brouckaert. 2005a. Key causes of drinking water quality failure in a rural small water supply of South Africa. In *Water Encyclopedia –Domestic, Municipal, and Industrial water supply and waste disposal* (Edt J.H. Lehr and J. Keeley). John Wiley & Son, Inc. Pages 221–227

- Momba, M. N. B., N. Makala, Z. Tyafa, and B. M. Brouckaert.** 2005b. A model partnership for sustainable production of safe drinking water for rural communities in South Africa. *SA J. Sci.*; **101**: 335–336.
- Momba, M. N. B., V. K. Malakate, and J. Theron.** 2006a. Abundance of pathogenic *Escherichia coli*, *Salmonella typhimurium* and *Vibrio cholerae* in Nkonkobe drinking water sources. *J. Water Health*; **04**: 289–296.
- Momba, M. N. B., Z. Tyafa, N. Makala, B. M. Brouckaert, and C. L. Obi.** 2006b. Safe drinking water still a dream in rural areas of South Africa. Case study: The Eastern Cape Province. *Water, SA*; **32**: 715–720.
- Montagnier, L.** 2002. Historical essay. A history of HIV discovery. *Sci.*; **298**: 1727–1728
- Morabito, S., G. Dell’omo, U. Agrimi, H. Schmidt, H. Karch, T. Cheasty, T., and A. Caprioli.** 2001. Detection and characterization of Shiga toxin-producing *Escherichia coli* in feral pigeons. *Vet. Microbiol.*; **82**: 275–283.
- Morita, M., Y. Tanji, K. Mizoguchi, T. Akitsu, N. Kijima, and H. Unno.** 2002. Characterization of a virulent bacteriophage specific for *Escherichia coli* O157 : H7 and analysis of its cellular receptor and two tail fiber genes. *Lett. Appl. Microbiol.*; **211**: 77–83.
- Morris, J. G. and M. Potter.** 1997. Emergence of new pathogens as a function of changes in host susceptibility. *Emerg. Infect. Dis.*; **3**: 435–441.
- Mossoro C., P. Glaziou, S. Yassibanda et al.** 2002. Chronic diarrhea, hemorrhagic colitis, and hemolyticuremic syndrome associated with HEp-2 adherent *Escherichia coli* in adults infected with human immunodeficiency virus in Bangui, Central African Republic. *J. Clin. Microbiol.*; **40**: 3086–3088.

- Mosupye F. and A. von Holy.** 1999. Microbiological quality and safety of ready-to-eat street-vended foods in Johannesburg, South Africa, *J. Food Prot.*; **62**: 1278–1284
- Moxley, R. A., D. Smith, T. J. Klopfenstein, G. Erickson, J. Folmer, C. Macken, S. Hinkley, A. Potter, and B. Finlay.** 2003. Vaccination and feeding a competitive exclusion product as intervention strategies to reduce the prevalence of *Escherichia coli* O157:H7 in feedlot cattle. Page 23 in Proc. 5th Int. Symp. on Shiga Toxin-Producing *Escherichia coli* Infections, Edinburgh, U.K.
- Msobi, N. and Z. Msumi.** 2002. HIV/AIDS and other chronic conditions: home based care cost study, Bigamy District – Tanzania. Available from: www.iaen.org/conferences/durbansym/papers/42Msobi.pdf. Accessed 29th November 2006
- Mukherjee, A., D. Speh, E. Dyck, and F. Diez-Gonzalez.** 2004. Pre-harvest evaluation of coliforms, *Escherichia coli*, *Salmonella* and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. *J. Food Prot.*; **67(5)**: 894–900
- Müller, E. E., Grabow, W. and M. M. Ehlers.** 2003. Immunomagnetic separation of *Escherichia coli* O157:H7 from environmental and wastewater in South Africa. *Water, SA*; **29**: 427–432.
- Murat, G., B. Ethem, and G. Abamüslüm.** 2004. The use of novobiocin and cefixime-tellurite in broth, and of cefixime-tellurite, salicin, rhamnose and MUG in agar medium for the Detection of *Escherichia coli* O157:H7 in ground beef. *Turkey's J. Vet. Anim. Sci.*; **28**: 753–759.
- Muyima, N. Y. O. and F. Ngcakani.** 1998. Indicator bacteria and regrowth potential of the drinking water in Alice, Eastern Cape. *Water, SA*; **24 (1)**: 29–34.

- NACMCF.** 1998. Hazard analysis and critical control point principles and application guidelines. *J. Food Prot.*; **61**:762–775.
- NACMCF.** 1999. In *HACCP: A Systematic Approach to Food Safety, 3rd Ed.* The Food Processors Institute, Washington, D.C. pg.5-23.
- NAS.** 1983. *Risk assessment in the federal government: Managing the process.* National Research Council, National Academy of Sciences. National Academy Press, Washington, DC.
- Nasinyama, G.W., S. A. McEwen, J. B. Wilson, D. Walter-Toews, C. L. Gyles, and J. Opuda-Asibo.** 2000. Risk factors for acute diarrhoea among inhabitants of Kampala District, Uganda. *SA Med.*; **90(9)**: 891–898.
- Nataro, J. P., and J. B. Kaper.** 1998. Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews.* Pp 142 – 201. University of Maryland School of Medicine, Baltimore, Maryland, 21201.
- Nath, K. J., S. F. Bloomfield, M. Jones.** 2006. Household water storage, handling and point-of-use treatment. A review commissioned by IFH. Available from: published on <http://www.ifh-homehygiene.org>. Accessed on 18 August, 2007.
- Nel, S., J. F. R. Lues, E. M. Buys and P. Venter.** 2004. The personal and general hygiene practices in the deboning room of a high throughput red meat abattoir, *Food Cont.*; **15**: 571–578.
- NHMRC.** 2004. National Health and Medical Research Council & Natural Resource Management Ministerial Council, 2004, *National Water Quality Management Strategy: Australian Drinking Water Guidelines: 6*, Australian Government. Available at: <http://www.nhmrc.gov.au/publications>. Accessed 18 July, 2007.

- NHS Fife.** 2006. *E. coli* O157 outbreak from. NHS Fife, press release 29 May 2006. Accessed 06th, June 2007. Available from <http://www.nhsfife.scot.nhs.uk/news2.aspx>
- Nicholson, F.A., S. J. Groves, and B. J. Chambers.** 2005. Pathogen survival during livestock manure storage and following land application. *Bio Res. Technol.*; **96**: 135–143.
- Nolan, T., R. E. Hands, and S. A. Bustin.** 2006. Quantification of mRNA using real-time RT-PCR. *Nat. Protoc.*; **1**: 1559–1582.
- Nutsch, A.L., R. K. Phebus, M. J. Riemann, D. E. Schafer, J. E. Boyer Jr. and R. C. Wilson et al.,** 1997. Evaluation of a steam pasteurization process in a commercial beef processing facility, *J. Food Prot.*; **60**: 485–492.
- Oberst, R. D., M. P. Hays, L. K. Bohra, R. K. Phebus, C. T. Yamashiro, C. Paszko-Kolva, S. J. A. Flood, and J. M. Sargeant.** 1998. PCR-based DNA amplification and presumptive detection of *Escherichia coli* O157:H7 with an internal fluorogenic probe and the 5' nuclease (TaqMan[®]) assay. *Appl. Environ. Microbiol.*; **64**: 3389–3396.
- Obi C. L., J. Ramalivhana, M. N. B. Momba, B. Onabulu, J. O. Igumbor, M. Lukoto, T. B. Mulaudzi, E. L. Jansen van Rensburg, E. Green, and S. Ndou.** 2007a. Antibiotic resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients with and without diarrhea and their household drinking water in rural communities in Limpopo Province South Africa. *Afri. J. Biotech.*; **6(8)**: 1035–1047.
- Obi C. L., J. Ramalivhana, M. N. B. Momba, B. Onabulu, J. O. Igumbor, M. Lukoto, T. B. Mulaudzi, E. L. Jansen van Rensburg, E. Green, and S. Ndou.** 2007a.

- Antibiotic resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients with and without diarrhea and their household drinking water in rural communities in Limpopo Province South Africa. *Afri. J. Biotec.*; **6(8)**: 1035–1047.
- Obi, C. L. J. Ramalivhana, M. N. B Momba and J. Igumbor.** 2007b. Scope and frequency of enteric bacterial pathogens isolated from HIV/AIDS patients and their household drinking water in Limpopo Province. *Water, SA*; **33(4)**: 539–538
- Obi, C. L., N. Potgieter, P. O. Bessong, E. O. Igumbor, and E. Green.** 2004. Gene encoding virulence makers among *Escherichia coli* isolates from diarrheic stools samples and river sources in rural Venda communities of South Africa. *Water, SA*; **30(1)**: 37–42.
- O'Brien, A. D., J. W. Newland, S. F. Miller, and R. K. Holmes.** 1984. Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. *Sci.*; **226**: 694–696
- O'Flynn, G., R. P. Ross, G. F. Fitzgerald, and A. Coffey.** 2004. Evaluation of a cocktail of three bacteriophages for biocontrol of *Escherichia coli* O157: H7. *Appl. Environ. Microbiol.*; **70**: 3417–3424.
- Ogden, I. D., M. Macrae, N. F. Hepburni, and N. J. C. Strachan.** 2000. Improved isolation of *Escherichia coli* O157 using large enrichment volumes for immunomagnetic separation. *Lett. Appl. Microbiol.*; **31**: 338–341.
- Okrend. A. J. G., B. E. Rose, and C.P. Lattuada.** 1990. Use of 5-bromo-4-chloro-3-indoxyl-b-D- glucuronide in MacConkey sorbitol agar to aid in the isolation of *Escherichia coli* O157:H7 from ground beef. *J. Food Prot.*; **53**: 941–943.

- Olorunshola, I. D., S. I. Smith, and A. O. Coker, A. O.** 2000. Prevalence of EHEC O157:H7 in patients with diarrhoea in Lagos, Nigeria. *APMIS.*; **108**: 761–763.
- Ongerth, J. E.** 1990. "Evaluation of treatment for removing Giardia cysts." *JAWWA.*; **82(6)**: 85–96.
- Osek J.** 2004. Phenotypic and genotypic characterization of *Escherichia coli* O157 strains isolated from humans, cattle and pigs. *J. Vet. Med.*; **49**: 317–326.
- Panos, G. Z., G. I. Betsi, and M. E. Falagas.** 2006. Systematic review: are antibiotics detrimental or beneficial for the treatment of patients with *Escherichia coli* O157: H7 infection? *Alimen. Pharmac. Therapeut.*; **24(5)**: 731-742.
- Paquet, C., W. Perea, P. Grimont, M. Collin, and M. Guillod.** 1993. Aetiology of haemorrhagic colitis epidemic in Africa. *Lancet*; **342**: 175
- Paton A. W. and J. C. Paton.** 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx*₁, *stx*₂, *eaeA*, enterohemorrhagic *E. coli* *hlyA*, *rfb*_{O111}, and *rfb*_{O157}. *J. Clin. Microbiol.*; **36**: 598 – 602.
- Patriquin, D. G.** 2000. Reducing risks from *E. coli* 0157 on the organic farm. Canadian Organic Growers (COG) Reference Series # 15.
- Pavlov, A. R. N. V. Pavlova, S. A. Kozyavkin, and A. I. Slesarev.** 2004. "Recent developments in the optimization of thermostable DNA polymerases for efficient applications". *Trends Biotech.*; **22**: 253–260.
- Pebody, R. G., C. Furtado, A. Rojas, N. McCarthy, G. Nylen, P. Ruutu, T. Leino, R. Chalmers, B. de Jong, M. Donnelly, I. Fisher, C. Gilham, L. Graverson, T. Cheasty, G. Willshaw, M. Navarro, R. Salmon, P. Leinikki, P. Wall, and C.**

- Bartlett.** 1999. An international outbreak of Vero cytotoxin-producing *Escherichia coli* O157 infection amongst tourists; a challenge for the European infectious disease surveillance network. *Epidemiol. Infect.*; **123**: 217–223.
- Pedley, S. and G. Howard.** 1997. The Public Health Implication of Groundwater Microbiology. *Q. J. Eng. Geolo.*; **30**(2): 179–188.
- Pennington, H.** 1997. The Pennington Group: Report on the circumstances leading to the 1996 outbreak of infection with *E. coli* O157 in Central Scotland, the implications for food safety and the lessons to be learned. Edinburgh: The Stationery Office.
- Perna, T. N., P. Guy, B. Valerie, et al.** 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Lett. Nature*; **409**: 529–533.
- Petridis, H., G. Kidder, and A. Ogram.** 2002. *Escherichia coli* O157:H7 A Potential Health. Fact sheets of the Soil and Water Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.
- Phaswana-Mafunya N. and N. Shukla.** 2005. Factors that could motivate people to adopt safe hygienic practices in the Eastern Cape Province, South Africa. *SA Health Sci.*; **5**(1): 21–28.
- Phaswana-Mafunya, N.** 2006. Hygiene status of rural communities in the Eastern Cape of South Africa. *Int. J. Environ. Health Res.*; **16**(4): 289–03.
- Pichner, R, A. Sander, H. Steinruck, and M. Gareis.** 2005. Occurrence of *Salmonella* spp. and shigatoxin-producing *Escherichia coli* (STEC) in horse faeces and horse meat products. *Berlin Munch Tierarzt Wochenschr*; **118**: 321–325.

- Pilipcinec, E., L. Tkacikova, H. T. Naas, R. J. Cabada, and I. Mikula.** 1999. Isolation of verotoxigenic *Escherichia coli* O157 from poultry. *Folia Microbiol.*; **44**: 455–456.
- Ponnuswamy, P. and M. Gromiha.** 1994. On the conformational stability of oligonucleotide duplexes and tRNA molecules. *J. Theor. Biol.*; **169(4)**: 419–432.
- Potter, M. E.** 1996. Risk assessment terms and definitions. *J. Food Prot. (Supplement)* S6–S9.
- Prescott, L.M., J. P. Harley, and D.A. Klein.** 1996. Microbiology. Third Edition. Wm. C. Brown Publishers, Dubuque, I. A.
- Presser, K. A., D. A. Ratkowsky, and T. Ross.** 1997. Modelling the growth rate of *Escherichia coli* as a function of pH and lactic acid concentration. *Appl. Environ. Microbiol.*; **63**: 2355 – 2360.
- Prier, R. and V. S. Jay.** 2000. Foodborne and waterborne infectious diseases: Contributing factors and solutions to new and reemerging pathogens. *The Pract. peer-Rev. J. Primary Care Phy.*; **107(4)**.
- Pritchard, G. C., S. Williamson, T. Carson, J. R. Bailey, L. Warner, G. Willshaw, and T. Cheasty.** 2001. Wild rabbits—novel vector for verocytotoxigenic *Escherichia coli* O157. *Vet. Res.*; **149**: 567.
- Prüss, A., D. Kay, L. Fewtrell, and J. Bartram.** 2002. Estimating the Burden of Disease from Water, Sanitation, and Hygiene at a Global Level. *Environ. Health Perspect.*; **110(5)**: 537–542.
- Pupo, G. M., D. K. Karaolis, R. Lan, and P. R. Reeves.** 1997. Evolutionary relationships among pathogenic and nonpathogenic *Escherichia coli* strains inferred from

- multilocus enzyme electrophoresis and *mdh* sequence studies. *Infect. Immun.*; **65**: 2685–2692
- Quick, R. E., A. Kimura, A. Thevos, M. Tembo, I. Shamputa, L. Hutwagner, and E. Mintz.** 2002. Diarrhea prevention through household level water disinfection and safe storage in Zambia. *Am. J. Trop. Med Hyg.*; **66**: 584–589.
- Quick, R. E., L. V. Venczel, E. D. Mintz, L. Soletto, J. Aparicio, M. Gironaz, L. Hutwagner, K. Greene, C. Bopp, Maloney, D. Chavez, M. Sobsey, and R. V. Tauxe.** 1999. Diarrhoea prevention in Bolivia through point-of-use water treatment and safe storage: a promising new strategy. *Epidemiol. Infect.*; **122**: 83–90.
- Quick, R., L. Venczel, O. Gonzalez, E. Mintz, A. Highsmith, A. Espada, E. Damiani, N. Bean, R. De Hannover, and R. Tauxe.** 1996. Narrow-mouthed water storage vessels and *in situ* chlorination in a Bolivian community: a simple method to improve drinking water quality. *Am. J. Trop. Med. Hyg.*; **54**: 511–516
- Quinn B. P. and N. G. Marriott.** 2002. HACCP plan development and assessment: a review. *J. Muscle Foods*; **13(4)**: 313–330. doi: 10.1111/j.1745-4573.2002.tb00339.x
- Radu, S., A. M. Shahilah, R. Gulam, A. Zainori, M. Tadaaki, A. Norio, B. K. Yung, O. Jun, and N. Mitsuaki.** 1998. Detection of *Escherichia coli* O157:H7 in beef marketed in Malaysia. *Appl. Environ. Microbiol.*; **64**: 1153–1156.
- Ralston, K., Y. Starke, P. Brent, and T. Riggins.** 2000. Awareness of risks: changing how hamburgers are cooked. *Food Rev.*; **23(2)**: 44–46.
- Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin, and D. L. Swerdlow.** 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002.

- Emerg. Infect. Dis.* Available from: <http://www.cdc.gov/ncidod/EID/vol11no04/04-0739.htm> Accessed May 07th 2007.
- Redmond, E.C. and C.J. Griffith.** 2003. Consumer food handling in the home: a review of consumer food safety studies, *J. Food Prot.*; **66(1)**: 130–161.
- Reisberg, R., S. Olsnes, and K. Eiklid.** 1981. The cytotoxic activity of shigella toxin. *J. Biol. Chem.*; **256**: 8739–8744.
- Reller, J., B. Lopez, M. A. Mejia, C. Mendoza, and S. Luby.** 2003. A novel technology to improve drinking water quality: a microbiological evaluation of in-home flocculation and chlorination in rural Guatemala. *J. Water Health*; **1**: 15–22.
- Rena, O.** 2000. The Prevalence of *Escherichia coli* O157:H7 in Cattle: <http://www.foodsafetynetwork.ca/animal/prevalence-0157-cattle.htm>
- Renter, D. G., J. M. Sargeant, R. D. Oberst, and M. Samadpour.** 2003. Diversity, frequency, and persistence of *Escherichia coli* O157 strains from range cattle environments. *J. Appl. Environ. Microbiol.*; **69**: 542–547.
- Republic of South Africa.** 1999. Regulations regarding the standards to which and requirements with which processing areas, facilities, apparatus and equipment or which or with which food, intended for use by the final consumer, is processed, handled or prepared for purposes of sale to the public, shall confirm, Government Gazette No. 20318. Department of Health, Government Printer, pp. 1–71.
- Restaino, L.** 1996. Accentuate the positive. *Food Quality (USA)*. **8**: 68–70
- Restaino, L. E. W. Frampton, K. M. Turner, and D. R. K. Allison.** 1999. A chromogenic plating medium for isolating *Escherichia coli* O157:H7 from beef. *Lett. Appl. Microbiol.*; **29**: 26–30

- Rice, D. H., D. D. Hancock, and I. E. Besser.** 2003. Faecal culture of wild animals for *Escherichia coli* O157:H7. *Vet. Res.*; **152**: 82–83.
- Rice, E. W., E. G. Soweres, C. H. Johnson *et al.*** 1992 Serological cross-reactions between *Escherichia coli* O157 and other species of the genus *Escherichia*. *J. Clin. Microbiol.*; **30**: 1315–1316
- Richards, H. A., D. Perez-Conesa, C. A. Doane, B. E. Gillespie, J. R. Mount, S. P. Oliver, P. Pangloli, and F. A. Draughon.** 2006. Genetic Characterization of a Diverse *Escherichia coli* O157:H7 Population from a Variety of Farm Environments. *Foodborne Path. Dis.*; **3(3)**: 259–265.
- Riley, L. W., R. S. Remis, and S. D. Helgerson.** 1983. Haemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New Eng. J. Med.*; **308**: 681-685.
- Ritchie, J. M., P. L. Wagner, D. W. Acheson, and M. K. Waldor.** 2003. Comparison of Shiga toxin production by hemolytic-uremic syndrome-associated and bovine-associated Shiga toxin-producing *Escherichia coli* isolates. *Appl. Environ. Microbiol.*; **69**:1059–1066.
- Roberts, D.** 1990. Sources of infection: food. *The Lancet*; **336(8719)**: 859–861.
- Roberts, L., Y. Chartier, O. Chartier, G. Malenga, M. Toole, and H. Rodka,** 2001. Keeping clean water clean in a Malawi refugee camp: a randomized intervention trial. *Bull World Health Organ.*; **79**: 280–87.
- Robertson, J. B. and S. C. Edberg.** 1997. Natural protection of spring and well drinking water against surface microbial contamination. *Crit. Rev. Microbiol.*; **23(2)**: 143– 178.

- Samadpour, M., J. Liston, E. O. Jerry, and I. P. Tarr.** 1990. Evaluation of DNA Probes for Detection of Shiga-Like-Toxin-Producing *Escherichia coli* in Food and Calf Fecal Samples. *Appl. Environ. Microbiol.*; **56(5)**: 1212–1215
- Sambrook, J., and D. W. Russel.** 2001. *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press. ISBN 0-87969-576-5.
- Samelis, J., N. S. John, A. K. Patricia, and C. S. Gary.** 2005. Survival or Growth of *Escherichia coli* O157:H7 in a model system of fresh meat decontamination runoff waste fluids and its resistance to subsequent lactic acid stress. *J. Appl. Environ. Microbiol.*; **71(10)**: 6228–6234
- Sanderson, M. W., J. M. Gay, D. D. Hancock, C. C. Gay, L. K. Fox, and T. E. Besser.** 1995. Sensitivity of bacteriological culture detection of *Escherichia coli* O157:H7 in bovine feces. *J. Clin. Microbiol.*; **33(10)**: 2616–1619.
- Sang, W. K., S. M., Saidi, H., Yamamoto, T., Ezaki, T., Iida, M., Yoh, et al.** 1996. Haemorrhagic colitis due to *Escherichia coli* O157:H7 in Kenya. *J. Trop. Pediatr.*; **42**:118-119.
- Sangiwa, G., M. Sweat, and S. Gregorich.** 2000. Cost-effectiveness of voluntary HIV-1 counseling and testing in reducing sexual transmission of HIV-1 in Kenya and Tanzania, *Lancet*; **356**: 113–121.
- SANHS.** 2005. South African National HIV Prevalence, HIV Incidence, Behavior and Communication Survey, 2005.

- Sargeant, J. M., D. J. Hafer, J. R. Gillespie, R. D. Oberst, and S. J. Flood.** 1999. Prevalence of *Escherichia coli* O157:H7 in white-tailed deer sharing rangeland with cattle. *J. Am. Vet. Med. Assoc.*; **215**: 792–794
- Sathaporn, M., T. Somsit, W. Darawan, S. Surachai, S. Parvinee, P. Saranjit, L. Somchal, E. Boonchuey, and K. Udom.** 1996. Causes of chronic diarrhea in patients with AIDS in Thailand: A prospective clinical and microbiological study. *J. Gastro.*; **31(4)**: 533–537.
- Savelkoul, P. H. M., H. J. M. Aarts, J. De Haas, L. Dijkshoorn, B. Duim, M. Otsen, L. Rademaker, J. L. W. Schouls, and J.A. Lenstra.** 1999. Amplified-Fragment Length Polymorphism Analysis: the State of an Art. *J. Clin. Microbiol.*; **37**: 3083 - 3091.
- Schmidt, H., J. Scheef, S. Morabito, A. Caprioli, L. H. Wieler, and A. Karch.** 2000. A new shiga toxin 2 variant (Stx2f) from *Escherichia coli* isolated from pigeons. *J. Appl. Environ. Microbiol.*; **66**: 1205–1208.
- Schoeni, J. L. and M. P. Doyle.** 1994. Variable colonization of chickens perorally inoculated with *Escherichia coli* O157:H7 and subsequent contamination of eggs. *J. Appl. Environ. Microbiol.*; **60**: 2958–2962.
- Schouten, J. M., A.W. Van De Giessen, K. Frankena, M. C. M. De Jong, and E.A. M. Graat.** 2005. *Escherichia coli* O157 prevalence in Dutch poultry, pig finishing and veal herds and risk factors in Dutch veal herds. *Prev. Vet. Med.*; **70**: 1–15.
- Scott, E. and S. F. Bloomfield.** 1990. The survival and transfer of microbial contamination via cloths, hands and utensils. *J. Appl. Bacteriol.*; **8**: 271–278.

- Seo K. H. and J. F. Frank.** 1999. Attachment of *Escherichia coli* O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy, *J. Food Prot.*; **62**: 3–9.
- Sharkey, J., E. R. Scalice, K. G. Christy Jr., S. M. Atwood, and J. L. Daiss.** 1994. "Antibodies as Thermolabile Switches: High Temperature Triggering for the Polymerase Chain Reaction". *Biotech.*; **12**: 506–509.
- Sharma, S. K., A. Mohan, and T. Kadiravan. 2005. HIV-TB co-infection: Epidemiology, diagnosis and management. *Indian J. Med. Res.*; **121**: 550–567.
- Shelton E. M., L. T. Nguyen, S. J. Ivey, R. A. Almeida, and S. P. Oliver.** 2002. Novel Single-Tube Agar-Based Test System for Motility Enhancement and Immunocapture of *Escherichia coli* O157:H7 by H7 Flagellar Antigen-Specific Antibodies. *J. Clin. Microbiol.*; **40**: 4685–4690.
- Slutsker, L., A. A. Ries, K. D. Greene, J. G. Wells, L. Hutwagner, and P. M. Griffin.** 1997. *Escherichia coli* O157:H7 diarrhea in the United States: clinical and epidemiologic features. *Ann. Int. Med.*; **126**: 505–513.
- Sobsey, M. D.** 2002. Managing water in the home: accelerated gains from improved water supply. World Health Organization, Geneva: WHO/SDE/WSH/02.07.
- Sobsey, M. D., T. Handzel, and L. Venczel.** 2003. Chlorination and safe storage of household drinking water in developing countries to reduce water-borne disease. *Water Sci Tech.*; **47**: 221–228.
- Soderlund, N., A. Zwi, A. Kinghorn, and G. Gray.** 1999. Prevention of vertical transmission of HIV: analysis of cost effectiveness of options available in South Africa, *BioMed.*; **318**: 1650–1656.

- Sohrab. 1999.** Risk assessment a pre-requisite for application of HACCP in food industries. *Indian Food Packer*; **53(4)**: 43–50.
- Solomon, E. B., S. Yaron, and K. R. Matthews. 2002.** Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Appl. Environ. Microbiol.*; **68**: 397–400.
- Solomon, E. B., C. J. Potenski, and K. R. Matthews. 2002.** Effect of irrigation method on transmission to and persistence of *Escherichia coli* O157:H7 on Lettuce. *J. Food Prot.*; **65**: 673–676.
- Son, W-G., T. A. Graham, and V. J. P. Gannon. 2002.** Immunological Characterization of *Escherichia coli* O157:H7 Intimin $\gamma 1$. *Clin. Diag. Lab. Immuno.*; **9(1)**: 46–53.
- Soomro A.H, M. A. Arain, M. Khaskheli and B. Bhutto. 2002.** Isolation of *Escherichia coli* from Raw Milk and Milk Products in Relation to Public Health Sold under Market Conditions at Tandojam. *Pakistan J. Nut.*; **1(3)**: 151–152.
- Southern, E. M. 1975.** Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.*; **98**: 503–517.
- Spano, G., L. Beneduce, V. Terzi, A. M. Stanca, and S. Massa. 2005.** Real-time PCR for the detection of *Escherichia coli* O157:H7 in dairy and cattle wastewater. *Lett. Appl. Microbiol.*; **40(3)**: 164–171.
- Spiegel, G. 1991.** Food safety in the home. *Nutrition and Food Science*: 14-15, November/December.
- Stewart, C. S. 1999.** Microbial Interactions in the Rumen and Their Potential Impact on the Survival of *Escherichia coli* O157. *Microbial Biosystems: New Frontiers Proceedings of the 8th International Symposium on Microbial Ecology Bell CR*,

Brylinsky M, Johnson-Green P (eds) Atlantic Canada Society for Microbial Ecology, Halifax, Canada,

Strockbine, N. A., L. R. M. Marques, J. W. Newland, H. W. Smith, R. K. Holmes, and A.

D. O'Brien. 1986. Two toxin-converting phages from *Escherichia coli* O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. *Infect. Immun.*; **53**: 135–140.

Su, C. and L. J. Brandt. 1995. *Escherichia coli* O157:H7 infection in humans. *Ann. Int. Med.*; **123**: 698 – 714.

Sun, W., F. Khosravi, H. Albrechtsen, L. Y. Brovko, and M. W. Griffiths. 2002. Comparison of ATP and Invivo bioilluminescence for assessing the efficiency of Immunomagnetic sorbents for live *Escherichia coli* O157:H7 cells. *J. Appl. Microbiol.*; **92**: 1021–1027.

Sutherland, J. P., G. K. Folkard, and W. D. Grant. 1990. Natural coagulants for appropriate water treatment: a novel approach. *Waterlines*; **8**: 30–32.

Szabo, R. A., E. C. Todd, and A. Ean. 1986. Method to isolate *E. coli* O157:H7 from food. *J. Food Prot.*; **10**: 768–772.

Tanji, Y., T. Shimada, M. Yoichi, K. Miyanaga, K. Hori, and H. Unno. 2004. Towards rational control of *Escherichia coli* O157:H7 by a phage cocktail. *Appl. Microbiol. Biotechnol.*; **64**: 270–274.

Tatsuno, I., M. Horie, H. Abe, T. Miki, K. Makino, H. Shinagawa et al. 2001. *tox*B gene on pO157 of enterohemorrhagic *Escherichia coli* O157:H7 is required for full epithelial cell adherence phenotype. *Infect. Immun.*; **69**: 6660–6669.

- Tauxe, R. V.** 1997. Emerging food borne diseases: an evolving public health challenge. *Emerg. Infect. Dis.*; **3(4)**: 425–441.
- Teal, C. J.** 2002. Antimicrobial resistance and the food chain. *J. Appl. Microbiol. Symp. Sup.*; **92**: S85–S89.
- Tenover, F. C., and E. R. Unger.** 1993. *Nucleic acid probes for detection and identification of infectious agents. In: Diagnostic molecular microbiology principles and applications.* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White, eds. American Society for Microbiology, Washington, D.C. pg. 3–20.
- Teunis, P. T. Katsuhisa, and S. Kunihiro.** 2004. Dose response for infection by *Escherichia coli* O157:H7 from outbreak data. *Risk Analy.*; **24(2)**: 401–407.
- Thayer, D. W. and G. Boyd.** 1993. Elimination of *Escherichia coli* O157:H7 in meat by gamma irradiation. *Appl. Environ. Microbiol.*; **59**: 1030–1034.
- Thompson, J. S., D. S. Hodge, and A. A. Borczyk.** 1990. Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* serotype O157. *J. Clin. Microbiol.*; **28(10)**: 2165–2168.
- Todar, K.** 2002. Online Textbook of Bacteriology: available on line at www.textbookofbacteriology.net
- Torres, J., C. López-Saucedo, J. F. Cerna, N. Villegas-Sepulveda, R., Thompson, and F. R. Velazquez.** 2003. Single multiplex polymerase chain reaction to detect diverse loci associated with diarrheagenic *Escherichia coli*. *Emerg. Infect. Dis.*; **9**: 127–131.
- Tsuboi, I., H. Ida, E. Yoshikawa et al.** 1998. Antibiotic susceptibility of enterohemorrhagic *Escherichia coli* O157:H7 isolated from an outbreak in Japan in 1996. *Ant. Agents Chem.*; **42**, 431–432.

- UNAIDS.** 2004. Report of the Global AIDS Epidemic. Available from: www.unaids.org/bangkok2004/GAR2004_html/GAR2004_00_en.htm. Accessed 10th May 2007.
- UNAIDS/WHO.** 2006 Report on the global AIDS epidemic. Available from: http://www.unaids.org/en/HIV_data/2006GlobalReport/default.asp. Accessed 11th May 2007.
- USDA: APHIS:VS.** 1994. *Escherichia coli* O157:H7 issues and ramifications. Centers for epidemiology and Animal Health, U.S Department of Agriculture, Fort Collins, CO. Available from: www.aphis.usda.gov/vs/ceah/ncas/nahms/dairy. Accessed 5th July 2004.
- Van Der Wende, E. and W. G. Characklis.** 1990. Biofilms in potable water distribution systems. In: McFeters GA (ed.) *Drinking Water Microbiology: Progress and Recent Developments*. Springer-Verlag. New York. 249–268.
- Van Donkersgoed, J., T. Graham, and V. Gannon.** 1999. The Prevalence of Verotoxins, *Escherichia coli* O157:H7 and *Salmonella* in the Feces and Rumen of Cattle at Processing *Canadian Vet.*; **40**: 332–338
- Vicki, F., W. L. Huan, and P. Polyxeni.** 2000. Communicating the Threat of Emerging Infections to the Public. *Emerg. Infect. Dis.*; **6(4)**: 337–347.
- Wallace, J. S., T. Cheasty, and K. Jones.** 1997. Isolation of vero cytotoxin-producing *Escherichia coli* O157 from wild birds. *J. Appl. Microbiol.*; **82**: 399 – 404.
- Walls, I., and V. N. Scott.** 1997. Use of predictive microbiology in microbial food safety risk assessment. *Int. J. Food Microbiol.*; **36**: 97–102.

- Walterspiel, J. N., A. L. Morrow, T. G. Cleary, and S. Ashkenazi.** 2003. "Effect of sub-inhibitory concentrations of antibiotics on extracellular shiga-like toxin I". *Infect.*; **20**: 25–29.
- Wang, G., G. C. Clifford, and G. R. Frank.** 2002. Detection in *Escherichia coli* of the Genes Encoding the Major Virulence Factors, the Genes Defining the O157:H7 Serotype, and Components of the Type 2 Shiga Toxin Family by Multiplex PCR. *J. Clin. Microbiol.*; **40**: 3613–3619.
- Ware, J. M., S. L. Abbott, and J. M. Janda.** 2000. A new diagnostic problem: isolation of *Escherichia coli* O157:H7 strains with aberrant biochemical properties. *Diag. Microbiol. Infect. Dis.* **38**: 185–187.
- Washington Post.** 2006. South Africa Panel: 336,000 Dead of AIDS", Washington Post, 29 August 2006
- Washington State Department of Health.** 2001. Confirmed recreational waterborne illness outbreaks 1991 through 2000.
- Wasteson, Y., J. M. Arnemo, B. K. Johansen, L. Vold, S. D. Mathiesen, M. A. Olsen, O. Wiig, and A. E. Derocher.** 1999. Analysis of faecal samples from wild animals for verocytotoxin producing *Escherichia coli* and *E. coli* O157. *Vet. Res.*; **144**: 646– 647.
- Watanabe, Y., O. Kotaro, H. Jonathan et al.** 1999. Factory Outbreak of *Escherichia coli* O157:H7 Infection in Japan. *Emerg. Infect. Dis.*; **5(3)**: 424–428.
- Welinder-Olsson, C., K. Stenqvist, M. Badenfors, A. Brandberg, K. Florén, M. Holm, L. Holmberg, E. Kjellin, S. Mårild, A. Studahl, and B. Kaijser.** 2004. EHEC outbreak among staff at a children's hospital – use of PCR for verocytotoxin

- detection and PFGE for epidemiological investigation. *J. Epidemiol. Infect.*; **132**: 43–49.
- Wells, J. G., L. D. Shipman, K. D. Greene, E. G. Sowers, J. H. Green, D. N. Cameron, F. P. Downes, M. L. Martin, P. M. Griffin, S. M. Ostroff, M. E. Potter, R. V. Tauxe, and I. K. Wachsmuth.** 1991. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like toxin-producing *E. coli* from dairy cattle. *J. Clin. Microbiol.*; **29**: 985–989.
- Whittam, T. S., I. K. Wachsmuth, and R. A. Wilson.** 1988. Genetic evidence of clonal descent of *Escherichia coli* O157:H7 associated with hemorrhagic colitis and hemolytic uremic syndrome. *J. Infect. Dis.*; **157**: 1124–1133.
- WHO.** 2006a. Guidelines for drinking-water quality incorporating first addendum. Available at: http://www.who.int/water_sanitation_health/. Accessed April 06th 2007.
- WHO.** 2006b. *Protecting Groundwater for Health: Managing the Quality of Drinking-water Sources*. Edited by O. Schmoll, G. Howard, J. Chilton and I. Chorus. ISBN: 1843390795. Published by IWA Publishing, London, UK.
- Wong, C. S., S. Jelacic, R. L. Habeeb, S. L. Watkins, and P. I. Tarr.** 2000. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N. Engl. J. Med.*; **342**: 1930–1936.
- Wright, J. G. S. and Conroy, R.** 2004. Household drinking water in developing countries: a systematic review of microbiological contamination between source and point-of- use. *Trop. Med. Int. Health*; **9**: 106–117.

- Yoshida, T., F. Masako, K. Naoki, I. Hiroshi, S. Tsuyoshi, K. Yutaka, I. Naohisa, and Y. Takashi.** 1999. Primary cultures of human endothelial cells are susceptible to low doses of Shiga toxins and undergo apoptosis. *J. Infect. Dis.*; **180**: 2048–2052.
- Yuk, H. G., and D. L. Marshall.** 2004. Adaptation of *Escherichia coli* O157:H7 to pH alters membrane lipid composition, verotoxin secretion, and resistance to simulated gastric fluid. *Appl. Environ. Microbiol.*; **70**: 3500–3505.
- Zafer G. and G. Gunes.** 2007. Influence of gamma irradiation on growth and survival of *Escherichia coli* O157:H7 and quality of cig kofte, a traditional raw meat product *Int. J. Food Sci. Techno.*; **42(9)**: 1067–1072.
- Zamxaka, M., G. Pironcheva, and N. Y. O. Muyima.** 2004. Microbiological and physicochemical assessment of the quality of domestic water resources in selected rural communities of the Eastern Cape Province. *Water, SA*; **30(3)**: 333–340.
- Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D. G. White, D. Wagner, and J. Meng.** 2001. Prevalence of *Campylobacter*, spp, *Escherichia coli* and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, DC area. *Appl. Environ. Microbiol.*; **67(12)**: 5431–5436.
- Zhao, S. H., S. E. Mitchell, J. H. Meng, S. Kresovich, M. P. Doyle, R. E. Dean, A. M. Casa and J. W. Weller.** 2000. Genomic typing of *Escherichia coli* O157:H7 by semi-automated fluorescent AFLP analysis. *Microbes and Infect.*; **2**: 107–113.
- Zhao, T., M. P. Doyle, J. Shere, and L. Garber.** 1995. Prevalence of entero-hemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. *Appl. Environ. Microbiol.*; **61**: 1290–1293.

Zweifel, C., S. Schumacher, L. Beutin, J. Blanco, and R. Stephan. 2006. Virulence profiles of Shiga toxin 2 producing *Escherichia coli* isolated from healthy pig at slaughter. *Vet. Microbiol.*; **117(2–4)**: 328–332.

CHAPTER 3

TABLE OF CONTENTS	Page №
Table of contents.....	209
List of Tables.....	211
List of Figures.....	212
Abstract.....	213
3.1 Introduction.....	214
3.2 Materials and methods.....	217
3.2.1 Study area.....	217
3.2.2 Collection of samples.....	217
3.2.2.1 Enumeration.....	218
3.2.2.2 Isolation of <i>E. coli</i> O157:H7.....	218
3.2.2.3 Identification of <i>E. coli</i> O157.....	219
3.2.3 Molecular characterization of <i>E. coli</i> O157:H7 using Polymerase	
Chain Reaction (PCR).....	220
3.2.3.1 Bacterial DNA Extraction.....	220
3.2.3.2 Amplification of <i>fliC_{H7}</i> ; <i>rfbE_{O157}</i> and <i>eaeA</i> genes.....	220
3.2.3.3 DNA Electrophoresis.....	221
3.2.4 Anti-microbial susceptibility test.....	222
3.2.6 Statistical Analysis.....	222
3.3 Results.....	223

3.3.1	Counts of presumptive <i>E. coli</i> O157 in the water samples.....	223
3.3.2	Prevalence of <i>E. coli</i> O157 in water samples.....	224
3.3.3	Molecular characterization of isolates.....	225
3.3.4	Statistical analysis of the results.....	226
3.3.5	Anti-microbial susceptibility.....	227
3.4	Discussion and Conclusion.....	228
	References.....	232

LIST OF TABLES

Table 3.1:	Summarized PCR amplified genes of <i>E. coli</i> O157:H7 isolated from drinking water from various distribution points in Amathole district.....	225
Table 3.2:	Distribution of susceptibility of <i>E. coli</i> O157:H7 isolated from water samples selected distribution points in Amathole district.....	228

LIST OF FIGURES

Figure 3.1:	Counts of presumptive <i>E. coli</i> O157 in drinking water from various distribution points in Amathole district.....	223
Figure 3.2:	Percentage prevalence of presumptive <i>E. coli</i> O157 in drinking water samples from the various distribution points in the Amathole district.....	224
Figure 3.3:	The amplified <i>fliC_{H7}</i> , <i>rfbE_{O157}</i> and <i>rfbE_{O157}</i> genes of <i>E. coli</i> O157:H7 isolated from water.....	226

CHAPTER 3

Abstract

Provision of potable water to the community will reduce the transmission of water borne infectious agents such as *E. coli* O157:H7, a known bacterial agent of diarrhoea and haemorrhagic colitis. Between February 2005 and August 2006, we investigated the prevalence and antimicrobial susceptibility of *E. coli* O157:H7 isolated from drinking water samples obtained from standpipes supplying water to communities of Fort Beaufort, Alice and Kwasiki, Ngwenya, Dimbaza and Mdantsane informal settlements in the Amathole District using standard microbiological methods. One hundred and eighty (180) samples of water were analyzed for *E. coli* O157:H7 using enrichment culture and confirmed using molecular techniques. Average presumptive *E. coli* O157:H7 counts for the water samples ranged between 3.3×10^4 and 1.7×10^5 CFU/ml. Ngwenya borehole water had higher prevalence of presumptive *E. coli* O157, 36.66% (11/30) whereas treated water from Mdantsane had the least prevalence, 16.66% (4/30). Molecular analysis of 27 representative presumptive *E. coli* O157 from these water revealed that 14.81% (4/27) of the water samples carried *E. coli* O157:H7 that was positive for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes. The *E. coli* O157:H7 isolates showed multiple resistances to one or more antibiotics that were tested. The findings of this study confirmed that waters from these areas harbour pathogenic *E. coli* O157:H7. The presence of *E. coli* O157:H7 in drinking waters suggest a possible health hazard from the drinking water samples obtained from these distribution points. The Eastern Cape Local Government should address issues around safe drinking water supplied to rural communities.

Keywords: Prevalence, Water, *Escherichia coli* O157:H7 and Antibiotic Resistance

3.1 Introduction

Access to safe drinking water is a basic human right and essential to people's health. This is well stated in the South African Constitution, which says that everyone has the right to have access to a constant supply of clean, safe drinking water (DWAF, 2005). Safe drinking water is water that is acceptable for humans to drink and use for other domestic purposes such as food preparation and bathing. Drinking water should contain no harmful concentrations of micro-organisms (DWAF, 2005). However, many rural and some peri-urban areas of South Africa still do not have access to potable water.

The poor microbiological quality of drinking water and especially the presence of pathogenic *E. coli* strains, including enterohemorrhagic *E. coli* O157:H7 in drinking water clearly explains why diarrhoeal diseases have become endemic in this region and continue to ravage the Eastern Cape communities (Bradshaw *et al.*, 2000). Since 1994, significant progress has been made in South Africa in the provision of water (Hodgson and Manus, 2006). However, investigations show that an unacceptably high incidence of poor drinking water quality occurs in non-metro South Africa (Momba *et al.*, 2003; 2004a, b; Zamxaka *et al.*, 2004; Momba 2005a, b; 2006; Obi *et al.*, 2007a, b). As recently as 2004, some 4 million people were still obtaining water from rivers, ponds and springs (Kasrils, 2004), which were usually not treated and were faecally contaminated (Muyima and Ngcakani, 1998; Momba and Kaleni, 2002; Obi *et al.*, 2002; Momba and Notshe, 2003). While the present South African Government has implemented many rural water supply schemes under the National Reconstruction and Development Programme, drinking

water is often of poor quality and considered unsafe for drinking in some communities (Mackintosh *et al.*, 2002; Momba *et al.*, 2003a, b; 2004a, b; 2005a, b; 2006; Obi *et al.*, 2007a, b). This means that consumers are at risk of contracting waterborne diseases even from treated water supplies.

By the year 2002, a large proportion of the Eastern Cape population (68%) still lived below the South African national poverty line (UNDP, 2004) and approximately 11% and 38% of the population lived in informal and traditional structures, respectively. Piped water distribution had reached only 62% of households and 31% of households had no toilet facilities (SSA, 2003).

The World Health Organization (WHO) estimated that about 88% of diarrhoeal diseases in the world are attributed to unsafe water, sanitation and hygiene. Approximately 3.1% of annual deaths (1.7 million) and 3.7% of the annual health burden disability burden (disability adjusted life years [DALYs]) worldwide (54.2 million) are attributed to unsafe water, sanitation and hygiene (WHO, 2003). The impact of diarrhoeal diseases is significant in South Africa, with an annual estimated deaths of about 50 000. Three million cases of illnesses and treatment cost the state about 3.4 billion Rand (Pegrum *et al.*, 1998; Mackintosh *et al.*, 2002). The most alarming situation is the death of about 20% of all children under 5 years of age living in settlements with rudimentary access to water supply and sanitation (Bourne and Coetzee, 1996).

Apart from lack of potable water, sanitation is still a problem in South Africa with the whole republic having sanitation coverage of only 65% by 2004 (Phaswana-Mafunya and Shukla, 2005; UNDP, 2006). The area is also characterized by unhygienic practices such as unsafe human excreta, solid and liquid waste disposal (Phaswana-Mafunya, 2006). Past studies (Swaddinwudhipong *et al.*, 1995; Kumar and Harada, 2002; Tumwine *et al.*, 2002) have

indicated that waterborne illnesses, particularly diarrhoea, occur when people live in conditions of poor water and sanitation facilities, poor health promotion, poor personal hygiene practices, and lack of safe water sources.

Studies on the microbiological quality of water supplied to communities residing within the Amathole District indicated that water consumption may be unsafe (Momba *et al.*, 2004; Zamxaka *et al.*, 2004). Other studies have shown that water may be contaminated at the point of use (Momba and Notshe, 2003; Momba and Kaleni, 2003). A recent study that investigated the compliance of some drinking water treatment plants revealed that only 18% of the plants were producing water of good quality (Momba *et al.*, 2006b)

Based on the studies conducted (Momba *et al.*, 2006a, b) most rural homes in the Eastern Cape Province still lack potable water supply. Although, *E. coli* O157:H7 is associated with contaminated drinking water and its incrimination in cases of *E. coli* O157:H7 causes hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) in humans (Dean-Nystrom *et al.*, 1998; Ritchie *et al.*, 2003), studies on the organism have received a cursory attention in the Eastern Cape Province. We, therefore, conducted the present study to ascertain the prevalence of *E. coli* O157:H7 in drinking water collected from selected distribution sources within the Amathole District of the Eastern Cape Province.

3.2 Materials and Methods

3.2.1 Study area

The study was conducted between March 2005 and August 2006. The Amathole District was selected based on the researchers' familiarity with the area. Fort Beaufort, Alice, Dimbaza, Mdantsane, Ngwenya and Kwasaki were chosen as water sampling points because potential pathogenic *E. coli* strains have been reported to be predominant in water sources used by these communities in these areas (Momba and Kaleni, 2002; Momba and Notshe, 2003; Momba *et al.*, 2004a, b; 2005a, b). The mentioned sampling areas fall within the geographical coordinates 32.77°S, 26.63°E for Fort Beaufort and 32.97°S, 27.87°E for Mdantsane both at the extreme ends when a linear stretch is presumed. Alice is the central sampling location at the coordinates 32.79°S, 26.83°E.

3.2.2 Collection of samples

In total, 180 water samples (30 for each site) were collected from the standpipes that supplied treated drinking water to the communities of Fort Beaufort, Alice (Fort Hare), Dimbaza, Mdantsane, and untreated groundwater to the communities of Ngwenya and Kwasaki, using internationally accepted techniques and principles. The water samples and stool swabs were transported on ice in a cooler box to the laboratory, for the isolation of *E. coli* O157:H7. Microbiological analyses were performed within 1 to 4 h of their collection.

3.2.2.1 Enumeration

Presumptive *E. coli* O157:H7 populations in the water samples were determined by direct plating. Ten millilitre of water (10 ml) was added into 90 ml of 1% (w/v) Phosphate Buffered Saline (PBS) (Merck, Johannesburg, SA). Subsequent serial dilutions of 10^{-2} , 10^{-4} and 10^{-6} were made and 1 ml of each respective dilution (including the 10^{-1}) was pour plated in duplicates onto Cefixime-Tellurite Sorbitol MacConkey (CT-SMAC) Agar (Merck, SA). All plates were incubated at 37°C for 24 h. Average presumptive *E. coli* O157:H7 counts were estimated and expressed as CFU/ml of water sample.

3.2.2.2 Isolation of *E. coli* O157:H7

Escherichia coli O157:H7 from the water samples were isolated using a pre-enrichment process. This was followed by immunomagnetic separation and spread plate procedure. For pre-enrichment, 1 ml of water sample was added to 99 ml of modified *E. coli* (mEC) broth containing 20 µg/ml of novobiocin (n) (Merck, SA) (Heuvelink *et al.*, 1998).

The samples were incubated for 8 h at 37 °C on a rotary shaker (143 × g) (Gallenkamp, Loughborough, England). Twenty microlitres (20 µl) of the Dynabead (Dyna, Oslo) suspensions were incubated in a 1.5 ml Eppendorf tubes (Eppendorf, SA) with 1 ml aliquots of the pre-enriched samples at room temperature for 10 min with continuous mixing by hand. This step was performed to allow the *E. coli* O157-specific antibodies coated onto the beads to bind to the target bacteria.

The bacterial-IMS bead complexes were separated using a magnetic particle concentrator, Dynal MPC-M (Dyna, Oslo) for 3 min (Dyna product brochure, 2006). After discarding the supernatants and washing the bead-particles using 0.02% (v/v) diluted (1:20) PBS-Tween 20 in

distilled water (pH 7.2) (Merck, SA), the complete immunomagnetic separation and washing procedure were repeated twice (Dynal product brochure, 2006). The final bacterial-IMS bead complexes were re-suspended in 1000 µl washing buffer (PBS-Tween 20) (Merck, SA). Fifty microlitres (50 µl) of the bacteria-IMS complex concentrate was transferred to *E. coli* O157 selective media cefixime (0.05 mg/l) and potassium tellurite (2.5 mg/l)-Sorbitol MacConkey (CT-SMAC) (Merck, SA) Agar and spread out into half of the plate using a sterile cotton swab. The swabbed portion using an inoculating loop was further streaked out onto the agar surface to achieve single isolated colonies (Dynal product brochure, 2006). The plates were then incubated at 37°C for 24 h. Sorbitol-non-fermenting colonies (up to 5 colourless colonies per plate per water sample) were randomly selected and further plated by streaking onto Eosin Methylene Blue (EMB) agar (Merck, SA) (Müller *et al.*, 2003; Cagney *et al.*, 2004). Colonies with characteristic greenish blue-black metallic sheen colour on EMB Agar (Merck, SA). The colonies were then subjected to confirmatory test using conventional biochemical tests (Cagney *et al.*, 2004).

3.2.2.3 Identification of *E. coli* O157

Escherichia coli O157 colonies were identified as described by Cagney *et al.* (2004). The Oxidase test was performed on the colonies that were Gram negative prior to the conventional indole-methyl red-Voges-Proskauer-citrate (IMViC) tests (Heuvelink *et al.*, 1998, Müller *et al.*, 2003). Colonies of presumptive *E. coli* O157 each representing the 46 water samples were subjected to IMViC test and further confirmed as *E. coli* with API 20E kits. The strips were read and final identification was secured using API LAB PLUS computer software (BioMérieux, Marcy-Etoile, France) (Momba *et al.*, 2006a). Of the 46 water samples, only 27-representative *E.*

coli O157 from these water samples, which had 99.9% profile for *E. coli* with API LAB PLUS computer software program were used for molecular characterization.

3.2.3 Molecular characterization of *E. coli* O157:H7 using PCR

3.2.3.1 Bacterial DNA Extraction

DNA was extracted from colonies identified as *E. coli* O157 and also from a positive control strain (*E. coli* O157:H7, ATCC 43895) purchased from the Microbiology Department of the National Health Laboratory Services (NHLS), Johannesburg, South Africa. The extraction was performed according to the method previously described by Torres *et al.* (2003).

Briefly, a loop-full of overnight culture of *E. coli* colonies was suspended in 200 µl of sterile Milli-Q PCR grade water (Merck, SA) and the cells were lysed using a Dri-block DB.2A (Techne, SA) for 15 min at 100 °C. The cell debris was removed by centrifugation at $20\,000 \times g$ for 2 min using a MiniSpin micro centrifuge (Merck, SA). The lysate supernatant was placed on ice for 5 min. Sterile Milli-Q PCR grade water (Merck, SA) was included in each PCR assay as a negative control.

3.2.3.2 Amplification of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes

Oligonucleotide primers specific for the targeted *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes used in the polymerase chain reaction (PCR) were similar to those used by Wang *et al.* (2002). The primers sequences that were used to identify the target genes were FliC-F 5'-TAC CAT CGC AAA AGC AAC TCC-3', FliC-R 5'-GTC GGC AAC GTT AGT GAT ACC-3' for *fliC_{H7}*. For *rfbE_{O157}*, the forward was RfbE-F 5'-CTA CAG GTG AAG GTG GAA TGG-3'; RfbE-R 5'-AATT CCT CTC TTT CCT CTG CGG-3'. The primers for *eaeA* gene were EAE-R 5'-ATG CTT AGT GCT GGT

TTA GG-3', EAE-R 5'-GCC TTC ATC ATT TCG CTT TC-3'. The expected amplification sizes for *fliC_{H7}*, *rfbE_{O157}*, and *eaeA* genes were 247, 328, and 248 base pairs, respectively. The PCR assays for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes were carried out in a 50 µl reaction volume. The reaction mixture contained 10X SuperTherm GOLD Buffer, 1.5 mM MgCl₂, each of the four deoxynucleoside triphosphates (dNTPs) (Southern Cross Biotechnology, SA) at a concentration of 0.25 mM, 100 pmol for each of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* specific primers, and 5 U of *Taq* DNA polymerase (Southern Cross Biotechnology, SA).

The PCR reaction was carried out in the Eppendorf model AG 22331 Thermocycler (Merck, SA). The following PCR conditions for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes optimized in our laboratory were similar to those previously used by Wang *et al.* (2002). Initial denaturation at 95 °C for 8 min followed by 30 cycles of amplification, denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s. The final extension cycle was followed by incubation at 72 °C for 7 min and cooling to 4 °C.

3.2.3.3 *DNA Electrophoresis*

The PCR products (10 µl aliquots) were visualized on a 2% (w/v) agarose gel (Merck, SA) in 1 U TAE buffer (40 mM Tris-HCl, 20 mM Na-acetate, 1 mM EDTA, pH 8.5) and stained with 0.5 µg/ml Ethidium Bromide (EtBr) (Merck, SA). A 100-bp DNA ladder (Promega, USA) was included on each gel as a molecular size standard. The electrophoresis was carried out at 76 V for 1 h. The products were photographed under the BioDoc-It System (UVP Upland, CA 91786, USA).

3.2.4 Anti-microbial susceptibility test

The Antimicrobial susceptibility test for *E. coli* O157:H7 isolates was determined using Bauer and Kirby disk diffusion technique on Mueller-Hinton Agar (MHA) (Merck, SA) (Jorgensen *et al.*, 1999). The following antibiotic discs were used: ampicillin (10 µg), ceftriaxone (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), and amikacin 30 µg) and erythromycin (15 µg) (Davies Diagnostics, SA). For the inocula development the bacterial isolates were grown overnight on nutrient broth at 37°C for reactivation.

The cells were harvested by centrifugation at $2200 \times g$ using a Beckman model TJ - 6 centrifuge (Great Britain) and washed twice with 0.85% (w/v) saline water (NaCl) (Merck, SA). The bacterial cells were re-suspended in sterile saline solution to a final density of 10^7 CFU/ml. The cell suspensions were then swabbed onto a freshly prepared Mueller-Hinton Agar (MHA) in order to achieve a lawn of growth on incubation. The antibiotic discs were placed on the surface of the inoculated Mueller-Hinton Agar (MHA) using a disc dispenser and the plates were incubated for 18 h at 37 °C (Jorgensen *et al.*, 1999). Zones of growth inhibition were measured in millimetres and results interpreted according to the guidelines of National Committee for Clinical Laboratory Standards for antimicrobial susceptibility testing (NCCLS, 1999). *Escherichia coli* ATCC 43895 was included as a positive control.

3.2.5 Statistical Analysis

The statistical analysis was done by use of SAS program (SAS Institute, Cary, NC). The *Chi* square test was used to establish the significance difference between the prevalence rates of *E. coli* O157:H7 in the water samples from the different sampling locations.

3.3 Results

3.3.1 Counts of presumptive *E. coli* O157 in the water samples

The average counts of presumptive *E. coli* O157 were lower for Fort Beaufort water (3.3×10^4 CFU/ml) but highest for Ngwenya borehole water (1.71×10^5 CFU/ml). Mdantsane, Dimbaza, Kwasaki and Alice had *E. coli* O157 counts of 3.5×10^4 CFU/ml; 4.11×10^4 CFU/ml; 4.8×10^4 CFU/ml and 1.05×10^5 CFU/ml, respectively. The probability values for the counts were > 0.05 ; therefore the counts of *E. coli* O157:H7 of the water samples from these three areas were not significantly different. The respective counts for the presumptive *E. coli* O157 are shown in Figure 3.1.

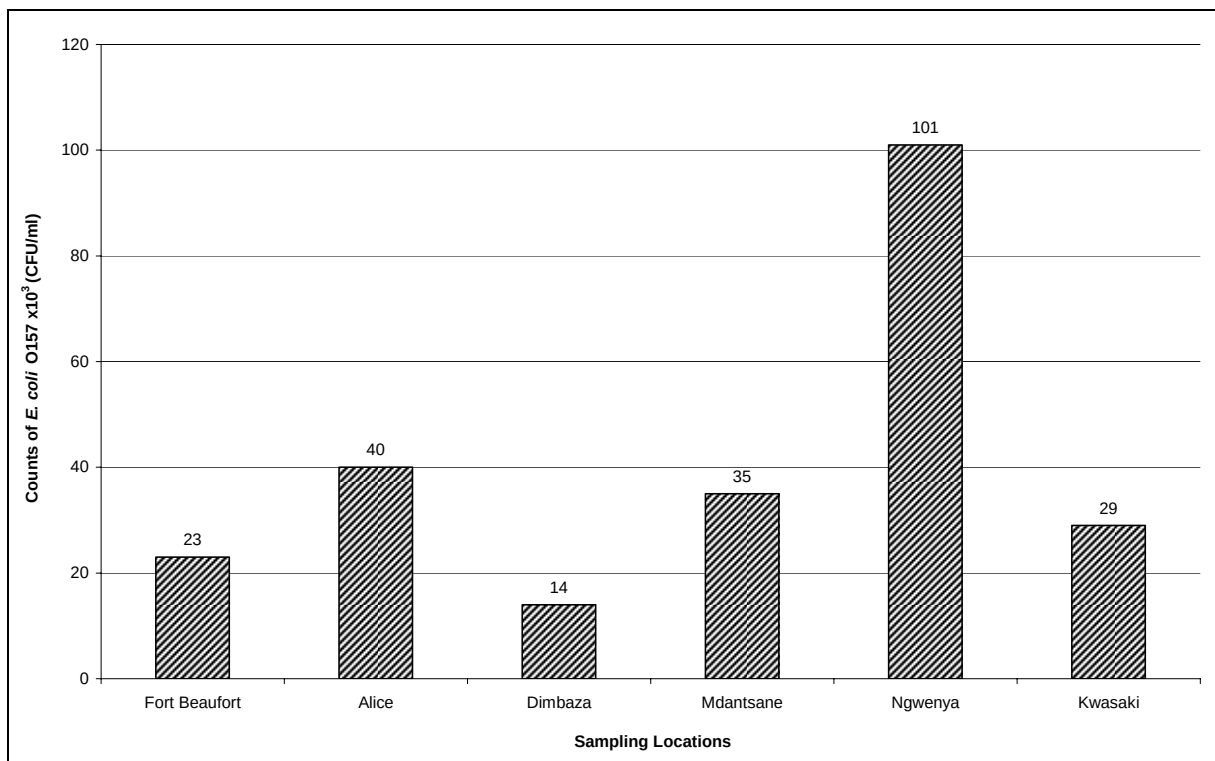


Figure 3.1: Counts of presumptive *E. coli* O157 in drinking water from various distribution points in Amathole District.

3.3.2 Prevalence of *E. coli* O157 in the water samples

Of the 180 drinking water samples, 46 (25.56 %) were presumptively positive for *E. coli* O157. Ngwenya borehole water had the highest prevalence of presumptive *E. coli* O157, 36.66% (11/30), followed by water samples from Alice and Dimbaza at 30% (9/30). Water samples from Kwasaki, Fort Beaufort and Mdantsane had prevalence of presumptive *E. coli* O157 at rates of 26.66% (8/30), 16.66% (5/30) and 13.33% (4/30), respectively. Percentage prevalence's of presumptive *E. coli* O157 in the waters are reflected in Figure 3.2.

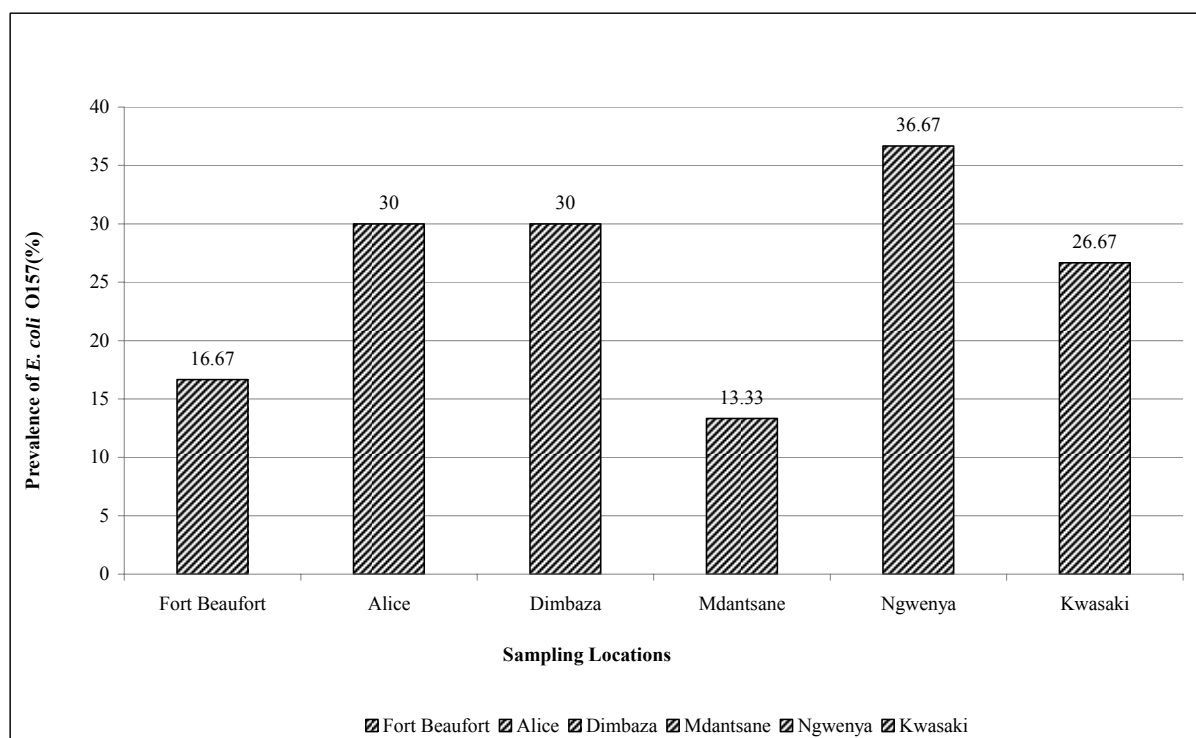


Figure 3.2: Percentage prevalence of presumptive *E. coli* O157 in drinking water samples from the various distribution points in the Amathole District. The “n” sample size was 30 for all the sampling locations.

3.3.3 Molecular characterization of isolates

Water samples isolates identified by biochemical profiles for *E. coli* O157 that were positive by PCR for *fliC_{H7}*, *rfbE_{O157}*, and *eaeA* genes, characteristics of *E. coli* O157:H7 are summarized in Table 3.1. Of the 27 water samples presumptively positive for *E. coli* O157 isolates, 4 (14.81%) carried *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes whereas 3.70% (1/27) was only positive for *fliC_{H7}* gene. The three target genes of *E. coli* O157:H7 (*fliC_{H7}*, *rfbE_{O157}*, and *eaeA*) under the present study were noticed in isolates obtained from Dimbaza, Fort Beaufort, Ngwenya and Mdantsane water samples. Representative gel electrophoresis profiles of amplified products of target genes for *E. coli* O157:H7 are illustrated in Figure 3.3.

Table 3.1: Summarized PCR amplified genes of *E. coli* O157:H7 isolated from drinking water from various distribution points in Amathole District.

Water Source	Amplified genes			Water samples positive for <i>E.</i>
	<i>fliC_{H7}</i>	<i>rfbE_{O157}</i>	<i>eaeA</i>	<i>coli</i> O157:H7
Alice*	+	–	–	0
Dimbaza	+	+	+	1
Fort Beaufort	+	+	+	1
Ngwenya	+	+	+	1
Mdantsane	+	+	+	1
Total number of water samples positive with <i>E. coli</i> O157:H7				4(1*)

"+" target gene present, "–" target gene absent. n = 27 Representative number of presumptive *E.*

coli O157 characterized by PCR. (*) represent water samples whose *E. coli* O157 only had either 1 or 2 of the targeted genes. These water samples were not considered as being positive for *E. coli* O157:H7 due to the absence of the other genes.

The entire three target genes of *E. coli* O157:H7 (*fliC_{H7}*, *rfbE_{O157}*, and *eaeA*) were noticed in the isolates obtained from Dimbaza, Fort Beaufort, Ngwenya and Mdantsane water samples.

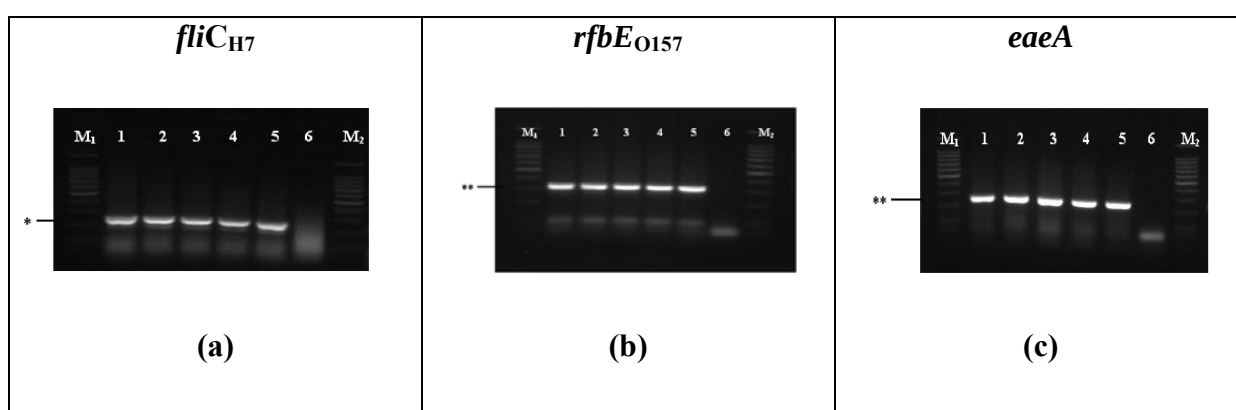


Figure 3.3: The amplified *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes of *E. coli* O157:H7 isolated from water. Lanes M₁ & M₂: 100 bp DNA ladder marker (Promega, USA), lane 1: Positive control (*E. coli* O157:H7, ATCC 43895), lane 2:NgweW₄, lane 3: DimW₉, lane 4: MdaW₂₇, lane 5: FbW₂₇, lane 6: Negative control. The expected molecular sizes of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* fragments were at 247bp, 327bp and 248bp respectively.

3.3.4 Statistical analysis of the results

The samples that had *E. coli* O157:H7 were compared with each other using chi square test to establish the significant difference. The logistic analysis was also run to determine which parameters were good predictors for infection by *E. coli* O157:H7. The probability values were >

0.05; suggesting that the prevalence of *E. coli* O157:H7 in the water samples from these different areas was not significantly different from one region to the other.

3.3.5 Anti-microbial susceptibility

Antibiotic susceptibility testing found that 100% (4/4) of *E. coli* O157:H7 that were isolated from the water samples were resistant to gentamicin and erythromycin, 50% (2/4) were resistant to ampicillin and tetracycline whereas 25% (1/4) was resistant to nalidixic acid. However, 100% (4/4) and 50% (2/4) of *E. coli* O157:H7 were susceptible to amikacin and ceftriaxone respectively. An intermediate susceptibility (S) to ampicillin, ceftriaxone and tetracycline was observed amongst 50% of the *E. coli* O157:H7 whereas 75% and 100% of the *E. coli* O157:H7 were intermediately susceptible (I) to chloramphenicol and nalidixic acid respectively (Table 3.2).

Table 3.2: Antimicrobial susceptibility of *E. coli* O157:H7 isolated from the water samples

Antibiotic agent	Antibiotic disc content (µg)	Distribution %		
		R	I	S
Gentamycin	10	100	0	0
Ampicillin	10	50	50	0
Chloramphenicol	30	0	100	0
Nalidixic acid	30	25	75	0
Amikacin	30	0	0	100
Ceftriaxone	30	50	50	0
Erythromycin	15	100	0	0
Tetracycline	30	50	50	0

^aR, Resistant; I, Intermediate; S, Susceptible. ^bThe interpretation to achieve the above susceptibility percentages was based on the breakpoints of zone diameters for individual antibiotic agents as outlined in NCCLS ninth informational supplement of 1999:document M100–S9.

3.4 Discussion and Conclusion

The present study revealed important findings on the prevalence of *E. coli* O157 in the drinking water supplied to the communities of Fort Beaufort, Alice, Dimbaza, Mdantsane, Ngwenya and

Kwasiki. A higher *E. coli* O157 prevalence rate was noted in water samples collected from Ngwenya groundwater samples (36.66%) while a lower prevalence rate was recorded in Mdantsane treated drinking water samples (13.33%) (Figure 3.2). With the exception of the water samples collected from Alice, which showed only one target gene (*fliC_{H7}*) (Table 3.3), the PCR successfully amplified the three target genes (*fliC_{H7}*, *rfbE_{O157}* and *eaeA*) of the *E. coli* O157:H7 from the isolates obtained from the drinking water samples (Table 3.3, Figure 3.3).

The results of this study confirm the poor microbiological quality of the drinking water that is produced by many water treatment plants in the Eastern Cape Province. From October 2004 to November 2004 and from July to September 2005, a survey of 55 water treatment plants was conducted in five District Municipalities (Cacadu, Chris Hani, Amathole, Ukhahlamba and O.R. Tambo) of the Eastern Cape Province by Momba *et al.* (2006b). Of these 55 water treatment plants, only 18% complied with the South African National Standards 241 Drinking Water Specification (SANS, 2001). Total and faecal coliforms were recorded at the points of treatment as well as at the consumers' taps. Among 26 bacterial species identified during the survey, *E. coli* was predominant in treated drinking water supplied to the communities of the above-mentioned five District Municipalities (Momba *et al.*, 2006a). In another study conducted between 2001 and 2002, polymerase chain reaction analysis using *UidaA*-specific primers revealed that, a genetic region homologous in size to the *E. coli* *UidaA* structural gene was present in Ngwenya and other groundwater sources used by the communities for domestic consumption (Momba *et al.*, 2006b). These studies and the present investigation gave conclusive evidence that the microbiological quality of drinking water supplied to the Eastern Cape communities poses a high risk to the health of these communities.

Although only representative *E. coli* O157 isolates identified by culture-based methods and biochemical tests were subjected to PCR assays, low prevalence rates of *E. coli* O157:H7 from the waters was noted (Table 3.2). It is interesting to note that the PCR assays were limited only to the amplification of the three target genes (*fliC_{H7}*, *rfbE_{O157}* and *eaeA*) characteristics of the enterohemorrhagic *E. coli* O157:H7 serotype. Most important is the fact that there are other verotoxigenic *E. coli* (VTEC) such as *E. coli* O157: H⁻ (Hussein and Stanley, 2003). Other virulence as well as putative genes that have been used in the characterization of *E. coli* O157:H7 include but are not limited to EHEC *hlyA*, *stx₁* and *stx₂*, which in some instances may also be referred to as *vt₁* and *vt₂* and *E. coli* 16s rRNA (Wang *et al.*, 2002; Cagney *et al.*, 2004).

Different variants of *stx₂* such as *stx_{2c}*, *stx_{2d}*, *stx_{2e}* and *stx_{2f}* have also been reported (Wang *et al.*, 2002). In addition, other virulence factors as well may be involved in *E. coli* O157 pathogenesis (Dean-nystrom *et al.*, 1998). Consequently, the high prevalence of presumptive *E. coli* O157 could be linked to the presence of other serotypes of *E. coli* O157 in the water samples.

Waterborne *E. coli* O157:H7 transmissions have been attributed to the ingestion of contaminated drinking water or recreational waters (Keene *et al.*, 1994; Armstrong *et al.*, 1996; Friedman *et al.*, 1999). This study therefore predicts a possible epidemiological link between *E. coli* O157:H7 isolated from the drinking water and diarrhoeal diseases in humans.

Epidemiological studies have identified that only small numbers of *E. coli* O157:H7 (e.g. 10 – 200 of organisms) is needed to cause diarrhoeal infections (Wilshaw *et al.*, 1994). Considering the microbiological quality of these drinking waters in the Eastern Cape in general and that of the Amathole District in particular and the profile of the molecular results of the present study, monitoring of drinking water quality in the province should be re-enforced. Although, the PCR assays were able to amplify the *E. coli* O157:H7 genes (*fliC_{H7}*, *rfbE_{O157}* and

eaeA) from the water *E. coli* O157:H7 isolates, it is of great concern for such levels of pathogenic bacteria to be present in drinking waters consumed by the community.

Resistance to the anti-microbial agents was noted in at least one of the *E. coli* O157:H7 isolates with all the four isolates showing complete resistance to gentamycin and erythromycin (Table 3.3). However, six out of the eight anti-microbial agents were effective against the *E. coli* O157:H7. The observed high resistance by *E. coli* O157:H7 isolates to most of the antibiotics, which are often considered to be of broad spectrum have also been reported by other researchers elsewhere (Ashraf *et al.*, 2005; Magwira *et al.*, 2005). Ashraf *et al.* (2005), reported antimicrobial resistance of *E. coli* O157:H7 isolates from a diarrhoeal patient in Japan. The *E. coli* O157:H7 that were isolated by Ashraf *et al.* (2005) showed multi-drug resistance against streptomycin, spectinomycin, co-trimoxazole, ampicillin and tetracycline. In their findings, Ashraf *et al.* (2005) explained that drug resistant gene in *E. coli* O157:H7 could be because of gene transfer.

Provision of safe drinking water is a basic human right and essential to the health of rural communities in the Eastern Cape in general and the Amathole District in particular. This study and other studies conducted in the province (Momba and Kaleni, 2002, Momba and Notshe, 2003, Momba *et al.*, 2004a, b; 2005a, b 2006a, b) therefore, points to the need of the Provincial and Local Government to address the safety of drinking water supplied to the rural communities for enhanced wellbeing.

References

- Armstrong, G. L., J. Hollingsworth, and J. G. Jr. Morris.** 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.*; **18**: 29–51.
- Ashraf, M. A., K. Hidekazu, I. Kumiko, H. Yoshiko, S. Miyoko, S. Masato, and S. Tadashi.** 2005. Genomic analysis of a multidrug-resistant strain of enterohaemorrhagic *Escherichia coli* O157: H7 causing a family outbreak in Japan. *J. Med. Microbiol.*; **54**: 867–872.
- Bourne, D. E. and N. Coetzee.** 1996. An atlas of potentially water-related diseases in South Africa. Pretoria, South Africa. WRC Report, № 584/1/96.
- Bradshaw, D., N. Nadine, L. Ria, G. Pam, J. Jané, N. Beatrice, N. Rosana, P. Desirée, P. and S. Michelle.** 2000. South African National Burden of Disease Study: Mortality Estimates for Eastern Cape Province. A publication OF Medical Research Council of South Africa: Burden of Disease Research Unit; 1–16. Available from: <http://www.mrc.ac.za>. Accessed 06th February, 2007.
- Cagney, C., H. Crowley, G. Duffy, J-J. Sheridan, S. O'Brien, E. Carney, W. M. Anderson, D. A. Dowell, I. S. Blair, and R. H. Bisho.** 2004. Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. *J. Food Microbiol.*; **21**: 203–212.
- Dean-Nystrom, E. A., T. B. Brad, W. M. Harley, and D. O. Alison.** 1998. *Escherichia coli* O157:H7 requires intimin for enteropathogenicity in calves. *Infect. Imm.*; **66(9)**: 4560–4563.

- DWAF (Department of Water affairs and Forestry).** 2005. A drinking water quality framework for South Africa; pp 1 – 69. Available from: <http://www.dwaf.gov.za>. Accessed April 09th 2007.
- Dynal Product Brochure.** 2006. *Dynabeads® anti-E coli*. For rapid selective enrichment of *Escherichia coli* O157:H7. Dynal A.S. Oslo, Norway.
- Friedman, M. S., T. Roels, J. E. Koehler, L. Feldman, L., Bibb, W. F. and P. Blake.** 1999. *Escherichia coli* O157:H7 outbreak associated with an improperly chlorinated swimming pool. *Clin. Infect. Dis.*; **29**: 298–303.
- Heuvelink, A. E., F. I. A. M Van Den Biggelaar, J. T. M. Zwartkruis-nahuis, R. G. Herbes, R. Huyben, N. Nagelkerkr, W. J. G. Melchers, L. A. H. Monnens, and E. De Boer.** 1998. Occurrence of verocytotoxin producing *Escherichia coli* O157:H7 on Dutch dairy farms. *J. Clin. Microbiol.*; **36(12)**: 3480–3487.
- Hodgson, K. And L. Manus.** 2006. A drinking water quality framework for South Africa. *Water, SA*; **32(5)**: 673 – 678.
- Hussein, S. H. and T. O. Stanley.** 2003. Introduction to the food safety concerns of verotoxin-producing *Escherichia coli*. *Exper. Biology and Med.*; **228**: 331–332.
- Jorgensen, J. H., J. D. Turnidge, and J. A. Washington.** 1999. *Antibacterial susceptibility tests: dilution and disk diffusion methods*. In: Murray, P. R., Tenover, F. C., Baron, E. J. and Tenover, F. C., Baron, E. J. and Tenover, F. C. ed. *Manual of clinical microbiology*, 7th ed. Washington, DC: American Society for Microbiology Press; pg. 1526–1543.
- Kasrils, R.** 2004. *A Decade of Delivery*. Minister of Water Affairs and Forestry.
- Keene, M. E., J. M. McAnulty, F. C. Hoesly, L. P. Jr. Williams, K Hedberg, G. L. Oxman, T. J. Barrett, M. A. Pfaller, and D. W. Fleming.** 1994. A swimming-

associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. *New Eng. J. Med.*; **331(9)**: 579–584

Kumar K, and H. Harada. 2002. Field survey on water supply, sanitation and associated health impacts in urban poor communities – a case from Mumbai city, India. *Water Sci. Techno.*; **46**: 269–275.

Mackintosh, G.S., P.F. De Souza, and E. Delpont. 2002. Addressing the sustainability of small-user rural water treatment systems in South Africa. *Water Supp.*; **2(2)**: 139–144

Magwira C.A., B. A. Gashe, and E. K. Collison. 2005. Prevalence and antibiotic resistance profiles of *Escherichia coli* O157:H7 in beef products from retail outlets in Gaborone, Botswana. *J. Food Prot.*; **68 (2)**: 403–406.

Momba M. N. B., Z. Tyafa, and N. Makala. 2004a. Rural water treatment plants fail to provide potable water to their consumers: Alice water treatment plant in the Eastern Cape Province of South Africa. *SA J. Sci.*; **100**: 307–310.

Momba, M. N. B., and P. Kaleni. 2003. Re-growth and survival of indicator microorganisms on the surfaces of household containers used for the storage of drinking water in rural communities of South Africa. *Water Res.*; **36**: 3023–3028.

Momba, M. N. B., and T. L. Notshe. 2003. The effect of long storage and household containers on the microbiological quality of drinking water in rural communities of South Africa. *J. Water Supp. Res. Techno.-AQUA.*; **52 (1)**: 67–76.

Momba, M. N. B., N Makala, B. Zani, and B. M. Brouckaert. 2005a. Key causes of drinking water quality failure in a rural small water supply of South Africa. In *Water Encyclopedia –Domestic, Municipal, and Industrial water supply and waste disposal* (Edt J.H. Lehr and J. Keeley). John Wiley & Son, Inc. Pages 221–227

- Momba, M. N. B., N. Makala, B. Brouckaert, Z. Tyafa, P. Thompson and A. C. Buckley.** 2004b. Improving the efficiency and sustainability of disinfection at a small rural water treatment plant. *Water, SA*. **30(5)**: 617–622
- Momba, M. N. B., N. Makala, Z. Tyafa, and B. M. Brouckaert.** 2005b. A model partnership for sustainable production of safe drinking water for rural communities in South Africa. *SA J. Sci.*; **101**: 335–336.
- Momba, M. N. B., S. Ndaliso, and M. A. Binda.** 2003a. Effect of a combined chlorine-monochloramine process on the inhibition of biofilm regrowth in potable water systems. *J. Water Supp.*; **3 (1-2)**: 215–221.
- Momba, M. N. B., V. K. Malakate, and J. Theron.** 2006a. Abundance of pathogenic *Escherichia coli*, *Salmonella typhimurium* and *Vibrio cholerae* in Nkonkobe drinking water sources. *J. Water Health*; **04**: 289–296.
- Momba, M. N. B., Z. Tyafa, N. Makala, B. M. Brouckaert, and C. L. Obi.** 2006b. Safe drinking water still a dream in rural areas of South Africa. Case study: The Eastern Cape Province. *Water, SA*; **32**: 715–720.
- Momba, M.N.B., N. Makala, and B. M. Brouckaert.** (2003b) Evaluation of factors affecting the performance of small water supplies for the removal of indicator bacteria in rural developing areas of South Africa. *International Water Association Health-Related Water Microbiology Symposium*. Cape Town/South Africa 14-19 September 2003.
- Müller, E. E., Grabow, W. and M. M. Ehlers.** 2003. Immunomagnetic separation of *Escherichia coli* O157:H7 from environmental and wastewater in South Africa. *Water, SA*; **29**: 427– 432.
- Muyima, N. Y. O. and F. Ngcakani.** 1998. Indicator bacteria and regrowth potential of the

drinking water in Alice, Eastern Cape. *Water, SA*; **24 (1)**: 29–34.

NCCLS, (National Committee for Clinical Laboratory Standards). Performance standards for antimicrobial susceptibility testing; ninth informational supplement. Wayne, Pennsylvania: NCCLS; document M100-S9, 1999: 19.

Obi C. L., J. Ramalivhana, M. N. B. Momba, B. Onabulu, J. O. Igumbor, M. Lukoto, T. B. Mulaudzi, E. L. Jansen van Rensburg, E. Green, and S. Ndou. 2007a. Antibiotic resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients with and without diarrhea and their household drinking water in rural communities in Limpopo Province South Africa. *Afri. J. Biotech.*; **6(8)**: 1035–1047.

Obi, C. L. J. Ramalivhana, M. N. B Momba and J. Igumbor. 2007b. Scope and frequency of enteric bacterial pathogens isolated from HIV/AIDS patients and their household drinking water in Limpopo Province. *Water, SA*; **33(4)**: 539–538

Obi, C. L., N. Potgieter, P. O. Bessong, and G. Matsaung. 2002. Assessment of the microbial quality of river water sources in rural Venda communities in South Africa. *Water, SA*; **28(3)**: 287–292.

Pegrum, G. C., N. Rollins, and Q. Espey. 1998. Estimating the cost of diarrhea and epidemic dysentery in KwaZulu-Natal and South Africa. *Water, SA*; **24(1)**.

Phaswana-Mafunya N. and N. Shukla. 2005. Factors that could motivate people to adopt safe hygienic practices in the Eastern Cape Province, South Africa. *SA Health Sci.*; **5(1)**: 21–28.

Phaswana-Mafunya, N. 2006. Hygiene status of rural communities in the Eastern Cape of South Africa. *Int. J. Environ. Health Res.*; **16(4)**: 289–03.

- Ritchie, J. M., P. L. Wagner, D. W. Acheson, and M. K. Waldor.** 2003. Comparison of Shiga toxin production by hemolytic-uremic syndrome-associated and bovine-associated Shiga toxin-producing *Escherichia coli* isolates. *Appl. Environ. Microbiol.*; **69**:1059–1066.
- SANS (South African National Standards),** 2001. *SANS 241-2001: Specification for Drinking Water*. Pretoria, South Africa.
- SSA, (Statistics South Africa).** 2003. Census 2001: Census in Brief. Report No. 03-02-03 2001. Pretoria: Statistics South Africa.
- Swaddinwudhipong, W., S. Karintraratana, and S. Kavinum.** 1995. A common source outbreak of Shigellosis involving a piped public water supply in northern Thai communities. *J. Trop. Med. Hyg.*; **98**:145–150.
- Torres, J., C. López-Saucedo, J. F. Cerna, N. Villegas-Sepulveda, R., Thompson, and F. R. Velazquez.** 2003. Single multiplex polymerase chain reaction to detect diverse loci associated with diarrheagenic *Escherichia coli*. *Emerg. Infect. Dis.*; **9**: 127–131.
- Tumwine, J. K., J. Thompson, M. Katna-Katna, M. Mujmajuzi, H. Johnstone, and I. Porras.** 2002. Diarrhea and effects of different water sources, sanitation and hygiene behavior in East Africa. *Trop. Med. and Int. Health*; **7**: 750–756.
- UNDP (United Nations Development Programme).** 2004. South Africa Human Development Report 2003. Cape Town: Oxford University Press Southern Africa.
- UNDP (United Nations Development Programme).** 2006. Human Development Report. Beyond scarcity: Power, poverty and the global water crisis. Pp 1 – 440. Palgrave Macmillan, New York. ISBN 0-230-50058-7.
- Wang, G., G. C. Clifford, and G. R. Frank.** 2002. Detection in *Escherichia coli* of the Genes

Encoding the Major Virulence Factors, the Genes Defining the O157:H7 Serotype, and Components of the Type 2 Shiga Toxin Family by Multiplex PCR. *J. Clin. Microbiol.*; **40**: 3613–3619.

WHO. 2003. *Emerging Issues in Water and Infectious Disease*, World Health Organisation, Geneva, Switzerland.

Wilshaw, G. A., J. Thirlwell, and A. P. Jones. 1994. Verocytotoxin -producing *Escherichia coli* O157 in beef burgers linked to an outbreak of diarrhea, haemorrhagic colitis and haemolytic uremic syndrome. *Lett. Appl. Microbiol.*; **19**: 304–307.

Zamxaka, M., G. Pironcheva, and N. Y. O. Muyima. 2004. Microbiological and Physico-chemical assessment of the quality of domestic water sources in selected rural communities of the Eastern Cape Province, South Africa. *Water, SA*; **30(3)**: 333– 340.

CHAPTER 4

TABLE OF CONTENTS	Page №
Table of contents.....	239
List of Tables.....	241
List of Figures	242
Abstract.....	243
4.1 Introduction.....	244
4.2 Materials and Methods.....	246
4.2.1 Study areas and sample collection.....	246
4.2.2 Culture-based enumeration, recovery and identification of <i>E. coli</i>	
O157:H7.....	247
4.2.2.1 <i>Enumeration of presumptive E. coli O157.....</i>	247
4.2.2.2 <i>Enrichment and IMS recovery of E. coli O157:H7.....</i>	247
4.2.2.3 <i>Identification of E. coli O157:H7.....</i>	248
4.2.3 Molecular characterization of <i>E. coli</i> O157:H7 using Polymerase	
Chain Reaction (PCR).....	249
4.2.3.1 <i>Bacterial DNA extraction.....</i>	249
4.2.3.2 <i>Amplification of fliC_{H7}, rfbE_{O157} and eaeA genes.....</i>	250
4.2.3.3 <i>DNA electrophoresis.....</i>	251
4.2.4 Anti-microbial susceptibility testing.....	251
4.2.5 Statistical analysis.....	252

4.3	Results.....	252
4.3.1	Counts of presumptive <i>E. coli</i> O157 for meat and meat products.....	252
4.3.2	Prevalence of <i>E. coli</i> O157 in the meat and meat products.....	253
4.3.3	Molecular analysis for <i>E. coli</i> O157:H7.....	254
4.3.4	Statistical analysis of results.....	256
4.3.5	Anti-microbial susceptibility.....	257
4.4	Discussion and Conclusion.....	258
	References.....	263

LIST OF TABLES

Table 4.1:	Summarized PCR amplified genes of <i>E. coli</i> O157:H7 isolated from meat and meat products samples obtained from Amathole district.....	256
Table 4.2:	Distribution of susceptibility of <i>E. coli</i> O157:H7 isolated from meat and meat products samples.....	258

LIST OF FIGURES

Figure 4.1:	Counts of presumptive <i>E. coli</i> O157 in meat and meat products purchased from supermarkets, shops and butcheries in Amathole district.....	253
Figure 4.2:	Percentage prevalence of <i>E. coli</i> O157 isolated from meat and meat products purchased from supermarkets, shops and butcheries in the Amathole district.....	254
Figure 4.3:	The amplified <i>fliC_{H7}</i> , <i>rfbE_{O157}</i> and <i>rfbE_{O157}</i> genes of <i>E. coli</i> O157:H7 isolated from meat and meat products.....	255

CHAPTER 4

Abstract

In the Amathole District Municipality of the Eastern Cape Province of South Africa, a large number of households consume meat and meat products daily. To indicate whether the meat and meat products sold in this district might be associated with *E. coli* O157:H7, we investigated the prevalence and molecular characteristics of this bacterium from 180 samples of meat products (45 samples each of biltong, cold meat, mincemeat and polony). Also investigated were the antibiogram profiles of this bacterium isolated from these foods. Strains of *E. coli* O157 were isolated by enrichment culture in phosphate-buffered saline followed by immunomagnetic separation and culture of magnetic beads onto Cefixime Tellurite Sorbitol MacConkey Agar. Strains of *E. coli* O157 were characterized by Polymerase Chain Reaction (PCR) using primers specific for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes. The mean counts of presumptive *E. coli* O157 for the meat and meat products ranged between 4.00×10^3 CFU/g and 5.04×10^6 CFU/g. Immunomagnetic separations revealed prevalence of presumptive *E. coli* O157 at average rates of 22.22% (10/45) for biltong, 8.88% (4/45) for cold meat, 53.33% (24/45) for mincemeat and 57.77% (26/45) for polony. Molecular analysis of 13 representative presumptive *E. coli* O157 from these meat and meat products revealed that 7.81% (5/64) of the meat and meat products had *E. coli* O157:H7. Over 80% of the *E. coli* O157:H7 isolates were resistant to gentamycin and erythromycin. To prevent *E. coli* O157: H7 infections and protect the health of the community, these meat and meat products should be properly handled and well cooked (in the case of mincemeat) before consumption.

Keywords: Prevalence, *Escherichia coli* O157:H7, meat and meat products, antimicrobials

4.1 Introduction

Escherichia coli serotype O157:H7 is a worldwide human pathogen associated with meat and meat products among other food items. These bacteria may cause infections resulting in haemolytic colitis (HC) and haemolytic uremic syndrome (HUS) (Browning *et al.*, 1990; Olorunshola *et al.*, 2000; Galane and Le Roux, 2001; Magwira *et al.*, 2005). Diarrhoeal outbreaks linked to *E. coli* O157:H7 infections are characterized by blood, cramping abdominal pain, fever, nausea, and vomiting (Calundungo *et al.*, 1994; Germani *et al.*, 1997; Yoh *et al.*, 1996; Olorunshola *et al.*, 2000; Koyange *et al.*, 2004). African people living in countries such as Central African Republic, Democratic Republic of Congo, Angola, Kenya, Nigeria, Swaziland and Cameroon, Côte d'Ivoire, Malawi and others have suffered from cases of diarrhoea linked to *E. coli* O157:H7 (Germani *et al.*, 1997,1998; Paquet *et al.*,1993; Isaacson *et al.*,1993). Most of the *E. coli* O157:H7 cases that have been reported in the continent have been traced and found to have a food or water link with meat and meat products being the most implicated foods (Browning *et al.*, 1990, Galane and Le Roux, 2001; Magwira *et al.*, 2005). The first case of *E. coli* O157:H7 infection in South Africa was in 1990 (Browning *et al.*, 1990). Since then, numerous sporadic cases of bloody diarrhoeal outbreaks have been reported in many parts of South Africa (Effler *et al.*, 1992, Galane and Le Roux, 2001). Some astonishing research have been done on water distribution systems in the rural parts of South Africa and have revealed worrying levels of water contamination by *E. coli* O157:H7 (Muller *et al.*, 2001; 2003).

Approximately 56% of goat meat in South Africa is sourced from the Eastern Cape and the province produces 12 000 tons of beef, 8000 tons of mutton and 76 000 tons of pork every year (SAG, 2000). This gives a brief overview of the availability of meat and meat products in the Eastern Cape Province. The co-existence of bovine and human requires sanitary and hygienic conditions from the farm to the table in order to enjoy the products obtained from these animals. Due to poverty in most African countries, and in the Eastern Cape Province of South Africa in particular, people continue with unsafe hygienic practices, such as unsafe human excreta disposal, unsafe solid and liquid waste disposal and unsafe drinking water (Phaswana-Mafuya and Shukla, 2005). There are also cultural practices that encourage communal eating in the Eastern Cape for example during funerals, weddings, political rallies and circumcision ceremonies. These occasions are good venues for contracting food-borne disease illnesses. Even though there haven't been any reported cases of food-borne disease outbreaks in the Eastern Cape, it could be because of lack of surveillance and poor reporting. The aforementioned unsafe practices are possible breeding avenues for pathogenic microorganisms such as *E. coli* O157:H7. The organisms contaminate meat and meat products and may finally infect humans. Infections commonly associated with consumption of contaminated meat and meat products can easily spread in the entire community and have negative impact on public health, mostly on people with reduced immune systems such as HIV/AIDS patients.

One of the major problems facing the human race today is that many of the disease-causing bacteria have become resistant to the effects of different antibiotics. The reason for the cause of this problem is indiscriminate use of antibiotics (Wong *et al.*, 2000; Okoli *et al.*, 2005). As a result, it was of great importance to assess the quality of some foods of bovine origin consumed in the Amathole District and determine the prevalence and molecular characteristics and

antibiogram profile of *E. coli* O157:H7 isolated from selected meat and meat products often consumed in the Province.

4.2 Materials and Methods

4.2.1 Study areas and sample collection

One hundred and eighty (180) samples of meat and meat products (45 samples of biltong, cold meat, mincemeat, and polony) were used in the study. The meat and meat products were purchased from butcheries, shops, supermarkets, and open air markets serving communities of Alice, Fort Beaufort, and Mdantsane from March 2005 to August 2006. Biltong is a South African product typically made from raw fillets of meat cut into strips following the grain of the muscle and dried. It is similar to beef jerky in that both are spiced dried meats but they differ significantly in typical ingredients, taste, and production process. The word *biltong* is from the Dutch word “*bil*” meaning rump and “*tong*” meaning strip or tongue (Stephanie, 2006). The samples were placed in sterile polyvinyl bags, put in a cooler box with ice blocks, and transported to the laboratory for analysis. *Escherichia coli* O157 enumeration and isolation was conducted within 24 h of sample collection.

4.2.2 Culture-based enumeration, recovery and identification of *E. coli* O157:H7

4.2.2.1 Enumeration of presumptive E. coli O157

Presumptive *E. coli* O157 counts in the meat and meat products were determined by direct plating (Cagney *et al.*, 2004). Ten grams (10 g), of biltong, cold meat, mincemeat or polony was homogenized separately by blending for 1 min in 90 ml of Phosphate-Buffered Saline (PBS) (Merck, SA). Aliquots of 1.0 ml of the undiluted homogenate of a respective meat and meat product were added to 9 ml of 1% (w/v) PBS solution (Merck, SA). Serial dilutions were made and 1 ml of the various dilutions was pour plated in duplicates onto Sorbitol MacConkey Agar (Merck, SA). All plates were incubated at 37°C for 24 h. Average presumptive *E. coli* O157 counts were estimated and expressed as CFU/g of every meat and meat product sample.

4.2.2.2 Enrichment and IMS recovery of E. coli O157:H7

For enrichment, 10 g of a meat and meat product sample was added to 90 ml of modified EC (mEC) broth containing 20 µg/ml novobiocin (n) (Merck, SA) (Cagney *et al.*, 2004). The samples were then incubated for 8 h at 37°C on a rotary shaker at $143 \times g$ (Gallenkamp, Loughborough, England). Twenty microlitres (20 µl) of the Dynabead (Dyna, Oslo) suspensions were incubated in a 1.5 ml Eppendorf tubes (Eppendorf, SA) with 1 ml aliquots of the pre-enriched samples at room temperature for 10 min with continuous mixing. This step was performed to allow the *E. coli* O157-specific antibodies coated onto the beads to bind to the target bacteria (*E. coli* O157).

The bacterial-IMS bead complexes were separated using a magnetic particle concentrator, Dynal MPC-M (Dyna, Oslo) for 3 min (Dyna product brochure, 2006). After aspirating the supernatants and washing the bacterial-IMS bead complexes using 0.02% (v/v) diluted (1:20)

PBS-Tween 20 in distilled water (pH 7.2) (Merck, SA), the complete immunomagnetic separation and washing procedure were repeated twice (DynaL product brochure, 2006). The final bacterial-IMS bead complexes were re-suspended in 1000 µl washing buffer (PBS-Tween) (Merck, SA). Fifty microlitres of the bacterial-IMS complex concentrate was transferred to *E. coli* O157 selective media, Cefixime Tellurite Sorbitol MaConkey Agar (cefixime [0.05 mg/l] and potassium tellurite [2.5 mg/l]) (CT-SMAC) (Merck, SA) and spread out into half of the plate using sterile cotton swab (Cagney *et al.*, 2004). The swabbed portion using an inoculating loop was further streaked out onto the agar surface in order to achieve single isolated colonies (DynaL product brochure, 2006).

The plates were then incubated at 37°C for 24 h (Cagney *et al.*, 2004). Five colourless, smooth, circular and entire edged colonies with brown centres were selected and sub-cultured onto Nutrient Agar (NA) (Merck, SA). The colonies were further plated onto Eosin Methylene Blue (EMB) Agar (Merck, SA) and colonies with characteristic greenish blue-black metallic sheen colour on EMB Agar (Merck, SA), were subjected to confirmatory test using conventional biochemical tests (Cagney *et al.*, 2004).

4.2.2.3 Identification of *E. coli* O157:H7

Typical colourless, smooth, circular and entire edge colonies with brown centres on Cefixime-Tellurite Sorbitol MacConkey (CT-SMAC) Agar (Merck, SA) were picked using an inoculating loop and streaked directly onto Eosin Methylene Blue (EMB) Agar (Merck, SA) (Müller *et al.*, 2003; Cagney *et al.*, 2004), and incubated overnight at 37 °C. Colonies displaying metallic greenish blue sheen colour exhibiting typical characteristics of *E. coli* O157 species were transferred onto Nutrient Agar (NA) plates, (Merck, SA) and incubated overnight. The pure

isolates were then gram stained and subjected to biochemical characterization. Biochemical characterisation was based on conventional indole-methyl red-Voges-Proskauer-citrate (IMViC) tests procedures (Heuvelink *et al.*, 1998; Radu *et al.*, 1998; Feng, 2000; Müller *et al.*, 2003). Further biochemical characterizations using API 20E kit was performed following the manufactures instructions (BioMérieux, Marcy-Etoile, France). The strips were read and final identification was secured using API LAB PLUS computer software (BioMérieux, Marcy-Etoile, France) (Momba *et al.*, 2006a). Thirteen (13) presumptive *E. coli* O157 (representing the 64 meat and meat products), which had 99.9% profile for *E. coli* with API PLUS computer software program were used for molecular characterization.

4.2.3 Molecular characterization of *E. coli* O157:H7 using Polymerase Chain Reaction (PCR)

4.2.3.1 Bacterial DNA Extraction

DNA was extracted from colonies identified as *E. coli* and from a positive control strain (*E. coli* O157:H7, ATCC 43895) purchased from the Microbiology Department of the National Health Laboratory Services (NHLS), (Johannesburg, SA). DNA extraction was performed according to the method previously described by Torres *et al.* (2003).

Briefly, a loop-full of overnight culture of *E. coli* colony was suspended in 200 µl of sterile Milli-Q PCR grade water (Merck, SA) and the cells were lysed using a Dri-block DB.2A (Techne, SA) for 15 min at 100°C. The cell debris was removed by centrifugation at $20\,000 \times g$ for 2 min using a MiniSpin micro centrifuge (Merck, SA). The lysate supernatant was placed on ice for 5 min. Sterile Milli-Q PCR grade water (Merck, SA) was included in each PCR assay as a negative control.

4.2.3.2 Amplification of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes

Oligonucleotide primers specific for the targeted *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes used in the polymerase chain reaction (PCR) were similar to those used by Wang *et al.* (2002). The primers sequences that were used to identify the target genes were FliC-F 5'-TAC CAT CGC AAA AGC AAC TCC-3', FliC-R 5'-GTC GGC AAC GTT AGT GAT ACC-3' for *fliC_{H7}*. For *rfbE_{O157}*, the forward was RfbE-F 5'-CTA CAG GTG AAG GTG GAA TGG-3'; RfbE-R 5'-AATT CCT CTC TTT CCT CTG CGG-3'. The primers for *eaeA* gene were EAE-R 5'-ATG CTT AGT GCT GGT TTA GG-3', EAE-R 5'-GCC TTC ATC ATT TCG CTT TC-3'. The expected amplification sizes for *fliC_{H7}*, *rfbE_{O157}*, and *eaeA* genes were 247, 328, and 248 base pairs, respectively. Three sets of primer mixtures were prepared according to the method used by Wang *et al.* (2002). A total volume of 10 µl genomic DNA was used in each PCR reaction. The PCR assays for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes were carried out in a 50 µl reaction volume. The reaction mixture contained 10X SuperTherm GOLD Buffer, 1.5 mM MgCl₂, each of the four deoxynucleoside triphosphates (dNTPs) (Southern Cross Biotechnology, Cape Town, SA) at a concentration of 0.25 mM, 100 pmol for each of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* specific primers, and 5 U of *Taq* DNA polymerase (Southern Cross Biotechnology, SA). The PCR reaction was carried out in the Eppendorf model AG 22331 Thermocycler (Merck, SA). The following PCR conditions for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes optimized in our laboratory were similar to those previously used by Wang *et al.* (2002). Initial denaturation at 95°C for 8 min followed by 30 cycles of amplification, denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. The final extension cycle was followed by incubation at 72 °C for 7 min and cooling to 4°C.

4.2.3.3 *DNA Electrophoresis*

The PCR products (10 µl aliquots) were visualized on a 2% (w/v) agarose gel (Merck, SA) in 1 U TAE buffer (40 mM Tris-HCl, 20 mM Na-acetate, 1 mM EDTA, pH 8.5) and stained with 0.5 µg/ml Ethidium Bromide (EtBr) (Merck, SA). A 100-bp DNA ladder (Promega, USA) was included on each gel as a molecular size standard. The electrophoresis was carried out at 76 V for 1 h. The products were photographed under the BioDoc-It System (UVP Upland, CA 91786, USA).

4.2.4 **Antimicrobial susceptibility testing**

The Antimicrobial susceptibility test for *E. coli* O157:H7 isolates was determined using Bauer and Kirby disk diffusion technique on Mueller-Hinton Agar (MHA) (Merck, SA) (Jorgensen *et al.*, 1999). The following antibiotic discs were used: ampicillin (10 µg), ceftriaxone (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), and amikacin 30 µg) and erythromycin (15 µg) (Davies Diagnostics, SA). For the inocula development the bacterial isolates were grown overnight on nutrient broth at 37 °C for reactivation.

The cells were harvested by centrifugation at $2200 \times g$ using a Beckman model TJ - 6 centrifuge (Great Britain) and washed twice with 0.85% (w/v) saline water (NaCl) (Merck, SA). The bacterial cells were re-suspended in sterile saline solution to a final density of 10^7 CFU/ml. The cell suspensions were then swabbed onto a freshly prepared Mueller-Hinton Agar (MHA) in order to achieve a lawn of growth on incubation. The antibiotic discs were placed on the surface of the inoculated Mueller-Hinton Agar (MHA) using a disc dispenser and the plates were incubated for 18 h at 37 °C (Jorgensen *et al.*, 1999). Zones of growth inhibition were measured

and results interpreted according to the guidelines of National Committee for Clinical Laboratory Standards for antimicrobial susceptibility testing (NCCLS, 1999). *Escherichia coli* ATCC 43895 was included as a positive control.

4.2.5 Statistical analysis

The statistical analysis was done using Statistical Analysis System (SAS) (SAS Institute, Cary, USA). Test of significance was run at a 0.05 level of significance.

4.3 Results

4.3.1 Counts of presumptive *E. coli* O157 for meat and meat products

The mean counts of presumptive *E. coli* O157 of all the meat and meat products was found to be higher in Fort Beaufort polony (5.04×10^6 CFU/g), whereas Alice and Mdantsane mincemeat had 2.58×10^6 and 1.95×10^5 CFU/g, respectively. Generally, meat and meat products from Mdantsane had the least presumptive *E. coli* O157 count.

The mean counts for presumptive *E. coli* O157 for meat and meat products from individual locations ranged from 1.77×10^4 CFU/g to 5.04×10^6 CFU/g for biltong and polony respectively, from Fort Beaufort, 4.00×10^3 CFU/g for cold meat to 2.58×10^6 CFU/g for mincemeat from Alice. For Mdantsane meat and meat products, the counts ranged from 0 CFU/g for cold meat and 1.95×10^5 CFU/g for mincemeat. Counts of presumptive *E. coli* O157 were found to be significantly different ($P = 0.0039$). Presumptive *E. coli* O157 counts are represented in Figure 4.1 below.

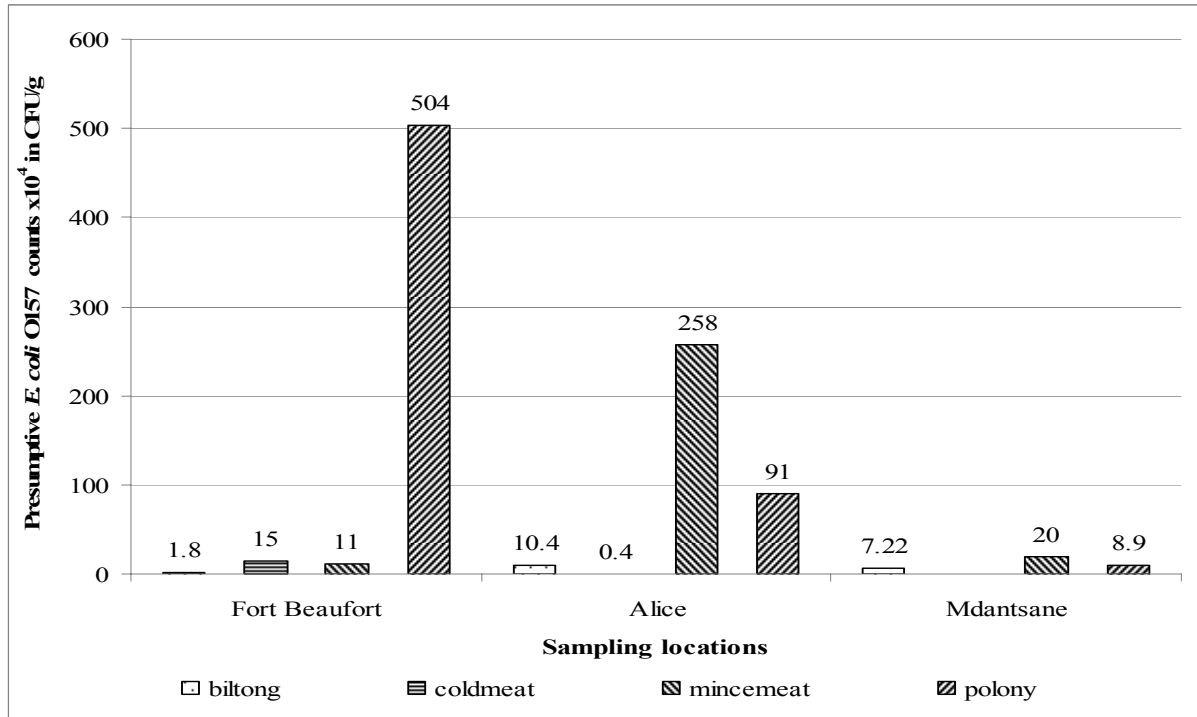


Figure 4.1: Counts of presumptive *E. coli* O157 in meat and meat products purchased from supermarkets, shops and butcheries in the Amathole District.

4.3.2 Prevalence of *E. coli* O157 in meat and meat products

The prevalence of *E. coli* O157 has been disaggregated by region and by meat and meat product type. The results indicated that the rate of prevalence of presumptive *E. coli* O157 for meat and meat products from Fort Beaufort was 20%, 26.66%, 46.66% and 60% for cold meat, biltong, mincemeat and polony, respectively. Cold meat, biltong, polony and mincemeat from Alice carried presumptive *E. coli* O157 at a prevalence rate of 6.6%, 26.66%, 53% and 73.33%, respectively. Whereas the meat and meat products from Mdantsane were found to be presumptively positive of *E. coli* O157 with biltong, mincemeat and polony having presumptive *E. coli* O157 in 13.33%, 53% and 60%, respectively (Figure 4.2).

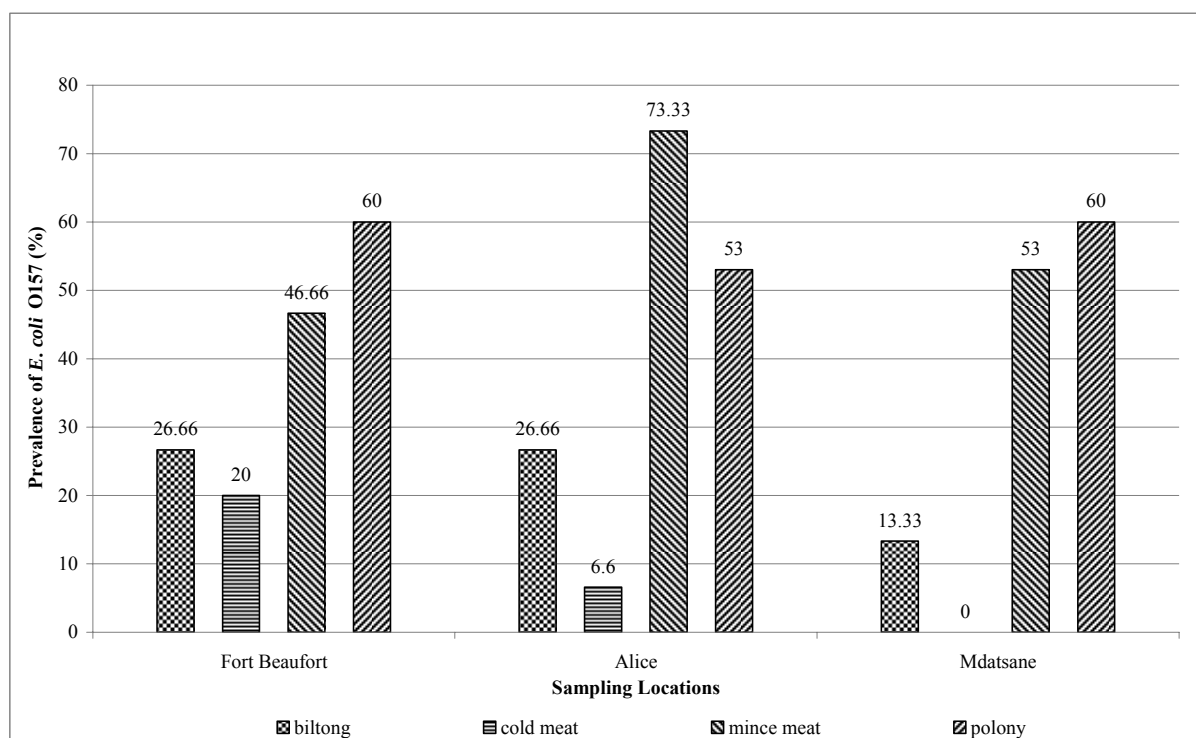


Figure 4.2: Percentage prevalence of presumptive *E. coli* O157 isolated from meat and meat products purchased from supermarkets, shops and butcheries in the Amathole District.

4.3.3 Molecular analysis for *E. coli* O157:H7

Four out of the 64 meat and meat products presumptively positive of *E. coli* O157, were found to carry *E. coli* O157:H7 that were positive for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes. These were meat and meat products such as slices of polony, minced meat, cold meat and biltong. Mincemeat and cold meat samples from Mdantsane were positive for *E. coli* O157:H7. One biltong sample from Alice and two polony samples (one from Fort Beaufort and another from Mdantsane) were also positive for *E. coli* O157:H7. Gel electrophoresis profiles of the amplified products of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes for these *E. coli* O157:H7 isolated from the meat and meat products are illustrated in Figure 4.3.

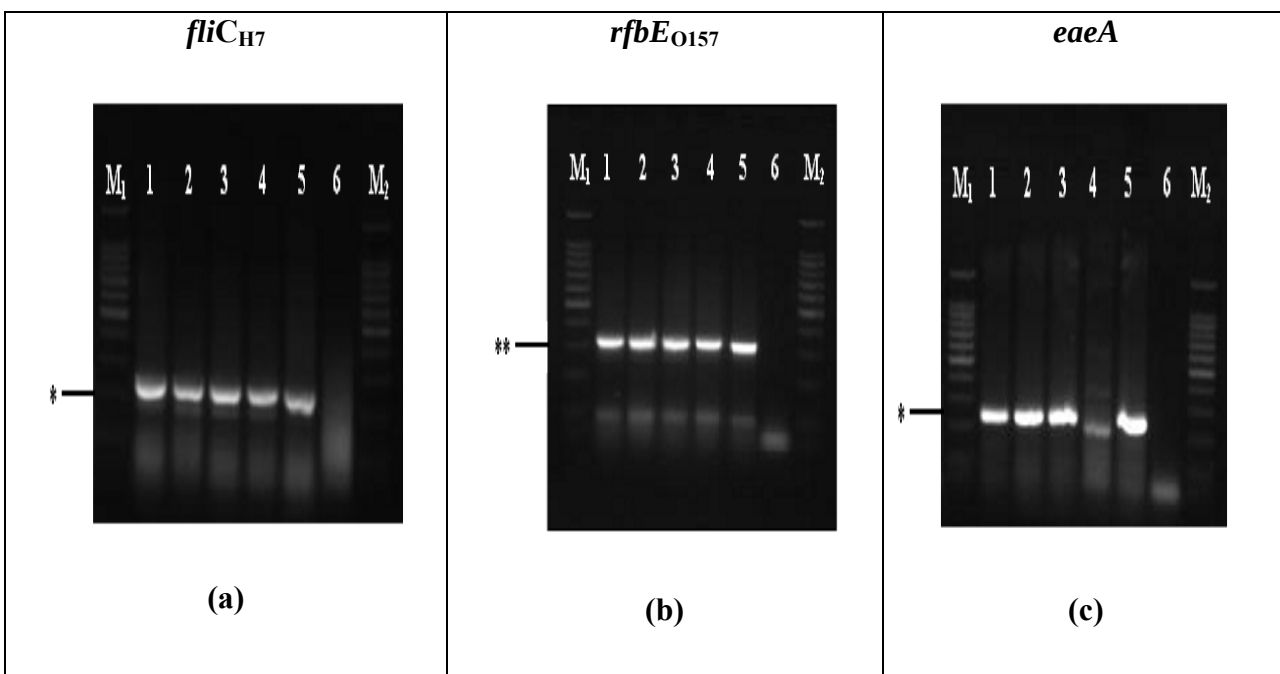


Figure 4.3: The amplified *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes of *E. coli* O157:H7 isolated from meat and meat products. Lanes M₁ & M₂: 100 bp DNA ladder marker (Promega, USA), lane 1: Positive control (*E. coli* O157:H7, ATCC 43895), lane 2: MdaM₁, lane 3: MdaM₂₃, lane 4: MdaM₃₄, lane 5: AlM₁₈, lane 6: FBM₂₈, lane 7: Negative control. The expected molecular sizes of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* fragments were at 247bp, 327bp and 248bp respectively.

Table 4.1: PCR amplified genes of *E. coli* O157:H7 isolated from the meat and meat products

Meat Product	Location	Amplified genes			№ positive with <i>E. coli</i> O157:H7
		<i>fliC_{H7}</i>	<i>rfbE_{O157}</i>	<i>eaeA</i>	
Biltong	Alice	+	+	+	1
Cold meat	Mdantsane	+	+	+	1
Mincemeat	Mdantsane	+	+	+	1
Polony	Mdantsane	+	+	+	1
Polony	Fort Beaufort	+	+	+	1
Total number of meat and meat products positive with <i>E. coli</i> O157:H7					5

n = 13, Representative number of *E. coli* O157 analyzed by PCR, (+) gene present, (–) gene absent.

4.3.4 Statistical analysis of results

Test of significance was run at a 0.05 level of significance on the average counts of presumptive *E. coli* O157. The average counts of the presumptive *E. coli* O157 of meat and meat products from Fort Beaufort were significantly different from those of meat and meat products purchased from Mdantsane (P = 0.0162). Likewise the average counts of presumptive *E. coli* O157 of meat and meat products from Fort Beaufort were significantly different from the average counts of presumptive *E. coli* O157 of meat and meat products from Alice (P = 0.0039).

4.3.5 Anti-microbial susceptibility

All *E. coli* O157:H7 isolates of meat and meat products were susceptible to amikacin. However, there was 100% resistance to erythromycin by the *E. coli* O157:H7 isolates. Resistance to gentamicin, ampicillin, nalidixic acid and tetracycline was found in 80%, 40%, 20% and 10% of the strains. Approximately 80% and 40% of the isolates were susceptible to tetracycline and chloramphenicol respectively with 20% of the isolates being susceptible to gentamicin, ampicillin and ceftriaxone. An intermediate susceptibility was observed in 80% of the isolates to ceftriaxone and nalidixic acid. Approximately 60% of the isolates had intermediate susceptibility to chloramphenicol, 40% to ampicillin and 10% to tetracycline (Table 4.2).

Table 4.2: Antimicrobial susceptibility of *E. coli* O157:H7 isolated from meat and meat products.

Antibiotic agent	Antibiotic disc content (µg)	Distribution %		
		R	I	S
Gentamycin	10	80	0	20
Ampicillin	10	40	40	20
Chloramphenicol	30	0	60	40
Nalidixic acid	30	20	80	0
Amikacin	30	0	0	100
Ceftriaxone	30	0	80	20
Erythromycin	15	100	0	0
Tetracycline	30	10	10	80

^aR, Resistant, I, Intermediate, S, Susceptible. ^bThe interpretation to achieve the above susceptibility percentages was based on the breakpoints of zone diameters for individual antibiotic agents as outlined in NCCLS ninth informational supplement of 1999: document M100–S9.

4.4 Discussion and Conclusion

The study has clearly shown that *E. coli* O157:H7 is present in meat and meat products (biltong, cold meat, mincemeat and polony) sold in Alice, Fort Beaufort, and Mdantsane at levels that are

unacceptable and potentially hazardous to public health. Considering individual counts, it is evidenced that polony had high counts of presumptive *E. coli* O157, followed by mincemeat whereas biltong and cold meat had the least counts of presumptive *E. coli* O157 (Figure 4.1). Polony from Fort Beaufort had the highest counts while on the other hand mincemeat from Alice was the second meat product with high counts. It was interesting to note that cold meat from Mdantsane did not carry any presumptive *E. coli* O157.

The prevalence of *E. coli* O157 also followed a similar trend. The prevalence was based on the number of meat and meat products that were presumptively positive for *E. coli* O157 relative to the total number of meat and meat products analyzed from a particular location. On average, mincemeat and polony from all the three locations had higher prevalence of presumptive *E. coli* O157 than all the other meat and meat products. The results indicate that 57.66% of all the mincemeat and polony analysed had presumptive *E. coli* O157 as opposed to 22.2% and 13.3% of biltong and cold meat, respectively.

The PCR results revealed that only 7.81% (5/64) were positive for *E. coli* O157:H7. These were the meat and meat products that had *E. coli* O157 isolates, which carried *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes. Two packs of sliced polony had *E. coli* O157:H7. These sliced polony were from Mdantsane and Fort Beaufort. Biltong purchased from Alice and cold meat and mincemeat purchased from Mdantsane contained *E. coli* O157:H7 species that were positive for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes (Figures 4.3 and Table 4.1).

The PCR analysis gave consistent results with previously published findings (Agin and Wolf, 1997; Wang *et al.*, 2002; Cagney *et al.*, 2004). The primers used in the study allowed for amplification of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes. The presence of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes in *E. coli* O157:H7 isolates from either environmental, clinical or food samples have as well been

reported in previous research works similar to this one (Mora *et al.*, 2007; Cagney *et al.*, 2004). The PCR results of the current study confirm the results of Cagney *et al.* (2004) in Ireland in which 95.35% (41/43) of *E. coli* O157 recovered from minced beef and beef burgers were positive for *eaeA*, *rfbE* and *fliC_{H7}* genes. Mora *et al.* (2007), while investigating by use of *rfbE*, *fliC_{H7}*, *eae* and *ehxA* *E. coli* O157:H7 specific genes proficiently characterized 35 *E. coli* O157:H7 that were isolated from ground beef (22.54%) and beef meat (14.71%).

A previous study carried out in South Africa found much higher levels (74.5%) of *E. coli* O157:H7 in meat and meat products (Vorster *et al.*, 1994). Although the study by Vorster *et al.* (1994) took samples from the Pretoria region of South Africa, it is unlikely that the difference observed in these two studies could be accounted for by geographical separation given the national nature of beef supply chain in South Africa.

The results of the current study confirm the most recent findings of similar research in other countries. In Botswana, Magwira *et al.* (2005) isolated *E. coli* O157:H7 in 5.22% of chunk meat, 3.76% of mincemeat and 2.26% of sausages. In France, Vernozy-Rozand *et al.* (2002) isolated *E. coli* O157:H7 in 0.12% of industrial minced meat. Similar studies in United Kingdom, Switzerland and Argentina have isolated *E. coli* O157:H7 from 1.1% of raw beef, 2.3% of mincemeat and 3.8% of retail meat, respectively (Chapman *et al.*, 2000; Chinen *et al.*, 2001; Fanteli and Stephan, 2001).

The probable reason for high *E. coli* O157 counts and even prevalence in polony could have been because most shop and butchery attendants slice polony and other food products using the same slicer without cleaning between slicing jobs. This practice carries the potential of cross-contamination of meat and meat products (Flores and Stewart, 2004).

Minced meat was the second meat and meat product with high counts of presumptive *E. coli* O157. Mincemeat has been identified as a source of *E. coli* O157:H7 in previous studies (Fantelli and Stephan, 2001; Vernozy-Rozand *et al.*, 2002; Magwira *et al.*, 2005). The most probable reason for high numbers of *E. coli* O157:H7 and even other bacteria in minced meat could be due to the spread of these bacteria during the mincing process and from cross contamination from the mincing blades (Flores and Stewart, 2004).

Escherichia coli O157:H7 may also be present in raw beef used in the manufacturing of other meat products, such as mincemeat. However, meat products like biltong and cold meat undergo a treatment process in which high levels of concentrated humectants are used that help lower the water activity (a_w) in them and for this reason growth of bacteria may not be encouraged. This explains the low counts of *E. coli* O157 in the biltong and cold meat samples as shown by the results.

Recent studies have revealed a trend towards increased antibiotic resistance by *E. coli* O157: H7 (Amornrut *et al.*, 2000; Magwira *et al.*, 2005). In the present study, *E. coli* O157:H7 isolates were resistant to 5 out of 8 antibiotics. The resistance profile for the *E. coli* O157:H7 isolates from the meat and meat products were in the order of erythromycin 100% (5/5), gentamicin 80% (4/5), ampicillin 40% (2/5), nalidixic acid, ceftriaxone and tetracycline at 20% (1/5). It was observed that a single *E. coli* O157:H7 that was isolated from a polony purchased from Fort Beaufort had the highest rate of resistance. It was resistant to five antimicrobial agents (gentamycin, ampicillin, nalidixic acid, erythromycin, and tetracycline) out of the eight antibiotics. The resistance trends observed amongst the *E. coli* O157:H7 indicated the variability in response to antimicrobial agents. This variability was equally noted to vary depending on the origin and source of the *E. coli* O157:H7 isolates.

Sein *et al.* (2005) also noted difference in antimicrobial resistance by organism depending on the geographical location. This trend can be useful in highlighting the degree of antibiotic use in animals since microorganism isolated from products of such animals may show increased antimicrobial resistance. Based on such an argument it would be correct to say that farmers from Fort Beaufort use antibiotics in rearing their beef animals unlike farmers from Mdantsane and Alice. However, such an argument would only be justifiable if the animals slaughtered and supplied to butcheries in Fort Beaufort, Mdantsane or Alice were actually reared by farmers from these areas.

Biodiversity of *E. coli* O157:H7 could be a contributing factor in the acquisition of genes that make them resistant to antibiotics. Enteric pathogens like *E. coli* O157:H7 have been widely reported to demonstrate resistance to several antibiotics (Magwira *et al.*, 2005). For example, in 2005, about 35% of *E. coli* O157:H7 strains isolated from meat and meat products in Gaborone, Botswana were resistant to cephalothin, sulphatriad, colistin sulphate and tetracycline (Magwira *et al.*, 2005). Antibiotic resistance may occur either spontaneously by selective pressure or because of antibiotic miss-use by humans or over-use by farmers on their animals (Schroeder *et al.*, 2002). Although antibiotic resistance is common, antibiotics are still indicated in the management of life threatening diseases like diarrhoea. However, the use of antibiotics in the management of *E. coli* O157:H7 infections in humans are still controversial due to the possible development of HUS (Wong *et al.*, 2000).

Noteworthy is the fact that the meat and meat products (biltong, polony, cold meat) a part from mincemeat used in this study, were ready to eat products and the presence of *E. coli* O157:H7 in such foods is a wake-up call of potential infection source. However, cooking meat products like mince meat to high enough temperature would inactivate *E. coli* O157:H7 cells.

References

- Agin, T.S. and M. K. Wolf.** 1997. Identification of a family of intimins common to *Escherichia coli* causing attaching-effacing lesions in rabbits, humans, and swine. *Infect. Imm.*; **65**: 320–326.
- Amornrut, L., P. Manthana, E. Boonchuay, M. Sathaporn, T. Samruay, S. Sontana, N. Kenichi, I. Tetsuya, N. Chaisit, and K. Podjanee.** 2000. Shiga toxin and enterotoxin-producing *Escherichia coli* isolated from subjects with bloody and nonbloody diarrhea in Bangkok, Thailand. *Diag. Microbiol. and Infect. Dis.*; **46(3)**: 173–180.
- Browning, N. G., J. Botha, H. Sacho, and P. J. Moore.** 1990. *Escherichia coli* O157:H7 haemorrhagic colitis. Report of the first South African case. *SA Surg. J.*; **28**: 28–29.
- Cagney, C., H. Crowley, G. Duffy, J-J. Sheridan, S. O'Brien, E. Carney, W. M. Anderson, D. A. Dowell, I. S. Blair, and R. H. Bisho.** 2004. Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. *J. Food Microbiol.*; **21**: 203–212.
- Calundungo, R., G. Maddau, P. Rappelli, P. Cappuccinelli, F. Leite, M. Francisco, and M. Colombo.** 1994. *Escherichia coli* and enteritis in Angola. *Lancet*; **344**: 538 – 539.
- Chapman, P. A.** 2000. Sources of *Escherichia coli* O157 and experiences over the past 15 years in Sheffield, UK. *J. Appl. Microbiol.*; **88**: 51S–60S.
- Cheville, A. M., K. W. Arnold, C. Buchrieser, C.-M. Cheng, and C. W. Kaspar.** 1996. *rpoS* regulation of acid, heat and salt tolerance in *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.*; **62**: 1822–1824.
- Chinen, I., J. D. Tanoro, E. Miliwebsky, L. H. Lound, G. Chillemi, S. Ledri, A. Baschkier, M. Scarpin, E. Manfredi, and M. Rivas.** 2001. Isolation and

- characterisation of *Escherichia coli* O157:H7 from retail meats in Argentina. *J. Food Prot.*; **64**: 1346–1351.
- Dynal Product Brochure.** 2006. *Dynabeads® anti-E coli*. For rapid selective enrichment of *Escherichia coli* O157:H7. Dynal A.S. Oslo, Norway.
- Effler, E., M. Isaacson, L. Arntzen, R. Heenan, P. Canter, and T. Barrett.** 2001. Factors contributing to the emergence of *Escherichia coli* O157:H7 in Africa. *Emerg. Infect. Dis.* **7**: 812–819.
- Fantelli, K. and R. Stephan.** 2001. Prevalence and characteristics of shigatoxin-producing *Escherichia coli* and *Listeria monocytogenes* strains isolated from minced meat in Switzerland. *Int. J. Food Microbiol.* **70**: 63–69.
- Feng, P.** 2000. *Escherichia coli* serotype O157:H7: novel vehicles of infection and emergence of phenotypic variants. *Emerg. Infect. Dis.* **1**: 9.
- Flores R. A. and T. E. Stewart.** 2004. Empirical Distribution Models for *Escherichia coli* O157:H7 in Ground Beef Produced by a Mid-size Commercial Grinder. *J. food Sci.* **69(5)**: M121–M126.
- Galane, P. M and M. Le Roux.** 2001. Molecular Epidemiology of *Escherichia coli* isolated from young South Africa children with diarrheal diseases. *J. Health. Pop. Nutr.* **19(1)**: 311–318.
- Germani, Y., B. Soro, M. Vohito, O. Morel, and J. Morvan.** 1997. Enterohaemorrhagic *Escherichia coli* in Central African Republic. *Lancet*; **349**: 1670.
- Germani, Y., Cunin, P., Tedjouka, E., Bou-Neharre, C., Morvan, J. and P. Martin.** 1998. Enterohaemorrhagic *Escherichia coli* in Ngoila (Cameroon) during an outbreak of bloody diarrhoea. *Lancet*; **352**: 625–626.

Heuvelink, A. E., F. I. A. M Van Den Biggelaar, J. T. M. Zwartkruis-nahuis, R. G.

Herbes, R. Huyben, N. Nagelkerkr, W. J. G. Melchers, L. A. H. Monnens, and E.

De Boer. 1998. Occurrence of verocytotoxin producing *Escherichia coli* O157:H7 on Dutch dairy farms. *J. Clin. Microbiol.*; **36(12)**: 3480–3487.

Isaacson, M., P. H. Canter, P. E. Effler, L. Arntzen, P. Bomans, and R. Heenon. 1993. Haemorrhagic colitis epidemic in Africa. *Lancet*; **341**: 961.

Jorgensen, J. H., J. D. Turnidge, and J. A. Washington. 1999. *Antibacterial susceptibility tests: dilution and disk diffusion methods*. In: Murray, P. R., Tenover, F. C., Baron, E. J. and Tenover, F. C., Baron, E. J. and Tenover, F. C. ed. *Manual of clinical microbiology*, 7th ed. Washington, DC: American Society for Microbiology Press; pg. 1526–1543.

Koyange, L., G. Ollivier, J. J. Muyembe, B. Kebela, M. Gouali, and Y. Germani. 2004. Enterohemorrhagic *Escherichia coli* O157, Kinshasa. *Emerg. Infect. Dis.* Available from: <http://www.cdc.gov/ncidod/EID>. Accessed 17th February 2007.

Magwira C.A., B. A. Gashe, and E. K. Collison. 2005. Prevalence and antibiotic resistance profiles of *Escherichia coli* O157:H7 in beef products from retail outlets in Gaborone, Botswana. *J. Food Prot.*; **68 (2)**: 403–406.

Mora, A., S. L. Leon, M. Blanco, J. E. Blanco, C. Lopez, G. Dahbi, A. Echeita, E. A. Gonzalez, and J. Blanco. 2007. Phage types, virulence genes and PFGE profiles of shigatoxin-producing *Escherichia coli* O157:H7 isolated from raw beef, soft cheese and vegetables in Lima (Peru). *Int. J. Food Microbiol.*; **114(2)**: 2004–210

Müller, E. E., Ehlers, M. M. and W. O. Grabow. 2001. The occurrence of *Escherichia coli* O157:H7 in South African water sources intended for direct and indirect human consumption. *Water Res.*; **35**: 3085–3088.

- Müller, E. E., Grabow, W. and M. M. Ehlers.** 2003. Immunomagnetic separation of *Escherichia coli* O157:H7 from environmental and wastewater in South Africa. *Water, SA*; **29**: 427– 432.
- NCCLS, (National Committee for Clinical Laboratory Standards).** Performance standards for antimicrobial susceptibility testing; ninth informational supplement. Wayne, Pennsylvania: NCCLS; document M100-S9, 1999: 19.
- Okoli, C. I., K. F. Chah, P. T. E. Ozoh, and A. B. I. Udedibie.** 2005. Anti Microbial Resistance Profile of *E. coli* isolates From Tropical Free Range Chickens. *J. Health Allied Sci.*; **4(3)**: 1–12.
- Olorunshola, I. D., S. I. Smith, and A. O. Cker, A. O.** 2000. Prevalence of EHEC O157:H7 in patients with diarrhoea in Lagos, Nigeria. *APMIS.*; **108**: 761–763.
- Paquet, C., W. Perea, P. Grimont, M. Collin, and M. Guillod.** 1993. Aetiology of haemorrhagic colitis epidemic in Africa. *Lancet*; **342**: 175
- Phaswana-Mafunya N. and N. Shukla.** 2005. Factors that could motivate people to adopt safe hygienic practices in the Eastern Cape Province, South Africa. *SA Health Sci.*; **5(1)**: 21–28.
- Radu, S., A. M. Shahilah, R. Gulam, A. Zainori, M. Tadaaki, A. Norio, B. K. Yung, O. Jun, and N. Mitsuaki.** 1998. Detection of *Escherichia coli* O157:H7 in beef marketed in Malaysia. *Appl. Environ. Microbiol.*; **64**: 1153–1156.
- SAG, (South African Government).** 2000. Opening address to the Eastern Cape emergent red meat producers' Organization annual general meeting, Dohne Research Station, stutterheim, 18 July 2000.

- Schroeder, C. M., C. Zhao, C. Debroy, J. Torcolini, S. Zhao, D. G. White, D. D. Wagner, P. F. McDermott, R. D. Walker, and J. Meng.** 2002. Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine, and food. *Appl. Environ. Microbiol.* 68: 576-581.
- Sein, P. P., A. A. Hoosen, H. H. Crew-Brown, Y. Hoovadia, M. G. Dove, O. Heid, H. J. Koornhof, S. Oliver, O. Perovi, B. Prinsloo, J. M. N. Van RENSBURG, J. Simpson, A. W. Sturm, J. Wadula and E. Wasserman.** 2005. Antimicrobial susceptibility profile of selected invasive pathogens from academic hospitals of South Africa for the years 2001 – 2004. *SA J. Epidemiol. infect.*; **20(3)**: 85– 89.
- Stephanie, H.** 2006. Biltong: much more than just a snack. *The Christian Science Monitor*
- Torres, J., C. López-Saucedo, J. F. Cerna, N. Villegas-Sepulveda, R., Thompson, and F. R. Velazquez.** 2003. Single multiplex polymerase chain reaction to detect diverse loci associated with diarrheagenic *Escherichia coli*. *Emerg. Infect. Dis.*; **9**: 127–131.
- Vernozy-Rozand, C., S. Ray-Gueniot, C. Ragot, C. Bavai, C. Mazuy, M. P. Montet, J. Bouvet, and Y. Richard.** 2002. Prevalence of *Escherichia coli* O157:H7 in industrial mince beef. *Lett. Appl. Microbiol.*; **35**: 7–11.
- Vorster, S. M., R. P. Greebe, and G. L. Nortje.** 1994. Incidence of *Staphylococcus aureus* and *Escherichia coli* in ground beef, broilers and processed meats in Pretoria, South Africa. *J. Food Prot.*; **57**: 305–310.
- Wang, G., G. C. Clifford, and G. R. Frank.** 2002. Detection in *Escherichia coli* of the Genes Encoding the Major Virulence Factors, the Genes Defining the O157:H7 Serotype, and Components of the Type 2 Shiga Toxin Family by Multiplex PCR. *J. Clin. Microbiol.*; **40**: 3613–3619.

- Wong, C. S., S. Jelacic, R. L. Habeeb, S. L. Watkins, and P. I. Tarr.** 2000. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N. Engl. J. Med.*; **342**: 1930 –1936.
- Yoh, M., W. K., Sang, S. M., Saidi, H., Yamamoto, T., Ezaki, and T. Lida.** 1996. Haemorrhagic colitis due to *Escherichia coli* O157:H7 in Kenya. *J. of Trop. Ped.* **42**: 118–119.

CHAPTER 5

TABLE OF CONTENTS	Page №
Table of contents.....	269
List of Tables.....	271
List of Figures.....	272
Abstract.....	273
5.1 Introduction.....	274
5.2 Materials and Methods.....	276
5.2.1 Study areas and sample collection.....	276
5.2.2 Culture-based enumeration, recovery and identification of <i>E. coli</i>	
O157:H7.....	277
5.2.2.1 Enumeration.....	277
5.2.2.2 Enrichment and IMS recovery of <i>E. coli</i> O157:H7.....	277
5.2.2.3 Identification of <i>E. coli</i> O157:H7.....	279
5.2.4 Molecular characterization of <i>E. coli</i> O157:H7 using Polymerase	
Chain Reaction (PCR).....	279
5.2.4.1 Bacterial DNA extraction.....	279
5.2.4.2 Amplification of <i>fliC_{H7}</i> ; <i>rfbE_{O157}</i> and <i>eaeA</i> genes.....	280
5.2.4.3 DNA electrophoresis.....	281
5.2.5 Anti-microbial susceptibility tests.....	281
5.2.6 Statistical analysis.....	282

5.3	Results.....	282
5.3.1	Counts of presumptive <i>E. coli</i> O157 for the vegetables.....	282
5.3.2	Prevalence of <i>E. coli</i> O157 in the vegetables.....	283
5.3.2.1	<i>Molecular analysis of E. coli O157:H7.....</i>	284
5.3.3	Antimicrobial susceptibility test.....	286
5.4	Discussions and Conclusion.....	288
	References.....	293

LIST OF TABLES

Table 5.1:	Vegetable samples that were positive for <i>E. coli</i> O157:H7.....	285
Table 5.2:	Anti-microbial Susceptibility of <i>Escherichia coli</i> O157:H7 isolated from the vegetable samples	287

LIST OF FIGURES

Figure 5.1:	Counts of presumptive <i>E. coli</i> O157 on the vegetable samples expressed in 10 ³ CFU/g of the vegetables.....	283
Figure 5.2:	Percentage prevalence of <i>E. coli</i> O157 in the vegetables samples.....	284
Figure 5.3:	The amplified <i>fliC_{H7}</i> , <i>rfbE_{O157}</i> and <i>rfbE_{O157}</i> genes of <i>E. coli</i> O157:H7 isolated from vegetable samples.....	286

CHAPTER 5

Abstract

Fresh vegetables have been implicated in outbreaks of *E. coli* O157:H7 infections in most parts of the world. Microbiological quality of vegetables used as recipe for the making of salads is very crucial as salads are always eaten without any further cooking. Residents of Amathole District consume salads frequently although the microbial quality of recipe vegetables is questionable due to poor pre and post harvest handling. The present study investigated the prevalence and anti-microbial susceptibility of *E. coli* O157:H7 isolated from cabbages, carrots, cucumbers, onions and spinach. One hundred and eighty (180) vegetable samples used in the study were purchased from shops, supermarkets and open-air markets within the Amathole District. Strains of *E. coli* O157 were isolated by enrichment culture in phosphate-buffered saline followed by immunomagnetic separation and culture of magnetic beads onto cefixime tellurite sorbitol MacConkey Agar. Strains were characterized by Polymerase Chain Reaction (PCR) using primers specific for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes. Results indicated that presumptive *E. coli* O157 counts ranged between 1.3×10^3 and 1.59×10^5 CFU/g for vegetables. Prevalence of presumptive *E. coli* O157 was noted at 21.66% (39/180) of the vegetables. Four out of thirty-nine (10.26%) vegetable samples that were presumptively positive of *E. coli* O157 were confirmed to carry *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes characteristic of *E. coli* O157:H7. All the four *E. coli* O157:H7 isolates were susceptible to at least one of the 8 anti-microbials tested against them. Even though the prevalence of *E. coli* O157:H7 was low and those isolated were more

susceptible to most of the anti-microbials, there is need for surveillance for *E. coli* O157:H7 in vegetables used in salad recipes both in urban centres and in rural areas of South Africa.

Keywords: *fliC_{H7}*, *rfbE_{O157}* and *eaeA*, *Escherichia coli* O157:H7, vegetables

5.1 Introduction

Escherichia coli O157:H7 is a well-known food and water-borne pathogen (Olsen *et al.*, 2002; Vogt and Dippold, 2005). Its vehicles of transmission include contaminated food products, especially of bovine origin (Liptakova *et al.*, 2004; Mohle-Boetani *et al.*, 2004). Other modes of transmission include person-to-person, contact with infected animals or their manure and fruits and vegetables irrigated with *E. coli* O157:H7 contaminated waters or bathing in and drinking such waters (Keene *et al.*, 1994; Olsen *et al.*, 2002; Josefa *et al.*, 2005).

Contamination of vegetables with *E. coli* O157:H7 may also occur during cultivation, harvesting, packaging and transportation of the vegetables to the consumers (Ackers *et al.*, 1998). There is always a chance of *E. coli* O157:H7 being present in vegetables grown in manure-fertilized soils (Islam *et al.*, 2004). Solomon *et al.* (Solomon *et al.*, 2002) demonstrated experimentally that *E. coli* O157:H7 could become internalized in lettuce tissue when lettuce seeds were sown in manure-amended soils. Vegetables such as spinach may also be contaminated by manure that is used as fertilizer or by irrigation or surface water that contains *E. coli* O157:H7 (Ingham *et al.*, 2004). Outbreaks of diseases caused by shiga toxin producing *E. coli* have been associated with the consumption of leafy lettuce (Ackers *et al.*, 1998), potatoes (Chapman *et al.*, 1997), radish sprouts (Michino *et al.*, 1999) and alfalfa sprouts (Taormina *et al.*, 1999).

The growing of crops in South Africa in general and the Eastern Cape Province in particular involves the use of varied farming practices ranging from the use of commercial fertilizers to the use of human waste as sources of nutrients to the plants (Mnkeni *et al.*, 2006). However, health hazards associated with human waste reuse in the cultivation of crops carries a high risk in that products contaminated via such wastes may subsequently transmit pathogenic microorganisms to persons who consume or handle such products (Höglund, 2001). Vegetables used for making of salads may act as possible routes of disease transmission if grown in soils fertilized using human waste. These vegetables carry varying populations of microorganisms as was reported by Mnkeni *et al.* (2006). When hands are not washed correctly and at appropriate times, pathogens such as *E. coli* O157:H7 can be transmitted to vegetables meant for consumption as ready-to-eat and eventually passed directly to the mouth, or to other household members (Beuchat and Ryu, 1997; Medeiros *et al.*, 2001).

Immunomagnetic separation and polymerase chain reaction (PCR) procedures have been used in combination to isolate and characterize *E. coli* O157:H7 from either clinical, environmental or food samples (Müller *et al.*, 2003; Cagney *et al.*, 2004). Cagney *et al.* (2004), employed immunomagnetic separation and PCR techniques to successfully isolate and characterize *E. coli* O157:H7 from mince beef and beef burgers. In their study, Cagney *et al.* (2004) found shiga toxin-producing genes that encode for shiga toxin 1 and 2 (*stx1* and *stx2*). They also detected the presence of *E. coli* attaching and effacing gene (*eaeA*) that encodes for the intimin protein and the gene that encodes for haemolysin protein (*hlyA*), a 60-MDa plasmid or *rfb* gene that encodes for the biosynthesis of the O-antigen (*rfb*_{O157}) and the gene encoding the synthesis of H7 flagella protein (*fliC_{H7}*) (2004). Müller *et al.* (2003) also used a similar method to

isolate and characterize *E. coli* O157:H7 from river and wastewater samples and detected the presence of the genes coding for *stx2*, *eaeA* and *hlyA*.

In this paper, we report on the prevalence and antibiotic susceptibility of *E. coli* O157:H7 isolated from some selected vegetables obtained from the Amathole District in the Eastern Cape Province of South Africa. The selected vegetables are often used as salad vegetables. People in this district, as in any other part of the globe, often consume these salads in their fresh (raw) form without any cooking. For this reason, these vegetables may serve as vehicles for the transmission of pathogenic microorganism such as *E. coli* O157:H7. With the emergence of incurable diseases like HIV/AIDS, it was imperative to assess the prevalence of these dreaded bacteria, as the Eastern Cape Province is home to an estimated 12% of people living with HIV/AIDS in South Africa (UNDP, 2004; Dorrington *et al.*, 2006).

5.2 Materials and Methods

5.2.1 Study areas and sample collection

A total of 180 vegetable samples made up of 36 samples each of cabbage, carrots, cucumber, onions and spinach were purchased from shops, supermarkets and open-air markets within Alice, Fort Beaufort town and Mdantsane settlements in the Amathole District between March 2005 and August 2006. These vegetables were chosen for the study in one part because they are used in the preparation of salads that are eaten without any cooking.

Secondly, in this part of South Africa, farmers apply bovine manure on their farms as fertilizer, even though such manure has been reported elsewhere to harbour *E. coli* O157:H7 that can contaminate vegetables (Islam *et al.*, 2004). The location for this study falls within the

geographical coordinates of 32.77°S, 26.63°E for Fort Beaufort and 32.97°S, 27.87°E for Mdantsane with Alice centrally located between these two points at 32.79°S, 26.83°E. The samples were placed in sterile polyvinyl bags, put in a cooler box with ice blocks and transported to the laboratory for analysis. Presumptive *E. coli* O157:H7 enumeration and isolation was conducted within 24 h of sample collection.

5.2.2 Culture-based enumeration, recovery and identification of *E. coli* O157:H7

5.2.2.1 Enumeration

Presumptive *E. coli* O157:H7 populations in vegetable samples were determined by direct plating. Twenty-five grams (25 g) each, of cabbage, carrot, cucumber, onion and spinach samples were homogenized separately by blending for 1 min in 225 ml of 1% (w/v) Phosphate Buffered Saline (PBS) (Merck, Johannesburg, SA). Aliquots of 10 ml of the undiluted homogenates of each vegetable were added to 90 ml of PBS solution (Merck, SA).

The homogenate (10^{-1}) and subsequent serial dilutions of 10^{-2} , 10^{-4} and 10^{-6} were made and 1 ml of each respective dilution was pour plated in duplicates onto Cefixime-Tellurite Sorbitol MacConkey (CT-SMAC) Agar (Merck, SA). All plates were incubated at 37°C for 24 h. Average presumptive *E. coli* O157:H7 counts were estimated and expressed as CFU/g of vegetable samples.

5.2.2.2 Enrichment and IMS recovery of *E. coli* O157:H7

For enrichment, another 25 g each of cabbage, carrot, cucumber, onion and spinach samples were added to 225 ml of modified EC (mEC) broth containing 20 µg/ml novobiocin (n) (Merck, SA) (Cagney *et al.*, 2004). The samples were then incubated for 8 h at 37 °C on a rotary shaker at

a relative centrifugal force of $143 \times g$ (Gallenkamp, Loughborough, England). Twenty microlitres of the Dynabead (Dynal, Oslo) suspensions were incubated in a 1.5 ml Eppendorf tubes (Eppendorf, SA) with 1 ml aliquots of the pre-enriched samples at room temperature for 10 min with continuous mixing by hand. This step was performed to allow the *E. coli* O157-specific antibodies coated onto the beads to bind to the target bacteria.

The bacterial-IMS bead complexes were separated using a magnetic particle concentrator, Dynal MPC-M (Dynal, Oslo) for 3 min (Dynal product brochure, 2006). After discarding the supernatants and washing the bead-particles using 0.02% (v/v) diluted (1:20) PBS-Tween 20 in distilled water (pH 7.2) (Merck, SA), the complete immunomagnetic separation and washing procedure were repeated twice (Dynal product brochure, 2006). The final bacterial-IMS bead complexes were re-suspended in 1000 μ l washing buffer (PBS-Tween 20) (Merck, SA). Fifty microlitres (50 μ l) of the bacterial-IMS bead complexes concentrate was transferred to *E. coli* O157 selective media cefixime (0.05 mg/l) and potassium tellurite (2.5 mg/l)-Sorbitol MacConkey (CT-SMAC) (Merck, SA) Agar and spread out into half of the plate using a sterile cotton swab. The swabbed portion using an inoculating loop was further streaked out onto the agar surface to achieve single isolated colonies (Dynal product brochure, 2006).

The plates were then incubated at 37°C for 24 h. Sorbitol-non-fermenting colonies (up to 5 colourless colonies per plate per vegetable sample) were randomly selected and further plated by streaking onto Eosin Methylene Blue (EMB) agar (Merck, SA) (Müller *et al.*, 2003; Cagney *et al.*, 2004). Colonies with characteristic greenish blue-black metallic sheen colour on EMB Agar (Merck, SA). The colonies were then subjected to confirmatory test using conventional biochemical tests (Cagney *et al.*, 2004).

5.2.3.1 Identification of *E. coli* O157:H7

Escherichia coli O157:H7 colonies were identified as described by Cagney *et al.* (2004). The Oxidase test was performed on the colonies that were Gram negative prior to the conventional indole-methyl red-Voges-Proskauer-citrate (IMViC) tests (Müller *et al.*, 2003). Of the 180 vegetable samples, 39 were positive for *E. coli* with the IMViC test. One colony representing each of these samples was further confirmed using API 20E kits. The strips were read and final identification was secured using API LAB PLUS computer software reader (BioMérieux, Marcy-Etoile, France) (Momba *et al.*, 2006). These 39 colonies scored 99.9% identification profile for *E. coli* with API LAB PLUS computer software reader and so were the ones used for PCR analysis.

5.2.4 Molecular characterization of *E. coli* O157:H7 using Polymerase Chain Reaction (PCR)

5.2.4.1 Bacterial DNA extraction

DNA was extracted from the colonies identified as *E. coli* by API LAB PLUS computer software and from positive control strain (*E. coli* O157:H7, ATCC 43895) following the method of Torres *et al.* (2003). A loop-full of overnight culture of *E. coli* colonies was suspended in 200 µl of sterile Milli-Q PCR grade water (Merck, SA) and the cells were lysed using a Dri-block DB.2A (Techne, Cape town, SA) for 15 min at 100°C. The cell debris was removed by centrifugation at 20 000 x g for 2 min using a MiniSpin microcentrifuge (Merck, SA). The lysate supernatant was placed on ice for 5 min.

5.2.4.2 Amplification of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes

Oligonucleotide primers specific for the targeted *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes used in the polymerase chain reaction (PCR) were similar to those used by Wang *et al.* (2002). The primers sequences that were used to identify the target genes were FliC-F 5'-TAC CAT CGC AAA AGC AAC TCC-3', FliC-R 5'-GTC GGC AAC GTT AGT GAT ACC-3' for *fliC_{H7}*. For *rfbE_{O157}*, the forward was RfbE-F 5'-CTA CAG GTG AAG GTG GAA TGG-3'; RfbE-R 5'-AATT CCT CTC TTT CCT CTG CGG-3'. The primers for *eaeA* gene were EAE-R 5'-ATG CTT AGT GCT GGT TTA GG-3', EAE-R 5'-GCC TTC ATC ATT TCG CTT TC-3'. The expected amplification sizes for *fliC_{H7}*, *rfbE_{O157}*, and *eaeA* genes were 247, 328, and 248 base pairs, respectively. The PCR assays for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* (A/E) genes were carried out in a 50 µl reaction volume. The reaction mixture contained 10X SuperTherm GOLD Buffer, 1.5 mM MgCl₂, each of the four deoxynucleoside triphosphates (dNTPs) (Southern Cross Biotechnology, Cape town, SA) at a concentration of 0.25 mM, 100 pmol each of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* specific primers, 5 U of *Taq* DNA polymerase (Southern Cross Biotechnology). The reaction was carried out in an Eppendorf model AG 22331 Thermocycler (Merck, SA). The following PCR conditions for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* (A/E) genes optimized in our laboratory were similar to those previously used by Wang *et al.* (2002). Initial denaturation was at 95 °C for 8 min followed by 30 cycles of amplification. Denaturation was at 95 °C for 30 s; annealing at 58 °C for 30 s; and extension at 72 °C for 30 s, ending with a final extension at 72 °C for 7 min to allow for the complete synthesis of the strands and cooling to 4 °C (Wang *et al.*, 2002). Sterile Milli-Q PCR grade water (Merck, SA) and reference *E. coli* O157:H7, ATCC 43895 strain were included in each PCR assay as negative and positive controls respectively.

5.2.4.3 *DNA electrophoresis*

The PCR products (10 µl aliquots) were visualized on a 2% (w/v) agarose gel (Merck, SA) in 1 U TAE buffer (40 mM Tris-HCl, 20 mM Na-acetate, 1 mM EDTA, pH 8.5) and stained with 0.5 µg/ml Ethidium Bromide (EtBr) (Merck, SA). A 100-bp DNA ladder (Promega, USA) was included on each gel as a molecular size standard. The electrophoresis was carried out at 76 V for 1 h. The products were photographed under the BioDoc-It System (UVP Upland, CA 91786, USA).

5.2.5 **Anti-microbial susceptibility test**

The Antimicrobial susceptibility test for *E. coli* O157:H7 isolates was determined using Bauer and Kirby disk diffusion technique on Mueller-Hinton Agar (MHA) (Merck, SA) (Jorgensen *et al.*, 1999). The following antibiotic discs were used: ampicillin (10 µg), ceftriaxone (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), and amikacin 30 µg) and erythromycin (15 µg) (Davies Diagnostics, SA). For the inocula development the bacterial isolates were grown overnight on nutrient broth at 37 °C for reactivation.

The cells were harvested by centrifugation at $2200 \times g$ using a Beckman model TJ - 6 centrifuge (Great Britain) and washed twice with 0.85% (w/v) saline water (NaCl) (Merck, SA). The bacterial cells were re-suspended in sterile saline solution to a final density of 10^7 CFU/ml. The cell suspensions were then swabbed onto a freshly prepared Mueller-Hinton Agar (MHA) in order to achieve a lawn of growth on incubation. The antibiotic discs were placed on the surface of the inoculated Mueller-Hinton Agar (MHA) using a disc dispenser and the plates were incubated for 18 h at 37 °C (Jorgensen *et al.*, 1999). Zones of growth inhibition were measured

and results interpreted according to the guidelines of National Committee for Clinical Laboratory Standards for antimicrobial susceptibility testing (NCCLS, 1999). *Escherichia coli* ATCC 43895 was included as a positive control.

5.2.6 Statistical analysis

The statistical analysis was done using the Statistical Analysis System (SAS) (SAS Institute, Cary, USA) program. The *Chi* square test was run at a statistical significance level of ($P \leq 0.05$).

5.3 Results

5.3.1 Counts of presumptive *E. coli* O157 for the vegetables

The vegetable samples from Fort Beaufort had the highest counts of presumptive *E. coli* O157 followed by vegetables from Mdantsane whereas vegetables from Alice had the lowest counts. The mean counts for vegetables from Fort Beaufort ranged from 9.0×10^3 CFU/g for carrots to 1.6×10^6 CFU/g for spinach. The mean counts of presumptive *E. coli* O157 isolated from the vegetables from Mdantsane ranged between 1.6×10^3 CFU/g for onions and 1.6×10^5 CFU/g for spinach whereas counts of presumptive *E. coli* O157 in vegetables from Alice ranged from 1.3×10^3 CFU/g for cucumber to 4.1×10^4 CFU/g for spinach (Table 2). The counts of presumptive *E. coli* O157 were not significantly different from one vegetable sample to another for all the vegetables ($P > 0.05$).

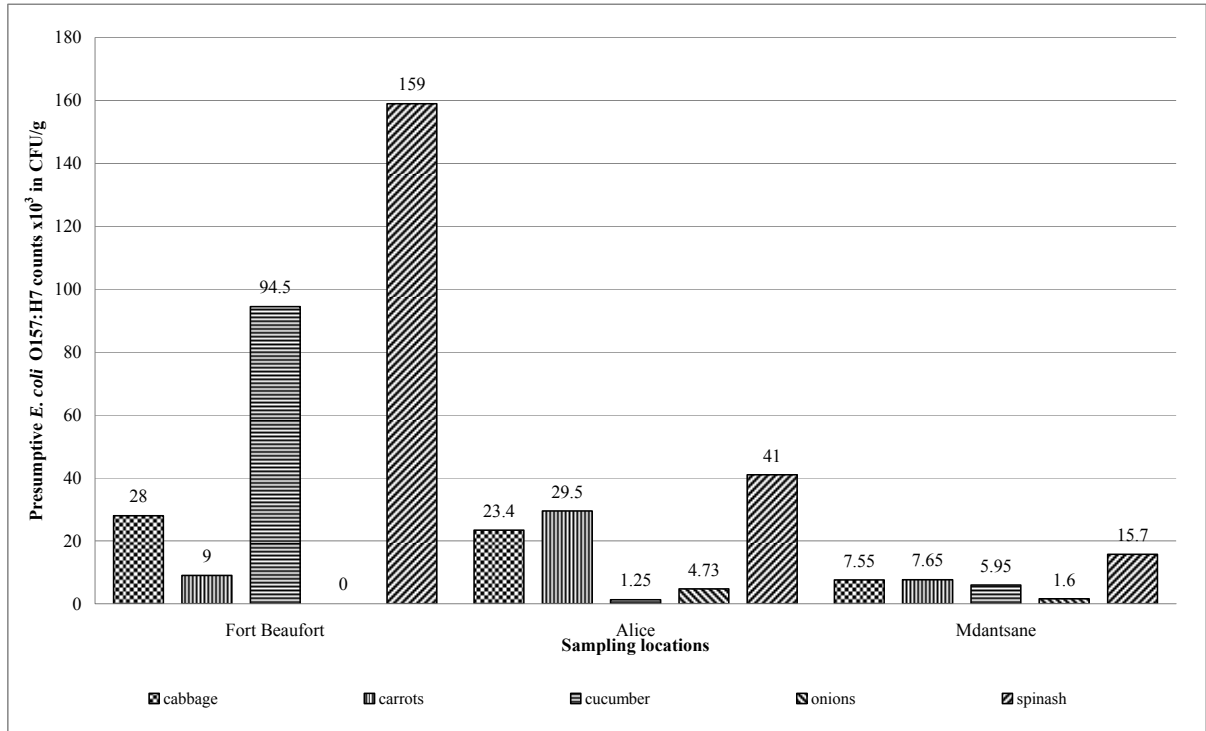


Figure 5.1: Counts of presumptive *E. coli* O157 on the vegetable samples

5.3.2 Prevalence of *E. coli* O157 in the vegetables

The prevalence of presumptive *E. coli* O157 was disaggregated by region and further by sample. The prevalence of presumptive *E. coli* O157 ranged from 0% to 33.3% respectively for onions and cabbages from Fort Beaufort. Also cabbages, carrots and cucumber from Mdantsane had *E. coli* O157 at a prevalence rate of 33.3%. Onions from Mdantsane had low prevalence rates. Prevalence of *E. coli* O157 in the Alice vegetables ranged between 16.7% for cabbage, cucumber and spinach to 25% for carrots and onions. Figure 2 below shows the percentage prevalence of presumptive *E. coli* O157 in the vegetable samples from the three study locations.

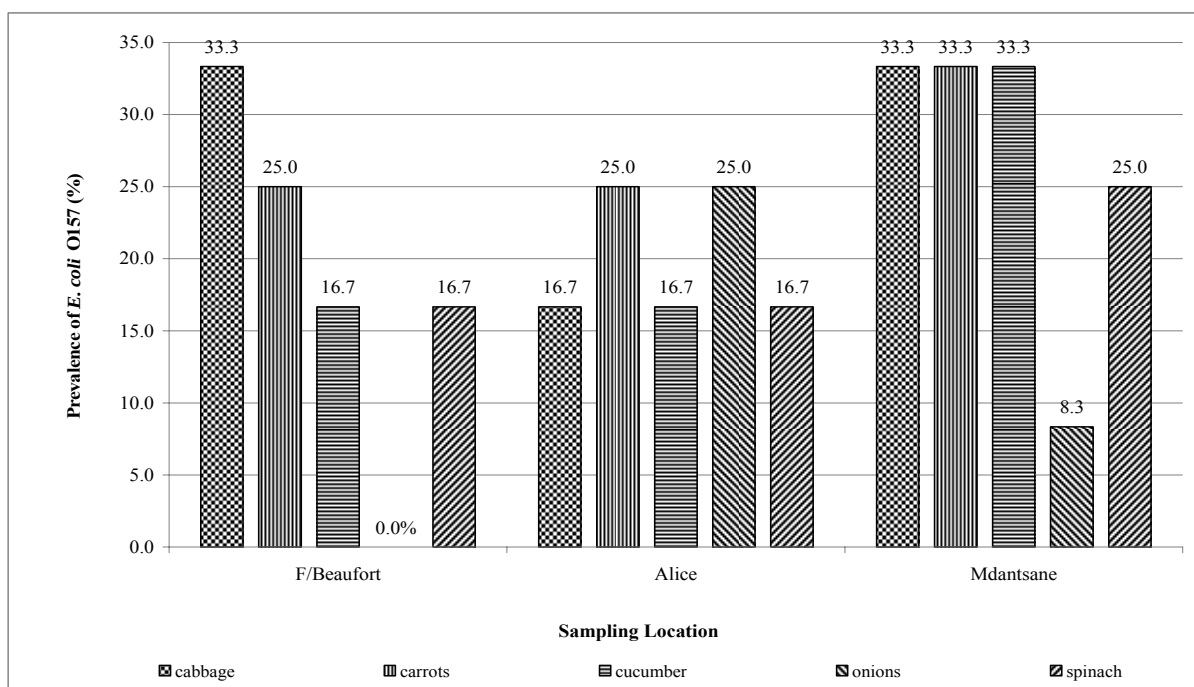


Figure 5.2: Percentage prevalence of *E. coli* O157 in the vegetables samples.

5.3.2.1 Molecular analysis of *E. coli* O157:H7

Results of PCR analysis revealed that four out of the thirty-nine (10.3%) vegetable samples that were presumptively positive for *E. coli* O157 carried the target genes (*fliC_{H7}*, *rfbE_{O157}*, and *eaeA*) characteristic of *E. coli* O157:H7. These included one cabbage and one cucumber sample both from Fort Beaufort. One spinach and one onion sample from Mdantsane were also positive for presumptive *E. coli* O157 that carried the three target genes. However, two carrot (5.1%) samples (one from Alice and the other from Mdantsane) carried *E. coli* O157 that were only positive for *fliC_{H7}* genes. The vegetables, which were positive for *E. coli* O157, carrying only *fliC_{H7}* but lacked the other two target genes (*rfbE_{O157}* and *eaeA*), were not considered as positive for *E. coli* O157:H7. A list of vegetables that were positive for *E. coli* O157:H7 are shown in Table 5.1 and representative gel electrophoresis profiles are illustrated in Figure 5.3.

Table 5.1: Vegetable samples that were positive for *E. coli* O157:H7

Vegetable	Location	Amplified genes			Samples with <i>E. coli</i> O157:H7
		<i>fliC_{H7}</i>	<i>rfbE_{O157}</i>	<i>eaeA</i>	
Cabbage	Fort Beaufort	+	+	+	1
Cucumber	Fort Beaufort	+	+	+	1
Spinach	Mdantsane	+	+	+	1
Onion	Mdantsane	+	+	+	1
Carrots	Alice & Mdantsane	+	–	–	2*
Total number of vegetables positive with <i>E. coli</i> O157:H7					4(2*)

*Samples that had *E. coli* O157 isolates, which only carried *fliC_{H7}* but lacked *rfbE_{O157}* and *eaeA*. These samples were not considered positive for *E. coli* O157:H7. n = 39, Representative number of *E. coli* O157 analyzed by PCR.

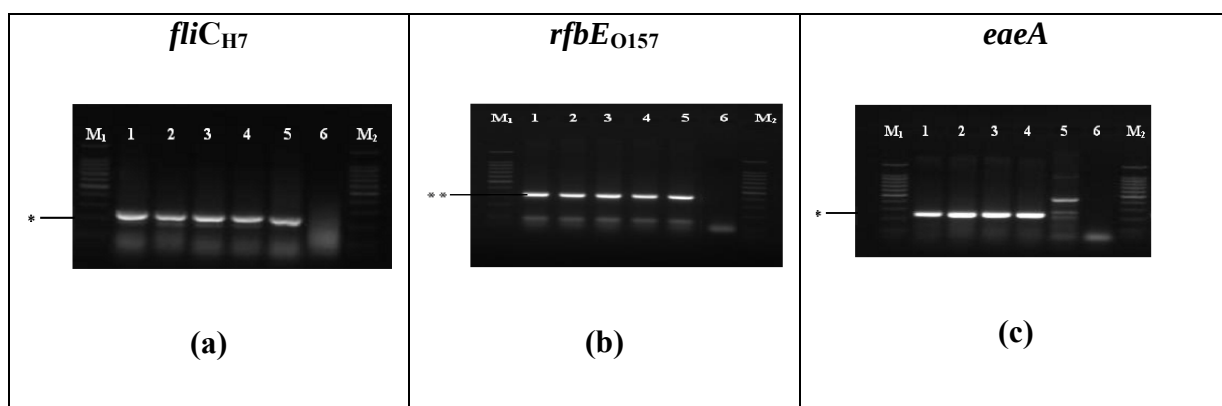


Figure 5.3: The amplified *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes of *E. coli* O157:H7 isolated from vegetable samples. Lanes M₁ & M₂: 100 bp DNA ladder marker (Promega, USA), lane 1: Positive control (*E. coli* O157:H7, ATCC 43895), lane 2: MdaV₂₅, lane 3: FbV₁₁, lane 4: FbV₉, lane 5: MdaV₃, lane 6: Negative control. The expected molecular sizes of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* fragments were at 247bp, 327bp and 248bp respectively.

5.3.3 Antimicrobial susceptibility test

All the four (100%) *E. coli* O157:H7 isolates were susceptible to amikacin and ceftriaxone but resistant to erythromycin. Some resistance to gentamycin was found for 50% (2/4) isolates. One (25%) isolate was susceptible to tetracycline and nalidixic acid whereas two (2/4) other isolates were susceptible to gentamycin, ampicilin, and chloramphenicol. Intermediate susceptibility to nalidixic acid and tetracycline was observed among 75% (3/4) of the *E. coli* O157:H7 isolates whereas 50% (2/4) showed intermediate susceptibility to ampicillin and chloramphenicol. Results on antibiotic susceptibility of the *E. coli* O157:H7 that were isolated from the vegetables is shown on Table 5.2.

Table 5.2: Distribution of antimicrobial susceptibility of *E. coli* O157:H7 isolated from

Antibiotic agent	Antibiotic disc content (µg)	Distribution %		
		R	I	S
Gentamycin	10	50	0	50
Ampicillin	10	0	50	50
Chloramphenicol	30	0	50	50
Nalidixic acid	30	0	75	25
Amikacin	30	0	0	100
Ceftriaxone	30	0	0	100
Erythromycin	15	100	0	0
Tetracycline	30	0	75	25

^aR, Resistant, I, Intermediate, S, Susceptible. ^bThe interpretation to achieve the above susceptibility percentages was based on the breakpoints of zone diameters for individual antibiotic agents as outlined in NCCLS ninth informational supplement of 1999: document M100–S9.

5.4 Discussions and Conclusion

The finding of this study sheds light on the prevalence of *E. coli* O157:H7 in selected vegetables consumed in a rural district of South Africa. Comparing the *E. coli* O157:H7 counts of the vegetables from the three areas, vegetables from Fort Beaufort and Mdantsane were contaminated with *E. coli* O157:H7 as opposed to vegetables from Alice of which no sample was found to carry all the three target genes under this investigation, characteristic of *E. coli* O157:H7. However, the presumptive colonies confirmed with PCR were few and so it cannot be certain that vegetables from Alice were free of *E. coli* O157:H7.

Separate studies have confirmed that fresh produce such as lettuce; alfalfa sprouts, and cabbage may become contaminated in the farms or during transportation and marketing (Como-Sabetti *et al.*, 1997; Ackers *et al.*, 1998; Wojtkunski *et al.*, 1999). Other studies have reported that these products become contaminated in the field from animal manure or irrigation water applied during farming (Josefa *et al.*, 2005). Contamination of vegetables may also occur during processing due to contaminated wash water, equipment, and poor handling practices; through contaminated storage equipment (Josefa *et al.*, 2005).

Washing fresh vegetables with water or a chlorine-based solution may not achieve complete elimination of *E. coli* O157:H7 from the vegetables or other fresh produces (Beuchat and Ryu, 1997). In this case *E. coli* O157:H7 may eventually be passed on to consumers of these vegetables. Comparing the *E. coli* O157:H7 counts of the vegetables from the three areas, vegetables from Fort Beaufort and Mdantsane were contaminated with *E. coli* O157:H7 as opposed to vegetables from Alice of which no sample was found to carry all the three target genes under this investigation, characteristic of *E. coli* O157:H7.

Identification of the *E. coli* O157:H7 was confirmed based on two putative genes, *fluC_{H7}* and *rfbE_{O157}* and a virulent gene *eaeA*. The amplification products confirmed that the *E. coli* O157:H7 isolated from the vegetables was similar to *E. coli* O157:H7 ATCC 43895 in genomic loci for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes with expected amplicon sizes at 247, 327 and 248 base pairs respectively. This confirms that indeed the *E. coli* O157:H7 that were isolated from the vegetables can be pathogenic and may be capable of causing human infections.

The genes that were used in the present study to characterize *E. coli* O157:H7 isolates have been used successfully in other studies to ascertain the identity of these bacteria (Wang *et al.*, 2002; Cagney *et al.*, 2004). This justifies the choice for the primers for characterization of the *E. coli* O157:H7 isolates from such published literature. The presence of *E. coli* O157:H7 in the vegetables as was revealed by the PCR results was not varied between the regions for the two regions whose vegetables were positive for the bacteria. Among those *E. coli* O157:H7 positive vegetables, the average counts were found to be significantly different ($P > 0.05$).

Although, only representative presumptive *E. coli* O157 was used to confirm the presence of *E. coli* O157:H7 in the vegetables, it sufficiently showed that these vegetables are actually contaminated with pathogenic *E. coli* O157:H7 serotype. The low prevalence of *E. coli* O157:H7 in the vegetables observed in this study is very much in agreement with previous studies in other parts of the world (Mora *et al.*, 2000). In 2003 and 2004, Mukherjee *et al.* (2004) in a microbiological survey of fresh produce with *E. coli* O157:H7 being one of their target pathogenic bacteria failed to isolate the bacteria from the vegetables.

Despite the low prevalence, pre- and post-harvest handling of vegetables in South Africa in general and Eastern Cape Province in particular is a great cause of concern regarding the microbiological quality of the vegetables. Due to the fact that infectious dose of *E. coli* O15:H7

is very low, it can cause infections even at unnoticeable levels (Wilshaw *et al.*, 1994). Farmers use bovine manure and untreated water on their farms to grow vegetables and the vegetables are in some cases sold in open-air markets. These vegetables are displayed either on top of wooden racks or on the ground in these open-air markets.

Vegetables such as cabbage heads are split into halves to meet customers' financial affordability. The cut cabbage surfaces in most cases are not covered to protect them from contaminants in the surrounding environments. Carrots are occasionally sold while packed in polyethylene films, however, most of the times the carrots are not packed and are exposed in the open air for the whole day. These practices expose the vegetables to open environments thus they may be easily contaminated by air-borne pathogens. However, contamination of vegetables with pathogenic *E. coli* O157:H7 has rarely been reported in the literature mostly from the African continent. Although, in western countries there have been studies on microbiological qualities of vegetables the majority of these studies have often been unsuccessful in isolating *E. coli* O157:H7 (Martinez *et al.*, 2000; Mukherjee *et al.*, 2004)

Another study in Norway, which attempted to detect *E. coli* O157:H7 in lettuce did not succeed (Loncarevic *et al.*, 2005). An investigation by Sagoo *et al.* (2001) that evaluated the microbiological quality of ready-to-eat vegetables, which included 86 carrots, mushrooms, cherry, pepper and alfalfa sprouts and 3200 samples of broccoli, cabbage, celery, lettuce, radish and others did not reveal the presence of *E. coli* O157:H7 in any of the vegetable samples they studied. This can be explained in part by assuming either that these bacteria are absent from the vegetables that were studied or that they are present in very low counts. However, recently *E. coli* O157:H7 were isolated from spinach that caused an outbreak in the US, which infected approximately 81 individuals (FDA News, 2007a, b).

The counts of presumptive *E. coli* O157 recorded in this study may not exclusively be due to *E. coli* O157:H7 as other serotypes of *E. coli* do often out-compete *E. coli* O157:H7 whenever they share a common growth environment (Ingham *et al.*, 2004). This may be the reason why in most studies aimed at revealing the microbiological qualities of fresh products with a focus on *E. coli* O157:H7 often end up with high counts of other *E. coli* strains instead of *E. coli* O157:H7. It has also been reported that *E. coli* O157:H7 is rapidly inactivated in the soils even if such soils were fertilized with bovine manures before the growing of vegetables and hence transmission of *E. coli* O157:H7 to the edible parts of vegetables may be minimal (Johannessen *et al.*, 2004). It was interesting to note that this was the first time that *E. coli* O157:H7 has been isolated from fresh vegetables in South Africa.

The most worrying observation made in this study was the resistance of the isolated *E. coli* O157:H7 to two of the anti-microbial agents that are commonly used in the treatment of bacterial infections in humans. This may imply that if by any chance these strains of *E. coli* were transmitted to humans, their treatment would be challenging. The resistance of *E. coli* O157:H7 to erythromycin has also been reported among human clinical isolates (Shebib *et al.*, 2003). Contrarily, in the Shebib *et al.* (2003), study, human *E. coli* O157:H7 isolates were observed to be susceptible to gentamicin. This shows that anti-microbial susceptibility by *E. coli* O157:H7 and other pathogens depend on the source of the isolate. However, all the four isolates were susceptible to at least one of the anti-microbial agents tested with the exception of erythromycin.

The anti-microbial agents that completely inhibited the growth of at least two of the *E. coli* O157:H7 were ceftriaxone and amikacin whereas gentamicin, ampicillin, chloramphenicol, nalidixic acid and tetracycline partially inhibited the growth of these bacteria. Acquisition of anti-microbial resistance by any bacteria in nature is due to forces that govern genetic changes in

the pathogens such as mutation of nucleotide sequences within their genomes and the horizontal transfer of existing gene sequences among bacterial species (LeClerc *et al.*, 1996). These forces are believed to increase the rate of genetic variation including the possession of drug resistance genes.

Even though it has been demonstrated in this study that some anti-microbial agents inhibited growth of *E. coli* O157:H7, it should be noted that it is not recommended to administer anti-microbial agents in the treatment of infections suspected of being from *E. coli* O157:H7 (Wong *et al.*, 2000). Wong *et al.* (2000) warn that the treating of *E. coli* O157:H7 infections with the use of anti-microbial agents may cause the release of shiga toxins into the system of patients upon the death of the bacterial cells causing haemolytic uremic syndrome. Contamination of vegetables with *E. coli* O157:H7 as was revealed in this study make stronger the need for surveillance for *E. coli* O157:H7 in the Amathole District and in South Africa in general. Control measures for preventing any practice that has the of potential to contamination of food items such as cabbage, carrots, cucumber, onions and spinach plus other food items that have not been covered in this research should be implemented. Consumers should be educated about the potential risk of consuming uncooked fresh vegetables. Regulatory and educational efforts are needed to improve the safety of fresh farm vegetables that are intended for use as ready-to-eat vegetables.

References

- Ackers, M. L., B. E. Mahon, E. Leahy, B. Goode, T. Damrow, P. S. Hayes, W. F. Bibb, D. H. Rice, T. J. Barret, L. Hutwagner, P. M. Griffin, and L. Slutsker. 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. *J. Infect. Dis.*; **177**: 1588–1593.
- Beuchat, L. R. and J. H. Ryu. 1997. Produce handling and processing practices. *Emerg. Infect. Dis.*; **3**(4): 459–465.
- Cagney, C., H. Crowley, G. Duffy, J-J. Sheridan, S. O'Brien, E. Carney, W. M. Anderson, D. A. Dowell, I. S. Blair, and R. H. Bisho. 2004. Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. *J. Food Microbiol.*; **21**: 203–212.
- Chapman, P. A., C. A. Siddons, A. T. Cerdan Malo, and M. A. Harkin. 1997. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. *Epidemiol. Infect.*; **119**: 245–250.
- Como-Sabetti, K., S. Reagan, S. Allaire, *et al.* 1997. Outbreaks of *Escherichia coli* O157:H7 infection associated with eating alfalfa sprouts—Michigan and Virginia, June–July, 1997. *MMWR.*; **46**:741–744.
- Dorrington, R. E., L. F. Johnson, D. Bradshaw, and T. Daniel. 2006. The Demographic Impact of HIV/AIDS in South Africa. National and Provincial indicators for 2006. Cape Town: Centre for Actuarial Research, South African Medical Research Council and Actuarial Society of South Africa. ISBNB 0-7992-2322-0
- Dynal Product Brochure. 2006. *Dynabeads® anti-E coli*. For rapid selective enrichment of *Escherichia coli* O157:H7. Dynal A.S. Oslo, Norway.

- FDA News.** 2006a Pacific Coast Fruit Company Voluntarily Recalls Spinach Salad Products and Pizza Because of Possible Health Risk. Available at: <http://www.fda.gov/oc/po/firmrecalls>. Accessed 1 September 2007.
- FDA News.** 2006b. Investigations of *E. coli* O157 Infections associated with taco bell restaurants in Northeast. Available at: <http://www.fda.gov/oc/opacom/hottopics>. Accessed 1 September 2007
- Höglund, C.** 2001. *Evaluation of microbial health risks associated with the reuse of source separated human urine*. PhD thesis, Department of Biotechnology, Royal Institute of Technology, Stockholm, Sweden. ISBN 91-7283-039-5.
- Ingham, S. C., J. A. Losinski, M. P. Andrews, J. E. Breuer, J. R. Breuer, T. M. Wood, and T. H. Wright.** 2004. *Escherichia coli* contamination of vegetables grown in soils fertilized with non-composted bovine manure: Garden-scale studies. *Appl. Environ. Microbiol.*; **70**:6420–6427.
- Islam, M., J. Morgan, M. P. Doyle, and X. P. Jiang.** 2004. Fate of *Escherichia coli* O157: H7 in manure compost-amended soil and on carrots and onions grown in an environmentally controlled growth chamber. *J. Food Prot.*; **67**:574–578.
- Johannessen, G. S., R. B., Froseth, L., Solemdai, J., Jarpe, Y., Wasteson, and L. M. Rorvik.** 2004. "Influence of bovine manure as fertilizer on the bacteriological quality of organic iceberg lettuce", *J. Appl. Microbiol.*; **96**:787–794.
- Jorgensen, J. H., J. D. Turnidge, and J. A. Washington.** 1999. *Antibacterial susceptibility tests: dilution and disk diffusion methods*. In: Murray, P. R., Tenover, F. C., Baron, E. J. and Tenover, R. H. ed. *Manual of clinical microbiology*, 7th ed. Washington, DC: American Society for Microbiology Press; pg. 1526–1543.

- Josefa, M. R., H. S., Phyllis, C. Collen, M. G., Patricia, and L. S. David.** 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerg. Infect. Dis.*; **11(4)**: 603–609.
- Keene, M. E., J. M. McAnulty, F. C. Hoesly, L. P. Jr. Williams, K. Hedberg, G. L. Oxman, T. J. Barrett, M. A. Pfaller, and D. W. Fleming.** 1994. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. *New Eng. J. Med.*; **331(9)**: 579–584
- LeClerc, J. E., B. Li, W. L. Payne, and T. A. Cebula.** 1996. High mutation frequencies among *Escherichia coli* and Salmonella pathogens. *Sci.*; 274:1208–1211
- Liptakova, A., L. Siegfried, J. Rosocha, L. Podracka, E. Bogyiova, and D. Kotulova.** 2004. A family outbreak of haemolytic uraemic syndrome and haemorrhagic colitis caused by verocytotoxigenic *Escherichia coli* O157 from unpasteurised cow's milk in Slovakia. *Clin. Microbiol. Infect.*; **10(6)**: 576 – 578.
- Loncarevic, S., G. S. Johannessen, and L. M. Rorvik.** 2005. Bacteriological quality of organically grown leaf lettuce in Norway. *Lett. Appl. Microbiol.*; **41**:186–189.
- Martinez, A., R. V. Diaz, and M. S. Tapia.** 2000. Microbial ecology of spoilage and pathogenic flora associated to fruits and vegetables, p. 43–62. In S. T. Alzamora, M. S. Tapia, and A. Lopez-Malo (ed.), Minimally processed fruits and vegetables—fundamental aspects and applications. Aspen Publishers, Inc., Gaithersburg, Md.
- Medeiros, L. C., V. N., Hillers, P. A. Kendall, and A. Mason.** 2001. Food safety education: what should we be teaching consumers? *J. Nutri. Edu.*; **33(2)**: 108–114.

- Michino, H., K. Andaraki, and S. Minami.** 1999. Massive outbreak of *Escherichia coli* O157:H7 infection in schoolchildren in Sakai City, Japan associated with consumption of white radish sprouts. *Am. J. Epidemiol.*; **150**:787–796.
- Mnkeni, P, N. S., B. J. Cisneros, M. C. Phasha, and L. M. Austin.** 2006. Use of human excreta from urine-diversion toilets in food gardens: Agronomic and health aspects. WRC Report № 1439/3/06. ISBN № 1-77005-455-3. *Water Res. Comm.*; **3**:1–40
- Mohle-Boetani, J. C., M. T. Jay, and V. Garrett.** 2004. A multistate outbreak of *Escherichia coli* O157:H7 infection linked to consumption of beef tacos at a fast-food restaurant chain. *J. Clin. Infect. Dis.*; **39**(1): 1–7.
- Momba, M. N. B., Z. Tyafa, N. Makala, B. M. Brouckaert, and C. L. Obi.** 2006. Safe drinking water still a dream in rural areas of South Africa. Case study: The Eastern Cape Province. *Water, SA*; **32**: 715–720.
- Mora, A., M.A. Usera, M. Blanco, J. E. Blanco, M. P. Alonso, G. Prats, A. Stirrat, F. M. Carter, and J. Blanco.** 2000. Bacteriophage typing and virulence genes of verocytotoxigenic *E. coli* (VTEC) O157:H7 strains isolated in Spain. In: Duffy G., Garvey, P., Coia, J., Wasteson, Y., McDowell, D.A. (Eds.), Verocytotoxigenic *E. coli* in Europe, 3. Pathogenicity and virulence of verocytotoxigenic *E. coli*. Concerted Action CT98-3935. Teagasc, The National Food Centre, Dublin, pp. 189.
- Mukherjee, A., D. Speh, E. Dyck, and F. Diez-Gonzalez.** 2004. Pre-harvest evaluation of coliforms, *Escherichia coli*, *Salmonella* and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. *J. Food Prot.*; **67**(5): 894–900.
- Müller, E. E., Grabow, W. and M. M. Ehlers.** 2003. Immunomagnetic separation of *Escherichia coli* O157:H7 from environmental and wastewater in South Africa. *Water,*

SA; **29**: 427– 432.

NCCLS, (National Committee for Clinical Laboratory Standards). Performance standards for antimicrobial susceptibility testing; ninth informational supplement. Wayne, Pennsylvania: NCCLS; document M100-S9, 1999: 19.

Olsen, S. J., G. Miller, T., Breuer, M., Kennedy, C., Higgins, J., Walford, G., McKee, K., Fox, W., Bibb, and P., Mead. 2002. A water-borne outbreak of *Escherichia coli* O157:H7 infections and hemolytic uremic syndrome: implications for rural water systems. *Emerg. Infect. Dis.*; **8**: 370–375.

Sagoo, S. K., C. L. Little, and R. T. Mitchell. 2001. The microbiological examination of ready-to-eat organic vegetables from retail establishments in the United Kingdom. *Lett. Appl. Microbiol.*; **33**: 434–439.

Shebib, Z. A. Z. G. A., Ghani, and L. K., Mahdi. 2003. First report of *Escherichia coli* O157 among Iraqi children. Available at: <http://www.emro.who.int/publications/>. Accessed 20 July 2007.

Solomon, E. B., S. Yaron, and K. R. Matthews. 2002. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Appl. Environ. Microbiol.*; **68**: 397–400.

Taormina, P. J., L. R. Beuchat, and L. Slutsker. 1999. Infections associated with eating seed sprouts: an international concern. *Emerg. Infect. Dis.*; **5**: 626–634.

Torres, J., C. López-Saucedo, J. F. Cerna, N. Villegas-Sepulveda, R., Thompson, and F. R. Velazquez. 2003. Single multiplex polymerase chain reaction to detect diverse loci associated with diarrheagenic *Escherichia coli*. *Emerg. Infect. Dis.*; **9**: 127–131.

UNDP (United Nations Development Programme). 2004. South Africa Human Development

Report 2003. Cape Town: Oxford University Press Southern Africa.

- Vogt, R. L., and L. Dippold.** 2005. *Escherichia coli* O157:H7 outbreak associated with consumption of ground beef, June-July 2002. *Public Health Rep.*; **120(2)**: 174–178.
- Wang, G., G. C. Clifford, and G. R. Frank.** 2002. Detection in *Escherichia coli* of the Genes Encoding the Major Virulence Factors, the Genes Defining the O157:H7 Serotype, and Components of the Type 2 Shiga Toxin Family by Multiplex PCR. *J. Clin. Microbiol.*; **40**: 3613–3619.
- Wilshaw, G. A., J. Thirlwell, and A. P. Jones.** 1994. Verocytotoxin -producing *Escherichia coli* O157 in beef burgers linked to an outbreak of diarrhea, haemorrhagic colitis and haemolytic uremic syndrome. *Lett. Appl. Microbiol.*; **19**: 304–307.
- Wojtkunski, C., E. D. Hilborn, J. H., Mermin, P. A., Mshar, J. L., Hadler, and A. Voetsch.** 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. *Arch. Int. Med.*; **159**: 1758–764.
- Wong, C. S., S. Jelacic, R. L. Habeeb, S. L. Watkins, and P. I. Tarr.** 2000. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N. Engl. J. Med.*; **342**: 1930–1936.

CHAPTER 6

TABLE OF CONTENTS	Page №
Table of contents.....	299
List of Tables.....	301
List of Figures.....	302
Abstract.....	303
6.1 Introduction.....	304
6.2 Materials and Methods.....	306
6.2.1 Sample size determination.....	306
6.2.2 Study population and area.....	307
6.2.3 Sampling of stool swabs.....	307
6.2.3.1 <i>Scientific ethics and informed consent</i>	308
6.2.4 Culture-based isolation and identification of <i>E. coli</i> O157:H7.....	309
6.2.4.1 <i>Enrichment and Immunomagnetic separation (IMS)</i>	
<i>Recovery.....</i>	309
6.2.4.2 <i>Identification of <i>E. coli</i> O157:H7.....</i>	310
6.2.5 Molecular characterization of <i>E. coli</i> O157:H7 using Polymerase	
Chain Reaction (PCR).....	311
6.2.5.1 <i>Bacterial DNA extraction.....</i>	311
6.2.5.2 <i>Amplification of <i>fliC_{H7}</i>; <i>rfbE_{O157}</i> and <i>eaeA</i></i>	

	<i>genes</i>	311
6.2.5.3	<i>DNA electrophoresis</i>	312
6.2.6	Anti-microbial susceptibility tests.....	313
6.2.7	Statistical analysis.....	313
6.3	Results and Discussion.....	314
6.3.1	Prevalence of <i>E. coli</i> O157 in confirmed and non-confirmed HIV/AIDS diarrheic patients.....	314
6.3.1.1	<i>Molecular analysis for E. coli O157:H7 from stools of confirmed and non-confirmed HIV/AIDS patients</i>	320
6.3.2	Statistical analysis of the results.....	324
6.3.3	Anti-microbial susceptibility of <i>E. coli</i> O157:H7 isolates	324
6.4	Conclusion and Recommendations	327
	References.....	328

LIST OF TABLES

Table 6.1(a): Summarized PCR amplified genes of <i>E. coli</i> O157:H7 isolated from diarrheic confirmed HIV/AIDS patients.....	321
Table 6.1(b): Summarized PCR amplified genes of <i>E. coli</i> O157:H7 isolated from diarrheic non-confirmed HIV/AIDS patients	322
Table 6.2: Distribution of susceptibility of <i>E. coli</i> O157:H7 isolated from stool swab suspension of confirmed HIV/AIDS positive and non-confirmed HIV/ AIDS negative patients to antibiotic agents.....	326

LIST OF FIGURES

Figure 6.1:	Percentage prevalence of presumptive <i>E. coli</i> O157 in confirmed Diarrhoeic and non-confirmed HIV/AIDS patients.....	315
Figure 6.2:	Prevalence of <i>E. coli</i> O157 in confirmed and non-confirmed diarrhoeic HIV/AIDS patients by gender.....	316
Figure 6.3:	Prevalence of <i>E. coli</i> O157 in confirmed and non-confirmed diarrhoeic HIV/AIDS patients by age.....	317
Figure 6.4:	Percentage prevalence of <i>E. coli</i> O157 in confirmed and non-confirmed diarrhoeic HIV/AIDS patients by race.....	318
Figure 6.5:	Prevalence of <i>E. coli</i> O157 in confirmed diarrhoeic HIV/AIDS and non-confirmed diarrhoeic HIV/AIDS patients visiting Frere Hospital, East London by locations.....	319
Figure 6.6:	The amplified <i>fliC_{H7}</i> , <i>rfbE_{O157}</i> and <i>eaeA</i> gene of <i>E. coli</i> O157:H7 isolated from stool swabs of (a) confirmed HIV/AIDS (b) non-confirmed HIV/AIDS patients all with diarrhoea.....	323

CHAPTER 6

Abstract

This study investigated the prevalence of *Escherichia coli* O157:H7 in the stool of confirmed and non-confirmed diarrhoeic HIV/AIDS patients. *Escherichia coli* O157:H7 was isolated by culture-based and immunomagnetic separation from three hundred and sixty stool swabs. Identification was by conventional IMViC, 20E API and molecular techniques. Confirmed and non-confirmed diarrhoeic HIV/AIDS patients had 56.5% (74/131) and 43.5% (57/131) respectively of *E. coli* O157:H7. Molecular results indicated that the prevalence of *E. coli* O157:H7 was 12.16% (9/74) and 8.77% (5/57) from stool swabs of confirmed and non-confirmed diarrhoeic HIV/AIDS patients. Antimicrobial resistance was higher for *E. coli* O157:H7 isolates from stools of confirmed HIV/AIDS than it was for non-confirmed HIV/AIDS patients. *Escherichia coli* O157:H7 might be a silent cause of diarrhoea in HIV/AIDS patients. It is recommended that HIV/AIDS patients with diarrhoea should be screened for *E. coli* O157:H7 and surveillance programmes for these bacteria should be established in both urban and rural areas of South Africa.

Keywords: Prevalence, *Escherichia coli* O157:H7, Diarrhoea and HIV/AIDS

6.1 Introduction

Diarrhoea is a universally occurring infectious disease found mostly in developing countries. Diarrhoeal diseases cause an estimated 5 483 deaths, mostly among children (CSE, 2006). A wide assortment of organisms cause diarrhoea and many of them have only been discovered in recent years. While *E. coli* is part of the normal faecal flora of humans and animals, some strains can cause life-threatening diarrhoea (Gray, 1995). Enterotoxigenic *E. coli*, mostly spread by means of contaminated food and water, remains a major public health problem of children and young infants and especially to the severely immuno-compromised (CSE, 2006). Of the well-known pathogenic enterotoxigenic *E. coli* is the serotype O157:H7, however, some non-O157:H7 have as well been reported to cause human disease outbreaks (CDC, 2004). Unlike non-O157:H7, which is mostly associated with cattle and other ruminants, *E. coli* O157:H7 is the only well known strain of *E. coli* associated with water and food-borne diarrhoea in humans (Renter *et al.*, 2003). Some *E. coli* O157:H7 are referred to as non-motile *E. coli* O157:H⁻, because they have lost their flagella characteristics due to deletion in the flagella regulatory gene *fliC_{H7}* (Feng *et al.*, 1996). Diarrhoeal outbreaks, which are linked to *E. coli* O157:H7 infections; are characterized by blood in the stools (not in all the cases); cramping abdominal pain, fever, nausea, and vomiting (Olorunshola, 2000; Koyange *et al.*, 2004).

Approximately 93% of HIV/AIDS patients develop significant gastrointestinal complications at some point during the course of their illness (Gazzard, 1988). As HIV progresses and the patient become more immune-compromised, the occurrence of gastrointestinal symptoms increases (May, 1993). In Sub-Saharan Africa, recent estimates suggest that 6 out of every 10 men, 5 out of every 10 women and 9 out of every 10 children are

positive for HIV/AIDS (Shisana *et al.*, 2005). South Africa is severely affected, being a home to 4.8 million people living with HIV/AIDS. This translates to an HIV prevalence of 10.8% among persons aged 2 years and older (Shisana *et al.*, 2005).

The Eastern Cape Province has one of the highest HIV growth rates increasing from 20.2% in 2000 to 29.5% in 2005 for antenatal clinic attendees (Shisana *et al.*, 2005). The predicted incidence of HIV infections in the Eastern Cape Province is at 1.3% and as at June 2006, 194 443 people had died of HIV/AIDS and another 64 095 people were already sick with the disease (Dorrington *et al.*, 2006). Diarrhoea is also another disease that threatens the lives of rural communities in the Eastern Cape (Bradshaw *et al.*, 2000). The situation is further exacerbated by the prevalence of extreme poverty in the rural areas. Awareness of the clinical and therapeutic aspects of watery diarrhoea suspected to have been caused by *E. coli* O157:H7 in HIV/AIDS patients is therefore vital in directing diagnostic evaluation of these patients and further research to improve human health. This strain of *E. coli* is a threat to human health, especially to immuno-compromised person such as HIV/AIDS patients, as the bacteria can be contracted from food and water consumed by these patients. Also because of a compromised immune system, such patients may develop diarrhoea attributed to *E. coli* O157:H7 than it would be the case for immuno-competent persons. This is the baseline on which this investigation of the prevalence of *E. coli* O157:H7 in confirmed and non-confirmed HIV/AIDS diarrhoeic patients was based.

One of the effective ways of managing bacterial diarrhoea in immuno-compromised individuals is the use of antibiotics (Kelly *et al.*, 1996). Antibiotic susceptibility profiles of microorganisms have been documented to vary considerably (Sein *et al.*, 2005). There is no study known to our knowledge that has elucidated the presence of *E. coli* O157:H7 in HIV/AIDS

patients, though recently Obi *et al.* (2007) isolated *E. coli* from stools of some HIV/AIDS patients with and without diarrhoea, *E. coli* O157:H7 was not investigated. The primary objective of this study was to investigate if *E. coli* O157:H7 is one of the bacteria involved in diarrhoea that habitually characterize HIV/AIDS patients. To guide clinicians in the Eastern Cape Province on the empirical treatment of diarrhoeal cases in HIV/AIDS patients, the antimicrobial susceptibility profiles of *E. coli* O157:H7 isolates obtained from the stools of these patients was also investigated.

6.2 Materials and Methods

6.2.1 Sample size determination

A two-group continuity corrected χ^2 test ($P = 0.050$) one-sided significance and 95% power was used to estimate the sample size. A sample size of 186 in each group when β_1 is 0.060 for group 1 (confirmed HIV/AIDS patients); and β_2 is 0.160 for group 2 (non-confirmed HIV/AIDS patients) with odds ratio of 2.984; was found to be significant. This was estimated as a sample size that could bring out significance difference for the prevalence of *E. coli* O157:H7 in confirmed and non-confirmed HIV/AIDS patients ($P \leq 0.05$) (Dixon and Massey, 1983). Hence equal sample sizes (180 for confirmed and 180 for non-confirmed diarrhoeic HIV/AIDS patients, which totals to 360) was chosen for the study (Fleiss *et al.*, 1980).

6.2.2 Study population and area

The study was conducted among confirmed HIV/AIDS and non-confirmed diarrhoeic HIV/AIDS patients visiting Frere Hospital for treatment of diarrhoea. Diarrhoea was diagnosed by the recruited nurse in the case of a patient experiencing 3 or more watery stools in 24 h. Frere Hospital is situated in the City of East London within the Amathole District in the Eastern Cape Province of South Africa. The Hospital basically caters for patients from the surrounding locations such as Mdantsane, King Williams Town, Berlin, Alice Butterworth, Umtata, City of East London, Stutterheim, Gomoa, Chulumba and other small villages. However, being a referral hospital, it also caters for special referral cases from other districts within Eastern Cape Province. The Amathole District has high levels of poverty and is one of the regions affected by the HIV/AIDS scourge, with about 8.6% of the population affected (Department of Health, 2006).

6.2.3 Sampling of stool swabs

Between March 2005 and August 2006, three hundred and sixty stool swabs were collected (180 from confirmed HIV/AIDS and another 180 from non-confirmed HIV/AIDS diarrhoeic patients). All the patients who consulted the hospital for diarrhoeal complaints and voluntarily consented by signing the informed consent form to give their stool specimen were recruited into the study. The confirmed HIV/AIDS patients had already been tested for HIV at the HIV/AIDS clinic of Frere Hospital and were known by the Hospital clinicians to be carriers of the HIV virus.

The stool swabs from non-confirmed HIV/AIDS diarrhoeic patients were used as controls. Sterile specimen bottles filled with 30 ml of sterile saline (Merck, SA) solution and sterile cotton swabs (Merck, SA) were taken to the HIV/AIDS and Outpatient clinics of Frere

Hospital. The patients' biographical information such as gender, age, race, location and their diarrhoea and HIV/AIDS status was recorded. Anonymity of the patients was protected as much as possible.

The HIV/AIDS clinic provided stool swabs of confirmed HIV/AIDS patients with diarrhoea whereas the Outpatient clinic provided stool swabs of non-confirmed HIV/AIDS diarrhoeic patients. The swabbing of stools was done by the patients themselves. The patients were instructed by the nurse recruited to conduct sampling to touch the stools using the cottoned side of the swab. The patient then returned the cotton swab having the stool to the nurse. The stool swabs were then dipped into the sterile saline solutions in the specimen bottles, put into a cooler box filled with ice blocks and transported to the laboratory for the isolation of *E. coli* O157:H7. Specimens were duly processed within 1-4 h after their collection.

6.2.3.1 Scientific ethics and informed consent

The University of Fort Hare's Govan Mbeki Research and Development Centre, the Provincial Department of Health (Bisho), and the Regional Eastern Cape Ethical Review Committees all approved the protocol that was used for the stool swab collection. Informed consent was obtained from patients or their guardians with the help of a nurse with Voluntary Counselling and Testing (VCT) skills and experience.

6.2.4 Culture-based isolation and identification of *E. coli* O157:H7

6.2.4.1 *Enrichment and immunomagnetic separation recovery*

One millilitre of each saline solution (1% w/v) containing stool swabs were inoculated into 99 ml of modified *E. coli* (mEC) broth containing 20 µg/ml novobiocin (Merck, SA) (Cagney *et al.*, 2004). The suspensions were incubated in a shaking incubator (Gallenkamp, Loughborough, England). The incubation was for 8 h at 37°C while rotating at $143 \times g$. Twenty microlitres (20 µl) of the Dynabead (Dynal, Oslo, Norway) suspensions were incubated in a 1.5 ml Eppendorf tubes (Merck, SA) with 1 ml aliquots of the pre-enriched samples at room temperature for 10 min with continuous mixing by hand. This step was performed to allow the *E. coli* O157-specific antibodies coated onto the beads to bind to the target bacteria.

The bacteria-IMS bead complexes were separated using a magnetic particle concentrator, Dynal MPC-M (Dynal, Oslo) for 3 min (Dynal product brochure, 2006). After discarding the supernatants and washing the bead-particles using 0.02% (v/v) diluted (1:20) PBS-Tween 20 in distilled water (pH 7.2) (Merck, SA), the complete immunomagnetic separation and washing procedure were repeated twice (Dynal product brochure, 2006). The final bacteria-IMS bead complexes were re-suspended in 1000 µl washing buffer (PBS-Tween 20) (Merck, SA). Fifty microlitres (50 µl) of the bacteria-IMS complex concentrate was transferred to *E. coli* O157 selective media cefixime (0.05 mg/l) and potassium tellurite (2.5 mg/l)-Sorbitol MacConkey (CT-SMAC) (Merck, SA) Agar and spread out into half of the plate using a sterile cotton swab. The swabbed portion using an inoculating loop was further streaked out onto the agar surface to achieve single isolated colonies (Dynal product brochure, 2006). The plates were then incubated at 37°C for 24 h. Non-Sorbitol fermenting colonies (up to 5 colourless colonies per plate per stool specimen) were randomly selected and further plated by streaking onto Eosin Methylene

Blue (EMB) agar (Merck, SA) (Müller *et al.*, 2003; Cagney *et al.*, 2004). Seventy-four (74) and 57 stools from confirmed and non-confirmed diarrhoeic HIV/AIDS patients, respectively, had presumptive *E. coli* O157 with greenish blue-black metallic sheen colour on EMB agar.

6.2.4.2 Identification of *E. coli* O157:H7

Presumptive *E. coli* O157 colonies were identified as described by Cagney *et al.* (2004). The Oxidase test was then conducted on colonies that were Gram negative before the conventional indole-methyl red-Voges-Proskauer-citrate (IMViC) test was performed (Feng, 1995; Heuvelink *et al.*, 1998; Radu *et al.*, 1998; Müller, 2003). Out of 360 stool swabs (180 from confirmed HIV/AIDS and 180 from non-confirmed HIV/AIDS), 74 and 57 stool swabs were presumptively positive of *E. coli* O157.

One colony of presumptive *E. coli* O157, each representing the 74 and 57 stools from confirmed and non-confirmed HIV/AIDS patients with diarrhoea respectively, were subjected to IMViC test and further confirmed as *E. coli* with API 20E kits. The strips were then read and the final identification was secured using API LAB PLUS computer software (BioMérieux, Marcy-Etoile, France) (Momba *et al.*, 2006). Out of the 74 and 57 stools specimens, 25 and 29 presumptive *E. coli* O157 isolated from the stools of confirmed and non-confirmed HIV/AIDS patients with diarrhoea, respectively, demonstrated an identification profile for *E. coli* with API LAB PLUS computer software reader. These were further characterized using PCR.

6.2.5 Molecular characterization of *E. coli* O157:H7 using Polymerase Chain Reaction (PCR)

6.2.5.1 Bacterial DNA Extraction

DNA was extracted from colonies identified as *E. coli* and from a positive control strain (*E. coli* O157:H7, ATCC 43895) using direct lysis method according to Torres *et al.* (2003). A loop-full of overnight culture of *E. coli* colonies was suspended in 200 µl of sterile Milli-Q PCR grade water (Merck, SA) and the cells were lysed using a Dri-block DB.2A (Techne, Cape Town, SA) for 15 minutes at 100°C. The cell debris was removed by centrifugation at $20\,000 \times g$ for 2 min using a MiniSpin microcentrifuge (Merck, SA). The lysate supernatant was placed on ice for 5 minutes.

6.2.5.2 Amplification of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes

Oligonucleotide primers specific for the targeted *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes used in the polymerase chain reaction (PCR) were similar to those used by Wang *et al.* (2002). The primers sequences that were used to identify the target genes were FliC-F 5'-TAC CAT CGC AAA AGC AAC TCC-3', FliC-R 5'-GTC GGC AAC GTT AGT GAT ACC-3' for *fliC_{H7}*. For *rfbE_{O157}*, the forward was RfbE-F 5'-CTA CAG GTG AAG GTG GAA TGG-3'; RfbE-R 5'-AATT CCT CTC TTT CCT CTG CGG-3'. The primers for *eaeA* gene were EAE-R 5'-ATG CTT AGT GCT GGT TTA GG-3', EAE-R 5'-GCC TTC ATC ATT TCG CTT TC-3'. The expected amplification sizes for *fliC_{H7}*, *rfbE_{O157}*, and *eaeA* genes were 247, 328, and 248 base pairs, respectively. A total volume of 10 µl genomic DNA was used in each PCR reaction.

The PCR assays for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* (A/E) genes were carried out in a 50 µl reaction volume. The reaction mixture contained 10X SuperTherm GOLD Buffer, 1.5 mM

MgCl₂, each of the four deoxynucleoside triphosphates (dNTPs) (Southern Cross Biotechnology, Cape Town, SA) at a concentration of 0.25mM, 100pmol each of *fliC*_{H7}, *rfbE*_{O157} and *eaeA* specific primers, 5U of *Taq* DNA polymerase (Southern Cross Biotechnology, Cape Town, SA). The reaction was carried out in an Eppendorf model AG 22331 Thermocycler (Merck, SA). The following PCR conditions for *fliC*_{H7}, *rfbE*_{O157} and *eaeA* (A/E) genes optimized in our laboratory were similar to those previously used by Wang *et al.* (2002): initial denaturation was at 95 °C for 8 min followed by 30 cycles of amplification. Denaturation was at 95 °C for 30 s; annealing at 58 °C for 30 s; and extension at 72 °C for 30 s, ending with a final extension at 72 °C for 7 min to allow for the complete synthesis of the strands and cooling to 4 °C (Wang *et al.*, 2002). Sterile Milli-Q PCR grade water (Merck, SA) and reference *E. coli* O157:H7, ATCC 43895 strain were included in each PCR assay as negative and positive controls respectively.

6.2.5.3 *DNA electrophoresis*

The PCR products (10 µl aliquots) were visualized on a 2% (w/v) agarose gel (Merck, SA) in 1 U TAE buffer (40 mM Tris-HCl, 20 mM Na-acetate, 1 mM EDTA, pH 8.5) and stained with 0.5 µg/ml Ethidium Bromide (EtBr) (Merck, SA). A 100-bp DNA ladder (Promega, USA) was included on each gel as a molecular size standard. The electrophoresis was carried out at 76 V for 1 h. The products were photographed under the BioDoc-It System (UVP Upland, CA 91786, USA).

6.2.6 Antimicrobial susceptibility test

The antimicrobial susceptibility test for *E. coli* O157:H7 isolates was determined using Bauer and Kirby disk diffusion technique on Mueller-Hinton Agar (MHA) (Merck, SA) (Jorgensen *et al.*, 1999). The following antibiotics were used: ampicillin (10 µg), ceftriaxone (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), and amikacin 30 µg) and erythromycin (15 µg) (Davies Diagnostics, Ltd). For the inocula development the bacterial isolates were grown overnight on nutrient broth at 37°C for reactivation.

The cells were harvested by centrifugation of 10 ml of the overnight culture at 1500 rpm for 2 min using a Beckman model TJ - 6 centrifuge (Great Britain) and washed twice with 5 ml saline water 0.85% (w/v NaCl) (Merck, SA). The bacterial cells were re-suspended in 5 ml sterile saline solution 0.85% (w/v NaCl) (Merck, SA) to a final density of 10^7 CFU/ml. The cell suspensions were then swabbed onto a freshly prepared Mueller-Hinton Agar (MHA) in order to achieve a lawn of growth on incubation. The antibiotic discs were placed on the surface of the inoculated Mueller-Hinton Agar (MHA) using a disc dispenser and the plates were incubated for 18 h at 37°C (Jorgensen *et al.*, 1999). Zones of growth inhibition were measured in millimetres and results interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards for antimicrobial susceptibility testing (NCCLS, 1999). *Escherichia coli* ATCC 43895 was included as a positive control.

6.2.7 Statistical analysis

The statistical analysis was done using the Statistical Analysis System (SAS) (SAS Institute, Cary, USA) program. The *Chi* square test was run on the data at a statistical significance level (P

≤ 0.05) to determine if there existed any associations between the prevalence of *E. coli* O157:H7 and patient's HIV/AIDS status, age, gender and race.

6.3 Results and Discussion

6.3.1 Prevalence of *E. coli* O157 in confirmed and non-confirmed HIV/AIDS diarrhoeic patients

HIV/AIDS patients have a higher likelihood of developing diarrhoea than people with competent immune systems (Mitchell *et al.*, 1998; Hayes *et al.*, 2003). The results obtained by the culture-based method using IMS and selective media coupled with IMViC test indicated that 131 presumptive *E. coli* O157 were detected out of 360 stool swabs analyzed. About 56.5% (74/131) were from stool swabs of confirmed HIV/AIDS patients and 43.5% (57/131) were from non-confirmed HIV/AIDS patients (Figure 6.1).

The prevalence of presumptive *E. coli* O157 in patients by gender was of profound interest. The level of presumptive *E. coli* O157 was found to be higher in females than in males in both confirmed and non-confirmed HIV/AIDS patients. Out of the 74 confirmed HIV/AIDS patients who were presumptively positive of *E. coli* O157, 73.3% (54/74) were females whereas 26.7% (20/74) were males.

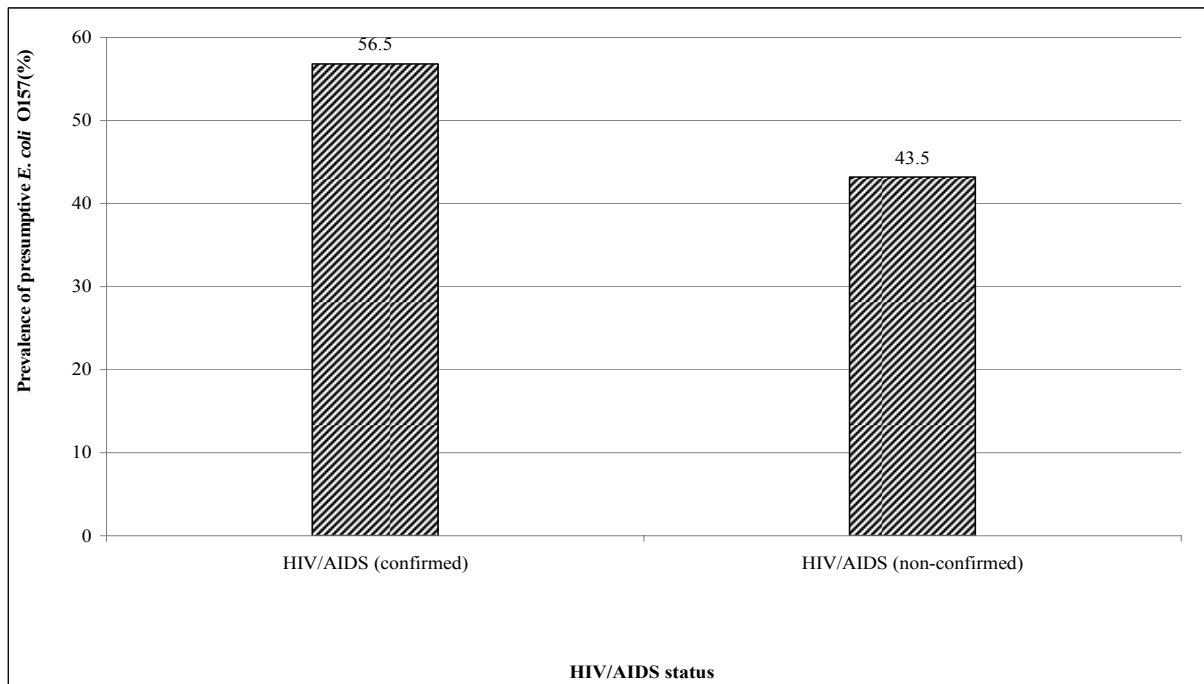


Figure 6.1: Percentage prevalence of presumptive *E. coli* O157 in confirmed and non-confirmed HIV/AIDS patients. (*) sample size per HIV group

Moreover, out of the 57 non-confirmed HIV/AIDS patients who were presumptively positive of *E. coli* O157, 84.2% (48/57) were females whereas 15.8% (9/57) were males (Figure 6.2). The high prevalence of *E. coli* O157 in females than in males has been observed in most epidemiological studies. A similar trend was reported in Cameroon in 1998 when an epidemic of diarrhoea due to *E. coli* O157 was investigated (Cunin *et al.*, 1999).

The non-confirmed diarrhoeic HIV/AIDS female patients had higher *E. coli* O157 prevalence (84.2%) than confirmed diarrhoeic HIV/AIDS females (73.3%). This observation was contrary to the expectation, based on the assumption that confirmed HIV/AIDS patients have a compromised immune system and could be more vulnerable to *E. coli* O157 infections than non-confirmed HIV/AIDS females. This discrepancy might be because non-confirmed HIV/AIDS patients were not tested for HIV/AIDS and so their HIV status was not known. Confirmed

HIV/AIDS males had a higher prevalence of *E. coli* O157 than non-confirmed diarrhoeic HIV/AIDS male patients did. This suggests that these confirmed diarrhoeic HIV/AIDS males might be more susceptible to *E. coli* O157 infections than non-confirmed diarrhoeic HIV/AIDS males.

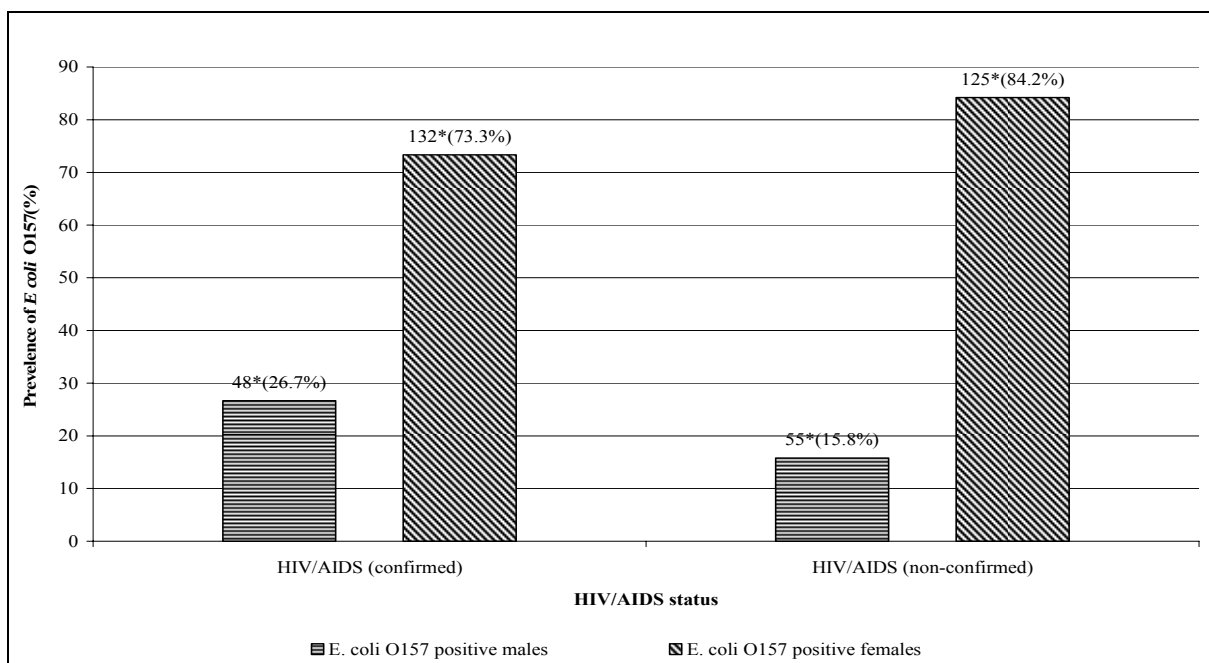


Figure 6.2: Prevalence of *E. coli* O157 in confirmed and non-confirmed HIV/AIDS patients by gender. (*) sample size per gender per HIV group.

The age distribution among *E. coli* O157 positive confirmed and non-confirmed HIV/AIDS patients indicated that confirmed and non-confirmed HIV/AIDS patients aged from 21 to 30 and 41 to 50 respectively had a higher prevalence of presumptive *E. coli* O157. There was a marked decrease in the prevalence of *E. coli* O157 in confirmed HIV/AIDS patients aged 21 to 30 from 41.9% down to 4.1% for those patients >50 years old. Contrarily for non-confirmed HIV/AIDS patients, there was an increase in the prevalence from 5.3% for those aged

21 to 30 up to 33.3% for those aged 41 to 50 (Figure 6.3). This trend was also observed by Cunin *et al.* (1999).

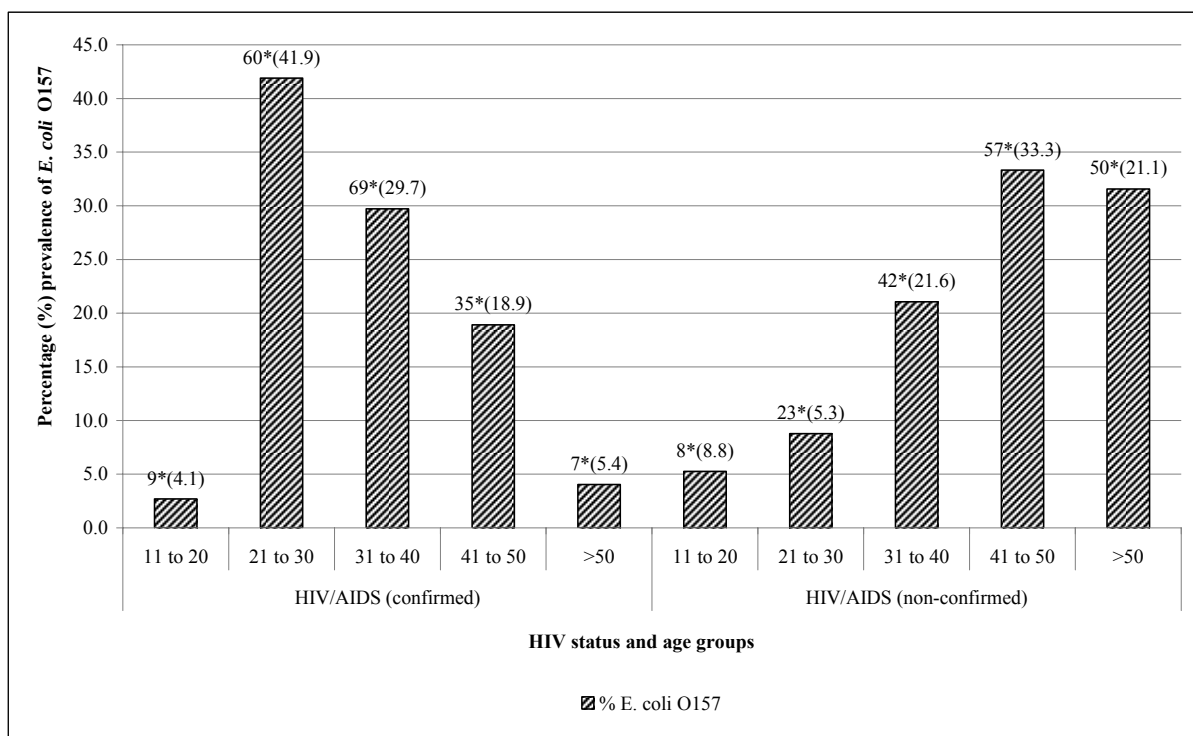


Figure 6.3: Prevalence of *E. coli* O157 in confirmed and non-confirmed HIV/AIDS patients by age. (*) Number of patients diagnosed with diarrhoea per age group.

The prevalence of presumptive *E. coli* O157 by race was found to be higher for patients of black race than the other races. The prevalence rate was 90.5% for the confirmed HIV/AIDS patients and 73.7% for the non-confirmed HIV/AIDS patients of the same race. The prevalence of presumptive *E. coli* O157 in patients of the Colored race was 9.5% for confirmed HIV/AIDS diarrhoeic patients and 12.3% for the non-confirmed HIV/AIDS diarrhoeic patients of white race. No *E. coli* O157 was noticed in stools from confirmed HIV/AIDS diarrhoeic patients of the White race. However, 12.3% of non-confirmed diarrhoeic HIV/AIDS patients of white race had

E. coli O157 (Figure 6.4). The increase in the prevalence of presumptive *E. coli* O157 could be due to a faster growing sexually active but yet immunocompromised individuals due to HIV/AIDS affliction. HIV/AIDS has been reported to be rife amongst the youths of ages between 15 and 30 yrs (Dorrington *et al.*, 2006). Nevertheless, the burden of *E. coli* O157 was felt across all the ages for both confirmed and non-confirmed HIV/AIDS patients.

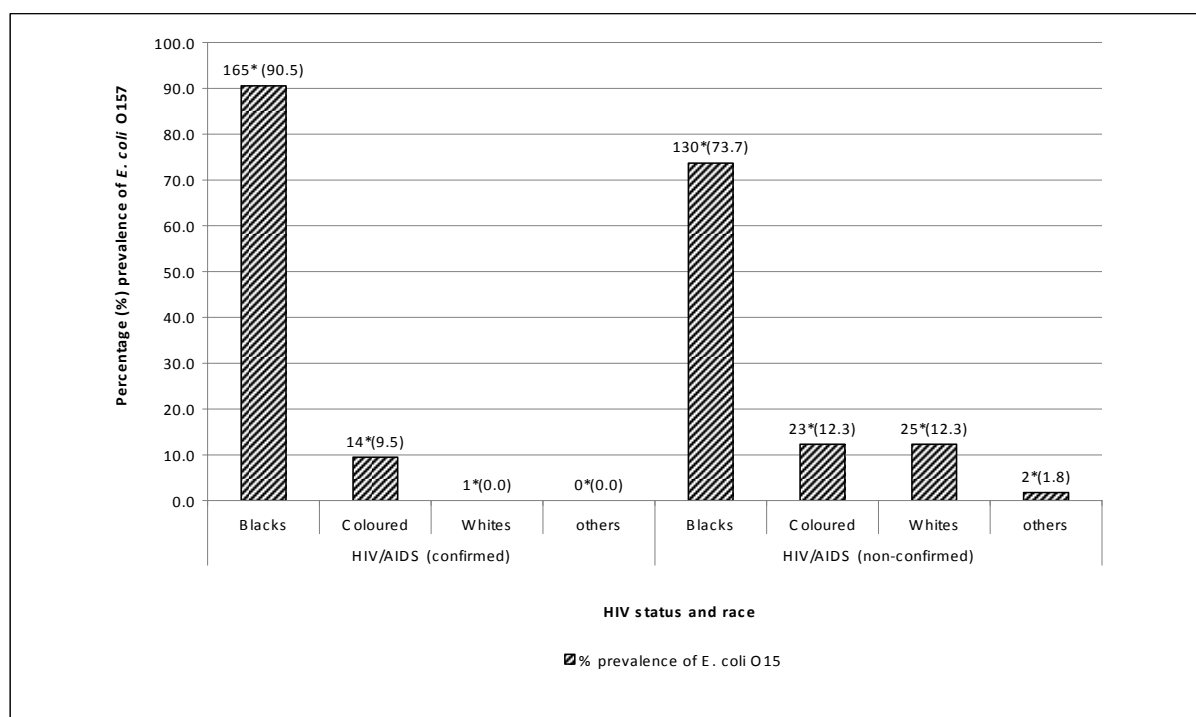


Figure 6.4: Percentage prevalence of *E. coli* O157 in confirmed and non-confirmed HIV/AIDS diarrhoeic patients by race. (*)Number of patients diagnosed with diarrhoea per race.

By location, *E. coli* O157 prevalence was higher in East London than it was for other locations. This was probably because of the close proximity of Frere Hospital to East London. Secondly, it could be a reflection of rural-urban migration, which is known to affect most cities universally. The prevalence rates of presumptive *E. coli* O157 was 75.7% (56/74) for confirmed HIV/AIDS

diarrhoeic patients and 73.6% (42/57) for non-confirmed HIV/AIDS diarrhoeic patients originating from East London. Other locations that had a noticeable prevalence of *E. coli* O157 for the confirmed HIV/AIDS patients were Stutterheim 4.05% (3/74) while for each of the other locations (Butterworth, Duncan Village and Mdantsane) the prevalence was 2.7% (2/74). The rates prevalence of *E. coli* O157 for the locations of the non-confirmed HIV/AIDS diarrhoeic patients was 5.26% (3/57) for Butterworth, 3.51% (2/57) for Duncan Village, King Williams Town and Stutterheim. Localities, which had very low numbers of patients, were categorized as “Others” and prevalence of presumptive *E. coli* O157 for confirmed HIV/AIDS diarrhoeic patients from such locations was 12.6% (9/74) whereas for the non-confirmed HIV/AIDS diarrhoeic patients the prevalence was 10.53% (6/57) (Figure 6.5).

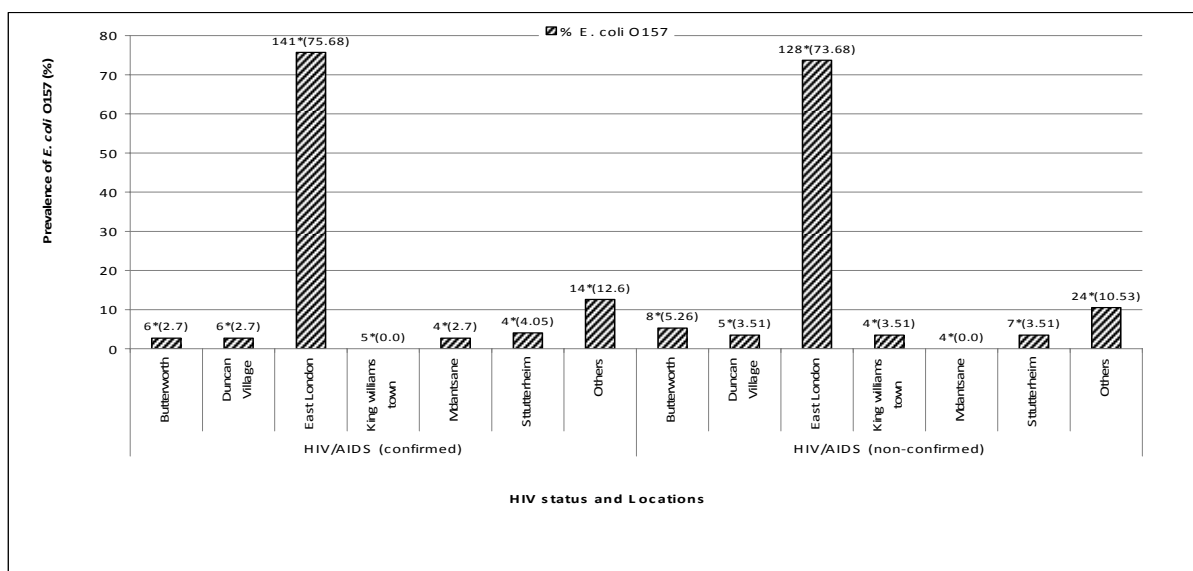


Figure 6.5: Prevalence of *E. coli* O157 in diarrhoeic confirmed and non-confirmed HIV/AIDS patients visiting Frere Hospital, East London from various locations. (*) Number of patients diagnosed with diarrhoea per location.

6.3.1.1 *Molecular analysis for E. coli O157:H7 from stools of confirmed and non-confirmed HIV/AIDS diarrhoeic patients.*

The use of PCR to characterize genes associated with *E. coli* O157:H7 has been documented (Gannon *et al.*, 1997a, b; Fratamico *et al.*, 2000; Feng and Monday, 2000; Wang *et al.*, 2002). *Escherichia coli* O157:H7 is often diagnosed based on its virulence factors. Some of the virulence factors are *stx1*, *stx2*, *hlyA*, *fliC_{H7}*, *rfbE_{O157}*, *eaeA* and many other genes (Feng and Monday, 2000; Wang *et al.*, 2002). During this study, we employed PCR method to characterize the presumptive *E. coli* O157 isolates from the stool swab suspensions. Subsequent PCR analysis of the isolates using primers specific for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes indicated the presence of *E. coli* O157:H7.

Approximately 14.86% of the representatives of presumptive *E. coli* O157 isolates from stool swabs of confirmed HIV/AIDS diarrhoeic patients were all positive for *fliC_{H7}* genes. However, only 12.16% of these isolates carried *rfbE_{O157}* and *eaeA* genes. The other 2.70% lacked *rfbE_{O157}* and *eaeA* genes. Such isolates were not considered as *E. coli* O157:H7. On the other hand, 12.28% of the representatives of presumptive *E. coli* O157 isolates from stool swabs of non-confirmed HIV/AIDS diarrhoeic patients that were positive for *fliC_{H7}*, only 8.77% carried *rfbE_{O157}* and *eaeA* genes. The other 3.51% lacked *rfbE_{O157}* and *eaeA* genes. Only the representatives of presumptive *E. coli* O157 that had the three target genes (*fliC_{H7}*, *rfbE_{O157}* and *eaeA*) were considered as *E. coli* O157:H7. This implies that the percentage proportion of confirmed diarrhoeic HIV/AIDS patients who had *E. coli* O157:H7 was higher than that for the non-confirmed diarrhoeic HIV/AIDS patients. Out of the nine patients whose presumptive *E. coli* O157 isolates were positive for genes specific for *E. coli* O157:H7, five were from East London, two from Stutterheim and one each from Chulumna and Duncan Village ((Tables 6.1a).

Table 6.1(a): Summarized PCR amplified genes of *E. coli* O157:H7 isolated from stool swabs of confirmed HIV/AIDS diarrhoeic patients

Patient location	Amplified genes			Patients positive for <i>E. coli</i> O157:H7
	<i>fliC_{H7}</i>	<i>rfbE_{O157}</i>	<i>eaeA</i>	
Cathcart	+	+	+	1
Duncan Village	+	+	+	1
East London	+	+	+	6
East London*	+	—	—	0
East London*	+	+	—	0
Gompo*	+	—	—	0
Stutterheim	+	+	+	1
Total number of patients positive for <i>E. coli</i> O157:H7				9(3*)

"+" target gene present, "—" target gene absent. n = 25 Representative number of presumptive *E. coli* O157 characterized by PCR. (*) represent patients whose *E. coli* O157 only had either 1 or 2 of the targeted genes. These patients were not considered as being positive for *E. coli* O157:H7 due to the absence of the other genes.

Five of the 57 (8.77%) presumptive *E. coli* O157 isolates from stool swabs of non-confirmed HIV/AIDS diarrhoeic patients carried *fliC_{H7}*, *rfbE_{O157}* and the attaching and effacing (A/E) (*eaeA*) genes. Out of these 5 patients, 4 of them were from East London and 1 was from Butterworth (Tables 6.1b).

Table 6.1(b): Summarized PCR amplified genes of *E. coli* O157:H7 isolated from stool swabs of non-confirmed HIV/AIDS diarrhoeic patients

Patient location	Amplified genes			Patients positive for <i>E. coli</i> O157:H7
	<i>fliC_{H7}</i>	<i>rfbE_{O157}</i>	<i>eaeA</i>	
East London	+	+	+	4
East London*	+	+	–	0
East London*	–	+	–	0
Butterworth	+	+	+	1
Total number of patients positive for <i>E. coli</i> O157:H7				5(2*)

"+" target gene present, "–" target gene absent. n = 29 Representative number of presumptive *E. coli* O157 characterized by PCR. (*) represent patients whose *E. coli* O157 only had either 1 or 2 of the targeted genes. These patients were not considered as being positive for *E. coli* O157:H7 due to the absence of the other genes.

The PCR detection of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes of *E. coli* O157:H7 is an excellent indication of the presence of *E. coli* O157:H7 (Wang *et al.*, 2002, Cagney *et al.*, 2004, Obi *et al.*, 2004). The PCR analysis using *fliC_{H7}*, *rfbE_{O157}* and *eaeA*-specific primers confirmed that a genetic region homologous in size to the *E. coli* O157:H7 *fliC_{H7}*, *rfbE_{O157}* and *eaeA* structural gene was present in the *E. coli* O157 isolates. It is therefore interesting to note that the PCR assay was successful in amplifying the 247 bp *fliC_{H7}*, 327 bp *rfbE_{O157}* and 248 bp *eaeA* fragments that were present in the genomic DNA of the isolates from stool swabs suspensions of confirmed

and non-confirmed HIV/AIDS diarrhoeic patients. Representative gel electrophoresis profiles of amplified products of target genes for *E. coli* O157:H7 are illustrated in Figure 6.6.

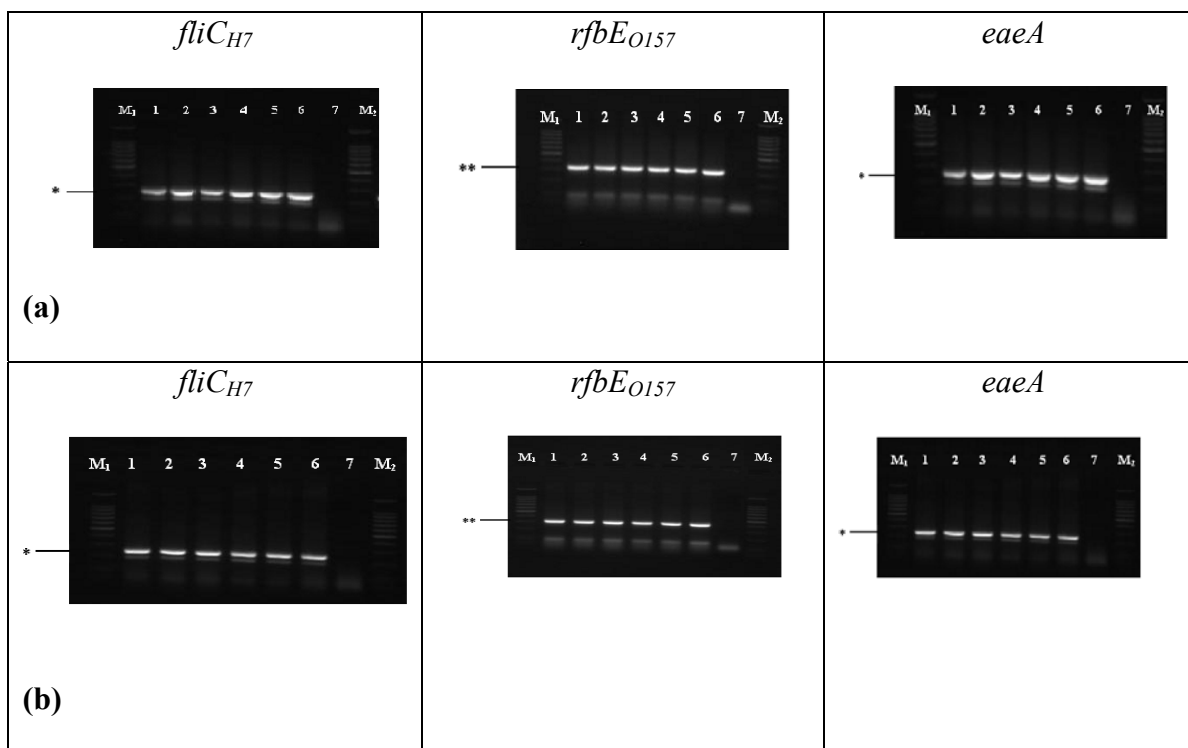


Figure 6.6: The amplified *fliCH7*, *rfbEO157* and *eaeA* gene of *E. coli* O157:H7 isolated from stool swabs of (a) confirmed HIV/AIDS diarrhoeic patients (b) non-confirmed HIV/AIDS diarrhoeic patients. Lanes M₁ & M₂: 100 bp DNA ladder marker (Promega, USA). All lanes 1 and 7: Positive (*E. coli* O157:H7, ATCC 43895) and negative control respectively. In (a) lanes 2: F₂₃₅, lanes 3: F₂₃₇, lanes 4: F₂₄₂, lanes 5: F₂₄₇, lanes 6: F₃₂₃ whereas in (b) lanes 2: F₇, lanes 3: F₁₁₆, lanes 4: F₁₁₇, lanes 5: F₁₂₅ and lanes 6: F₃₅₅. Expected amplifications were at 247, 327 and 248 bp as indicated by *, ** and * respectively

6.3.2 Statistical analysis of results

The χ^2 test predicted that gender was a significant variant for *E. coli* O157:H7 infection and that females were more likely to be infected with *E. coli* O157:H7 than males. However, age and race were not significant in determining the prevalence of *E. coli* O157:H7 in confirmed and non-confirmed HIV/AIDS patients ($P \leq 0.05$).

6.3.3 Anti-microbial susceptibility of *E. coli* O157:H7 isolates

Formerly curable bacterial diseases have developed antibiotic resistance and new bacterial pathogens have emerged. This problem has been compounded by the global spread of HIV and AIDS as reported by Paine and Flower (2002). The duo also blames this phenomenon primarily on antibiotic mishandling by both health practitioners and patients (Paine and Flower, 2002). Resistance to antibiotics by bacterial pathogens infecting HIV/AIDS patients, just as in non-HIV/AIDS patients, may spread through the resident bacteria developing resistant genes against drugs that are being used on these patients. The pathogenic bacteria may then acquire the resistance genes through lateral or horizontal gene transfer (Ochman *et al.*, 2000; Paine and Flower, 2002).

The present study equally investigated anti-microbial susceptibilities of the *E. coli* O157:H7 isolates towards some commonly used antibiotics. All the fourteen (100%) *E. coli* O157: H7 isolates from the stools of confirmed and non-confirmed HIV/AIDS patients were susceptible to amikacin. Susceptibility (S) to ampicillin was amongst 62.97% of the *E. coli* O157:H7 isolates from the stools of confirmed HIV/AIDS patients and to 80% of *E. coli* O157:H7 isolates from the stools of non-confirmed HIV/AIDS patients were susceptible to ceftriaxone. All the nine out of nine (9/9) *E. coli* O157:H7 isolates from stools of confirmed

HIV/AIDS patients were resistant to gentamycin, erythromycin and tetracycline whereas the isolates from stools of non-confirmed HIV/AIDS patients had 100% resistance against gentamycin and erythromycin only. Approximately 33.33% and 40% of *E. coli* O157:H7 isolates from stools of confirmed and non-confirmed HIV/AIDS patients respectively were resistant to ampicillin. An intermediate susceptibility to chloramphenicol was observed from 88.88% of the isolates from stool swabs of confirmed HIV/AIDS patients whereas 77.8% of the isolates were intermediately susceptible (I) to both nalidixic acid and ceftriaxone (Table 6.2).

The high rate of resistance against the antibiotics by *E. coli* O157:H7 isolates from stools of confirmed and non-confirmed HIV/AIDS diarrhoeic patients could be due to the use of antibiotics by these patients to control their diarrhoeal conditions (Kelly *et al.*, 1996). However, the type of antibiotics, which were prescribed to the patients, was not established, even though, most HIV/AIDS patients are often assumed registered for antiretroviral drugs. The present study could assist medical practitioners in the Eastern Cape in the administration of antibiotics to patients, taking into account their HIV/AIDS status. During a diarrhoea outbreak in Cameroon, the *E. coli* O157:H7 that were isolated from the stool specimen were found to be resistant to amoxicillin and chloramphenicol, but were sensitive to tetracycline and nalidixic acid. The antibiotic profile of the present study and the Cameroon one; show how antibiotic profile of organisms can vary from country to country.

Table 6.2: Distribution of susceptibility of *E. coli* O157:H7 isolated from stool swab suspension of confirmed and non-confirmed diarrhoeic HIV/AIDS patients to antimicrobial agents.

Antibiotic agent	Antibiotic disc content (µg)	Distribution %		
		R	I	S
Gentamycin	10	100	0	0
Ampicillin	10	64.29	7.13	28.57
Chloramphenicol	30	0	85.71	14.29
Nalidixic acid	30	0	78.57	21.43
Amikacin	30	0	0	100
Ceftriaxone	30	0	78.57	21.53
Erythromycin	15	100	0	0
Tetracycline	30	78.57	0	21.43

^aR, Resistant, I, Intermediate, S, Susceptible. ^bThe interpretation to achieve the above susceptibility percentages was based on the breakpoints of zone diameters for individual antibiotic agents as outlined in NCCLS ninth informational supplement of 1999: document M100–S9.

6.4 Conclusion and Recommendations

Escherichia coli O157:H7 was more prevalent in the stools of confirmed HIV/AIDS patients than in those of non-confirmed HIV/AIDS patients. However, the prevalence was much more dependent on gender than the age, race and HIV/AIDS status of the patients. The distribution of the *E. coli* O157 strains with regard to the age of the patients showed that *E. coli* O157:H7 infects all ages. The prevalence of *E. coli* O157:H7 was ubiquitous with respect to patients' locations; however, it was noted mostly in urban patients. Although some of the *E. coli* O157:H7 isolates were sensitive to some of the commonly used antibiotics, previous studies have opposed the use of antibiotics in the treatment of diarrhoea linked to *E. coli* O157:H7 (Wong *et al.*, 2000). It is recommended that HIV/AIDS patients with prolonged diarrhoea should be routinely screened for this bacterium. The Eastern Cape Department of Health should implement strategies to curb possible outbreaks of *E. coli* O157:H7.

References

- Bradshaw, D., N. Nadine, L. Ria, G. Pam, J. Jané, N. Beatrice, N. Rosana, P. Desirée, P. and S. Michelle.** 2000. South African National Burden of Disease Study: Mortality Estimates for Eastern Cape Province. A publication OF Medical Research Council of South Africa: Burden of Disease Research Unit; 1–16. Available from: <http://www.mrc.ac.za>. Accessed 06th February, 2007.
- Cagney, C., H. Crowley, G. Duffy, J-J. Sheridan, S. O'Brien, E. Carney, W. M. Anderson, D. A. Dowell, I. S. Blair, and R. H. Bisho.** 2004. Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. *J. Food Microbiol.*; **21**: 203–212.
- CDC (Centers for Disease Control).** 2004: FoodNet Surveillance Report for 2004 pp 203. Available at: www.cdc.gov/foodnet/annual/2004/Report.pdf. (Accessed: 20th September, 2007).
- CSE.** 2006. Water pollution and health: A deadly burden. In CSE draft dossier: Health and environment, environment and diseases. Available from: www.cseindia.org/programme. Accessed 29 March 2007.
- Cunin, P., T. Etienne, G. Yves, N. Chouaïbou, B. Raymond, M. Jacques, and M. V. M. Paul.** 1999. An Epidemic of Bloody Diarrhea: *Escherichia coli* O157 Emerging in Cameroon? *Emerg. Infect. Dis.*; **5(2)**: 285–290.
- Department of Health.** 2006. HIV/AIDS and STI Report, 2005/2006; 1 – 9.
- Dixon, W.J. and F.J. Massey.** 1983. Introduction to Statistical Analysis. 4th edition. McGraw-Hill pp.105–107.
- Dorrington, R. E., L. F. Johnson, D. Bradshaw, and T. Daniel.** 2006. The Demographic

- Impact of HIV/AIDS in South Africa. National and Provincial indicators for 2006. Cape Town: Centre for Actuarial Research, South African Medical Research Council and Actuarial Society of South Africa. ISBNB 0-7992-2322-0
- Dynal Product Brochure.** 2006. *Dynabeads® anti-E coli*. For rapid selective enrichment of *Escherichia coli* O157:H7. Dynal A.S. Oslo, Norway.
- Feng P.** 1995. *Escherichia coli* serotype o157:h7: novel vehicles of infection and emergence of phenotypic variants. Ph.D. U.S. Food and Drug Administration, Washington, D.C., USA. *Emerg. Infect. Dis.* **1**: 46–52.
- Feng, P. and S. R. Monday.** 2000. Multiplex PCR for detection of trait and virulence factors in enterohemorrhagic *Escherichia coli* serotypes. *Mol. Cell Prob.*; **14**: 333–337.
- Feng, P., I. F. Patricia, S. Bala. and T.S. Whittam.** 1996. Characterization of Nonmotile Variants of *Escherichia coli* O157 and Other Serotypes by Using an Antiflagellin Monoclonal Antibody. *J. of Clin Microbiol.* **34(11)**: 2856-2859.
- Fleiss, J. L., A. Tytun, and S.H.K. Ury.** 1980. A simple approximation for calculating sample sizes for comparing independent proportions. *Biomet.* **36(2)**: 343–346.
- Fratamico, P. M., L. K. Bagi, and T. Pepe.** 2000. A multiplex polymerase chain reaction assay for rapid detection and identification of *Escherichia coli* O157:H7 in foods and bovine feces. *J. Food Prot.*; **63**: 1032–1037.
- Gannon, V. P., S. D'souza, T. Graham, and R. K. King.** 1997b. Specific identification of *Escherichia coli* O157:H7 using a multiplex PCR assay. *Adv. Exper. Med. Biol.*; **412**: 81– 82
- Gannon, V. P., S. D'souza, T. Graham, R. K. King, K. Rahn, and S. Read.** 1997a. Use of the flagella H7 gene as a target in multiplex PCR assays and improved specificity in

- identification of enterohemorrhagic *Escherichia coli* strains. *J. Clin. Microbiol.*; **35**: 656–662.
- Gazzard, B. G.** 1988. HIV disease and the gastroenterologist. *Gut*; **29**:1497–1505.
- Gray, L. D.** 1995. *Escherichia, Salmonella, Shigella, and Yersinia*. In: Murray P. R, Baron E. J, Pfaller M. A, Tenover F. C, Yolken R. H, editors. Manual of clinical microbiology. 6th ed. Washington, D.C.: American Society for Microbiology; Press; pg 450–456.
- Hayes, C. E. Elliot, E. Krales, D. Goulda.** 2003. Food and Water Safety for Persons Infected with Human Immunodeficiency Virus. *Clin. Infect. Dis.*; **36(S2)**: S106–S109.
- Heuvelink, A. E., F. I. A. M Van Den Biggelaar, J. T. M. Zwartkruis-nahuis, R. G. Herbes, R. Huyben, N. Nagelkerkr, W. J. G. Melchers, L. A. H. Monnens, and E. De Boer.** 1998. Occurrence of verocytotoxin producing *Escherichia coli* O157:H7 on Dutch dairy farms. *J. Clin. Microbiol.*; **36(12)**: 3480–3487.
- Jorgensen, J. H., J. D. Turnidge, and J. A. Washington.** 1999. *Antibacterial susceptibility tests: dilution and disk diffusion methods*. In: Murray, P. R., Pfaller, M. A., Tenover, F. C., Baron, E. J. and Yolken, R. H. ed. *Manual of clinical microbiology*, 7th ed. Washington, DC: American Society for Microbiology Press; pg. 1526–1543.
- Kelly, P., F. Lungu, K. Eileen, B. Rachel, F. Kazembe, J. Pobee, and M. Farthing.** 1996. Albendazole chemotherapy for treatment of diarrhoea in patients with AIDS in Zambia: a randomised double blind controlled trial. *J. Biomed.*; **312**: 1187–1191
- Koyange, L., G. Ollivier, J. J. Muyembe, B. Kebela, M. Gouali, and Y. Germani.** 2004. Enterohemorrhagic *Escherichia coli* O157, Kinshasa. *Emerg. Infect. Dis.* Available from: <http://www.cdc.gov/ncidod/EID>. Accessed 17th February 2007

- May, G. R., M. J. Gill, and D. L. Church.** 1993. Gastrointestinal symptoms in ambulatory HIV-infected patients. *Dig. Dis. Sci.*; **138**: 1388–1394.
- Mitchell, D. K., J. Snyder, and L. K. Pickering.** 1998. *Gastrointestinal infections*. In: Pizzo, P. A. and C. M. Wilfert, (Ed.) *Pediatric AIDS: the challenge of HIV infection in infants, children and adolescents 3rd ed.* Baltimore, MD: Williams and Wilkins; pg 267– 291.
- Momba, M. N. B., Z. Tyafa, N. Makala, B. M. Brouckaert, and C. L. Obi.** 2006. Safe drinking water still a dream in rural areas of South Africa. Case study: The Eastern Cape Province. *Water, SA*; **32**: 715–720.
- Müller, E. E., Grabow, W. and M. M. Ehlers.** 2003. Immunomagnetic separation of *Escherichia coli* O157:H7 from environmental and wastewater in South Africa. *Water, SA*; **29**: 427– 432.
- NCCLS, (National Committee for Clinical Laboratory Standards).** Performance standards for antimicrobial susceptibility testing; ninth informational supplement. Wayne, Pennsylvania: NCCLS; document M100-S9, 1999: 19.
- Obi C. L., J. Ramalivhana, M. N. B. Momba, B. Onabulu, J. O. Igumbor, M. Lukoto, T. B. Mulaudzi, E. L. Jansen van Rensburg, E. Green, and S. Ndou.** 2007. Antibiotic resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients with and without diarrhea and their household drinking water in rural communities in Limpopo Province South Africa. *Afri. J. Biotech.*; **6(8)**: 1035–1047.
- Obi, C. L., N. Potgieter, P. O. Bessong, E. O. Igumbor, and E. Green.** 2004. Gene encoding virulence makers among *Escherichia coli* isolates from diarrheic stools

- samples and river sources in rural Venda communities of South Africa. *Water, SA*; **30** (1): 37–42.
- Ochman, H., J. G. Lawrence, and E. A. Groisman.** 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature*; **405**: 299–304.
- Olorunshola, I. D., S. I. Smith, and A. O. Cker, A. O.** 2000. Prevalence of EHEC O157:H7 in patients with diarrhoea in Lagos, Nigeria. *APMIS*.; **108**: 761–763.
- Paine, K, and D. R. Flower.** 2002. Bacterial bioinformatics: Pathogenesis and the genome. *J. Mol. Microbiol. Biotech.*; **4**(4): 357–365.
- Radu, S., A. M. Shahilah, R. Gulam, A. Zainori, M. Tadaaki, A. Norio, B. K. Yung, O. Jun, and N. Mitsuaki.** 1998. Detection of *Escherichia coli* O157:H7 in beef marketed in Malaysia. *Appl. Environ. Microbiol.*; **64**: 1153–1156.
- Renter, D. G., J. M. Sargeant, R. D. Oberst, and M. Samadpour.** 2003. Diversity, frequency, and persistence of *Escherichia coli* O157 strains from range cattle environments. *J. Appl. Environ. Microbiol.*; **69**: 542–547.
- Sein, P. P., A. A. Hoosen, H. H. Crew-Brown, Y. Hoovadia, M. G. Dove, O. Heid, H. J. Koornhof, S. Oliver, O. Perovi, B. Prinsloo, J. M. N. Van RENSBURG, J. Simpson, A. W. Sturm, J. Wadula and E. Wasserman.** 2005. Antimicrobial susceptibility profile of selected invasive pathogens from academic hospitals of South Africa for the years 2001 – 2004. *SA J. Epidemiol. Infect.*; **20**(3): 85– 89.
- Shisana, O., T. Rehle, L. Simbayi, W. Parker, K. Zuma, A. Bhana, C. Connolly, S. Jooste and V. Pillay.** 2005: *South African National HIV Prevalence, HIV Incidence, Behaviour and Communication Survey; 2005*. Cape Town: HSRC Press. pp 157.

- Torres, J., C. López-Saucedo, J. F. Cerna, N. Villegas-Sepulveda, R., Thompson, and F. R. Velazquez.** 2003. Single multiplex polymerase chain reaction to detect diverse loci associated with diarrheagenic *Escherichia coli*. *Emerg. Infect. Dis.*; **9**: 127–131.
- Wang, G., G. C. Clifford, and G. R. Frank.** 2002. Detection in *Escherichia coli* of the Genes Encoding the Major Virulence Factors, the Genes Defining the O157:H7 Serotype, and Components of the Type 2 Shiga Toxin Family by Multiplex PCR. *J. Clin. Microbiol.*; **40**: 3613–3619.
- Wong, C. S., S. Jelacic, R. L. Habeeb, S. L. Watkins, and P. I. Tarr.** 2000. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N. Engl. J. Med.*; **342**: 1930–1936.

CHAPTER 7

	Page
Table of contents.....	334
List of Figures	336
Abstract.....	337
7.1 Introduction.....	338
7.2 Materials and Methods.....	341
7.2.1 Study population.....	341
7.2.2 Sampling of water, meat and meat products and vegetables.....	341
7.2.3 Sampling of stool swabs.....	342
7.2.3.1 <i>Scientific ethics and informed consent.....</i>	342
7.2.4 Culture-based isolation and identification of <i>E. coli</i> O157:H7.....	343
7.2.4.1 <i>Enrichment and immunomagnetic separation (IMS)</i>	
<i>Recovery.....</i>	343
7.2.4.2 <i>Identification of presumptive <i>E. coli</i> O157.....</i>	343
7.2.5 Molecular characterization of <i>E. coli</i> O157:H7 using PCR.....	344
7.2.5.1 <i>Bacterial DNA Extraction.....</i>	344
7.2.5.2 <i>Amplification of <i>fliC_{H7}</i>; <i>rfbE_{O157}</i> and <i>eaeA</i> genes.....</i>	344
7.2.5.3 <i>DNA electrophoresis.....</i>	345
7.2.6 Statistical Analysis.....	346
7.3 Results.....	346

7.3.1	Prevalence of presumptive <i>E. coli</i> O157.....	346
7.3.2	Molecular analysis for <i>E. coli</i> O157:H7 from water, meat and meat products and vegetables.....	347
7.3.3	Molecular analysis for <i>E. coli</i> O157:H7 from stools of confirmed and non-confirmed HIV/AIDS patients.....	349
7.4	Discussion.....	351
7.4.1	Conclusion and recommendation.....	354
	References.....	355

LIST OF FIGURES

Figure 7.1:	The amplified <i>fliC_{H7}</i> , <i>rfbE_{O157}</i> and <i>rfbE_{O157}</i> genes of <i>E. coli</i> O157:H7 isolated from water.....	347
Figure 7.2:	The amplified <i>fliC_{H7}</i> , <i>rfbE_{O157}</i> and <i>rfbE_{O157}</i> genes of <i>E. coli</i> O157:H7 isolated from meat and meat products.....	348
Figure 7.3:	The amplified <i>fliC_{H7}</i> , <i>rfbE_{O157}</i> and <i>rfbE_{O157}</i> genes of <i>E. coli</i> O157:H7 isolated from vegetable samples.....	349
Figure 7.4:	The amplified <i>fliC_{H7}</i> , <i>rfbE_{O157}</i> and <i>eaeA</i> genes of <i>E. coli</i> O157:H7 isolated from stool swabs of (i) confirmed HIV/AIDS (ii) non- confirmed HIV/AIDS patients with diarrhoea.....	351

CHAPTER 7

Abstract

Water and food-borne *E. coli* O157:H7 has an epidemiological link with diarrhoea, though the link between water and food-borne *E. coli* O157:H7 and diarrhoeic conditions in HIV/AIDS patients is only vaguely understood. The current chapter investigated the molecular relatedness between *E. coli* O157:H7 isolated from water, meat, and meat products and vegetables and from stools of confirmed and non-confirmed HIV/AIDS patients with diarrhoea. Culture-based and polymerase chain reaction techniques were used to identify *E. coli* O157:H7. Thirty five percent (35.5%) of meat products, 25.5 % of water, and 21.7% of vegetables as well as 56.5% and 43.5% of stools of confirmed and non-confirmed HIV/AIDS patients, were presumptively positive with *E. coli* O157. Molecular results indicated that 10.3%, 8.6% and 7.8% of the vegetables, water and meat products examined carried *E. coli* O157:H7, which had homologous *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genetic loci to the genes of some *E. coli* O157:H7 isolated from 12.2% and 8.8% of the stools of confirmed, and non-confirmed HIV/AIDS patients, respectively. Water, meat, and meat products and vegetables are likely sources of *E. coli* O157:H7 potentially capable of causing diarrhoea in humans especially HIV/AIDS patients. Great care should be exercised to ensure that water and foods consumed by HIV/AIDS patients are safe, as contaminated water, and foods can cause secondary infections in these patients.

Keywords: Molecular relatedness, Drinking water, meat and vegetables, HIV/AIDS, stool specimens, *E. coli* O157:H7 and PCR.

7.1 Introduction

The microbiological safety of drinking water and foods remains a challenging problem globally, especially in this era of Human Immune Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) (Hayes *et al.*, 2003). Morbidity and mortality resulting from the consumption of water and foods contaminated by enteric pathogens may significantly increase in patients infected with HIV/AIDS (Hayes *et al.*, 2003). The major cause of morbidity and mortality due to water and food-borne related disease outbreaks is diarrhoea, which is also a common illhealth in HIV/AIDS patients. This situation can be worse in rural areas where communities consume waters and foods of an unacceptable quality as well as living under unsanitary conditions (Obi and Bessong, 2002; Momba *et al.*, 2003a, b; Phaswana-Mafunya 2006).

In the rural areas of South Africa, just as in any other developing country in the world, people usually obtain their drinking water from rivers, ponds, boreholes and springs as well as standpipes. These waters in some cases maybe untreated and hence are not suitable for human consumption (Momba *et al.*, 2004a, b; 2005a, b; 2006a, b).

Foods may also be contaminated with pathogenic bacteria during transportation, vending or cooking in kitchens due to poor handling practices (Vorster *et al.*, 1994; Mosupye and Von Holy, 1999; Haysom and Sharp, 2004; Du toit and Irma, 2005). Water and foods have been implicated in *E. coli* O157:H7 outbreaks since its first recognition in 1982 (Grimm *et al.*, 1995; Ihekweazu *et al.*, 2006).

The first case of *E. coli* O157:H7 in South Africa was reported in 1990. The bacterium was isolated from a man in the city of Johannesburg (Browning *et al.*, 1990). Ten years later, an outbreak of diarrhoeal infections suspected of being linked to *E. coli* O157:H7 occurred in the

Gauteng region (Galane and Le Roux, 2000). However, no cases of *E. coli* O157:H7 have been reported in South Africa since 2001 even though studies on environmental samples such as water have yielded some suspicious findings (Müller *et al.*, 2001, 2003, Momba *et al.*, 2006b). A study on the prevalence of enteric bacterial pathogens in the stools of HIV/AIDS patients among the Venda community in the Limpopo Province of South Africa revealed a high prevalence of enteric bacterial pathogens such as *Salmonella enteritidis*, *Salmonella typhimurium*, *Shigella dysenteriae*, and verotoxigenic *E. coli* though *E. coli* O157:H7 was not investigated (Obi and Bessong, 2002). Recently, Obi *et al.* (2007) reported the isolation of enteric bacterial pathogens from stools of HIV/AIDS patients as well as from drinking waters obtained from the households of these patients. Again no *E. coli* O157:H7 was investigated in this study.

Molecular techniques such as polymerase chain reaction have been used successfully to characterise *E. coli* O157:H7 from drinking waters (Muller *et al.* 2001; Bopp *et al.* 2003; Muller *et al.*, 2003). The technique has also been utilized to confirm the identity of *E. coli* O157:H7 isolated from animals and humans (Wang *et al.* 2002; Ramachandran *et al.* 2003) and from foods such as meat and meat products as well as from vegetables (Chinen *et al.*, 2001; Schroeder *et al.*, 2003; Stampi *et al.*, 2004).

Escherichia coli O157:H7 possess genetic markers that are essential for their survival as well as pathogenicity. Such genetic markers include known and putative virulence genes found in genomic DNA of these bacteria. The major virulence properties are usually shigatoxins *stx1* and *stx2* (Gyles, 1992; Bopp *et al.*, 2003; Durso *et al.*, 2005). In addition to these, other virulence-associated factors expressed by *E. coli* O157:H7 include proteins called intimin that are encoded by the *eaeA* gene and are responsible for causing attaching and effacing (A/E) lesions in the intestinal mucosa of the infected patients (Pradel *et al.*, 2000). Enterohemolysin

(*Ehly*), also called enterohemorrhagic *E. coli* haemolysin encoded by the *ehxA* gene has also been used as a virulence factor to characterise *E. coli* O157:H7 (Bopp *et al.*, 2003; Jin *et al.*, 2005). Other genes, though may not necessarily indicate the virulent nature of these bacteria, are very helpful in differentiating them from other *E. coli* strains. These include but are not limited to *fliC_{H7}*, *rfbE_{O157}* and 16S rRNA (Wang *et al.*, 2000, 2002; Jin *et al.*, 2005). These markers have served as excellent fingerprints in the identification of *E. coli* O157:H7.

Even though there have been no reports of water and food-borne *E. coli* O157:H7 outbreaks documented in South Africa in the recent years, the situation could change due to the rise in the number of HIV/AIDS cases in the country and the loss of immunity (Dorrington *et al.*, 2006). The Eastern Cape Province of South Africa where this study was conducted is predominantly rural and is one of the poorest provinces in South Africa with over 68% of the province's population living below the National poverty line, and an estimated 10% prevalence rate of HIV/AIDS (UNDP, 2004; Dorrington *et al.*, 2006). The province relies heavily on meat and meat products, which globally have a historical link to *E. coli* O157:H7, as the communities' daily foods. The quality of drinking water supplied to the communities living in this province is poor as was evidenced by the works of Momba *et al.* (2003b, 2006b).

The establishment of a molecular link between *E. coli* O157:H7 isolated from different hosts and environments enables the tracing of the sources of *E. coli* O157:H7 that may act as potential causes of disease outbreaks in humans. This study reports on the molecular relatedness that exists between *E. coli* O157:H7 isolated from drinking water, meat and meat products and vegetables as well as from the stools of confirmed and non-confirmed HIV/AIDS patients who visited Frere Hospital, in the Amathole District, for the treatment of diarrhoea. Published *fliC_{H7}*, *rfbE_{O157}* and *eaeA* gene primers were used to characterize the *E. coli* O157:H7 isolates.

7.2 Materials and Methods

7.2.1 Study population

The study was conducted between March 2005 and August 2006. The Amathole District was selected based on familiarity with the area, the high HIV prevalence and the presence of a referral hospital, Frere Hospital, which is situated in the City of East London, and caters for HIV/AIDS patients of various races, genders and age groups from the rural areas of the district. Fort Beaufort, Alice, Dimbaza, Mdantsane, Ngwenya and Kwasaki were chosen because potential pathogenic *E. coli* strains have been isolated from water sources used by these communities (Momba and Kaleni, 2003; Momba and Notshe, 2003; Momba *et al.*, 2004a, b; 2005a, b). The meat and meat products (biltong, cold meat, mincemeat and polony) and vegetables (cabbages, carrots, cucumbers, onions and spinach) were chosen due to their frequency of consumption by people living in this area.

7.2.2 Sampling of water, meat and meat products and vegetables

A total of 180 water samples (30 for each site) were collected from the standpipes that supplied treated drinking water to the communities of Fort Beaufort, Alice (Fort Hare), Dimbaza, Mdantsane, and from untreated water from boreholes that are used by the communities of Ngwenya and Kwasaki. Internationally accepted techniques and principles were used during the collection of the water samples (Momba and Makala, 2004). Another 180 samples of meat and meat products (45 samples each of biltong, cold meat, mincemeat and polony) and 180 vegetable samples (36 each of cabbages, carrots, cucumbers, onions and spinach) were purchased either from supermarkets, butcheries or shops serving the communities living in Fort Beaufort, Alice

and Mdantsane.

7.2.3 Sampling of stool swabs

Three hundred and sixty stool swabs were obtained from confirmed HIV/AIDS (180 stool swabs) and non-confirmed HIV/AIDS (180 stool swabs) patients with diarrhoea, who were visiting Frere Hospital for treatment; using sterile cotton swabs (Merck, Johannesburg, SA) dipped in sterile specimen bottles filled up with 30 ml of sterile saline solutions (Merck, SA). The confirmed HIV/AIDS patients had already been tested for HIV at the HIV/AIDS clinic of Frere Hospital and were known by the Hospital clinicians to be carriers of HIV. The patients' locations and their diarrhoeal conditions as well as their HIV/AIDS status were recorded. Anonymity of the patients was protected as much as possible. Diarrhoeic condition in this study was diagnosed by the nurse in charge in the case of patients experiencing 3 or more watery stools in 24 h. All the samples were transported to the laboratory for the isolation of *E. coli* O157:H7 on ice in their respective cooler boxes. Microbiological analyses were performed within 1 to 4 h of sample collection.

7.2.3.1 *Scientific ethics and informed consent*

The University of Fort Hare's Govan Mbeki Research and Development Centre, the Provincial Department of Health (Bisho), and the Regional Eastern Cape Ethical Review Committees all approved the protocol that was used for the stool swab collection. Informed consent was obtained from patients or their guardians with the help of a nurse with Voluntary Counselling and Testing (VCT) skills and experience.

7.2.4 Culture-based isolation and identification of *E. coli* O157:H7

7.2.4.1 Enrichment and immunomagnetic separation (IMS) recovery

For water and stool specimens of confirmed and non-confirmed HIV/AIDS patients, one millilitre of a water sample or stool suspension was added to 99 ml of modified *E. coli* (mEC) broth containing novobiocin (n) (Merck, SA) (Heuvelink *et al.*, 1998). The meat and meat product and vegetable samples were also enriched by suspending 10 g or 25 g of a meat or vegetable sample, respectively, in 90 or 225 ml of modified *E. coli* (mEC) broth containing 20 µg/ml of novobiocin (n) (Merck, SA) respectively, and were homogenized by blending for 1 min (Cagney *et al.*, 2004). The inoculated broths for the respective samples were incubated in a rotatory incubator shaker (Gallenkamp, Loughborough, England) at 37°C for 8 h at 143 × g. The separation of *E. coli* O157:H7 was done as described in the Dynal product brochure (Dynal Product Brochure, 2006). The bacteria-IMS bead complex was re-suspended into 1 ml of 1% (w/v) of sterile Phosphate Buffered Saline (PBS) (Merck, SA). Fifty microlitres (50 µl) of this bacteria-IMS concentrate was spread in duplicate onto Sorbitol-MacConkey agar supplemented with 0.05 mg/l of cefixime and 2.5 mg/l of potassium tellurite (CT-SMAC) (Merck, SA) (Müller *et al.*, 2003). The plates were then incubated at 37°C for 24 h. Non-Sorbitol fermenting colonies (up to 5 colourless colonies with black centres per plate per sample) were randomly selected and further plated by streaking onto Eosin Methylene Blue agar (EMBA) (Merck, SA).

*7.2.4.2 Identification of presumptive *E. coli* O157*

Presumptive *Escherichia coli* O157 colonies were identified as described by Cagney *et al.* (2004). The Oxidase test was performed on the colonies that were Gram negative prior to the conventional indole-methyl red-Voges-Proskauer-citrate (IMViC) tests (Feng, 1995; Feng *et al.*,

2000; Müller *et al.*, 2003). Colonies were subjected to IMViC test and further confirmed as *E. coli* with API 20E kits. The strips were read and the final identification was secured using API LAB PLUS computer software (BioMérieux, Marcy-Etoile, France) (Momba *et al.*, 2006b). Representatives of presumptive *E. coli* O157 isolates that scored 99.9% identification profile with API LAB PLUS computer software reader were used for molecular characterization using PCR technique.

7.2.5 Molecular characterization of *E. coli* O157:H7 using PCR

7.2.5.1 Bacterial DNA extraction

DNA was extracted from colonies identified as *E. coli* O157 and from a positive control strain (*E. coli* O157:H7, ATCC 43895) purchased from the Microbiology Department of the National Health Laboratory Services (NHLS), Johannesburg, South Africa. The extraction of DNA was performed as previously described by Torres *et al.* (2003). Briefly, a loop-full of overnight culture of the presumptive *E. coli* O157 colonies was suspended in 200 µl of sterile Milli-Q PCR grade water (Merck, SA) and the cells were lysed using a Dri-block DB.2A (Techne, Cape Town, SA) for 15 min at 100°C. The cell debris was removed by centrifugation at $20\,000 \times g$ for 2 min using a MiniSpin micro centrifuge (Merck, SA). The lysate supernatant was placed on ice for 5 min. Sterile Milli-Q PCR grade water (Merck, SA) was included in each PCR assay as a negative control.

7.2.5.2 Amplification of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes

Oligonucleotide primers specific for the targeted *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes used in the polymerase chain reaction (PCR) were similar to those used by Wang *et al.* (2002). The primers

sequences that were used to identify the target genes were FliC-F 5'-TAC CAT CGC AAA AGC AAC TCC-3', FliC-R 5'-GTC GGC AAC GTT AGT GAT ACC-3' for *fliC_{H7}*. For *rfbE_{O157}*, the forward was RfbE-F 5'-CTA CAG GTG AAG GTG GAA TGG-3'; RfbE-R 5'-AATT CCT CTC TTT CCT CTG CGG-3'. The primers for *eaeA* gene were EAE-R 5'-ATG CTT AGT GCT GGT TTA GG-3', EAE-R 5'-GCC TTC ATC ATT TCG CTT TC-3'. The expected amplification sizes for *fliC_{H7}*, *rfbE_{O157}*, and *eaeA* genes were 247, 328, and 248 base pairs, respectively. A total volume of 10 µl genomic DNA was used in each PCR reaction. The PCR assays for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes were carried out in a 50 µl reaction volume. The reaction mixture contained 10X SuperTherm GOLD Buffer, 1.5 mM MgCl₂, each of the four deoxynucleoside triphosphates (dNTPs) (Southern Cross Biotechnology, Cape Town, SA) at a concentration of 0.25 mM, 100 pmol each of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* specific primers, and 5 U of *Taq* DNA polymerase (Southern Cross Biotechnology, Cape Town, SA). The reaction was carried out in the Eppendorf model AG 22331 Thermocycler (Merck, SA). The following PCR conditions for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes optimized in our laboratory were similar to those previously used by Wang *et al.* (2002): initial denaturation at 95°C for 8 min followed by 30 cycles of amplification, denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. The final extension cycle was followed by incubation at 72°C for 7 min and cooling to 4°C.

7.2.5.3 DNA electrophoresis

Ten microlitre (10 µl) aliquots of the amplicons were visualized on a 2% (w/v) agarose gel (Merck, SA) in 1 U TAE buffer (40 mM Tris-HCl, 20 mM Naacetate, 1 mM EDTA, pH 8.5) and stained with 0.5 µg/ml Ethidium Bromide (EtBr) (Merck, SA). The amplified products were photographed under the BioDoc-It System (UVP Upland, CA 91786, USA). A 100 bp DNA

ladder (Promega, Madison, USA) was included in each gel as a molecular size standard. The electrophoresis was carried out at 76 V for 1 h.

7.2.6 Statistical analysis

The statistical analysis was done by use of SAS program (SAS Institute, Cary, NC). The *Chi* square test was used to establish the significance difference between the prevalence rates of *E. coli* O157:H7 in water, meat and meat products and vegetables and in the stool samples of confirmed and non-confirmed HIV/AIDS patients. The *Chi* square test was run at a statistical significance level of ($P \leq 0.05$).

7.3 Results

7.3.1 Prevalence of presumptive *E. coli* O157

Of the 180 drinking water samples, 46 (25.56 %); 64 (35.55%) of meat and meat products, and 39 (21.67%) of the vegetable samples were presumptively positive with *E. coli* O157. Prevalence of presumptive *E. coli* O157 in the stools was at 36.39% (131/360) of which 56.5% (74/131) and 43.5% (57/131) were from stools of confirmed and non-confirmed HIV/AIDS patients, respectively. The prevalence of presumptive *E. coli* O157 in the water, meat and meat products and vegetables were not significant different. This was also noted for the prevalence of presumptive *E. coli* O157 in the stools of confirmed and non-confirmed HIV/AIDS patients ($P \leq 0.05$).

7.3.2 Molecular analysis for *E. coli* O157:H7 from water, meat and meat products and vegetables

PCR analysis revealed that 8.70% (4/46) of the water samples had *E. coli* O157:H7 through confirmation of the presence of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes in the presumptive *E. coli* O157 isolated from these water samples. The water samples that were found positive for *E. coli* O157:H7 included treated tap waters from Mdantsane, Dimbaza and Fort Beaufort standpipes and borehole water from Ngwenya. One water sample from Alice only carried *E. coli* O157 that was positive for *fliC_{H7}* (Figure 7.1).

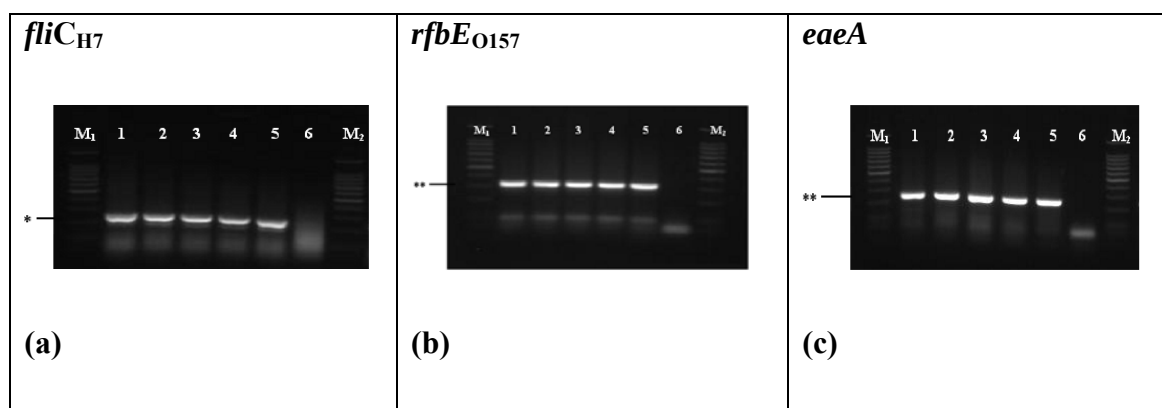


Figure 7.1: The amplified *fliC_{H7}*, *rfbE_{O157}* and *rfbE_{O157}* genes of *E. coli* O157:H7 isolated from water. Lanes M₁ & M₂: 100 bp DNA ladder marker (Promega, USA), lane 1: Positive control (*E. coli* O157:H7, ATCC 43895), lane 2: NgweW₄, lane 3: DimW₉, lane 4: MdaW₂₇, lane 5: FbW₂₇, lane 6: Negative control. The expected molecular sizes of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* fragments were at 247bp, 327bp and 248bp respectively.

In total 7.80% (5/64) of the meat and meat products had *E. coli* O157:H7. Figure 7.2 shows the gel electrophoresis profiles for the amplified target genes. For the meat and meat

products, two polony samples from Alice and Fort Beaufort, cold meat and mincemeat both from Mdantsane and biltong from Alice all carried *E. coli* O157:H7 that were positive for the targeted genes (*fliC_{H7}*, *rfbE_{O157}* and *eaeA*).

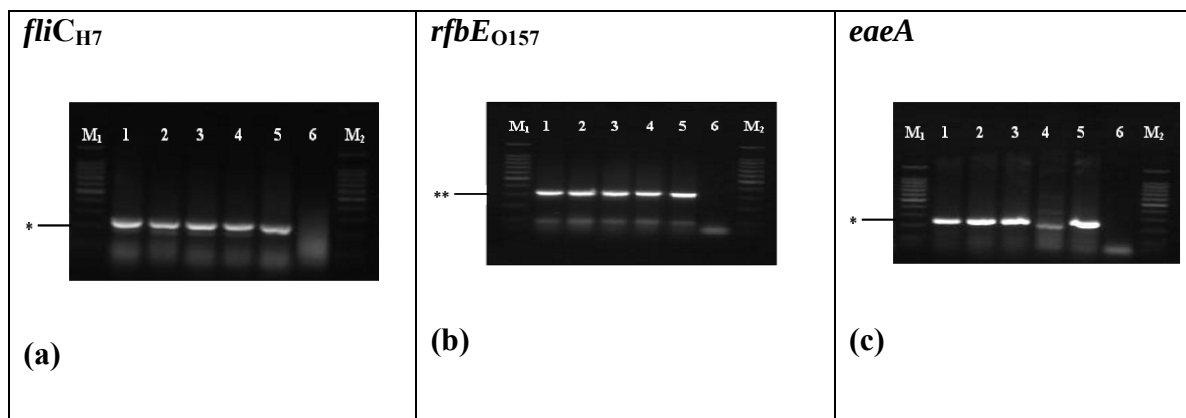


Figure 7.2: The amplified *fliC_{H7}*, *rfbE_{O157}* and *rfbE_{O157}* genes of *E. coli* O157:H7 isolated from meat and meat products. Lanes M₁ & M₂: 100 bp DNA ladder marker (Promega, USA), lane 1: Positive control (*E. coli* O157:H7, ATCC 43895), lane 2: MdaM₁, lane 3: MdaM₂₃, lane 4: MdaM₃₄, lane 5: AIM₁₈, lane 6: FBM₅₈, lane 7: Negative control. The expected molecular sizes of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* fragments were at 247bp, 327bp and 248bp respectively.

Four out of the thirty-nine (10.26%) vegetable samples that were presumptively positive of *E. coli* O157 were confirmed to carry *fliC_{H7}*, *rfbE_{O157}* and *eaeA*, which are characteristic of *E. coli* O157:H7. These included one cabbage and one cucumber sample both from Fort Beaufort, spinach and one onion sample from Mdantsane. However, two carrot (5.12%) samples (one from Alice and another from Mdantsane) carried *E. coli* O157 that were only positive for *fliC_{H7}*. Electrophoresis profiles for the target genes of *E. coli* O157:H7 isolated from the vegetables is

shown in Figure 7.3.

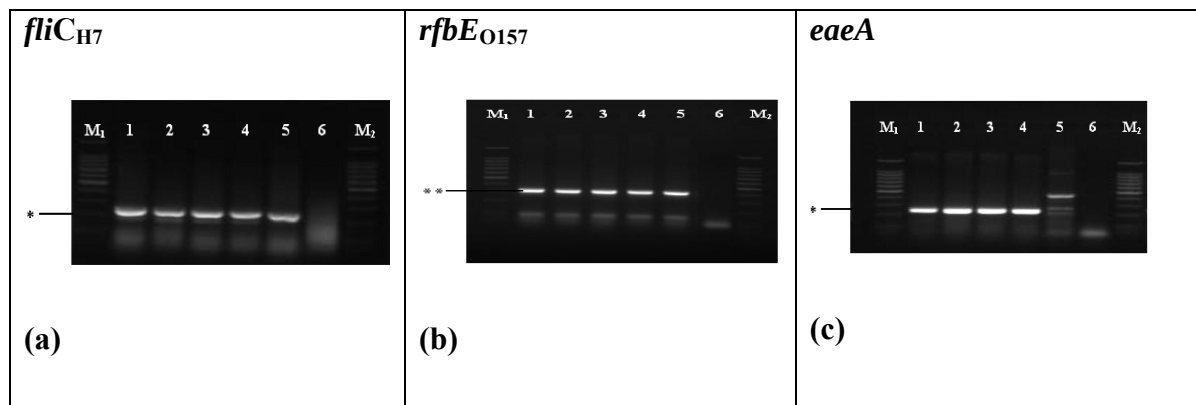


Figure 7.3: The amplified *fliC_{H7}*, *rfbE_{O157}* and *rfbE_{O157}* genes of *E. coli* O157:H7 isolated from vegetable samples. Lanes M₁ & M₂: 100 bp DNA ladder marker (Promega, USA), lane 1: Positive control (*E. coli* O157:H7, ATCC 43895), lane 2: MdaV₂₅, lane 3: FbV₁₁, lane 4: FbV₉, lane 5: MdaV₃, lane 6: Negative control. The expected molecular sizes of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* fragments were at 247bp, 327bp and 248bp respectively.

7.3.3 Molecular analysis for *E. coli* O157:H7 from stools of confirmed and non-confirmed HIV/AIDS patients

Molecular results indicated that 12.16% (9/74) and 8.77% (5/57) of stools from confirmed and non-confirmed HIV/AIDS patients with diarrhoea were positive for *E. coli* O157:H7. The confirmed HIV/AIDS patients with diarrhoea who were positive for *E. coli* O157:H7 included 6 patients from East London and one patient each from Duncan village, Stutterheim and Cathcart. On the other hand, all the stools from the non-confirmed HIV/AIDS patients with diarrhoea were from East London. Representative gel electrophoresis profiles of amplified products for the target genes (*fliC_{H7}*, *rfbE_{O157}* and *eaeA*) for the *E. coli* O157:H7 isolated from the stools of

confirmed and non-confirmed diarrhoeic HIV/AIDS patients are illustrated in Figure 7.4.

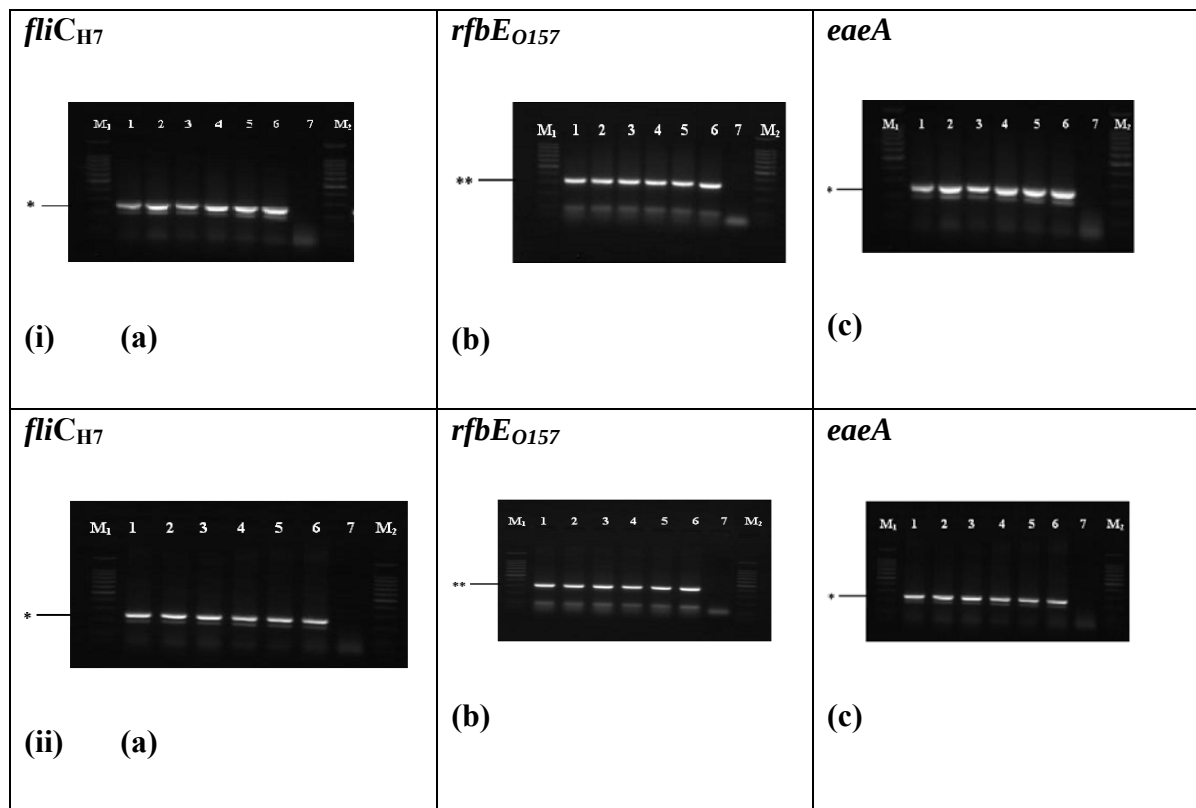


Figure 7.4: The amplified *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes of *E. coli* O157:H7 isolated from stool swabs of (i) confirmed diarrhoeic HIV/AIDS (ii) non-confirmed diarrhoeic HIV/AIDS patients with diarrhoea. Lanes M₁ & M₂: 100 bp DNA ladder marker (Promega, USA). All lanes 1 and 7: Positive (*E. coli* O157:H7, ATCC 43895) and negative control respectively. In (i) lanes 2: F₂₃₅, lanes 3: F₂₃₇, lanes 4: F₂₄₂, lanes 5: F₂₄₇, lanes 6: F₃₂₃ whereas in (ii) lanes 2: F₇, lanes 3: F₁₁₆, lanes 4: F₁₁₇, lanes 5: F₁₂₅ and lanes 6: F₃₅₅. The expected molecular sizes of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* fragments were at 247bp, 327bp and 248bp indicated by *, ** and * respectively.

7.4 Discussion

The molecular relatedness between the *E. coli* O157:H7 isolated from water, meat and meat products and vegetables as well as stools from confirmed and non-confirmed HIV/AIDS patients has been demonstrated using the target genes amplified by the PCR procedure. In all the 27 *E. coli* O157:H7, *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes were clearly amplified at the expected locations within the gene. The expected gene sizes for *fliC_{H7}*, *rfbE_{O157}* and *eaeA* were achieved at 247 bp for *fliC_{H7}*, 327 bp for *rfbE_{O157}* and 248 bp for *eaeA* genes. The PCR amplification products for the *E. coli* O157:H7 isolates from water, meat and meat products and vegetables when compared to those for the isolates from the stools of confirmed and non-confirmed HIV/AIDS patients showed a similarity in the amplified regions. These results showed that there might be an epidemiological link between *E. coli* O157:H7 isolated from water and foods and those isolated from the stools of confirmed and non-confirmed HIV/AIDS patients with diarrhoea. However, this linkage was not confirmed as only PCR technique was used to amplify the target genes. It was also noted that the patients whose stools were positive for *E. coli* O157:H7 did not come from any of the areas where either the waters or the foods were sampled. However, given that Alice, Fort Beaufort and Dimbaza; Mdantsane, Kwasiki and Ngwenya are locations within the Amathole District, confirmed and non-confirmed diarrhoeic HIV/AIDS patients could move from one place to another within the district thus increasing the possibility for these individuals to drink the water or consume meat and meat products or vegetables under investigation. This therefore calls for a more detailed molecular analysis of *E. coli* O157:H7 of water and food origin in order to establish their epidemiological linkage to diarrhoea in humans. Such studies can highlight the transmissibility of *E. coli* O157:H7 between different host environments such

as water and foods to humans. The χ^2 test ($P \geq 0.058$) predicted that HIV/AIDS status was not significant variant for *E. coli* O157 infection.

Several studies on the microbiological quality of drinking water in South African rural areas have always revealed the poor quality of waters and foods (Mosupye and von Holy, 1999; Momba *et al.*, 2004a, b; 2005a, b, 2006a, b). Momba *et al.* (2003a) reported that the Alice Water Treatment Plant (AWTP) had failed to produce water of good quality to its communities. The microbiological quality of the finished water from this plant and of samples drawn at various points in the distribution system did not meet the South African Guidelines for drinking water (DWAf, 1996; Momba *et al.*, 2003a, b). Various potential pathogenic microorganisms such as *E. coli*, *Aeromonas hydrophila*, *Salmonella arizonae*, *Vibrio fluvialis*, *Serratia odifera*, *Serratia liquefaciens*, *Serratia marcescens*, *Pseudomonas fluorescens*, and *P. aeruginosa* have been isolated from the water samples collected from on-site reservoirs and from various points in the distribution systems (Momba *et al.*, 2003a).

Water and food safety have a direct effect on the health of persons with compromised immune systems (Hayes *et al.*, 2003; Obi and Bessong, 2002; Obi *et al.*, 2007). Obi *et al.* (2007) confirmed the existence of a link between the presence of enteric bacterial pathogens such as *Salmonella*, *spp.*, *Shigella* *spp.*, *Aeromonas* *spp.*, *Campylobacter jejuni* and *C. coli* as well as *Plesiomonas* *spp.* isolated from drinking water to those isolated from stools of HIV/AIDS patients with and without diarrhoea in the Limpopo province of South Africa. Although *E. coli* was one of the pathogenic bacteria reported in that study; *E. coli* O157:H7 was not investigated.

Murinda *et al.* (2004) compared the pathogenic profiles of verotoxigenic *E. coli* O157:H7 isolated from human, foods and their animal companions using a multiplex PCR procedure and

suggested that there was a possibility of interspecies transmissibility between humans and these animal companions. Their study also indicated that these bacteria are capable of existing in and moving between different host species and host environments.

Even though only representative presumptive *E. coli* O157 isolates were used in the present study to confirm the presence of *E. coli* O157:H7, it sufficiently verified that the waters, meat and meat products and the vegetables were contaminated with these bacteria. The presence of *fliC_{H7}* and *rfbE_{O157}* genes in *E. coli* O157 has been used to confirm the serology of presumptive *E. coli* O157 isolates, however, these genes are not indicators of *E. coli* O157:H7 pathogenicity (Jin *et al.*, 2005). Separate studies have employed this dogma to confirm the identity of *E. coli* O157:H7 isolates (Wang *et al.*, 2002; Jin *et al.*, 2005). In the present study, greater emphasis was placed on the presence of *eaeA* gene in the *E. coli* O157:H7 isolates.

The presence of *eaeA* in *E. coli* O157:H7 has been linked to diarrhoeal cases in most disease outbreaks associated with this bacterium (Galane and Le Roux, 2001; Ramachandran *et al.*, 2003). This is because this gene is responsible for the attachment and effacing of the enterocytes lining the intestinal wall (Sandhu *et al.*, 1996; Gyles *et al.*, 1998; Boerlin *et al.*, 1999). A study in Spain on *E. coli* O157:H7 (87 isolates from bovine and ovine, 47 from humans and 7 from beef) that aimed at establishing the presence of *eae* revealed that all the 141 *E. coli* O157:H7 isolates carried the *eae* gene (Mora *et al.*, 2007; Blanco *et al.*, 2003). A study by Galane and Le Roux, (2001) on the molecular epidemiology of *E. coli* isolated from some South African children with diarrhoea indicated the presence of *eaeA* gene in 7.7% of *E. coli* isolates of serotype O157.

7.4.1 Conclusion and recommendation

Comparatively, the waters, meat and meat products and vegetable samples had a more or less equal prevalence of *E. coli* O157:H7. Confirmed HIV/AIDS patients were observed to have higher prevalence of *E. coli* O157:H7 than non-confirmed HIV/AIDS patients. The interesting finding in this study was the homology in the genetic loci of *fliC_{H7}*, *rfbE_{O157}* and *eaeA* genes in all the 27 *E. coli* O157:H7 isolates. The present study illustrates that water and foods are potential sources of pathogenic microorganisms that may infect humans. In conclusion, substantial genetic similarity exists among *E. coli* O157:H7 strains isolated from water, meat and meat products and vegetables as well as those isolated from the stools of confirmed and non-confirmed HIV/AIDS patients with diarrhoea. The revealing of such a genetic link suggests the presence of similar clones of *E. coli* O157:H7 in the waters and foods and that these clones can be potential sources of infection. We recommend an intensified surveillance of *E. coli* O157:H7 in waters and foods consumed by HIV/AIDS patients living in both urban and rural areas of South Africa.

References

- Blanco, J., Blanco, M., Blanco, J. E., et al.** 2003. Verotoxin-Producing *Escherichia coli* in Spain: Prevalence, Serotypes, and Virulence Genes of O157:H7 and Non-O157 VTEC in Ruminants, Raw Beef Products, and Humans. *Exp. Biol. Med.*; **228**: 345–351.
- Boerlin, P., S. A. McEwen, F. Boerlin-Petzold, J. B. Wilson, R. P. Johnson, and C. L. Gyles.** 1999. Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. *J. Clin. Microbiol.*; **37**: 497–503
- Bopp, D. J., B. D. Sauders, A. L. Waring, J. Ackelsberg, N. Dumas, E. Braun-Howland, D. Dziewulski, B. J. Wallace, M. Kelly, T. Halse, K. A. Musser, P. F. Smith, D. L. Morse, and R. J. Limberger.** 2003. Detection, Isolation, and Molecular Subtyping of *Escherichia coli* O157:H7 and *Campylobacter jejuni* Associated with a Large Waterborne Outbreak. *J. Clin. Microbiol.*; **41**: 174–180.
- Browning, N. G., J. Botha, H. Sacho, and P. J. Moore.** 1990. *Escherichia coli* O157:H7 haemorrhagic colitis. Report of the first South African case. *SA Surg. J.*; **28**: 28–29.
- Cagney, C., H. Crowley, G. Duffy, J-J. Sheridan, S. O'Brien, E. Carney, W. M. Anderson, D. A. Dowell, I. S. Blair, and R. H. Bisho.** 2004. Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. *J. Food Microbiol.*; **21**: 203–212.
- Chinen, I., J. D. Tanoro, E. Miliwebsky, L. H. Lound, G. Chillemi, S. Ledri, A. Baschkier, M. Scarpin, E. Manfredi, and M. Rivas.** 2001. Isolation and

- characterisation of *Escherichia coli* O157:H7 from retail meats in Argentina. *J. Food Prot.*; **64**: 1346–1351.
- Dorrington, R. E., L. F. Johnson, D. Bradshaw, and T. Daniel.** 2006. The Demographic Impact of HIV/AIDS in South Africa. National and Provincial indicators for 2006. Cape Town: Centre for Actuarial Research, South African Medical Research Council and Actuarial Society of South Africa. ISBNB 0- 7992- 2322-0
- Du Toit L. and V. Irma.** 2005. Food practices associated with increased risk of bacterial food-borne disease of female students in self-catering residences at the Cape Peninsula University of Technology. *J. Fam. Ecol. Cons. Sci.*; **33**: 73–88
- Durso, L. M., K. Reynolds, N. Bauer, and J. E. Keen.** 2005. Shiga-toxigenic *Escherichia coli* O157:H7 infections among livestock exhibitors and visitors at a Texas county fair. *Vector Borne Zoo. Dis.*; **5**: 193–201.
- Dynal Product Brochure.** 2006. *Dynabeads® anti-E coli*. For rapid selective enrichment of *Escherichia coli* O157:H7. Dynal A.S. Oslo, Norway.
- Feng P.** 1995. *Escherichia coli* Serotype O157:H7: Novel Vehicles of Infection and Emergence of Phenotypic Variants. Ph.D. U.S. Food and Drug Administration, Washington, D.C., USA. *Emerg. Infect. Dis.* **1**: 46–52.
- Feng, P. and S. R. Monday.** 2000. Multiplex PCR for detection of trait and virulence factors in enterohemorrhagic *Escherichia coli* serotypes. *Mol. Cell Prob.*; **14**: 333–337.
- Galane, P. M and M. Le Roux.** 2001. Molecular Epidemiology of *Escherichia coli* isolated from young South Africa children with diarrheal diseases. *J. Health Popl. Nutr.* **19(1)**: 311–318

- Gale.** 2001. Developments in microbiological risk assessment for drinking water. *J. Appl. Microbiol.* **91**: 191–205.
- Grimm, L. M., M. Goldoft, J. Kobayashi, J. H. Lewis, D. Alfi, A. M. Perdichizzi, P. I. Tarr, J. E. Ongerth, S. L. Moseley, M. Samadpour.** 1995. Molecular epidemiology of a fast-food restaurant-associated outbreak of *Escherichia coli* O157:H7 in Washington State. *J. Clin. Microbiol.*; **33**: 2155–2158.
- Gyles, C., R. Johnson, A. Gao, K. Ziebell, D. Piérard, S. Aleksic, and P. Boerlin.** 1998. Association of enterohemorrhagic *Escherichia coli* hemolysin with serotypes of Shiga-like toxin-producing *Escherichia coli* of human and bovine origins. *Appl. Environ. Microbiol.*; **64**: 4134–4141
- Hayes, C. E. Elliot, E. Krales, D. Goulda.** 2003. Food and Water Safety for Persons Infected with Human Immunodeficiency Virus. *Clin. Infect. Dis.*; **36(S2)**: S106–S109.
- Haysom, I and Sharp, K.** 2004. Cross contamination from raw chicken during meal preparation. *J. British Food* **106(1)**: 38–50
- Heuvelink, A. E., F. I. A. M Van Den Biggelaar, J. T. M. Zwartkruis-nahuis, R. G. Herbes, R. Huyben, N. Nagelkerkr, W. J. G. Melchers, L. A. H. Monnens, and E. De Boer.** 1998. Occurrence of verocytotoxin producing *Escherichia coli* O157:H7 on Dutch dairy farms. *J. Clin. Microbiol.*; **36(12)**: 3480–3487.
- Ihekweazu, C., M. Barlow, S. Roberts, H. Christensen, B. Guttridge, D. Lewis.** 2006. Outbreak of *E. coli* O157 infection in the South west of the UK: risks from streams crossing seaside beaches. *Euro. Surveill.*; **11(4)**: 128–130
- Jin, H. Y., Tao, K. H., Li, Y. X., Li, F. Q. and S. Q. Li.** 2005. Microarray analysis of *Escherichia coli* O157:H7. *World J. Gastro.*; **11(37)**: 5811–5815

- Momba, M. N. B., and P. Kaleni.** 2003. Re-growth and survival of indicator microorganisms on the surfaces of household containers used for the storage of drinking water in rural communities of South Africa. *Water Res.*; **36**: 3023–3028.
- Momba, M. N. B., and T. L. Notshe.** 2003. The effect of long storage and household containers on the microbiological quality of drinking water in rural communities of South Africa. *J. Water Supp. Res. Techno.-AQUA.*; **52 (1)**: 67–76
- Momba, M. N. B., S. Ndaliso, and M. A. Binda.** 2003a. Effect of a combined chlorine-monochloramine process on the inhibition of biofilm regrowth in potable water systems. *J. Water Supp.*; **3 (1-2)**: 215–221.
- Momba, M.N.B., N. Makala, and B. M. Brouckaert.** (2003b) Evaluation of factors affecting the performance of small water supplies for the removal of indicator bacteria in rural developing areas of South Africa. *International Water Association Health-Related Water Microbiology Symposium*. Cape Town/South Africa 14-19 September 2003.
- Momba, M.N.B. and N. Makala.** 2004. Comparing the effect of various pipe materials on biofilm formation in chlorinated and combined chlorine-chloraminated water systems. *Water SA* **30**: 175–182
- Momba M. N. B., Z. Tyafa, and N. Makala.** 2004a. Rural water treatment plants fail to provide potable water to their consumers: Alice water treatment plant in the Eastern Cape Province of South Africa. *SA J. Sci.*; **100**: 307–310.
- Momba, M. N. B., N. Makala, B. Broukaert, Z. Tyafa, P. Thompson and A. C. Buckley.** 2004b. Improving the efficiency and sustainability of disinfection at a small rural water treatment plant. *Water, SA*. **30(5)**: 617–622

- Momba, M. N. B., N Makala, B. Zani, and B. M. Brouckaert.** 2005a. Key causes of drinking water quality failure in a rural small water supply of South Africa. In *Water Encyclopedia –Domestic, Municipal, and Industrial water supply and waste disposal* (Edt J.H. Lehr and J. Keeley). John Wiley & Son, Inc. Pages 221–227
- Momba, M. N. B., N. Makala, Z. Tyafa, and B. M. Brouckaert.** 2005b. A model partnership for sustainable production of safe drinking water for rural communities in South Africa. *SA J. Sci.*; **101**: 335–336.
- Momba, M. N. B., V. K. Malakate, and J. Theron.** 2006a. Abundance of pathogenic *Escherichia coli*, *Salmonella typhimurium* and *Vibrio cholerae* in Nkonkobe drinking water sources. *J. Water Health*; **04**: 289–296.
- Momba, M. N. B., Z. Tyafa, N. Makala, B. M. Brouckaert, and C. L. Obi.** 2006b. Safe drinking water still a dream in rural areas of South Africa. Case study: The Eastern Cape Province. *Water, SA*; **32**: 715–720.
- Mora, A., M.A. Usera, M. Blanco, J. E. Blanco, M. P. Alonso, G. Prats, A. Stirrat, F. M. Carter, and J. Blanco.** 2007. Bacteriophage typing and virulence genes of verocytotoxigenic *E. coli* (VTEC) O157:H7 strains isolated in Spain. In: Duffy G., Garvey, P., Coia, J., Wasteson, Y., McDowell, D.A. (Eds.), Verocytotoxigenic *E. coli* in Europe, 3. Pathogenicity and virulence of verocytotoxigenic *E. coli*. Concerted Action CT98-3935. Teagasc, The National Food Centre, Dublin, pp. 189.
- Mosupye F. and A. von Holy.** 1999. Microbiological quality and safety of ready-to-eat street-vended foods in Johannesburg, South Africa, *J. Food Prot.*; **62**: 1278–1284.

- Müller, E. E., Ehlers, M. M. and W. O. Grabow.** 2001. The occurrence of *Escherichia coli* O 157:H7 in South African water sources intended for direct and indirect human consumption. *Water Res.*; **35**: 3085–3088.
- Müller, E. E., Grabow, W. and M. M. Ehlers.** 2003. Immunomagnetic separation of *Escherichia coli* O157:H7 from environmental and wastewater in South Africa. *Water, SA*; **29**: 427– 432.
- Murinda, S. E. T. L. Nguyen, T. L. Landers, F. A. Draughon, A. G. Mathew, J. S. Hogan, K. L. Smith, D. D. Hancock, and S. P. Oliver.** 2004 Comparison of *Escherichia coli* isolates from humans, food, and farm and companion animals for the presence of Shiga toxin-producing *E. coli* virulence markers. *Foodborne Path. Dis.*; **1**: 178–184.
- Obi, C. L. and P. O. Bessong.** 2002. Diarrhoeagenic bacterial pathogens in HIV positive patients with diarrhea in rural communities of Limpopo Province, South Africa. *J. Health Pop. Nut.*; **20(3)**: 230–234.
- Obi C. L., J. Ramalivhana, M. N. B. Momba, B. Onabulu, J. O. Igumbor, M. Lukoto, T. B. Mulaudzi, E. L. Jansen van Rensburg, E. Green, and S. Ndou.** 2007. Antibiotic resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients with and without diarrhea and their household drinking water in rural communities in Limpopo Province South Africa. *Afri. J. Biotech.*; **6(8)**: 1035–1047.
- Phaswana-Mafunya, N.** 2006. Hygiene status of rural communities in the Eastern Cape of South Africa. *Int. J. Environ. Health Res.*; **16(4)**: 289–03.
- Pradel, N., V. Liverelli, C. Champs, J. Palcoux, A. Reynaud, F. Scheytz, J. Sirot, B.**

- Joly, and C. Forestier.** 2000. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. *J. Clin. Microbiol.*; **38**: 1023–1031
- Ramachandran, V., K. Brett, M. A. Hornitzky, M. Dowton, K. A. Bettelheim, M. J. Walker, and S. P. Djordjevic.** 2003. Distribution of intimin subtypes among *Escherichia coli* isolates from ruminant and human sources. *J. Clin. Microbiol.*; **41**: 5022–5032.
- Sandhu, K. S., R. C. Clarke, K. McFadden, A. Brouwer, M. Louie, J. Wilson, H. Lior, and C. L. Gyles.** 1996. Prevalence of the *eaeA* gene in verotoxigenic *Escherichia coli* strains from dairy cattle in Southwestern Ontario. *Epidemiol. Infect.*; **116**: 1–7
- Schroeder, C. M., D. G. White, G. E. B. Zhang, P. F. McDermott, S. Ayers, S. Zhao, and J. Meng,** 2003. Isolation of antimicrobial-resistant *Escherichia coli* from retail meats purchased in Greater Washington, DC, USA. *Int. J. Food Microbiol.*; **85**: 197–202.
- Stampi, S., A. Caprioli, G. De Luca, P. Quaglio, R. Sacchetti, and F. Zanetti.** 2004. Detection of *Escherichia coli* O157 in bovine meat products in northern Italy. *Int. J. Food Microbiol.*; **90**: 257–262.
- Torres, J., C. López-Saucedo, J. F. Cerna, N. Villegas-Sepulveda, R., Thompson, and F. R. Velazquez.** 2003. Single multiplex polymerase chain reaction to detect diverse loci associated with diarrheagenic *Escherichia coli*. *Emerg. Infect. Dis.*; **9**: 127–131.
- UNDP (United Nations Development Programme).** 2004. South Africa Human Development Report 2003. Cape Town: Oxford University Press Southern Africa.

- Vorster, S. M., R. P. Greebe, and G. L. Nortje.** 1994. Incidence of *Staphylococcus aureus* and *Escherichia coli* in ground beef, broilers and processed meats in Pretoria, South Africa. *J. Food Prot.*; **57**: 305–310.
- Wang, L., D. Rothmund, H. Curd, and P. R. Reeves.** 2000. Sequence diversity of the *Escherichia coli* H7 *fliC* genes: implication for a DNA-based typing scheme for *E. coli* O157:H7. *J. Clin. Microbiol.*; **38**: 1786–1790.
- Wang, G., G. C. Clifford, and G. R. Frank.** 2002. Detection in *Escherichia coli* of the Genes Encoding the Major Virulence Factors, the Genes Defining the O157:H7 Serotype, and Components of the Type 2 Shiga Toxin Family by Multiplex PCR. *J. Clin. Microbiol.*; **40**: 3613–3619.

CHAPTER 8

TABLE OF CONTENTS	Page №
Table of content.....	363
List of Tables.....	365
List of Figures.....	366
Abstract.....	367
8.1 Introduction.....	368
8.2 Materials and Methods.....	370
8.2.1 Study population and samples.....	370
8.2.2 Scientific ethics.....	370
8.2.3 Risk characterization.....	370
8.3 Results and Discussion.....	374
8.3.1 Probability of <i>E. coli</i> O157:H7 infections from drinking water.....	374
8.3.2 Probability of <i>E. coli</i> O157:H7 infections from consumption of meat and meat products.....	376
8.3.3 Probability of <i>E. coli</i> O157:H7 infections from consumption of Vegetables.....	379
8.3.4 The effect of variability on risk estimates of <i>E. coli</i> O157:H7 infections associated with consumption of water, meat and meat products, and vegetables.....	382

8.3.5	The effect of uncertainty on risk estimates of <i>E. coli</i> O157:H7 infections associated with consumption of water, meat, and meat products, and vegetables.....	389
8.3.6	Overall confidence in risk estimates of <i>E. coli</i> O157:H7 infections associated with consumption of water, meat, and meat products and vegetables presented in this study.....	391
8.4	Conclusion.....	393
	References.....	395

LIST OF TABLES

Table 8.1:	The average water, meat and meat products and vegetable intake in South Africa in ml/person/day or g/capita/day.....	372
Table 8.2:	Average individual risks of <i>E. coli</i> O157:H7 infection associated with consumption of 1.5l/person/day of water from various distribution points. .	375
Table 8.3:	Average individual risks of <i>E. coli</i> O157:H7 infection and expected number of infections per 100 confirmed HIV/AIDS or non–confirmed HIV/AIDS patients associated with consumption of various meat and meat products.....	378
Table 8.4:	Average individual risks of <i>E. coli</i> O157:H7 infection and expected infections per 100 HIV-positive or HIV-negative patients associated with consumption of various vegetables.....	380

LIST OF FIGURES

Figure 8.1:	95 th Percentiles (Pinf.) for <i>E. coli</i> O157:H7 infections due to ingestion of 1500 ml/person/day of water to confirmed and non-confirmed HIV/AIDS Patients.....	376
Figure 8.2:	95 th Percentiles (Pinf.) for <i>E. coli</i> O157:H7 infections due to consumption of meat and meat products to confirmed and non-confirmed HIV/AIDS patients.....	379
Figure 8.3:	95 th Percentiles (Pinf.) for <i>E. coli</i> O157:H7 infections due to consumption of vegetables to confirmed and non-confirmed HIV/AIDS patients.	381
Figure 8.4:	Sample output distributions of cumulative frequency of individual risk (single exposure) of <i>E. coli</i> O157:H7 infection among (a) confirmed HIV/AIDS and (b) non-confirmed HIV/AIDS patients with diarrhoea, drinking water.....	384
Figure 8.5:	Sample output distributions of cumulative frequency of individual risk (single exposure) of <i>E. coli</i> O157:H7 infection among (a) confirmed HIV/AIDS and (b) non-confirmed HIV/AIDS patients with diarrhoea, consuming meat and meat products.....	386
Figure 8.6:	Sample output distributions of cumulative frequency of individual risk (single exposure) of <i>E. coli</i> O157:H7 infection among (a) confirmed HIV/AIDS and (b) non-confirmed HIV/AIDS patients with diarrhoea, consuming vegetables.....	388

CHAPTER 8

Abstract

People suffering from HIV/AIDS may acquire water and food borne *E. coli* O157:H7 more easily than HIV/AIDS negative individuals. The current study explored the health risk of *E. coli* O157:H7 to diarrheic confirmed and non-confirmed HIV/AIDS patients due to their exposure to presumed ingestion of water, meat and meat products, and vegetables ostensibly contaminated with *E. coli* O157:H7. Strains of *E. coli* O157:H7 were isolated by enrichment culture and on Cefixime-Telurite Sorbitol MacConkey (CT-SMAC) Agar. Average counts of presumptive *E. coli* O157 were used for dose-response assessment. Probability of infection in confirmed and non-confirmed HIV/AIDS patients was 0.27 and 0.20 from meat and meat products, 0.21 and 0.15 from vegetables and 1.0 due to ingestion of 1500 ml/person/day of water. Drinking water had a higher probability of transmitting *E. coli* O157:H7 infections than meat and meat products and vegetables. The probability of *E. coli* O157: H7 infection was higher for confirmed HIV/AIDS patients than for non-confirmed patients. Water and foods consumed by HIV/AIDS patients should be safe from any microbial contaminant. These waters and foods should as well be investigated for other enteric pathogens to establish their safety.

Keywords: Risk Assessment; *Escherichia coli* O157:H7; Water; Meat and meat products; Vegetables; HIV/AIDS; Diarrhoea.

8.1 Introduction

Risk Assessment is a method used to organize and analyze scientific information to help estimate the probability and severity of an adverse effect because of a hazard (Cassin *et al.*, 1998). Applied to microbial water and food safety, the method can help to identify the stages in the operation, distribution, handling, and consumption of foods and water that contribute to an increased risk of food and water-borne illnesses. The method can help focus resources and efforts on the most effective way of reducing the risk of contracting such water and food-borne pathogens (Cassin *et al.*, 1998). The probable reason why risk assessments related to the presence of pathogens presents a key problem in most countries is the lack of data on which to base estimates of disease burdens.

To specify a risk assessment with regard to disease causing pathogens, one needs to identify the pathogen of concern, the product with which it is associated (water or food), a pathway, risk factors and the population at risk (Nauta *et al.*, 2001).

For this study, the levels of *E. coli* O157:H7 in water, meat and meat products and vegetables consumed by the South African population residing in the Amathole district in the Eastern Cape Province, with a specific focus on diarrhoeic confirmed and non-confirmed HIV/AIDS patients, was investigated. *Escherichia coli* O157:H7 was chosen because of its association with water and foods, especially those of animal origin and with vegetables (Vorster *et al.*, 1994; Magwira *et al.*, 2005; Josefa *et al.*, 2005).

An important reason for conducting a risk assessment for pathogenic *E. coli* O157:H7 is its potential impact on public health either directly or indirectly. There is considerable evidence of persistent diarrhoea among adults and young children who are immunocompromised due to

diseases such as HIV/AIDS (Ashton and Ramasha, 2002). However, the risk that *E. coli* O157:H7 poses to HIV/AIDS patients and its possible link to the diarrhoeic conditions in such patients has not been established. This was the foundation for the current study. In the study, we assessed the health risks that may result from the exposure of confirmed HIV/AIDS and non-confirmed HIV/AIDS patients with diarrhoea due to ingestion of water, meat and meat products and vegetables that in one way or another were contaminated with *E. coli* O157:H7.

Risk assessment consists of four main steps, namely hazard identification, exposure assessment, dose-response assessment, and risk characterization (Cassin *et al.*, 1998). The knowledge in each step is combined to represent a cause-and-effect chain from the prevalence and population of a given microbial agent (in the present case *E. coli* O157:H7) to the probability and magnitude of health effects. In risk assessment, both the probability and impact of a disease are considered. The quantities of daily intakes of water and/or foods by the population are estimated using a published data (Helena and Steyn, 2002).

A risk assessment model is therefore developed, which describes the pathogen population in the water and/or food product (Hass *et al.*, 1999). The variability and uncertainty in the model are accommodated using probabilistic representations of the parameters (Potter *et al.*, 1996). To generate a representative distribution of risk, the model is simulated with values selected from the probability distributions of the risk from the hazard (Thompson *et al.*, 1992; Vose, 1996; Cassin *et al.*, 1998). The direct output of the model is a distribution of health risk from ingesting water or eating a given food product by a particular group of population (in the present theoretical study, confirmed and non-confirmed HIV/AIDS patients with diarrhoea).

8.2 Materials and Methods

8.2.1 Study population and samples

The exposure assessment in the present study was at the consumer level and the items that were considered for risk assessment included water, meat and meat products and vegetables. The meat and meat products were biltong, cold meat, mincemeat, and polony whereas the vegetable samples comprised of cabbage, carrot, cucumber, onions, and spinach. Water samples were obtained from stand-pipes supplying water to communities living in the informal settlements of Fort Beaufort, Alice, Dimbaza, Kwasaki, Mdantsane and Ngwenya.

The meat and meat products and vegetables were sampled from Fort Beaufort, Alice, and Mdantsane. The consumer demographics and health status was of confirmed HIV/AIDS and non-confirmed HIV/AIDS patients who were attending a referral hospital (Frere Hospital) East London for treatment. The ages of the patients ranged between ≥ 10 and > 50 years old.

8.2.2 Scientific ethics

The University of Fort Hare Research Office (Govan Mbeki Research and Development Centre), the Provincial Department of Health (Bisho), and Regional Eastern Cape Ethical Review Committees granted ethical approval for the study.

8.2.3 Risk characterization

For the purposes of risk characterization, the hypothetical immunity of the patients and the extent of vulnerability of the study cohorts to *E. coli* O157:H7 was based on a study done in Japan (Teunis *et al.*, 2004). Teunis *et al.* (2004) had developed model parameters for the risk of illness

associated with a confirmed *E. coli* O157:H7 outbreak in children and their teachers after consuming a lunch demonstrated to be contaminated by *E. coli* O157:H7 bacteria (Teunis *et al.*, 2004). There are no published studies on risk analysis of *E. coli* O157:H7 in HIV – positive individuals. The only study that showed difference in risk of *E. coli* O157:H7 infection between such-groups of persons of differing susceptibility to *E. coli* O157:H7 infection was one in Japan (Teunis *et al.*, 2004), which demonstrated that children were more susceptible to *E. coli* O157:H7 than their teachers.

As mentioned in chapter seven, the presumptive *E. coli* O157:H7 counts determined for water, meat and meat products and vegetables samples from several sites within the Amathole District, were used to estimate the potential risk of *E. coli* O157:H7 infections. Risk of *E. coli* O157:H7 infection to confirmed HIV/AIDS patients was modeled using parameters developed for children. On the other hand, risk of *E. coli* O157:H7 infections to non-confirmed HIV/AIDS patients was modeled using parameters developed for teachers (Teunis *et al.*, 2004). Presumed daily average quantities of water, meat, and meat products and vegetables used for calculating exposure were adopted from a study on food consumptions in South Africa (Helena and Steyn, 2002) (Table 8.1).

Table 8.1: The average water, meat and meat products and vegetable intake in South Africa in ml/person/day or g/capita/day (Helena and Steyn, 2002)

Food/Water	Quantity consumed	% population	Comments
Water	1500	100	1500 ml/person/day
Cabbage	17.4	73.8	cooked
Cucumber	2.5	12.8	used raw tomato data as substitute
Onions	2.5	12.8	used raw tomato data as substitute
Spinach	9.2	27.4	cooked
Carrots	3.9	30.8	cooked, flesh and skin
Biltong	4.6	46	beef sausage
Cold meat	4.4	34.6	meat products and dishes
Mince	9.7	38.7	beef stew
Polony	4.4	34.6	meat products and dishes

A beta-Poisson model was used to calculate the probability of infection from a single exposure using the formula:

$$P_{inf} = 1 - (1 + d/\beta)^{-\alpha} \text{ (Haas } et al., 1999).$$

where:

P_{inf} = probability of infection

d = infectious dose

β = beta, model parameter

α	=	alpha, model parameter
Dose d	=	cfu $\times$ g/capita/day for food items, or cfu $\times$ 1500 ml/person/day for water, assuming an average daily water intake of 1500 ml per day
Cfu	=	counts of presumptive <i>E. coli</i> O157 (cfu/g OR ml)

The values used for β and α were adopted from the report by Teunis *et al.* (2004), and were used to represent β and α values for confirmed and non-confirmed HIV/AIDS patients with diarrhoea. Based on the reasoning that children have a less well-developed immune system than adults; the β and α values for children (0.0844 and 1.442, respectively) were used for confirmed HIV/AIDS patients (compromised immune system), and the β and α values for teachers (0.0496 and 1.001, respectively) was used for non-confirmed HIV/AIDS patients. The non-confirmed HIV/AIDS patients were assumed to have normal immune function.

Average individual risks due to a single exposure, and prospective infections per 100 confirmed and non-confirmed HIV/AIDS patients with diarrhoea in a theoretical outbreak were calculated. The model parameters, incorporating the average daily per capita quantities of water, vegetables, meat, and meat products consumed (Helena and Steyn, 2002); and numbers of presumptive *E. coli* O157, (used to represent counts for presumptive *E. coli* O157:H7), actually cultured from these products sampled at various locations in the Amathole District, were used to calculate the risks.

8.3 Results and Discussion

The risk of *E. coli* O157:H7 infection was found to be higher for confirmed HIV/AIDS patients than it was for the non-confirmed HIV/AIDS patients for water, meat and meat products and vegetables (Tables 8.2 to 8.4). Nevertheless, the risk varied widely between and within samples of water, meat and meat products and vegetables for both confirmed and non-confirmed HIV/AIDS patients with diarrhoea.

8.3.1 The probability of *E. coli* O157:H7 infections from drinking water

The prospective risk of *E. coli* O157:H7 infection for both confirmed and non-confirmed HIV/AIDS patients was higher for water (Table 8.2) than for meat and vegetable samples. The average individual risk for confirmed HIV/AIDS patients due to ingestion of 1500 ml/person/day of water ranged from 0.75 for Dimbaza water to 0.81 for Mdantsane water, with 95th percentile values of 0.8 for both. Average individual risk for non-confirmed HIV/AIDS patients was slightly lower for Dimbaza waters (0.57), but was higher for Mdantsane water (0.63) with 95th percentile values above 0.6 for both. Table 8.2 and Figure 8.1, respectively illustrate average individual probability of *E. coli* O157:H7 infection and 95th percentiles due to ingestion of 1500 ml/person/day of water in a single exposure for both confirmed and non-confirmed HIV/AIDS patients.

The probability of *E. coli* O157:H7 infections per 100 persons for both confirmed and non-confirmed HIV/AIDS patients population drinking approximately 1500 ml/person/day of water from any of the distribution point was estimated at 1.00 (in other words, certainty of infection). This estimate is less likely to be reflective of true risk of infection than it is to be

indicative of the high level of uncertainty associated with the assumptions made in this preliminary risk assessment exercise.

The exaggerated probability of *E. coli* O157:H7 infection due to intake of water could be attributable to the high-assumed daily intake of water (1500 ml/person/day), and high counts of presumptive *E. coli* O157:H7, which may include other strains of *E. coli* besides strain O157:H7.

Table 8.2: Average individual risks of *E. coli* O157:H7 infection associated with consumption of 1500 ml/person/day of water from various distribution points.

Water Source	Average Probability of infection (Pinf.)
Fort Beaufort	0.79(0.61)
Alice	0.76(0.58)
Dimbaza	0.75(0.57)
Mdantsane	0.81(0.63)
Ngwenya	0.77(0.59)
Kwasaki	0.78(0.59)

Note: unparenthesised numbers represent average risks of *E. coli* O157:H7 infections to confirmed HIV/AIDS patients, whereas numbers in parenthesis represent average risks to non-confirmed HIV/AIDS patients.

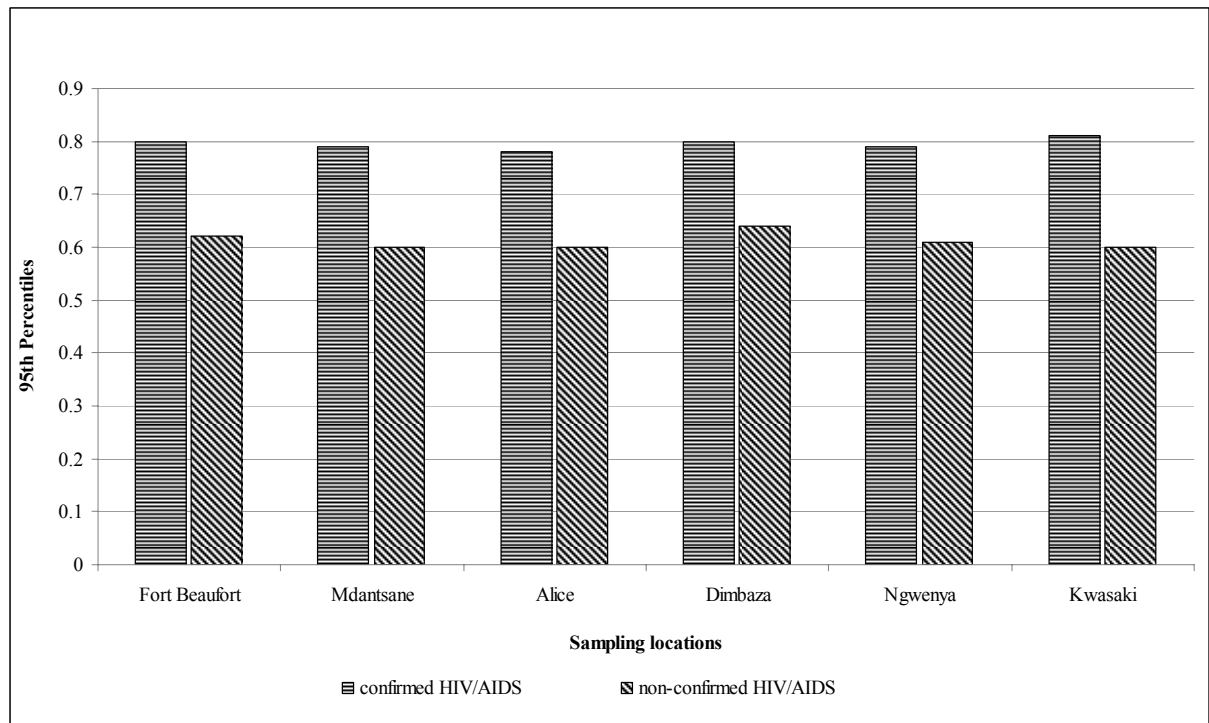


Figure 8.1: 95th percentiles (P_{inf}) for *E. coli* O157:H7 infections due to ingestion of 1500 ml/person/day of water in confirmed and non-confirmed HIV/AIDS patients

8.3.2 The probability of *E. coli* O157:H7 infections from consumption of meat and meat products

The probability of *E. coli* O157:H7 infection associated with consumption of meat and meat products was also estimated for both patient categories (Table 8.3). The risk estimates for meat and meat products ranged from 0.64 to 0.78 for biltong from Fort Beaufort (consumption of 9.7 g/capita/day) and mincemeat from Alice (consumption of 4.6 g/capita/day), respectively, for the confirmed HIV/AIDS patient. The 95th percentile values were noted at 0.68 for biltong and 0.79 for mincemeat to this group of patients. On the other hand, risk estimates for non-confirmed HIV/AIDS patients were lower. The risk ranged from 0.46 to 0.60 for biltong from Fort Beaufort

and mincemeat from Alice, respectively. The 95th percentile value of 0.5 was noted for biltong and 0.6 for mincemeat (Figure 8.2), at a similar consumption rate.

When risks of *E. coli* O157: H7 infection were ranked by type of meat product, mincemeat was associated with the highest individual risk of infection in a single exposure followed closely by polony, biltong and lastly by coldmeat. It was not surprising that mincemeat had the highest associated individual risk. Mincemeat has been reported to harbour high levels of *E. coli* O157:H7 because of the way the meat is ground, which serves to inoculate the whole batch of mincemeat during mincing (Flores and Stewart, 2004).

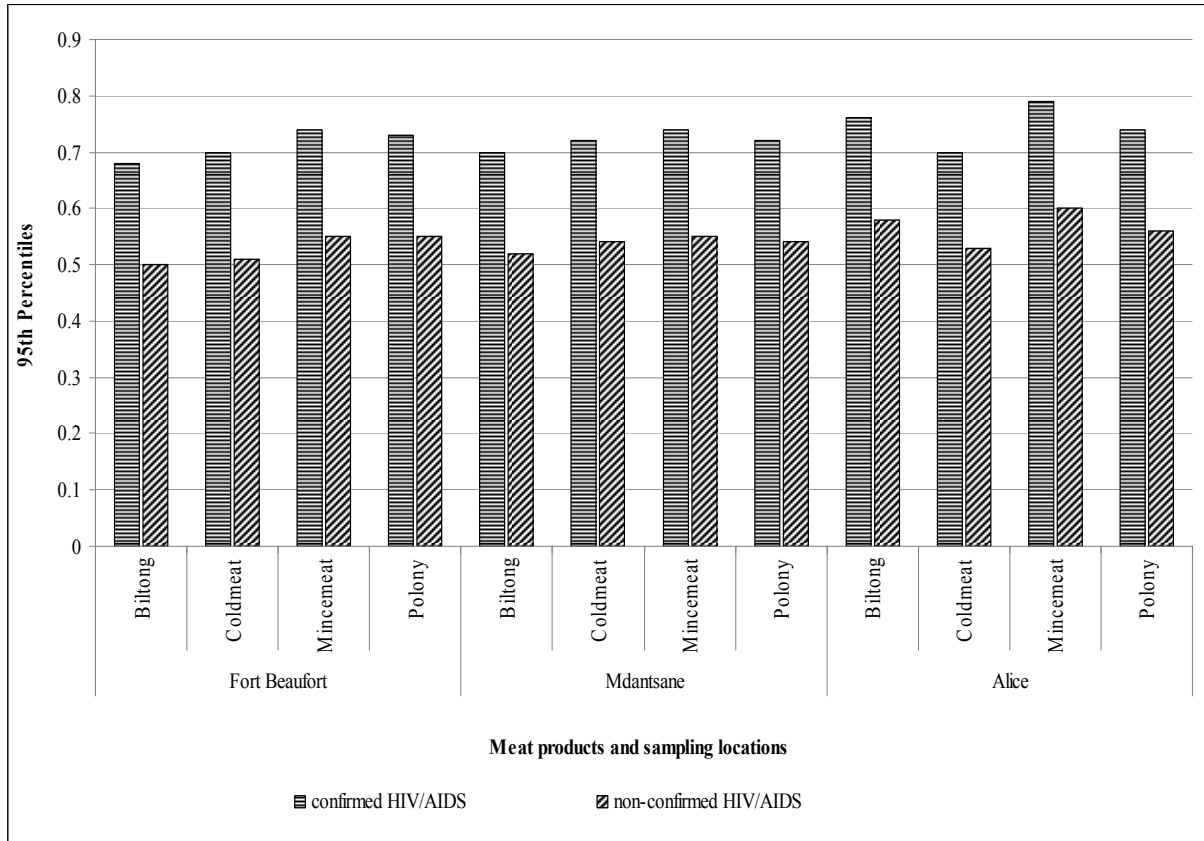
An astonishing observation was that biltong had the highest expected infection rate per population of 100 (an indication of population risk as opposed to individual risk). Biltong was associated with population risk estimates of 32 and 23 per population of 100 among confirmed and non-confirmed HIV/AIDS patients, respectively. This observation could be at least partially the result of high consumption of biltong by South Africans. Even though the quantities of biltong consumed per day (4.6 g/capita/day) by South Africans was found to be lower than that of mincemeat (9.7 g/capita/day), the number of people consuming biltong (46 persons per every 100 population) was higher than those consuming mincemeat (38 person per every 100 population) (Helena and Steyn, 2002).

The predicted consumption rate for coldmeats and polony were similar, and this is reflected in similar values for the associated individual risk of probability of *E. coli* O157:H7 infections and expected infections per 100 confirmed HIV/AIDS or non-confirmed HIV/AIDS patients. The average expected infections per 100 confirmed HIV/AIDS patients' consuming any of these meat products was predicted at 0.27 whereas average expected infection per 100 non-confirmed HIV/AIDS patients was estimated at 0.20.

Table 8.3: Average individual risks of *E. coli* O157:H7 infection and expected number of infections per 100 confirmed HIV/AIDS or non-confirmed HIV/AIDS patients associated with consumption of various meat and meat products

<u>Sample</u>	<u>HIV/AIDS</u>	<u>Av. Probability of infection (Pinf.)</u>			<u>Exposed/100</u>	<u>Av. infected/100</u>
	Status	FB	AL	MD	Population	Population
Biltong	+ve	0.64(29)	0.76(35)	0.68(31)	46	32
	–ve	0.46(21)	0.57(26)	0.50(23)	46	23
Cold meat	+ve	0.67(23)	0.67(23)	0.69(24)	35	23
	–ve	0.51(18)	0.49(17)	0.51(18)	35	17
Mincemeat	+ve	0.76(27)	0.78(30)	0.71(28)	39	28
	–ve	0.52(20)	0.60(23)	0.53(21)	39	21
Polony	+ve	0.71(25)	0.71(25)	0.71(25)	35	25
	–ve	0.52(18)	0.53(18)	0.50(17)	35	17

Note: Unparenthesised numbers (in average Probability of infection) represent average individual risks of *E. coli* O157:H7 infections to confirmed HIV/AIDS or non-confirmed HIV/AIDS patients. Numbers in parenthesis represent expected infections per 100 persons in a single outbreak for each location and each meat and meat product. FB = Fort Beaufort, AL = Alice, MD = Mdantsane.



Fi

Figure 8.2: 95th percentiles (Pinf.) for *E. coli* O157:H7 infections due to consumption of meat and meat products in confirmed and non-confirmed HIV/AIDS patients

8.3.3 The probability of *E. coli* O157:H7 infections from consumption of vegetables

Estimate of individual risk of *E. coli* O157:H7 infection because of consumption of vegetables ranged from 0.58 to 0.71 for confirmed HIV/AIDS patients. The estimated 95th percentile values were at 0.6 for onions and 0.72 for the cabbages for confirmed HIV/AIDS patients (Figure 8.3). This was observed for Mdantsane onions and Alice cabbage, for a single serving of 17.4 and 2.5 g/capita/day, respectively. Conversely, probability of *E. coli* O157:H7 infections in non-confirmed HIV/AIDS patients attributable to consumption of vegetables ranged between 0.41 and 0.52 on a single serving of the same quantities of cabbage and cucumber as above,

respectively (Table 8.4). The estimated 95th percentile values were at 0.4 for onions and 0.5 for the cabbages to non-confirmed HIV/AIDS patients. The average expected infections per 100 populations of confirmed HIV/AIDS patients consuming vegetables was estimated at 0.21. The average expected infections per 100 non-confirmed HIV/AIDS patients were considerably lower, at 0.15, which was almost equivalent to that of confirmed HIV/AIDS patients.

Table 8.4: Average individual risks of *E. coli* O157:H7 infection and expected infections per 100 confirmed or non-confirmed HIV/AIDS patients associated with consumption of various vegetable

<u>Sample</u>	<u>HIV/AIDS</u>	<u>Av. Probability of infection (Pinf.)</u>			<u>Exposed/100</u>	<u>Av. infected/100</u>
	Status	FB	AL	MD	Population	Population
Cabbage	+ve	0.68(53)	0.71(52)	0.66(49)	78	51
	–ve	0.50(39)	0.52(38)	0.48(35)	78	37
Carrot	+ve	0.66(20)	0.61(19)	0.62(19)	31	19
	–ve	0.47(14)	0.44(14)	0.44(14)	31	14
Cucumber	+ve	0.67(9)	0.59(8)	0.61(8)	13	8
	–ve	0.49(6)	0.41(5)	0.43(6)	13	6
Onion	+ve	0.66(8)	0.59(8)	0.58(8)	13	8
	–ve	0.47(6)	0.41(5)	0.41(5)	13	5
Spinach	+ve	0.68(19)	0.65(18)	0.62(17)	27	18
	–ve	0.50(14)	0.47(13)	0.45(12)	27	13

Note: Unparenthesised numbers (in Source and Risk Estimate columns) represent average individual risks of *E. coli* O157:H7 infections in confirmed or non-confirmed HIV/AIDS patients. Numbers in parenthesis represent expected infections per 100 confirmed or non-confirmed HIV/AIDS patients in a single outbreak for each location and for a particular vegetable. FB = Fort Beaufort, AL = Alice, MD = Mdantsane.

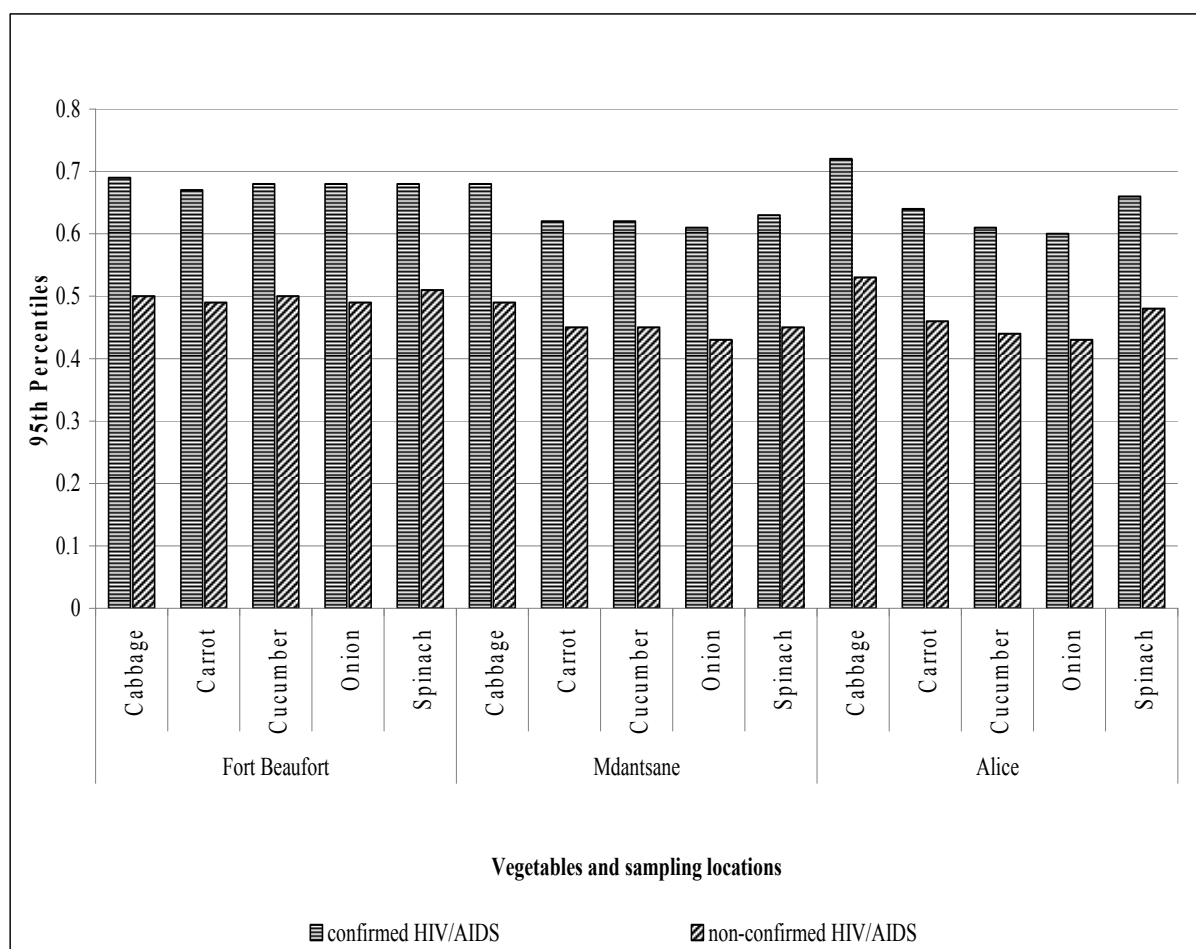


Figure 8.3: 95th percentiles (P_{inf.}) for *E. coli* O157:H7 infections due to consumption of vegetables to confirmed and non-confirmed HIV/AIDS patients

8.3.4 The effect of variability on risk estimates of *E. coli* O157:H7 infections associated with consumption of water, meat and meat products, and vegetables.

Figures 8.4 to 8.6 below show representative cumulative frequency distributions of risk estimates calculated by Monte Carlo simulation model sampling from the input distribution of *E. coli* O157:H7 counts for water, meat and meat products and vegetables at several sampling locations and supermarkets, shops and open air–markets. The estimated mean risks are indicated on the cumulative frequency curves, while the 5th and 95th percentiles of estimated risks are shown on the bar below the x-axis. By reading off the percentiles corresponding to the mean risks, an estimate of the percentage of the exposed population, which is exposed to risks lower or higher than the mean risk, was derived. When cumulative frequency distributions with similar mean risk estimates are compared, a wider spread of risk and a higher 95th percentile is indicative of the exposure scenario associated with a greater risk.

The variability of the estimated risk represented graphically by the spread of the cumulative frequency distribution; was greatly influenced by the counts of presumptive *E. coli* O157. This is because the presumptive counts obtained for the water, meat and meat products and vegetables from the areas were used to derive the cumulative distribution frequency curves. Comparing the cumulative distribution frequency curves presented in Figures 8.4 to 8.6, it is evidenced that the risk associated with drinking water (Figure 8.4) showed the greatest variability, followed closely by meat and meat products (Figure 8.5) and lastly by vegetables (Figure 8.6). Coincidentally, this order also corresponded to the extent of risk of *E. coli* O157:H7 infections associated with water, meat, and meat products and vegetables for both confirmed and non-confirmed HIV/AIDS patients with diarrhoea.

The variability of the estimated risk from drinking water as shown by the cumulative frequency distributions as illustrated in figure 8.4, were more spread than those for meat and meat products and vegetables for both confirmed and non-confirmed HIV/AIDS patients. The cumulative frequency distribution for water from Mdantsane was more spread with a higher 95th percentile value (0.8), for confirmed HIV/AIDS patients than for non-confirmed HIV/AIDS patients, (95th percentile value of 0.6), making it the source of drinking water with a greater variability, hence high individual risk of *E. coli* O157:H7 infections in a single exposure.

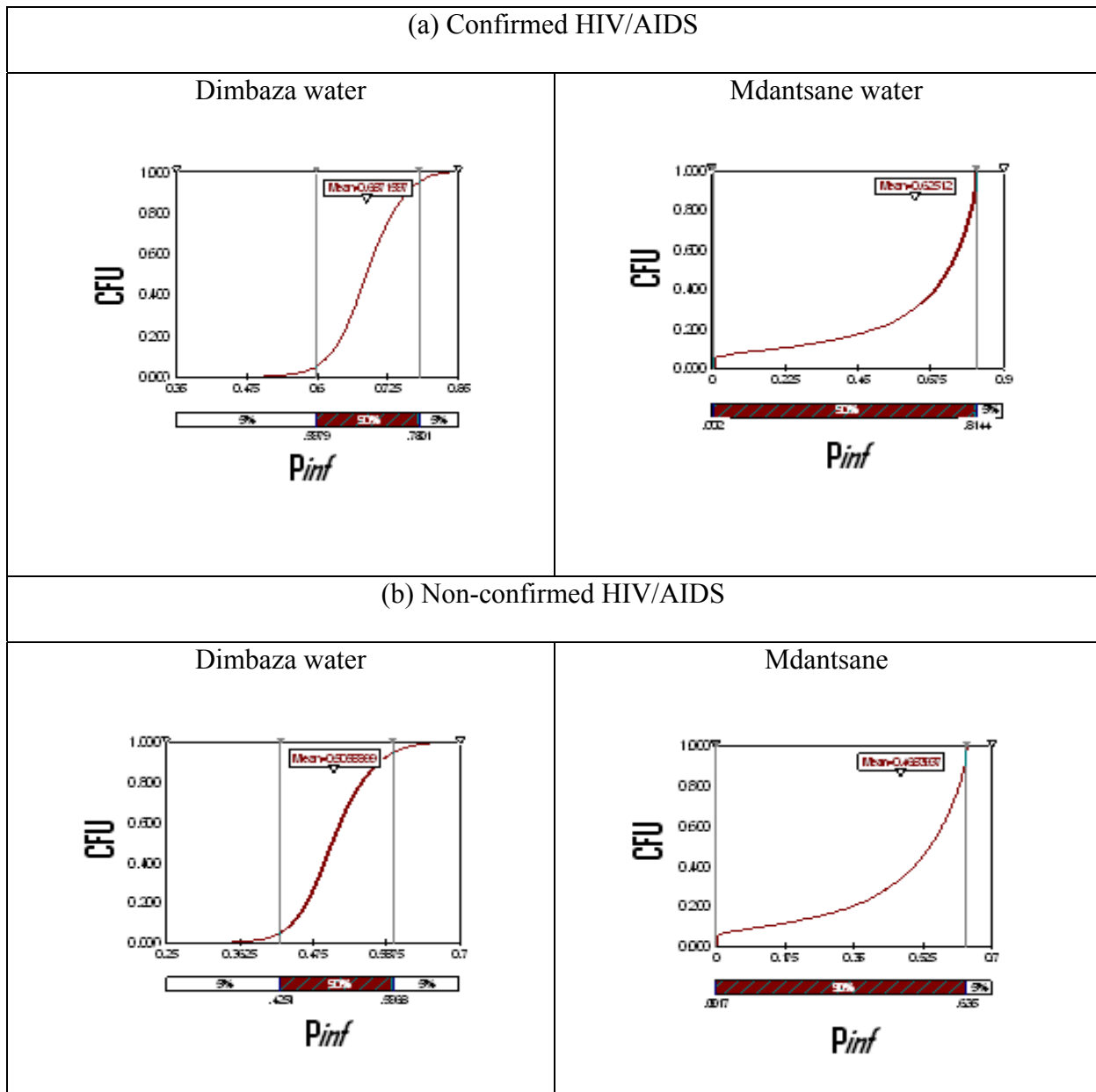


Figure 8.4: Sample output distributions of cumulative frequency of individual risk (single exposure) of *E. coli* O157:H7 infection among (a) confirmed HIV/AIDS and (b) non-confirmed HIV/AIDS patients with diarrhoea, drinking water from the indicated sampling locations. The 5th and 95th percentiles of calculated risk are shown on the bar below the x-axis. Mean risks are indicated on the cumulative frequency curve. Risk estimated by Monte Carlo simulation.

Conversely, water from Dimbaza showed a narrower spread. The 95th percentile value was noted at 0.78 for confirmed HIV/AIDS patients, implying that it had a lesser variability. However, this was still high compared to variability in estimated individual risk to non-confirmed HIV/AIDS patients drinking water from the same source (Dimbaza) with a 95th percentile value of 0.58. Therefore, a low individual risk of *E. coli* O157:H7 for non-confirmed HIV/AIDS than for confirmed HIV/AIDS patients due to drinking of water from Dimbaza was recorded.

The variability of the estimated risk from consumption of meat and meat products as shown by the cumulative frequency distribution were neither as spread as those for water nor as those for vegetables for both confirmed and non-confirmed HIV/AIDS patients (Table 8.3). As illustrated in figure 8.5, the cumulative frequency distribution for mincemeat from Alice was more spread with a higher 95th percentile value (0.78), for confirmed HIV/AIDS patients than for non-confirmed HIV/AIDS patients, (95th percentile value of 0.6), making it the meat and meat product with a greater variability, hence high risk of *E. coli* O157:H7 infections. Conversely, biltong showed a narrower spread. The 95th percentile value due to consumption of biltong was 0.68 for confirmed HIV/AIDS patients. This implied that biltong had a lesser variability and low risk of *E. coli* O157:H7 infection to confirmed HIV/AIDS patients, though this was still high compared to variability in estimated individual risk for non-confirmed HIV/AIDS patients consuming biltong (95th percentile value of 0.5).

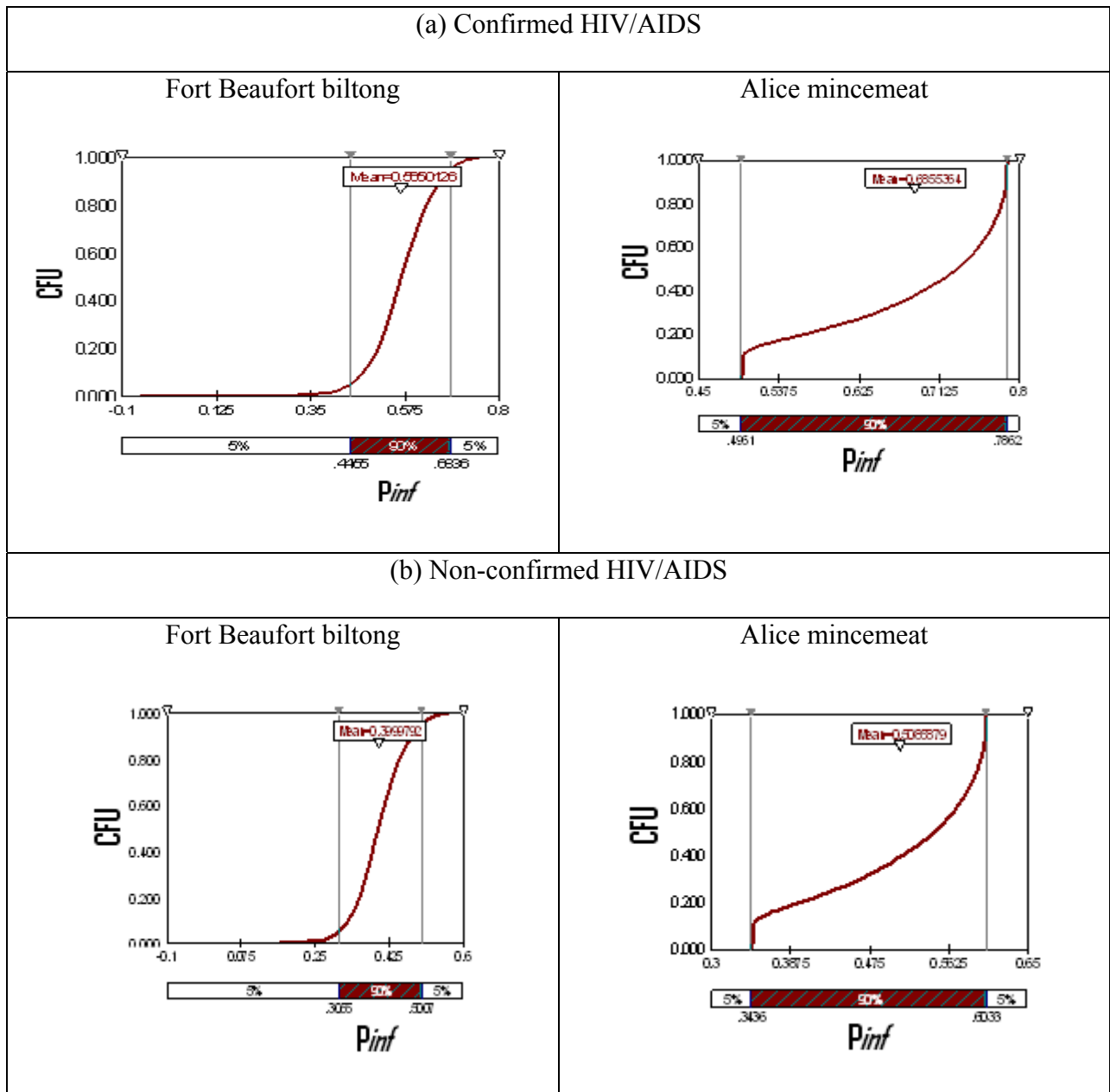


Figure 8.5: Sample output distributions of cumulative frequency of individual risk (single exposure) of *E. coli* O157:H7 infection among (a) confirmed HIV/AIDS and (b) non-confirmed HIV/AIDS patients with diarrhoea, consuming meat and meat products from the indicated sampling locations. The 5th and 95th percentiles of calculated risks are shown on the bar below

the x-axis. Mean risks are indicated on the cumulative frequency curve. Risk estimated by Monte Carlo simulation.

The variability of the estimated risk from consumption of vegetables as shown by the cumulative frequency distribution (Figure 8.6), were not as spread as those for water or meat and meat products for both confirmed and non-confirmed HIV/AIDS patients (Table 8.4). The cumulative frequency distribution for vegetables from Alice was more spread with a higher 95th percentile (0.6) value for confirmed HIV/AIDS patients than it was for non-confirmed HIV/AIDS patients (95th percentile value of 0.5), making it the vegetable sample with a greater variability, hence high risk of *E. coli* O157:H7 infections. Conversely, onions from Mdantsane showed a narrow spread, even though it had a higher 95th percentile value (0.67), the variability was less spread and so a low individual risk of *E. coli* O157:H7 for confirmed HIV/AIDS patients was predicted. However, onions from Alice had a lesser variability with low percentile value (0.4) demonstrating a low risk of *E. coli* O157:H7 infection for non-confirmed HIV/AIDS patients (Figure 8.6).

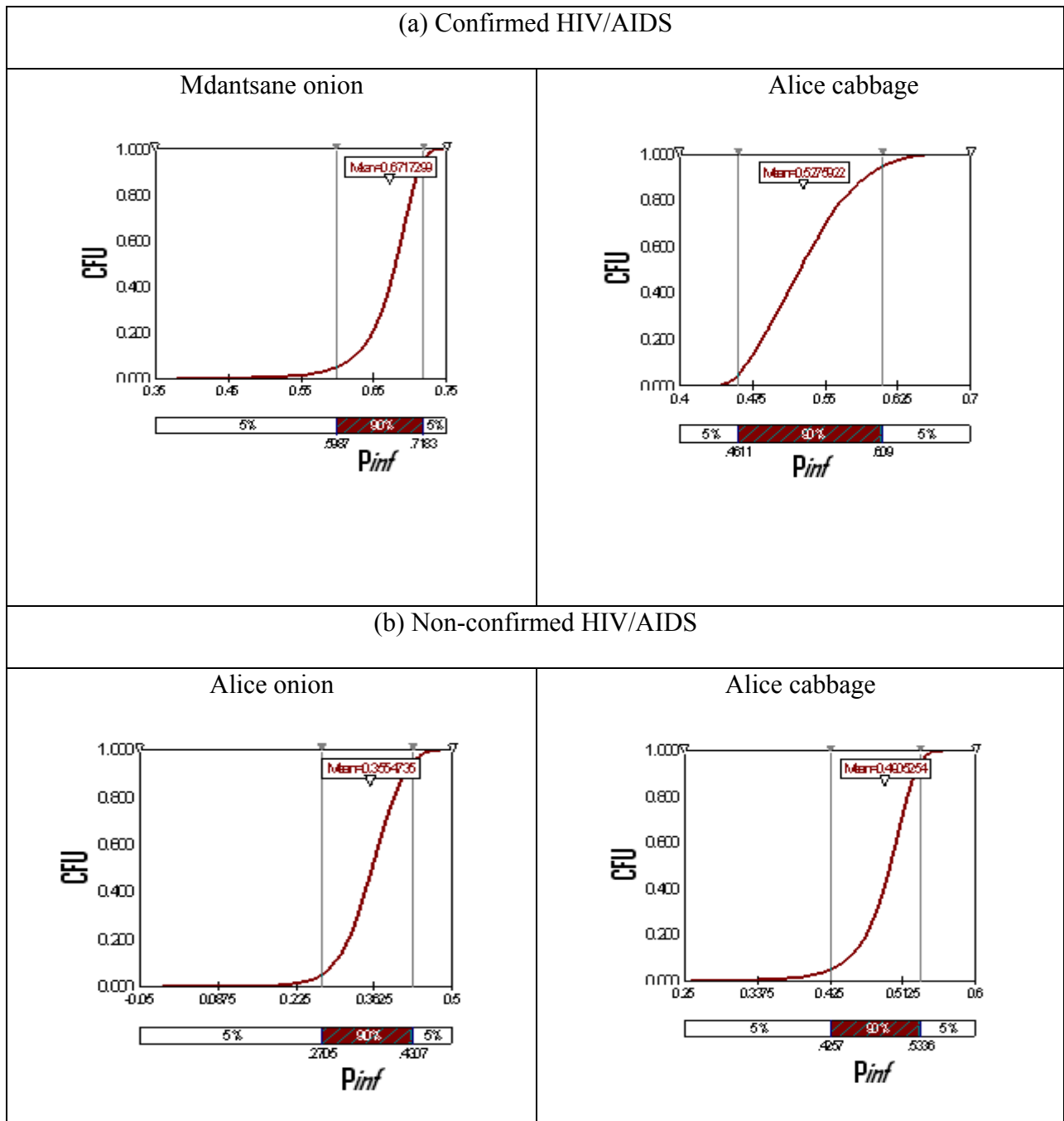


Figure 8.6: Sample output distributions of cumulative frequency of individual risk (single exposure) of *E. coli* O157:H7 infection among (a) confirmed HIV/AIDS and (b) non-confirmed HIV/AIDS patients with diarrhoea, consuming vegetables from the indicated sampling locations.

The 5th and 95th percentiles of calculated risks are shown on the bar below the x-axis. Mean risks are indicated on the cumulative frequency curve. Risk estimated by Monte Carlo simulation.

8.3.5 The effect of uncertainty on risk estimates of *E. coli* O157:H7 infections associated with consumption of water, meat, and meat products, and vegetables.

One of the major sources of uncertainty in any risk model is the suitability of the model parameters. Model parameters must be chosen from published studies, which vary in exposure conditions from the data to which the model is applied. The type of model parameters chosen may introduce uncertainty in the applicability of the model outputs in the context of the specific exposure under investigation. Comparing the findings of the present investigation to that conducted by Teunis *et al.* (2004) in Japan on which the risk model used in the present study was based, the rate of infection in children in a confirmed *E. coli* O157:H7 outbreak (used to model risk to confirmed HIV/AIDS patients in the present study), was 0.25. This is only 2% lower than the overall risk of infection estimated for confirmed HIV/AIDS patients (0.27) in the present study. The infection rate to the teachers, which was used to model risk to non-confirmed HIV/AIDS patients in the current study, was 0.16. This attack rate is 4% lower than the estimated risk of infection for non-confirmed HIV/AIDS patients in the present study (0.20). It therefore suggests that; the model parameters developed by Teunis *et al.* (2004) for the *E. coli* O157:H7 outbreak among schoolchildren and their teachers in Japan; can reasonably be used to estimate risk of *E. coli* O157:H7 infection among confirmed and non-confirmed HIV/AIDS patients with diarrhoea due to consumption of meat and meat products. However, risk of *E. coli* O157:H7 infection to confirmed and non-confirmed HIV/AIDS patients in the present study is slightly overestimated relative to actual attack rates observed in the Japanese study. Since it is preferable

to adopt a conservative approach when estimating risk, especially when the underlying data are subject to considerable uncertainty, the estimated risk values observed in the current study are acceptable.

The infection rates for confirmed and non-confirmed HIV/AIDS patients consuming vegetables were low (0.21 for confirmed and 0.15 for non-confirmed HIV/AIDS patients) than observed attack rates in the Japanese study (0.21 for confirmed HIV/AIDS patients vs 0.25 for children in the Japanese study; 0.15 for non-confirmed HIV/AIDS patients vs 0.16 for teachers in the Japanese study). This suggests that the model parameters developed by Teunis *et al.* (2004) may not be suitable for estimating *E. coli* O157:H7 risk of infection suspected on consumption of vegetables under South African conditions. However, there were various uncertainties associated with the choice for the vegetable consumption data (g/capita/day instead of g/serving as was the case in the Japanese study) used in the derivation of the risks of *E. coli* O157:H7 infections. The second source of uncertainty was the use of counts for presumptive *E. coli* O157 to represent counts for *E. coli* O157:H7. The study population was also selected based on the patients experiencing diarrhoea, rather than total exposed population. The estimated risks in the present study would have been lower if the risk of *E. coli* O157:H7 infection to confirmed and non-confirmed HIV/AIDS patients with diarrhoea was being compared to the risk of acquiring the bacteria by the general public who even though hypothetically could have been having diarrhoea but never reported to the clinics for treatment.

8.3.6 Overall confidence in risk estimates of *E. coli* O157:H7 infections associated with consumption of water, meat, and meat products and vegetables presented in this study

The overall estimated distribution of *E. coli* O157:H7 in the water, meat and meat products and vegetables and its risk of infection to confirmed and non-confirmed HIV/AIDS patients as predicted by the exposure model does not compare reasonably well with other published surveys. This is because currently there are no data on risk of *E. coli* O157:H7 infections from water, meat, and meat products, or vegetables to HIV/AIDS patients in South Africa or anywhere in the world that is documented in literature.

Cassin *et al.* (1998) estimated variability of *E. coli* O157:H7 risk using a beta-binomial model to determine the probability of illness following ingestion of hamburgers contaminated with *E. coli* O157:H7. The population was assumed essentially uniform in terms of susceptibility to infections from these bacteria. The study of Cassin *et al.* (1998) is similar to the current study in terms of hazard characterization. Cassin *et al.* (1998) also used Monte Carlo simulation, as in the present study, although their study differed in terms of the exposed population and the assumed susceptibility of the population to the hazard (*E. coli* O157:H7). The patients in the present study comprised of persons with weakened immune system, which was presumed to be either due to diarrhoea or due to HIV/AIDS infections. However, Cassin *et al.* (1998) were able to establish that children aged 5 years and below in their study populations were more susceptible to *E. coli* O157:H7 infections by virtue of their under-developed immune systems.

In comparison, in the current study, risk of *E. coli* O157:H7 infection was modeled using data from a study, which fit a beta-Poisson model to actual infection data from an outbreak of *E. coli* O157:H7 in a school in Japan. Different values were derived for α and β model parameters

for the two sub-populations: children and teachers (Teunis *et al.*, 2004). This corresponds with the study of Cassin *et al.* (1998) in recognizing children as a sub-population with a relatively weaker immune system. It therefore supports the use of the model parameters for children to substitute for values for confirmed HIV/AIDS patients in the present study, since no values for HIV/AIDS patients were available.

All the samples (water, meat, and meat products and vegetables) displayed a range of probability of propagating risk of *E. coli* O157:H7 infection resulting from a presumed ingestion of these bacteria. Cassin *et al.* (1998) made a similar observation when they modeled for the probability of *E. coli* O157:H7 infection resulting from a single hamburger meal. They argued that the probability of illness resulting from ingestion of *E. coli* O157:H7 described by individual simulation output was normally infinitesimal because of reduction in microorganism load during cooking.

The USEPA limit for acceptable risk is no more than 1 in 10 000 excess infections per year, in the case of drinking water (USEPA, 1994), the estimated risks in the present study are exceedingly high, even for single exposures. This could be due to the uncertainties in the data attributable to high intake of water and foods and the high presumptive *E. coli* O157:H7 counts that were used to derive the risks. The hypothetical analysis of possible risk of *E. coli* O157:H7 infection among both confirmed and non-confirmed HIV/AIDS patients with diarrhoea suggest that exposures of these populations under investigation were unacceptably high. Nevertheless, it should not be ignored that in the present study, all risks were modeled for the food products in their ready-to-eat forms that required no further cooking (boiling in the case of water). No adjustment such as washing or cooking was made for the reduction in *E. coli* O157:H7 levels for two reasons: (a) the study represented a theoretical exploration of possible impacts of *E. coli*

O157:H7 contamination of food and drinking water, rather than trying to fit a risk model to actual outbreak data. Mostly, the conservative assumptions were used to represent worst-case scenario. (b) Food preparation practices in the exposed population were also not surveyed and were likely to include both cooked (boiled in the case of water) and uncooked forms of most of the products tested. Using values for uncooked food products/unboiled water represented the most conservative approach in the current study. If reduction in *E. coli* O157:H7 during cooking had been taken into account, estimated individual risks and risks per 100 populations would have been lower.

The water and food consumption data could have also been over estimated, which may have resulted in higher risk estimation. A single exposure to *E. coli* O157:H7 in drinking water at a consumption rate of 1500 ml/person/day was found to have higher risk to both confirmed and non-confirmed HIV/AIDS patients than the risk associated with a single exposure from ingestion of meat and meat products, and vegetables. This might be the result of the high-assumed daily intakes of water. It should also be noted that cross contamination, especially for meat and meat products and vegetables as well as temperature shifts and even cross contamination of water from sampling bottles was not assessed. These may increase the uncertainty, and consequently the variability of the risk estimates.

8.4 Conclusion

The precise relationship between the numbers of *E. coli* O157:H7 consumed and resulting adverse effects in HIV/AIDS patients is not well known. Generally the probability of risk of *E. coli* O157:H7 infection was higher for confirmed HIV/AIDS patients than for the non-confirmed

HIV/AIDS patients. Distribution of risk was prevalent across all the samples of water, meat and meat products and vegetables. However, probability of infection due to water was higher than for meat and meat products and vegetables for both confirmed and non-confirmed HIV/AIDS patients.

This chapter comes to a conclusion that confirmed and non-confirmed HIV/AIDS patients have varied vulnerability to infections due to *E. coli* O157:H7, however, confirmed HIV/AIDS patients are at higher risk of developing *E. coli* O157:H7 induced diarrhoea than non-confirmed HIV/AIDS patients. The level of risk also varied with the type of food one consumes and that meat and meat products carried greater risk of infection than vegetables. Risk due to water ingestion also varied with source of water.

Future research is required to focus on handling of meat and meat products and farming practices in the growing of vegetables. The role of retail and household handling of meat and meat products and vegetables and storage and handling of drinking water as possible risk factors for *E. coli* O157:H7 risks of infection to the public should be investigated.

References

- Ashton, P. and V. Ramasha.** 2002. Water and HIV/AIDS: Some strategic considerations in Southern Africa. In: Turton AR and Henwood R (eds.) *Hydropolitics in the Developing World: A Southern African Perspective*. African Water Issues Research Unit, Pretoria. 217–235
- Cassin, M. H., M. L. Anna, C. D. Ewen, R. William, and R. S. McColl.** 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. *Int. J. Food Microbiol.* **41(1)**: 21–44.
- Flores R. A. and T. E. Stewart.** 2004. Empirical Distribution Models for *Escherichia coli* O157:H7 in Ground Beef Produced by a Mid-size Commercial Grinder. *J. food Sci.* **69(5)**: M121–M126.
- Haas, C. N., J. Rose, and C. Gerba.** 1999. *Quantitative microbial risk assessment*, John Wiley & Sons
- Helena J. N. and P. Steyn.** 2002. Report on South African food consumption studies undertaken amongst different population groups (1983-2000): Average intakes of foods most commonly consumed. Available from: <http://www.doh.gov.za/docs/reports/2002/>. Accessed April 16th 2007.
- Josefa, M. R., H. S., Phyllis, C. Collen, M. G., Patricia, and L. S. David.** 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerg. Infect. Dis.*; **11(4)**: 603–609.
- Magwira C.A., B. A. Gashe, and E. K. Collison.** 2005. Prevalence and antibiotic resistance profiles of *Escherichia coli* O157:H7 in beef products from retail outlets in Gaborone, Botswana. *J. Food Prot.*; **68 (2)**: 403–406.

- Nauta, M. J. E. G. Evers, K. Takumi, and A. H. Havelaar.** 2001. Risk assessment of Shiga-toxin producing *Escherichia coli* O157 in steak tartare in the Netherlands. RIVM Rapport 257851003. Available at: <http://www.rivm.nl/>. Accessed 05 April 2007.
- Potter, M. E.** 1996. Risk assessment terms and definitions. *J. Food Prot. (Supplement)* S6–S9.
- Teunis, P. T. Katsuhisa, and S. Kunihiro.** 2004. Dose response for infection by *Escherichia coli* O157:H7 from outbreak data. *Risk Analy.*; **24(2)**: 401–407.
- Thompson, K. M., D. E. Burmaster, and E. A. C. Crouch.** 1992. Monte Carlo techniques for quantitative uncertainty analysis in public health risk assessments. *Risk Analysis*; **12**: 53–63.
- USEPA,** 1994. National primary drinking water regulations: enhanced surface water treatment requirements; proposed rule. Fed. Reg. 59: 38, 832 – 38, 858.
- Vorster, S. M., R. P. Greebe, and G. L. Nortje.** 1994. Incidence of *Staphylococcus aureus* and *Escherichia coli* in ground beef, broilers and processed meats in Pretoria, South Africa. *J. Food Prot.*; **57**: 305–310.
- Vose, D.** 1996. Quantitative risk analysis: a guide to Monte Carlo simulation modelling. John Wiley & Sons, Inc. New York: USA.

CHAPTER 9

TABLE OF CONTENTS	Page №
Table of contents.....	397
General discussions	398
Conclusions.....	416
Future prospect.....	418
Recommendations.....	419
References.....	421

GENERAL DISCUSSIONS

There is considerable potential for water and foods meant for human consumption to be contaminated with microorganisms from their immediate environment (Acar and Röstel, 2001). Most water and/or food-borne diarrhoea are self-limiting in immune competent persons; however, this may not be the case for immune compromised persons such as those suffering from HIV/AIDS. The situation may be exacerbated in cases where such food or water borne diarrhoea-causing agents involves *E. coli* O157:H7 that are not supposed to be treated by antibiotic therapy (Wong, 2000; Nuermberger, 2005). *In vitro* studies have shown that certain antibiotics cause induction of shigatoxin release within the patients system upon death of *E. coli* O157:H7 cells (Nuermberger, 2005).

There is no study to our knowledge that has reported on the linkage and health risk of *E. coli* O157:H7 on HIV/AIDS positive patients with diarrhoea. However, studies on the prevalence of other enteric pathogenic bacteria in the stools of HIV/AIDS patients have been reported in South Africa and other countries (Prier *et al.*, 2000; Obi and Bessong, 2002; Obi *et al.*, 2005, 2007). The current study investigated the prevalence of *E. coli* O157:H7 in drinking water, meat and meat products and vegetables, and its health risk on confirmed and non-confirmed HIV/AIDS patients with diarrhoea. The high levels of presumptive *E. coli* O157 in drinking water from Alice and other water samples (water from Dimbaza, Mdantsane, Ngwenya, Kwasiki and Fort Beauport) within the Amathole District corroborate the finding of other studies (Momba 2003a, b, 2004a, b, 2005a, b, 2006a, b). The counts that were recorded for the water samples exceeded the acceptable limits of 0 CFU/100 ml for drinking waters (DWAF, 2005; WHO, 2006). This therefore suggests that the drinking water supplied to the communities within the

Amathole District does not comply with the South African National Standards for drinking water specifications (SANS, 2001).

The presence of any strain of *E. coli* in drinking water is an indication of either faecal contamination of the water sources supplying the drinking water treatment plants or re-growth of these bacteria in the water after treatment. However, separate reports have shown that re-growth of bacteria in finished drinking water may not necessarily be due to faecal contamination (Muyima and Ngcakani, 1998; Momba and Kaleni, 2002). Re-growth of bacteria in finished water may be due to the presence of bacterial biofilms within the water distribution system or inadequate treatment of the drinking water, hence residual disinfectant is below the recommended limit of at least 1 mg/l of finished drinking water (Momba *et al.*, 2003b). This may lead to the development of bacterial biofilms within the distribution pipes; hence, the water from such pipes will always be contaminated (Momba *et al.*, 2000).

Previously, Momba *et al.* (2003b) had recommended the combined use of chlorine and monochloroamine to control the development of bacterial biofilms on the surfaces of stainless and mild steels used for making drinking water distribution pipes. They concluded that dosing of water with chlorine-monochloroamine that resulted into a residual monochloramine of at least 0.35 mg/l in the finished water was sufficient in ensuring that biofilm build up within the distribution system was controlled and that chances of re-growth of bacterial contaminants in the finished waters would be negligible (Momba *et al.*, 2003b). The most probable explanation that was offered for the high bacteria counts noted in the waters was due to insufficient coagulants and chlorine dosing hence there was little residual chlorine in the waters at the points of use. In assessing the quality of drinking water from Alice Water Treatment Plant, Momba *et al.* (2003b) revealed that the waters were characterized by overdosing of coagulants whereas chlorine dosing

was patchy. Due to this improper water treatment performance, bacterial contaminants of *Aeromonas*, *Salmonella*, *Vibrio*, *Serratia* and *Pseudomonas* species were isolated from these waters. Following such an observation, the research team proposed that the remedy for the contaminated waters was only feasible if proper dosing of coagulants and chlorine were espoused.

In an attempt to remedy the above situation, Momba *et al.* (2004a) recommended that chlorine residual of at least 0.3 mg/l at the point of use was sufficient to control re-growth of bacterial contaminants in the distribution system. Usually a specific level of disinfectant residual, normally 1 mg/l in the case of chlorine, is generally recommended to be available in finished water for a certain period, to be able to kill contaminating microorganisms and prevent re-growth (Momba *et al.*, 2003a).

Human activities may also contribute to the contamination of waters, especially surface and underground waters. This could have been the case for Ngwenya and Kwasaki borehole waters. Ngwenya borehole water is situated close to a pit-latrine whereas Kwasaki borehole water borders cattle shed. Such amenities may act as sources of pathogenic microbial contaminants to underground water sources. Reports have likewise shown that water bodies supplying raw water to the drinking water treatment plants may be contaminated with human and animal faeces, birds droppings and dead insects (Lehloesa and Muyima, 2000; Obi *et al.*, 2002).

Intermittent water supply may also be a contributing factor in promoting bacterial growth in the water distribution system. Ayoub and Malaeb (2005) reported that intermittent water supply in Beirut, Lebanon led to re-growth of water contaminating bacteria in the distribution system. A case of intermittent water supply was noticed with the Dimbaza water distribution system during the study period as the standpipes were occasionally without water.

A study that investigated (among other parameters) the microbiological quality of selected water sources used by the rural communities of Nkonkobe and Gogogo villages near Alice, also reported that the waters used by these communities were not of acceptable microbiological quality (Zamxaka *et al.*, 2004). The waters were found to contain faecal coliforms of between 0.5 to 5 log₁₀ CFU/ml. The authors concluded that the waters required treatment prior to use by the communities. Similar observations have been made on waters serving other rural communities in the Eastern Cape and other provinces of South Africa (Obi *et al.*, 2002; Momba *et al.*, 2003a, b; 2006a, b; Obi *et al.*, 2007).

Momba *et al.* (2006b) assessed the compliance of water treatment plants in five District Municipalities within the Eastern Cape Province. The results of their study indicated that 82% of the plants surveyed did not comply with the South African National Standards 241 Drinking Water Specification (SANS, 2001). Dissolved organic and inorganic constituents in the drinking water were high. Usually microorganisms and such dissolved organic and inorganic matters react with free chlorine hence imposing demand on the available residuals and eventually leading to depletion of the disinfectant in the finished drinking water (Chandy and Angles, 2001).

A report in the US demonstrated the presence of *E. coli* O157:H7 in drinking water, which led to the Walkerton *E. coli* O157:H7 outbreak in 2000 (GAP-ES, 2000). One hundred and seventy four of the 1, 346 suspected cases were presumptively positive for *E. coli* O157 but only 167 were confirmed to be *E. coli* O157:H7 using polymerase chain reaction (GAP-ES, 2000). Run-off water from yards and animal sheds to wells was suspected to be the primary cause of contamination to the water supplies.

Adopting the recommendations of these earlier studies by water treatment operators in the Amathole District and other water treatment plants within South Africa would be of great

help in minimizing bacterial and other microbial contaminants in drinking waters. This approach may ensure that the quality of water supplied to the rural communities meets acceptable standards as prescribed by DWAF (1996) and SANS (2001).

The high counts of presumptive *E. coli* O157 that were observed for the meat and meat products, mostly for polony could be attributable to the poor handling and uncontrolled slicing of the polony loaves in most shops and butcheries. This was the main reason for choosing this type of polonies for analysis following a preliminary observation. In most shops and butcheries where polony was sampled, the polonies were stripped from their wrappers, sliced into pieces, which are then sold separately to customers. Subtle lapses in food-handling procedures might be sufficient to result in contamination of the meat and meat products as was noted by Altekruuse *et al.* (1996). In some shops and butcheries before a prospective customer arrived, the sliced polonies were kept inside temperature controlled glass cabinets. However, in other shops and butcheries, once the polonies were stripped off their wrapper and sliced into pieces, the pieces were left exposed on the shelves without a protective cover. This kind of practice is believed to expose these meat products to flies and other environmental contaminants.

In the other shops and butcheries, the polonies are packed by use of polyvinyl wrappers and displayed on the shelves. These kinds of packages do not provide a controlled atmosphere but rather offer a favourable environment for continued growth and multiplication of bacterial contaminants due to the presence of oxygen, moisture and favourable growth temperature within the packages. Packaging of meat under controlled or uncontrolled atmospheres prolongs the shelf life of meat but has no effect in controlling growth of *E. coli* O157:H7 in the meat and meat products (Uyttendaele *et al.*, 2001; Cagney *et al.*, 2004).

The other disturbing observation that was made during the study was the use of bare hands in handling of meat and meat products by shop and butchery attendants. The attendants also handled interchangeably different meat and meat products, a practice that has high potential for cross contamination of the products as was argued by Du Toit and Irma, (2005). During the slicing of the polonies, cold meat and biltong, the same slicer was observed to be used without cleaning the blades in between the slicing jobs.

The prevalence of presumptive *E. coli* O157 for the meat and meat products in the current study are similar to previous findings of other similar studies (Vorster *et al.*, 1994; Heuvelink *et al.*, 1999, Magwira *et al.*, 2005), but contradict sharply with a study that was done in South Africa by Charimba, (2004). A previous study carried out in South Africa found much higher levels (74.5%) of *E. coli* O157:H7 in meat and meat products (Vorster *et al.*, 1994). Although the study by Vorster *et al.* (1994) took samples from the Pretoria region of South Africa only, it is improbable to accept as true that the differences observed between the two studies could be accounted for by regional dissimilarity given the national nature of beef supply chain in South Africa. Magwira *et al.* (2005) while investigating the prevalence of *E. coli* O157:H7 in beef products from retail outlets in Gaborone, Botswana, managed to isolate *E. coli* O157:H7 from 5.22% of chunk meat, 3.76% of minced meat and 2.26% of sausages. Flores and Stewart (2004) argued that mincing of meat encourages contamination, as the grinding process appears to inoculate the whole mincemeat product in the case of a contaminated chunk meat used for mincing. *Escherichia coli* O157:H7 have also been isolated from Hamburgers and dairy products that were implicated in an *E. coli* O157:H7 outbreak in Egypt (WHO, 1997). Comparing with findings in other countries outside the African continent, the prevalence reported in the current study is higher than figures reported in France (0.12%) (Vernozy-Rozard *et al.*, 2002), UK

(1.1%) (Chapman *et al.*, 2000), Switzerland (2.3%) (Fantelli and Stephen, 2000), Argentina (3.8%) (Chinen *et al.*, 2001) and Ireland (2.8%) (Cagney *et al.*, 2004). Heuvelink *et al.* (1999) noted that *E. coli* O157:H7 were present in 1.1% of raw minced beef samples they had examined in the Netherlands. Prevalence of *E. coli* O157:H7 in meat and meat products differs from one country to another and is dependent on several factors. Some of the factors, which seem to regulate the prevalence of these bacteria in meat and meat products, include seasonal variation and more importantly the handling of beef cattle and meat and meat products during and after the slaughter. Cagney *et al.* (2004) while investigating the prevalence of *E. coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland managed to isolate the bacteria from 2.8% of the study samples. They argued that the rate of prevalence of *E. coli* O157:H7 as was noted for the beef and beef burgers were much higher, however, this cannot compare with the rates that were obtained in the present study.

There are no data on the prevalence of *E. coli* O157:H7 in fresh vegetables in South Africa. However, there are clear evidence of unsafe farming practices and poor postharvest handling of vegetable products (Du Toit and Irma, 2005; Mnkeni *et al.*, 2006). Fresh vegetables have been identified as vehicles of *E. coli* O157:H7 infections in the United States (Olsen *et al.*, 2000; DeWall *et al.*, 2002). However, most studies have been unsuccessful in isolating any *E. coli* O157:H7 from vegetables. In the current study, presumptive *E. coli* O157 were isolated from some selected vegetables consumed by the rural communities in the Amathole District.

Prevalence of presumptive *E. coli* O157 in the vegetables as was noted in the present study is very much in agreement with an earlier report in Lima, Peru (Mora *et al.*, 2007). Mora *et al.* (2007) obtained a relatively similar prevalence (39.60%) of *E. coli* O157:H7 from some 101 fresh vegetables analyzed. Viable *E. coli* O157:H7 have also been isolated from radish sprouts

(Itoh *et al.*, 1998). Mukherjee *et al.* (2004) also reported higher prevalence of *E. coli* in some leafy greens, lettuces, and cabbages. Isolation of *E. coli* O157:H7 from fresh vegetables is challenging, as some researchers argue that the bacteria normally become inactivated in soils and so chances of contamination of vegetables in the farms are minimal (Jiang *et al.*, 2002; Solomon *et al.*, 2002).

The use of raw manure or manure slurry in the growing of vegetables to be eaten raw may be a possible route by which vegetables are contaminated with *E. coli* O157:H7 as these bacteria can persist in manure and soils for several months. Reports have shown that the bacteria can eventually be internalized into the vegetables tissues (Bolton *et al.*, 1999; Tauxe *et al.*, 1999; DeWall *et al.*, 2002; Solomon *et al.*, 2002). These have raised anxiety as regards the microbiological safety of manure-grown vegetables in South Africa and elsewhere in the world.

While there are no reported cases of *E. coli* O157:H7 outbreaks associated with any form of vegetables consumed in South Africa, results of investigations elsewhere suggest that vegetable related *E. coli* O157:H7 outbreaks are ascribed to changes in farming practices, food preparation and consumption patterns (Mukherjee *et al.*, 2004; Du Toit and Irma, 2005, Mnkeni *et al.*, 2005). Contaminated irrigation waters are also possible sources of *E. coli* O157:H7 contamination to vegetables (Solomon *et al.*, 2002). In the United States, contaminated lettuce was associated with an outbreak of *E. coli* O157:H7 (Ackers *et al.*, 1998).

The major factors that may also contribute to postharvest contamination of fresh vegetables is poor handling, washing with contaminated waters, cross-contamination by transport vessels and temperature abuse in retail stores (Beuchat and Ryu, 1997; Du Toit and Irma, 2005). Handling of vegetables, which include preparation at food service levels and in the homes can

equally introduce *E. coli* O157:H7 into the vegetables (Beuchat and Ryu, 1997, Du Toit and Irma, 2005).

The high counts and prevalence of presumptive *E. coli* O157 recorded in this study may not exclusively be due to *E. coli* O157:H7 as other serotypes of *E. coli* do often out-compete *E. coli* O157:H7 whenever they share a common growth environment (Johannessen *et al.*, 2005). It was interesting to note that this was the first time that *E. coli* O157:H7 is isolated from fresh vegetables in South Africa. It therefore stresses the need for routine surveillance for *E. coli* O157:H7 in clinical, environmental and food laboratories in South Africa in order to monitor *E. coli* O157:H7 that may be of potential threat to public health.

Escherichia coli O157:H7 has a long history of causing diarrhoeal illnesses in persons with competent as well as those with incompetent immune systems. This include infants, the elderly, beneficiaries of solid organs transplantation (liver, kidney, lung, and heart), persons under immunosuppressive therapy and those suffering from chronic diseases such as cancer (UNOS, 1999; Devesa *et al.*, 2000; Prier *et al.*, 2000). Emerging and re-emerging water and food borne diseases is disquieting. Worse still is the crippled global health care infrastructure due to the emergence of incurable diseases such as HIV/AIDS (MMWR, 1981a, b, Fauci, 2003). Human Immunodeficiency virus/Acquired immune syndrome (HIV/AIDS) manifests itself in myriad facets of infirmity conditions. Diarrhoea is one of such many ill-health conditions that characterize HIV/AIDS infection (Obi and Bessong, 2002; Hayes *et al.*, 2003; Obi *et al.*, 2007).

Studies elsewhere have demonstrated the existence of an intricate association between water and food borne pathogenic microorganisms and diarrhoea in HIV/AIDS patients (Prier *et al.*, 2002; Hayes *et al.*, 2003). In South Africa, varied species of enteric bacterial pathogens have been isolated from stools of HIV/AIDS patients with and without diarrhoea (Obi and Bessong,

2002; Obi *et al.*, 2007). An array of such bacterial pathogens included but not limited to *Salmonella spp.*, *Campylobacter spp.*, *Aeromonas spp.*, *Vibrio spp.* and *Shigella spp.* However, water and food borne enterohemorrhagic *E. coli* O157:H7 have not been well thought-out as a possible diarrhoea-causing agent in HIV/AIDS patients.

The results of the present study indicated that majority of confirmed HIV/AIDS patients with diarrhoea harboured *E. coli* O157:H7 than their non-confirmed HIV/AIDS counterparts. The prevalence of presumptive *E. coli* O157 in the patients observed by gender was of profound interest. Considered by gender and HIV status, it was noted that non-confirmed HIV/AIDS positive females had high prevalence of presumptive *E. coli* O157 than their confirmed HIV/AIDS colleagues. This was contrary to the expectation based on the premise that HIV/AIDS patients have a compromised immune system and may be more vulnerable to *E. coli* O157:H7 and other secondary infections that may be acquired from water and food borne pathogens than their non-confirmed HIV/AIDS colleagues.

The above discrepancy could be because the non-confirmed HIV/AIDS patients were not actually tested to substantiate their HIV/AIDS status. This uncertainty in one way or another may have permitted for the inclusion of some HIV/AIDS positive patients into this group. However, the confirmed HIV/AIDS males had higher prevalence of presumptive *E. coli* O157 than their non-confirmed HIV/AIDS counterparts did. This trend suggests that HIV/AIDS male patients may be more vulnerable to *E. coli* O157 infections than their non-confirmed HIV/AIDS counterparts may.

According to studies by Obi *et al.* (2007) percentage prevalence of enteric bacterial pathogens in HIV/AIDS positive patients were high in females than in the males. Roughly, 65.1% of the females were positive with *E. coli* species compared to 34.9% of the males. Other

bacterial species that were isolated from these patients included *Salmonella* at 57.9% for females and 42% for males; *Campylobacter spp.* at 51.2% for females and 48.8% for males. An analogous trend was observed for HIV negative patients too. Females were still depicted as the gender that was more vulnerable to enteric bacterial pathogens. The results of Obi *et al.* (2007) are very much in agreement with the observations made in the present study, even though their investigation was not on *E. coli* O157:H7, they demonstrated the vulnerability of females to infectious pathogens than their male counterparts.

A similar trend was also noted in Cameroon when a case of epidemic diarrhoea due to *E. coli* O157:H7 was investigated and more females were diagnosed with *E. coli* O157:H7 than males (Cunin *et al.*, 1999). The most conceivable explanation why females are often exposed to various infectious diseases is because of their position and responsibilities in the society, especially for those staying in the rural areas, which typifies the area of the current study. Females are often charged with tasks of caring for the sick, washing their soiled clothes, bathing them and preparing foods for them. This in a way exposes females to pathogenic microorganisms especially if the patient they are caring for is suffering from an illness caused by a transmittable pathogen such as those causing diarrhoea.

The distribution of presumptive *E. coli* O157 by age among those presumptively positive indicated that confirmed HIV/AIDS patients in their early ages (21 and 30) were more vulnerable to *E. coli* O157. However, this was not the case for the non-confirmed HIV/AIDS patients. This trend was equally observed by Obi *et al.* (2007) for other enteric pathogens other than *E. coli* O157:H7. When Obi *et al.* (2007) stratified their study cohorts by age, there was a marked increase in the prevalence of enteric pathogens from 4.7% for HIV positive patients aged between 0 and 10 up to 32% for the patients aged between 21 and 30 in the same group. The

study by Obi *et al.* (2007) depicted a drop (from 32% to 7%) in the prevalence of the enteropathogens for the patients who were over 50 yrs old.

This study reveals an increase in the prevalence of presumptive *E. coli* O157 because of a faster growing sexually active but yet immunocompromised individuals due to HIV/AIDS affliction. HIV/AIDS has been reported to be rife amongst the youths of ages between 15 and 30 yrs (Dorrington *et al.*, 2006). Nevertheless, the burden of *E. coli* O157 was felt across all the ages for both confirmed and non-confirmed HIV/AIDS patients.

The high prevalence of *E. coli* O157 in patients of black race may be attributable to poverty. The reason for high numbers of patients of black race with *E. coli* O157:H7 as shown in the current study is probably as a result of the fact that the study location is dominated by black people. It would have been rational to argue that race was a determinant if the numbers of patients from the race that were registered for the study were equal and that the results portrayed only blacks as having higher prevalence of presumptive *E. coli* O157 than the other races. However, separate studies on the prevalence of HIV/AIDS by race have often reported blacks as having higher infection rates of HIV/AIDS than other races (Groenewald *et al.*, 2005; Dorrington *et al.*, 2006).

Isaacson *et al.* (1993) isolated *E. coli* O157:H7 from faeces of some patients from South Africa and Swaziland where thousands of people were infected following ingestion of surface waters purportedly contaminated with the bacteria. In 2002, a study in the Central Republic of Africa failed to isolate any enterohemorrhagic *E. coli* strains from HIV/AIDS patients who had persistent diarrhoea, however, this research team managed to isolate a strain of enteroaggregative *E. coli* that was identified by the presence of *eaeA*, *astA* and *stx2* genes (Mossoro *et al.*, 2002). Reporting of *E. coli* O157:H7 infections in both developed and developing countries, South

Africa included have been predicted to be at a regularity of only 10% and 1% respectively (Warriss *et al.*, 2000).

The high occurrence of *E. coli* O157:H7 in confirmed HIV/AIDS as opposed to non-confirmed HIV/AIDS patients with diarrhoea in the present study strongly shows that the immune status of HIV/AIDS patients could be a contributing factor in their colonization with these bacteria. The use of non-confirmed HIV/AIDS patients as a control group in the current study makes it rather tricky for comparison with other studies. This is due to the suspicion that some HIV/AIDS positive patients could have been accidentally included in the group. However, the present study has made known the association between waters and foods and *E. coli* O157:H7, even though, the link between these bacteria and diarrhoeal conditions in HIV/AIDS patients still remains ambiguous.

Other studies in South Africa have demonstrated the presence of linkage between some enteric bacterial pathogens isolated from water and stools of HIV/AIDS patients (Obi *et al.*, 2007). An endeavour was made during the current study to bring to light the link that may be present between water and food borne *E. coli* O157:H7 and diarrhoea in HIV/AIDS patients. This link was demonstrated by an investigation that revealed the presence of *fliC_{H7}*, *rfbE_{O157}* and attaching and effacing (*eaeA*) genes specific to *E. coli* O157:H7, in *E. coli* O157:H7 that were isolated from the waters, meat and meat products and vegetables as well as those from the stools of confirmed and non-confirmed HIV/AIDS patients with diarrhoea.

PCR amplification using *fliC_{H7}*, *rfbE_{O157}* and *eaeA*-specific primers confirmed that a genetic region homologous in size to *fliC_{H7}*, *rfbE_{O157}* and *eaeA* structural genes of *E. coli* O157:H7 was also present in the *E. coli* O157:H7 isolated from water, meat and meat products as well as those isolated from the vegetables. This was equally observed for the *E. coli* O157:H7

that were isolated from stools of confirmed and non-confirmed HIV/AIDS patients with diarrhoea. The fact that the primers, which are specific to *fliC_{H7}*, *rfbE_{O157}* and *eaeA* were able to amplify the genes of these *E. coli* O157:H7 was an apparent substantiation that water and foods consumed in the Amathole District may be probable sources of life threatening *E. coli* O157:H7; more than ever to HIV/AIDS patients.

The imprecision that was noted in the present study was that the waters and foods that were analyzed for *E. coli* O157:H7 were not as a point of fact from the households of confirmed or non-confirmed HIV/AIDS patients whose stools were screened for the bacteria. Nonetheless, the waters were from the standpipes; and foods were from the shops, and supermarkets serving the localities where most of the HIV/AIDS patients were assumed to be dwelling. It was convincing that the waters and foods might be consumed by such patients living in these areas (Alice, Dimbaza, Fort Beaufort, Kwasiki, Mdantsane, Ngwenya, and Kwasiki).

It is not peculiar that *E. coli* O157:H7 were isolated from the stools of these patients but the cause of concern is the presence of these bacteria in the waters and foods consumed by humans, particularly HIV/AIDS positive individuals. This is for the simple reason that diarrhoea, which is mostly seen in HIV/AIDS patients, has always been associated with water and food borne enteric pathogens (Archer *et al.*, 1988; Antony *et al.*, 1988; Guerrant *et al.*, 1991; Hayes *et al.*, 2003).

Albeit *E. coli* O157:H7 were isolated from both confirmed and non-confirmed HIV/AIDS patients, it is of great fear that HIV/AIDS patients are at an increased risk of acquiring secondary infections from contaminated waters and foods compared to HIV/AIDS negative persons. This is simply because HIV/AIDS patients have a compromised immune system and for that reason may be more vulnerable to a wider range of disease causing agents. Infections of *E. coli* O157:H7

may lead to severe conditions such as passing out of bloody diarrhoea (hemorrhagic colitis), non-bloody diarrhoea, haemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Gerber *et al.*, 2002). Such infections can be asymptomatic and may involve extra intestinal sites that may in due course grow to be fatal. Most patients have severe abdominal cramps; fever is predictable in less than half of the cases (Gerber *et al.*, 2002). These conditions may be fatal if they happen to be in HIV/AIDS positive individuals.

The risk of water and food borne *E. coli* O157:H7 to HIV/AIDS patients cannot be underestimated. The magnitude to which *E. coli* O157:H7 can exacerbate the health of HIV/AIDS patient have been demonstrated by the findings of the current study. With reference to the counts and prevalence of presumptive *E. coli* O157:H7 recorded in the current study, one would say with confidence that it is a case of underreporting in South Africa.

South Africa, being a country in Sub-Saharan Africa, a region that is worst hit by HIV/AIDS pandemic with over 2.8 million new infections (UNAIDS/WHO, 2006), needs to do a lot to improve the quality and safety of the waters and foods consumed by the public. The severity of *E. coli* O157:H7 infections can only be highlighted by revisiting incidences of *E. coli* O157:H7 outbreaks in countries with proper surveillance systems. In Japan 208 school children and 43 of their teachers were infected after consuming pumpkin salads and seafood sauce (Shinagawa *et al.*, 1997). Another outbreak occurred in the same country in 1997 in which 28 children and 4 adults became ill with *E. coli* O157:H7 after consuming contaminated melon (Uchimura *et al.*, 1997).

The only country that has continually reported *E. coli* O157:H7 outbreak is the US (Barrett *et al.*, 1994; CDC, 2002; Josefa *et al.*, 2005; CFIA, 2006). This could be attributable to improved surveillance programs. The recent outbreak that shook the US is the one that involved

spinach that infected close to 200 people (Maki, 2006). Approximately 101(51%) of the victims were hospitalized, 31 (16%) suffered acute renal failure because of haemolytic uremic syndrome and three persons lost their lives (Maki, 2006). The picture portrayed by the US and Japan statistics, not to mention other countries like UK (Parry *et al.*, 1998; Duffell *et al.*, 2003; Ihekweazu *et al.*, 2006), France (Deschenes *et al.*, 1996) and Germany and Austria (Gerber *et al.*, 2002); justifies the scourge of water and food borne *E. coli* O157:H7 infection. However, the safety of water and foods consumed in Africa with regard to *E. coli* O157:H7, especially South Africa, which is viewed as the only African country catching up with the Western world in terms of development remains questionable.

Lack of data on the prevalence of *E. coli* O157:H7 in South Africa raise questions. Such as how safe are the waters, meat and meat products and vegetables consumed in South Africa? What is the level of risk attributable to pathogens such as *E. coli* O157:H7 that may be present in these waters, meat and meat products and vegetables consumed by the public, especially people with compromised immune system such as those suffering from HIV/AIDS, the old and the very young? The answer to these questions may not be in the records since documentations on the prevalence of *E. coli* O157:H7 in the waters and foods consumed in South Africa is not traceable. It cannot be confirmed whether this is due to improved illness recognition and infection control, safer waters and food handling and finely tuned preventive measures, or simply a case of underreporting of *E. coli* O157:H7 cases in South Africa.

Data on *E. coli* O157:H7 infection from Japan and the US though do not specify the category of patients, but assuming such data were based on HIV/AIDS negative patients, then one would wonder what would happen to HIV/AIDS positive patients. Studies have shown that

patients suffering from HIV/AIDS are more vulnerable to water and food borne pathogens (Connolly *et al.*, 1990; Prier *et al.*, 2000; Hayes *et al.*, 2003).

Recent investigations on the microbiological quality of waters in the Eastern Cape Province and other provinces in South Africa confirmed the fears raised by the current study (Momba *et al.*, 2006a, b, Obi *et al.*, 2007). Even though no *E. coli* O157:H7 were isolated from these studies, other enterobacteriaceae were found to be contaminating the waters. Obi *et al.* (2007) further demonstrated that there exists an intricate link between water borne enteric pathogens and diarrhoea in HIV/AIDS patients. The presence of *E. coli* O157:H7, which is a member of the larger enterobacteriaceae family, in the waters and foods consumed by HIV/AIDS patients cannot be dismissed. However, further investigations need to be conducted in this area to ascertain the fear of the unknown.

The probability of *E. coli* O157:H7 infections recorded in the current study are way beyond the probability of *E. coli* O157:H7 infection that was recorded in Japan, (0.16) (Teunis *et al.*, 2004). Reason being that the counts used to predict the probability of infection in the Japan case were for cooked food products and heat may have reduced levels of *E. coli* O157:H7.

The South African Department of Health maintains that there should be 0 CFU of *E. coli* (not *E. coli* O157:H7) in 1 g or 250 ml of food or drinking water, respectively (DoH, 2001). The counts of presumptive *E. coli* O157 that were recorded for the waters and meat and meat products and vegetables analyzed in the present study were far beyond the acceptable limits.

Previous studies in South Africa on the microbiological quality of foods have equally reported the presence of pathogenic and non-pathogenic bacterial contaminants (Vorster *et al.*, 1994; Mosupye and Von Holy, 1999; Mosupye and Von Holy 2000; Kubheka *et al.*, 2001). However, not all these studies isolated *E. coli* O157:H7 from the foods and the same reason of *E.*

coli O157:H7 inactivation may have played a role in the absence of *E. coli* O157:H7 in these previous studies.

In the current study, the vegetables were in their raw forms, while the meat and meat products (except mincemeat) were in their ready-to-eat forms and therefore there was no reduction in the numbers of *E. coli* O157:H7 by heat. This could be the reason for the high risk estimates for *E. coli* O157:H7 infections as shown in the current study. *Escherichia coli* O157:H7 is often eliminated if the food is cooked properly (Murphy *et al.*, 2004; Shin *et al.*, 2006). Murphy *et al.* (2004) reported that the *D*-values for *E. coli* O157:H7 ranged between 19.05 to 0.038 and 21.55 to 0.055 for ground turkey and beef subjected to temperatures of between 55 and 70°C. Hence, it is not surprising that *E. coli* O157:H7 related water and food borne infections are few in South Africa.

The high resistance by *E. coli* O157:H7 isolates to antibiotics, which are regularly considered as of broad spectra, have also been reported by other researchers (Magwira *et al.*, 2005; Ashraf *et al.*, 2005). The resistance profile observed amongst the *E. coli* O157:H7 indicated the unpredictability in response to antimicrobial agents. Multiple antibiotic resistance patterns by *E. coli* O157:H7 isolates as observed in the current study is a cause for concern for its health impact. Kim *et al.* (1994) also noted increased *E. coli* O157:H7 resistance to antibiotics such as streptomycin and tetracycline. A comparable resistance pattern to tetracycline was observed amongst *E. coli* O157:H7 isolates in the current study.

Ashraf *et al.* (2005) reported antimicrobial resistance by *E. coli* O157:H7 isolated from a diarrhoeal patient in Japan. The *E. coli* O157:H7 that were isolated by Ashraf *et al.* (2005) showed multi-drug resistance against streptomycin, spectinomycin, co-trimoxazole, ampicillin and tetracycline. Biyela *et al.* (2004) suggested that the widespread and indiscriminate use of

antibiotics could be the prime factor causing the recent development of antibiotic resistance in pathogenic as well as commensal microorganisms. They (Biyela *et al.*, 2004) argued that genes may be transferred horizontally or vertically between bacterial communities in the environment and the recipient bacterial species may then act as reservoirs of such resistance genes (Biyela *et al.*, 2004).

In support to the above explanation, Loncarevic *et al.* (2005) explained that acquisition of anti-microbial resistance by any bacteria in nature is governed by genetic changes in these bacteria such as mutation of nucleotide sequences within their genomes and the horizontal transfer of existing gene sequences among bacterial species. These forces are believed to increase the rate of genetic variation including the possession of drug resistance genes.

Conclusions

Diarrhoeal morbidity in HIV/AIDS patients have emerged as one of the most life threatening conditions after tuberculosis. However, *E. coli* O157:H7 have not been linked to cases of diarrhoea in HIV/AIDS patients in the Eastern Cape Province, even though it has been a culprit for diarrhoea in immune competent persons. The disproportion nature of information on this issue may be a function of intermittent surveillance for this organism. Most *E. coli* O157:H7 outbreaks have implicated drinking and bathing waters, foods mostly those of animal origin and fresh vegetables.

The comparison in the current investigation was based on results from cultural and molecular analysis. Our results indicated that *E. coli* O157:H7 was present in water, meat and meat products and vegetables as well as in the stool swab suspensions of confirmed and non-

confirmed HIV/AIDS patients with diarrhoea. However, the percentage prevalence was noted to be higher in the stool swabs of confirmed HIV/AIDS patients, than was the case for non-confirmed HIV/AIDS patients, and suggests that confirmed HIV/AIDS patients may be vulnerable to *E. coli* O157:H7 infections and that water and foods may be the potential sources of these bacteria.

Also the prevalence of *E. coli* O157:H7 between the two patient groups was gender dependent in favour of female patients. However, *E. coli* O157:H7 was distributed across all the age groups. Nevertheless, confirmed HIV/AIDS patients in their active ages 20 and 30 years of age were heavily affected than those who were <20 and >50 years of age.

The average counts of presumptive *E. coli* O157 were low for Fort Beaufort water but high for Ngwenya borehole water. The population of *E. coli* O157:H7 was higher in meat products with polony and minced meat having the highest counts. Vegetable samples were equally found to harbour high levels of *E. coli* O157:H7 but not as high as those for meat and meat products. The range of presumptive *E. coli* O157 counts observed in the water, meat and meat products and vegetable samples are similar to previously reported observations.

People with weakened immune systems, including HIV/AIDS patients, might be at a higher risk of contracting *E. coli* O157:H7 infections than the general population when they drink such waters and consume meat and meat products and vegetables contaminated with *E. coli* O157:H7. The probability of *E. coli* O157:H7 infections due to water were rather exaggerated than those of meat and meat products as well as for vegetables. This must have been a function of differences in risk variability for water and quantity of daily intakes of water compared to quantities of daily intakes of meat and meat products and vegetables.

Risk of infection with *E. coli* O157:H7 was ubiquitous for both confirmed and non-confirmed HIV/AIDS patients. However, the conclusive observation was that confirmed HIV/AIDS patients were at a higher risk of contracting *E. coli* O157:H7 infections than non-confirmed HIV/AIDS patients. Antibiotic resistance was high for *E. coli* O157:H7 that were isolated from stool swabs of confirmed HIV/AIDS patients than was observed for those isolated from stools of non-confirmed HIV/AIDS patients and even for *E. coli* O157:H7 isolated from water, meat and meat products and vegetables. The resistance profiles were also much lower for water and vegetable isolates but were rather elevated for those isolated from meat and meat products.

In closure of this research effort, we believe the findings of this study will help to address the problems of water borne *E. coli* O157:H7 related diarrhoea and in particular create awareness amongst retailers and even farmers as well as those in the food service industry. It would also assist medical practitioners in taking into account HIV/AIDS status of diarrhoeic patients prior to prescription of antibiotics to control their diarrhoeic conditions.

The issues addressed in this study can motivate for the inclusion of *E. coli* O157:H7 surveillance as a water and food safety program to the benefit of HIV/AIDS patients and the public. Water and food safety and sanitation as well as personal hygiene education for HIV/AIDS positive patients and the public would be a very vital overture.

Future prospect

There is poor surveillance and reporting of *E. coli* O157:H7 cases in most African countries hence most cases are not realized by the relevant authorities. Information on the prevalence of *E.*

coli O157:H7 in South Africa's drinking water, beef and dairy animals as well as in fresh farm produce is limited.

The effects of animal husbandry practices on the prevalence of *E. coli* O157:H7 in animals are also unknown in South Africa. There is an urgent need for research in these areas. Definitive information is particularly needed on what the current *E. coli* O157:H7 levels are in beef cattle in South Africa prior to their slaughter. This will then allow for risk assessment on farms and reduction measures can be implemented to minimize the risk of infections from *E. coli* O157:H7 and other enteropathogens. Health risk of other enteropathogens to HIV/AIDS patients, especially those that are potentially transmittable by water and foods should be investigated and appropriate measures to control them formulated.

Recommendations

- Adequate training should be offered to personnel involved in water treatment and maintenance of water distribution system should be upgraded.
- Different shop and/or butchery attendants should be assigned for different food products to avoid cross contamination. In the event that a shop or butchery have only one slicer for different meat products, the slicer should be cleaned in between the slicing of different meat and meat products.
- Shop and/or butchery attendants should not handle meat and meat products with their bare hands. A protective powder less gloves or polyvinyl hand protective should be worn to avoid transfer of microorganisms from hands to food products. Scooping spoons should be available for different meat products such as mincemeat and biltong instead of using the same scooping spoon for different meat products.

- Monitoring of food production, processing and retail establishments and cross-contamination control strategies should be introduced by the medical, veterinary, department of public health and consumer authorities to safeguard public health.
- In order to reduce *E. coli* O157:H7 risks associated with meat and meat products and vegetables, consumers should be taught on health implications of *E. coli* O157:H7 and care for suspected *E. coli* O157:H7 infections.
- Growers of vegetables to be eaten raw should be advised on the correct handling of organic wastes if they are to be applied for growing vegetables. All vegetables to be eaten in their raw forms should be properly washed prior to sale and consumption.
- There is need for education program that target traders, caterers and consumers mostly those with compromised immune system such as HIV/AIDS patients residing in both rural and urban areas on the benefits of personal hygiene.

Reference

- Acar, J. and B. Röstel.** 2001. Antimicrobial resistance: an overview. Office International des Epizooties (OIE); **20 (3):** 797–810.
- Ackers, M. L., B. E. Mahon, E. Leahy, B. Goode, T. Damrow, P. S. Hayes, W. F. Bibb, D. H. Rice, T. J. Barret, L. Hutwagner, P. M. Griffin, and L. Slutsker.** 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. *J. Infect. Dis.* **177:** 1588–1593.
- Altekruse, S. F., D. A. Street, S. B. Fein, and A. S. Levy.** 1996. Consumer knowledge of foodborne microbial hazards and food-handling practices. *J. Food Prot.* **59(3):** 287–294.
- Antony, M. A., L. J. Brandt, R. S. Klein, and L. H. Klein.** 1988. Infectious diarrhea in patients with AIDS. *Dig. Dis. Sci.* **33:** 1141–1146.
- Archer, D, and F. Young.** 1988. Contemporary issues: diseases with a food vector. *Clin Microbiol. Rev.* **1:** 377–398.
- Ashraf, M. A., K. Hidekazu, I. Kumiko, H. Yoshiko, S. Miyoko, S. Masato, and S. Tadashi.** 2005. Genomic analysis of a multidrug-resistant strain of enterohaemorrhagic *Escherichia coli* O157: H7 causing a family outbreak in Japan. *J. of Medical Microbiol.* **54:** 867–872.
- Ayoub, G. M. and L. Malaeb.** 2005. Impact of intermittent water supply on water quality in Lebanon. *Internat. J. of Environment and Pollution.* **26(4):** 379–397.
- Barrett, T. J., H. Lior, J. H. Green, R. Khakhria, J. G. Wells, B. P. Bell, K. D. Greene, J. Lewis, and P. M. Griffin.** 1994. Laboratory investigation of a multistate food-borne outbreak of *Escherichia coli* O157:H7 by using pulsed-field gel electrophoresis and phage typing. *J. Clin. Microbiol.* **32:**3013-3017.

- Beuchat, L. R. and Ryu, J. H.** 1997. Produce handling and processing practices. *Emerging Infect (eaeA) ious Diseases*; **3(4)**: 459–465.
- Biyela, P. T., J. Lin, and H. Bezuiden.** 2004. The role of aquatic ecosystems as reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. *Water Science and Techno.* **50**: 45–50.
- Bolton, D.J., C. M. Byrne, J. J. Sheridan, D. A. McDowell, and I. Blair.** 1999. The survival characteristics of a non-toxigenic strain of *Escherichia coli* O157:H7. *J. of Applied Microbiol.* **86**: 407–411.
- Cagney, C., H. Crowley, G. Duffy, J. J. Sheridan, S. O'Brien, E. Carney, W. M. Anderson, D. A. Dowell, I. S. Blair, and R. H. Bisho.** 2004. Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. *J. of Food Microbiol.* **21**: 203–212.
- CDC.** 2002. Multistate Outbreak of *Escherichia coli* O157:H7 Infections Associated With Eating Ground Beef—United States, June-July 2002. *JAMA.* **288(6)**: 637–639.
- CFIA (The Canadian Food Inspection Agency).** 2006. *E. coli* O157:H7 Outbreak in the United States Associated with Bagged Fresh Spinach. *CCDR*, **32(22)**: 272–274.
- Chandy, J. P. and M.L. Angles.** 2001. Determination of nutrients limiting biofilm formation and the subsequent impact on disinfectant decay, *Water Res.*; **35(11)**: 2677–2682.
- Chapman, P.A., C. A. Siddons, C. A. T. Malo, and M. A. Harkin.** 2000. A one year study of *Escherichia coli* O157 in raw beef and lamb products. *Epidemiol. and Infect.* **124**: 207–213.

- Charimba, G.** 2004. The Incidence, Growth and Survival of Diarrhoeagenic *Escherichia coli* in South African Meat Products. Masters Thesis. Pp 1-146. University of the Free State, South Africa.
- Chinen, I., J. D. Tanoro, E. Miliwebsky, L. H. Lound, G. Chillemi, S. Ledri, A. Baschkier, M. Scarpin, E. Manfredi, and M. Rivas.** 2001. Isolation and characterisation of *Escherichia coli* O157:H7 from retail meats in Argentina. *J. of Food Prot.* **64**: 1346–1351.
- Connolly, G. M., A. Forbes, B. G. Gazzard.** 1990. Investigation of seemingly pathogen-negative diarrhoea in patients infected with HIV1. *Gut*; **31**: 886–889.
- Cunin, P., T. Etienne, G. Yves, N. Chouaïbou, B. Raymond, M. Jacques, and M. V. M. Paul.** 1999. An Epidemic of Bloody Diarrhea: *Escherichia coli* O157 Emerging in Cameroon? *Emerg. Infect. Dis.* **5(2)**: 285–290.
- Deschenes, G. C. Casenave, F. Grimont, J. C. Desenclos, S. Benoit, M. Collin, S. Baron, P. Mariani, E A.-D. Grimont, and H. Nivet.** 1996. Cluster of cases of haemolytic uraemic syndrome due to unpasteurised cheese. *Paediatric Nephrol.* 10:203-205.
- Devesa SS, Grauman DJ, Blot WJ, et al.** Atlas of cancer mortality in the United States: 1950-94. Washington, DC: US Government Printing Office, 1999; NIH publication No. (NIH) 99-4564. Available from: <http://www-dceg.ims.nci.nih.gov/atlas/index.html>. Accessed 24th August 2007.
- DoH.** 2001. Guidelines for environmental health officers on the interpretation of microbiological analysis data for food. Available from: <http://www.Doh.gov.za/doc/factsheets/microguides>. Accessed 1 September, 2007.

- Dorrington, R. E., L. F. Johnson, D. Bradshaw, and T. Daniel.** 2006. The Demographic Impact of HIV/AIDS in South Africa. National and Provincial indicators for 2006. Cape Town: Centre for Actuarial Research, South African Medical Research Council and Actuarial Society of South Africa. ISBNB 0-7992-2322-0
- Du Toit L. and V. Irma.** 2005. Food practices associated with increased risk of bacterial food-borne disease of female students in self-catering residences at the Cape Peninsula University of Technology. *J. of Family Ecology and Consumer Sciences*, **33**: 73–88
- Duffell, E., E. Espiè, T. Nichols, G. Adak, H. De Valk, and J. Stuart.** 2003. Investigation of an outbreak of *E. coli* O157 infections associated with a trip to France of schoolchildren from Somerset, England. *Eur. Surv.* **8(4)**: 81–86.
- DWAF (Department of Water Affairs and Forestry).** 1996. *South African Water Quality Guidelines for Domestic Use* (2nd edn.) Pretoria. pg 1–197.
- DWAF (Department of Water affairs and Forestry).** 2005. A drinking water quality framework for South Africa; pp 1 – 69. Available on: <http://www.dwaf.gov.za/Documents/>. Accessed on 09 April 2007.
- Fantelli, K. and R. Stephan.** 2001. Prevalence and characteristics of shigatoxin-producing *Escherichia coli* and *Listeria monocytogenes* strains isolated from minced meat in Switzerland. *Int. J. of Food Microbiol.* **70**: 63–69.
- Fauci, A. S.** 2003. HIV and AIDS: 20 years of science. *Nature Medicine*; **9**: 839 – 843.
- Flores R. A. and T. E. Stewart.** 2004. Empirical Distribution Models for *Escherichia coli* O157:H7 in Ground Beef Produced by a Mid-size Commercial Grinder. *J. of food Sci.* **69(5)**: M121–M126
- GAP ES Inc.** 2000. Investigations to trace the source of contamination and monitor disinfection of the drinking water system in Walkerton, Ontario In: *The Ontario Clean Water Agency's*

Report to the Walkerton Public Utilities Commission on the Operational Measures Taken to Address the E. coli Water Contamination in the Town of Walkerton. October 17, 2000.

- Gerber, A., H. Karch, F. Allerberger, H. M. Verweyen, and L. B. Zimmerhack.** 2002. Clinical Course and the Role of Shiga Toxin–Producing *Escherichia coli* Infection in the Hemolytic-Uremic Syndrome in Pediatric Patients, 1997–2000, in Germany and Austria: A Prospective Study. *The J. of Infect. Dis.* **186**: 493-500.
- Groenewald, P., N. Nannan, D. Bourne, R. Laubscher, and D. Bradshaw.** 2005. Identifying deaths from AIDS in South Africa. South African National HIV Prevalence Incidence Behavior and Communication Survey, 2005.
- Guerrant, R. L. and D. A. Bobak.** 1991. Bacterial and protozoal gastroenteritis. *New Eng. J. of Med.* **325**: 327–337.
- Hayes, C. E. Elliot, E. Kroles, D. Goulda.** 2003. Food and Water Safety for Persons Infected with Human Immunodeficiency Virus. *Clin. Infect. Dis.* **36(S2)**: S106–S109.
- Heuvelink, A. E., J. T. Zwartkruis-nahuis, B. R. Beumen, E. De Boer.** 1999. Occurrence and survival of verocytotoxin-producing *Escherichia coli* O157 in meat obtained from retail outlets in The Netherlands. *J. of Food Prot.* **62**: 1115-1122.
- Ihekweazu, C., M. Barlow, S. Roberts, H. Christensen, B. Guttridge, D. Lewis.** 2006. Outbreak of *E. coli* O157 infection in the south west of the UK: risks from streams crossing seaside beaches. *Euro Surveill*; **11(4)**: 128–130.
- Isaacson, M., P. M. Canter, P. E. Effler, L. Arntzen, P. Bomans, and R. Heenon.** 1993. Haemorrhagic colitis epidemic in Africa. *Lancet*; **341**: 961.
- Itoh, Y., Y. Sugita-Konishi, F. Kasuga, M. Iwaki, Y. Hara-Kudo, N. Saito, Y. Noguchi, H. Konuma, and S. Kumagai.** 1998. Enterohemorrhagic *Escherichia coli* O157:H7 present in radish sprouts. *Appl. Environ. Microbiol.* **64**:1532-1535.

- Jiang, X., J. Morgan, and M. P. Doyle.** 2002. Fate of *Escherichia coli* O157:H7 in manure-amended soil. *Appl. Environ. Microbiol.* **68**:2605–2609.
- Johannessen, G. S., G. B. Bengtsson, B. H. Heier, S. Bredholt, Wasteson, Y. and L. M. Rørvik.** 2005. Potential uptake of *Escherichia coli* O157:H7 from organic manure into crisphead lettuce. *Appl Environ Microbiol.* **71**: 2221–2225.
- Josefa, M. R., H. S., Phyllis, C. Collen, M. G., Patricia, and L. S. David.** 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerg. Infect. Dis.* **11**(4): 603–609.
- Kim, H. H. M. Samandpour, L. Grim, G. R. Claussen, T. N. Bessen, M. Bailer, J. M. Kobayashi, M. A. Neil, F. D. Schoenknecht, and P. I. Tarr.** 1994. Characteristics of antibiotic -resistant *Escherichia coli* O157:H7 in Washington State, 1984–1991. *J. of Infect. Dis.* **170**: 1606–1609.
- Kubheka, I. C., F.M. Mosupye, and A. Von-Holy.** 2001. Microbial survey of street-vended salad and gravy in Johannesburg City, South Africa. *Food Control*, **12**: 127–131.
- Lehloesa, L. J. and N. Y. O. Muyima.** 2000. Evaluation of the impact of household treatment procedures on the quality of groundwater supplies in the rural community of the Victoria district, Eastern Cape. *Water SA*; **26**(2): 285–290.
- Loncarevic, S., G. S. Johannessen, and L. M. Rorvik.** 2005. Bacteriological quality of organically grown leaf lettuce in Norway. *Lett. Appl. Microbiol.* **41**:186–189.
- Magwira, C. A., B. A. Gashe, and E. K. Collison.** 2005. Prevalence and antibiotic resistance profiles of *Escherichia coli* O157:H7 in beef products from retail outlets in Gaborone, Botswana. *J. of Food Prot.* **68** (2): 403 – 406.

- Maki, D. G.** 2006. Don't eat the spinach--controlling foodborne infectious disease. *New Engl J. Med.* **355(19)**:1952–1955.
- MMWR.** 1981. Karposi's sarcoma and pneumocystis pneumonia among homosexual men-New York City and California. *Morb. Mortal. Wkly. Rep.* **30**: 305–308.
- MMWR.** 1981. Pneumocystis pneumonia in Los Angeles. *Morb. Mortal. Wkly. Rep.* **30**: 250–252.
- Mnkeni, P, N. S., B. J. Cisneros, M. C. Phasha, and L. M. Austin.** 2006. Use of human excreta from urine-diversion toilets in food gardens: Agronomic and health aspects. WRC Report № 1439/3/06. ISBN № 1-77005-455-3. *Water Res. Comm.* **3**:1–40.
- Momba, M. N. B., S. Ndaliso, and M. A. Binda.** 2003a. Effect of a combined chlorine-monochloramine process on the inhibition of biofilm regrowth in potable water systems. *J. Water Supply*; **3 (1-2)**: 215–221.
- Momba, M.N.B., N. Makala, and B. M. Brouckaert.** (2003b) Evaluation of factors affecting the performance of small water supplies for the removal of indicator bacteria in rural developing areas of South Africa. *International Water Association Health-Related Water Microbiology Symposium*. Cape Town/South Africa 14-19 September 2003.
- Momba, M. N. B, V. K. Malakate, and J. Theron.** 2006a. Abundance of pathogenic *Escherichia coli*, *Salmonella typhimurium* and *Vibrio cholerae* in Nkonkobe drinking water sources. *J. of Water Health* **04**: 289–296.
- Momba, M. N. B., and P. Kaleni.** 2003. Re-growth and survival of indicator microorganisms on the surfaces of household containers used for the storage of drinking water in rural communities of South Africa. *Water Research*; **36**: 3023–3028.

- Momba, M. N. B., N Makala, B. Zani, and B. M. Brouckaert.** 2005a. Key causes of drinking water quality failure in a rural small water supply of South Africa. In *Water Encyclopedia –Domestic, Municipal, and Industrial water supply and waste disposal* (Edt J.H. Lehr and J. Keeley). John Wiley & Son, Inc. Pages 221–227.
- Momba, M. N. B., N. Makala, Z. Tyafa, and B. M. Brouckaert.** 2005b. A model partnership for sustainable production of safe drinking water for rural communities in South Africa. *SA J. of Science*, **101**: 335–336.
- Momba, M. N. B., R. Kfir, S. N. Venter, and T. E. Cloete.** 2000. An overview of biofilm formation in potable water distribution systems and its impact on the deterioration of water quality. *Water, SA*; **26(1)**: 59–66.
- Momba, M. N. B., V.K. Malakate, and J. Theron.** 2004a. Abundance of pathogenic *Escherichia coli*, *Salmonella typhimurium* and *Vibrio cholerae* in Nkonkobe drinking water sources. *J. Water and Health*. **04**: 289–296.
- Momba, M. N. B., Z. Tyafa, and N. Makala.** 2004b. Rural water treatment plants fail to provide potable water to their consumers: the Alice water treatment plant in the Eastern Cape Province of South Africa. *SA J. of Sci.*; **100**: 307–310.
- Momba, M. N. B., Z. Tyafa, N. Makala, B. M. Brouckaert, and C. L. Obi.** 2006b. Safe drinking water still a dream in rural areas of South Africa. Case study: The Eastern Cape Province. *Water, SA*; **32**: 715–720.
- Mora, A., S. L. Leon, M. Blanco, J. E. Blanco, C. Lopez, G. Dahbi, A. Echeita, E. A. Gonzalez, and J. Blanco.** 2007. Phage types, virulence genes and PFGE profiles of shigatoxin-producing *Escherichia coli*O157:H7 isolated from raw beef, soft cheese and vegetables in Lima (Peru). *Int. J. of Food Microbiol.* **114(2)**: 2004–210.

- Mossoro C., P. Glaziou, S. Yassibanda et al.** 2002. Chronic diarrhea, hemorrhagic colitis, and hemolyticuremic syndrome associated with HEP-2 adherent *Escherichia coli* in adults infected with human immunodeficiency virus in Bangui, Central African Republic. *J Clin Microbiol.* **40**: 3086–3088.
- Mosupye F. and A. von Holy.** 1999. Microbiological quality and safety of ready-to-eat street-vended foods in Johannesburg, South Africa, *J. of Food Prot.* **62**: 1278–1284.
- Mosupye F. and A. von Holy.** 2000. Microbiological hazard identification and exposure assessment of street food vending in Johannesburg, South Africa. *Int J Food Microbiol.* **61(2-3)**:137–145.
- Shin, J.-H., S.-Y. Lee, R.H. Dougherty, B. Rasco, and D.-H. Kang.** 2006. Combined effect of mild heat and acetic acid treatment for inactivating *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium* in an asparagus puree *J. of Applied Microbiol.* **101(5)**: 1140–1151.
- Murphy, R. Y., E. M. Martin, L. K., Duncan, B. L., Beard, J. A. Marcy.** 2004. Thermal process validation for *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in ground turkey and beef products. *J. of Food Prot.* **67(7)**: 1394–1402.
- Mukherjee, A., D. Speh, E. Dyck, and F. Diez-Gonzalez.** 2004. Pre-harvest evaluation of coliforms, *Escherichia coli*, *Salmonella* and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. *J. Food Prot.* **67(5)**: 894–900.
- Muyima, N. Y. O. and F. Ngcakani.** 1998. Indicator bacteria and regrowth potential of the drinking water in Alice, Eastern Cape. *Water SA* **24 (1)**: 29–34.
- Nuermberger, E.** 2005. Current Issues in the Diagnosis, Evaluation, and Management of Gastrointestinal Infections. *J. of Gastro. Infect.* **5(2)**: 90–97.

- Obi C. L., J. Ramalivhana, M. N. B. Momba, B. Onabulu, J. O. Igumbor, M. Lukoto, T. B. Mulaudzi, E. L. Jansen van Rensburg, E. Green, and S. Ndou.** 2007. Antibiotic resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients with and without diarrhea and their household drinking water in rural communities in Limpopo Province South Africa. *Afri. J. Biotec.* **6(8)**: 1035–1047.
- Obi, C. L. and P. O. Bessong.** 2002. Diarrhoeagenic bacterial pathogens in HIV positive patients with diarrhea in rural communities of Limpopo Province, South Africa. *J. of Health and Pop. Nut.* **20(3)**: 230–234.
- Obi, C. L., M. N. B. Momba, B. Onabolu, E. J. Van Rensburg, M. Lukoto, E. Green, J. Ramalivahna, J. O. Igumbor, S. Ndou, P. O. Bessong, and T. B. Mulaudzi.** 2005. Molecular relatedness of enteric bacterial pathogens isolated from water sources and HIV/AIDS patients with diarrhoea in Rural communities in Limpopo Province; WRC Report (K5/1633/3).
- Obi, C. L., N. Potgieter, P. O. Bessong, and G. Matsaung.** 2002. Assessment of the microbial quality of river water sources in rural Venda communities in South Africa. *Water SA*; **28(3)**: 287–292.
- Olsen, S. J., G. Miller, T., Breuer, M., Kennedy, C., Higgins, J., Walford, G., McKee, K., Fox, W., Bibb, and P., Mead.** 2002. A water-borne outbreak of *Escherichia coli* O157:H7 infections and hemolytic uremic syndrome: implications for rural water systems. *Emerg. Infect. Dis.* **8**: 370–375.
- Parry, S. M., R. L. Salmon, G. A. Willshaw, and T. Cheasty.** 1998. Risk factors for and prevention of sporadic infections with vero cytotoxin (shiga toxin) producing *Escherichia coli* O157. *Lancet*; **351**: 1019 – 1022.

- Prier, R. and V. S. Jay.** 2000. Foodborne and waterborne infectious diseases: Contributing factors and solutions to new and reemerging pathogens. *The Pract. peer-Rev. J. of Primary Care Phy.* **107(4)**.
- SANS (South African National Standards).** 2001. *SABS 241-2001: Specification for Drinking Water*. Pretoria, South Africa.
- Shinagawa, K., D. Y. Hu, and S. Yoshida.** 1997. Correspondence and problem for enterohemorrhagic *E. coli* O157 outbreak in Morioka city, Iwate. *Bull. Natl. Inst. Public Health*, **46**: 104–112.
- DeWaal, C., K. Barlow, L. Alderton, and M. F. Jacobson.** 2002. Outbreak alert! Center for Science in the Public Interest. Available from: <http://www.cspinet.org/reports>. Accessed 23rd August 2007.
- Solomon, E. B., S. Yaron, and K. R. Mathews.** 2002. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Appl. Environ. Microbiol.* **68**:397–400.
- Tauxe, R., H. Kruse, C. Hedberg, M. Potter, J. Madden, and K. Wachsmuth.** 1997. Microbial hazards and emerging issues associated with produce a preliminary report to the national advisory committee on microbiological criteria for foods. *J. Food Prot.* **60**:1400–1408.
- Teunis P., T. Katsuhisa, and S. Kunihiro.** 2004. Dose response for infection by *Escherichia coli* O157:H7 from outbreak data. *Risk Analysis*; **24(2)**: 401 – 407.
- Uchimura, M., K. Kishida, and K. Yoda.** 1997. An outbreak of enterohemorrhagic *E. coli* O157: H7 in a daycare—Chiba Prefecture. *Infect. Agents Surv. Rep.* **18**: 309–310.

- UNAIDS/WHO.** 2006 Report on the global AIDS epidemic. Available from: http://www.unaids.org/en/HIV_data/2006GlobalReport/default.asp. Accessed 11th May 2007.
- UNOS. (United Network for Organ Sharing).** 1999. Number of US transplants: 1988 to June 30, 1999, by organ and donor type. Available from: <http://www.unos.org/Newsroom>. Accessed 24th August, 2007.
- Uyttendaele, M., E. Jozwik, A. Tutenel, L. De Zutter, J. Uradzinski, D. Pierard, and J. Debevere.** 2001. Effect of acid resistance of *Escherichia coli* O157:H7 on efficacy of buffered lactic acid to decontaminate chilled beef tissue and effect of modified atmosphere packaging on survival of *Escherichia coli* O157:H7 on red meat. *J. Food Prot.* **64**: 1661–1666.
- Vernozy-Rozand, C., S. Ray-Gueniot, C. Ragot, C. Bavai, C. Mazuy, M. P. Montet, J. Bouvet, and Y. Richard.** 2002. Prevalence of *Escherichia coli* O157:H7 in industrial mince beef. *Lett. in Applied Microbiol.* **35**: 7–11.
- Vorster, S. M., R. P. Greebe, and G. L. Nortje.** 1994. Incidence of *Staphylococcus aureus* and *Escherichia coli* in ground beef, broilers and processed meats in Pretoria, South Africa. *J. Food Prot.* **57**: 305–310.
- Warriss, P. D.** 2000. Meat Science: An Introductory Text. 1st edn. CABI Publishers, New York.
- WHO.** 2006. Guidelines for drinking-water quality incorporating first addendum. Available at: http://www.who.int/water_sanitation_health/. Accessed on 06 April 2007.
- WHO.** Press Release, 58. 1997. Foodborne diseases possibly 350 times more frequent than reported. Geneva, Switzerland.

- Wong, C. S., S. Jelacic, R. L. Habeeb, S. L. Watkins, and P. I. Tarr.** 2000. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *New Eng J. of Med.* **342**: 1930–1936.
- Zamxaka, M., G. Pironcheva, and N. Y. O. Muyima.** 2004. Microbiological and Physico-chemical assessment of the quality of domestic water sources in selected rural communities of the Eastern Cape Province, South Africa. *Water, SA*; **30(3)**: 333–340.

APPENDICES

APPENDIX A1

Informed consent form used during the study English version

This informed consent form has been designed to be signed by patients who willingly agree to give their faecal swabs as test samples for the research project titled “Prevalence and impact of *Escherichia coli* O157:H7 in HIV positive and HIV negative patients with diarrhoea in Amathole District in the Eastern Cape Province of South Africa”.

Faecal swab will be used for research purposes only. All information shall be kept confidential.

I, the undersigned, agree to participate in the study for the “Prevalence and impact of *Escherichia coli* O157:H7 in HIV positive and HIV negative patients with diarrhoea in Amathole District in the Eastern Cape Province of South Africa” by giving my faecal swab as the test sample.

Patient’ Signature..... Date.....

I agree that the patient willingly gave his/her faecal sample for the above mentioned study.

Health Personnel’s Name..... Signature..... Date.....

Consent form №.....

NOTE: The consent form number on the form should be reflected on the specimen bottle to avoid sample duplication.

APPENDIX A2

Ifomu yokuvumela emakwenziwe emva kokucacelwa - isiXhosa version

Le fomu yokuvumela emakwenziwe emva kokucacelwa iye yaqulunqelwa okokuba isayinwe zizigulane ezithi zivume ngokunokwazo okokuba zinikezele ngelindle/ituwa yazo njengento ekuza kuvavanywa ngayo kumsebenzi wophando olwenziwayo phantsi kwesihloko esithi “Ubukho be-*Escherichia coli* O157:H7 Kwisithili sase-Amathole kwiPhondo leMpumaKoloni kweloMzantsi Afrika kwanegalelo lazo kwiimeko zorhudo lwezigulane ezinentsholongwane kagawulayo nezingenayo le ntsholongwane”

Iindle/ituwa yazo iya kusetyenziselwa iinjongo zophando. Phantsi kwazo zonke iimeko, aliya kwaziswa igama lomntu.

Mna, osayine apha ngezantsi, ndiyavuma ukuthabatha inxaxheba kwizifundo zophando phantsi kwesihloko esithi ““Ubukho be-*Escherichia coli* O157:H7 Kwisithili sase-Amathole kwiPhondo leMpumaKoloni kweloMzantsi Afrika kwanegalelo lazo kwiimeko zorhudo lwezigulane ezinentsholongwane kagawulayo nezingenayo le ntsholongwane”.

Isignitsha yesigulane..... Umhla.....

Ndiyavuma okokuba isigulane siye sanikezela ngelindle/ngetuwa yaso ngokuzithandela, ngaphandle kwesinyanzelo ngeenjongo zolu phando lukhankanywe apha ngasentla.

Igama leGosa lezeMpilo.....

Isignitsha..... Umhla.....

Inombolo yeFomu yeMvume.....

Qaphela: Inombolo yeFomu yeMvume kufuneka iboniswe kwibhotilana enelo lindle/ituwa ukuze kungabikho kuphinda-phindwa kwemizekelo yelo lindle/ituwa eza kuvavanywa.

APPENDIX B:

Data recording sheet used at Frere Hospital to record patients' information

Data Recording Sheet №-----

General Information

Date (DD)/ (MM)/ (YYYY)

-----/-----/-----

Time: - - : - -

Sample type-----Sample code-----

HIV Status (tick where appropriate)

Positive-----

Negative-----

Demographical information

Location of patient-----

Gender

M-----F----- Age-----Race-----

Hospital staff collecting sample

Name-----Signature-----Date-----

APPENDIX C1: Research Ethical approval from Department of Health Bisho



F 040 602 1319

EASTERN CAPE DEPARTMENT OF HEALTH

Enquiries: Dhlamini Thomas

Tel No: 040 609 3409

Date: 17 November 2004

Fax No: 040 639 1440

Dear Mr. Bernard Omondi

RE: PREVALENCE OF E. COLI 0157:H7 IN WATER AND BOVINE AND VEGETABLE PRODUCTS SOLD IN EASTERN CAPE & ITS IMPACT ON DIARRHEAGENIC CONDITIONS OF HIV/AIDS PATIENTS

The Department of Health would like to inform you that your application for conducting a research on the abovementioned topic has been approved based on the following conditions:

1. During your study, you will follow the submitted protocol with ethical approval and can only deviate from it after having a written approval from the Department of Health in writing
2. You are advised to ensure that you will respect the rights of the participants and maintain confidentiality of their identities
3. At the end of your study, you will inform the Department of Health about your findings and your recommendations based on the study. Note that the recommendations should be clear(easy-to-understand), concise and implementable
4. Your results on the findings of your study conducted in Eastern Cape will not be presented anywhere unless you have shared them with the Department of Health

Your interest in conducting a study in our province is highly appreciated because it will play a vital role in improving the health of our people in the province

We would like really appreciate your cooperation in this regard

DEPUTY DIRECTOR: EPIDEMIOLOGICAL RESEARCH & SURVEILLANCE MANAGEMENT

DATE: 18/11/2004

**APPENDIX C2: Research Ethical approval Govan Mbeki Research and Development
Centre**



University of Fort Hare
Together in Excellence

Govan Mbeki Research & Development Centre

P/Bag X1314, Alice, 5700. E-mail: agilbert@ufh.ac.za Tel & Fax: 04060-22319

TO WHOM IT MAY CONCERN

Ethical protocol for faecal sample

Student: Bernard Omondi
Student No: 200440969
PhD Microbiology

As the designated authority for Research Ethics I have considered the researcher's ethical protocol.

I note:

1. That the procedure carries no risk to the patient;
2. That confidentiality of the patient will be ensured as there will be no identified particulars recorded on the specimen bottles. In addition the researcher will have no face-to-face contact with the patient;
3. The procedure for collecting the samples is standard medical practice;
4. That all that is needed to identify the specimen is the name of the hospital, the date of the sample and a sequence number to be able to ascertain how many samples have been attained from each clinic.

Provided the nurse obtains consent from the patient for the sample to be used for research purposes, it is my opinion that the proper ethical procedures are in place and the process is approved by the university.

Signed:

PROF ANDY GILBERT
ACTING DEAN OF RESEARCH

Date:

11/11/08

APPENDIX D1: The mean counts of presumptive *E. coli* O157 in the study samples

Table D1a: *Escherichia coli* O157 average counts in CFU/ml of the water samples

Location of water samples	Counts
Fort Beaufort	3.3×10^4
Alice	1.1×10^5
Dimbaza	4.1×10^4
Mdantsane	3.5×10^4
Ngwenya	1.71×10^5
Kwasaki	4.8×10^4

Table D1b: *Escherichia coli* O157 average counts in CFU/g of the meat and meat product samples

Meat product	Fort Beaufort	Alice	Mdantsane
Biltong	1.8×10^4	10.4×10^5	7.22×10^4
Cold meat	1.5×10^5	4.0×10^3	0
Mincemeat	1.1×10^1	2.6×10^2	2.0×10^5
Polony	5.0×10^6	9.1×10^5	8.9×10^4

Table D1c: *Escherichia coli* O157 average counts in (10^3) CFU/g of the vegetable samples

Vegetable samples	F/Beaufort	Alice	Mdantsane
Cabbage	2.8×10^4	2.34×10^4	7.55×10^3
Carrots	9×10^3	2.95×10^4	7.65×10^3
Cucumber	9.45×10^4	1.25×10^3	5.95×10^3
Onions	0	4.73×10^3	1.6×10^3
Spinach	1.59×10^5	4.1×10^4	1.57×10^4

APPENDIX D2: Prevalence of presumptive *E. coli* O157 in the study samples

Table D2a: Percentage prevalence of presumptive *E. coli* O157 in drinking water samples from various distribution points in Amathole District.

Location/water	% Prevalence of <i>E. coli</i> O157
Fort Beaufort	16.67
Alice	30
Dimbaza	30
Mdantsane	13.33
Ngwenya	36.67
Kwasaki	26.67

Table D2b: Percentage prevalence of presumptive *E. coli* O157 in meat product samples sold in supermarkets, shops and butcheries in Amathole District.

Meat products	Fort Beaufort	Alice	Mdantsane
Biltong	4.43	86.89	8.67
Mincemeat	40.72	53.64	5.63
Cold meat	4.96	54.06	40.98
Polony	72.96	18.29	8.75

Table D2b: Percentage prevalence of presumptive *E. coli* O157 in vegetable samples sold in supermarkets, shops and butcheries in the Amathole District.

Vegetable samples	F/Beaufort	Alice	Mdantsane
Cabbage	33.3	16.7	33.3
Carrots	25.0	25.0	33.3
Cucumber	16.7	16.7	33.3
Onions	0.0	25.0	8.3
Spinach	16.7	16.7	25.0

APPENDIX E: Prevalence of presumptive *E. coli* O157 in the confirmed HIV/AIDS positive and non-confirmed HIV/AIDS negative patients.

Table E1a: Percentage prevalence of presumptive *E. coli* O157 in the stool swab suspensions of confirmed HIV/AIDS positive and non-confirmed HIV/AIDS negative patients with diarrhoea

HIV/AIDS Status	% <i>E. coli</i> O157 positive
HIV+ (confirmed)	56.81
HIV- (non-confirmed)	43.18

Table E1b: Percentage prevalence of presumptive *E. coli* O157 in the stool swab suspensions of confirmed HIV/AIDS positive and non-confirmed HIV/AIDS negative patients with diarrhoea visiting Frere Hospital, East London categorized by gender

Gender	HIV+ (confirmed) (%)	HIV- (non-confirmed) (%)
<i>E. coli</i> +ve (Males)	26.7	15.8
<i>E. coli</i> +ve (Females)	73.3	84.2

Table E1c: Percentage prevalence of presumptive *E. coli* O157 in the stool swab suspensions of confirmed HIV/AIDS positive and non-confirmed HIV/AIDS negative patients with diarrhoea visiting Frere Hospital, East London categorized by age.

Patients category	Age Range	% <i>E. coli</i> O157
HIV + (confirmed)	11 to 20	2.7
	21 to 30	41.9
	31 to 40	29.7
	41 to 50	18.9
	>50	4.1
HIV non-confirmed	11 to 20	5.3
	21 to 30	8.8
	31 to 40	21.1
	41 to 50	33.3
	>50	31.6

Table E1d: Percentage prevalence of presumptive *E. coli* O157 in the stool swab suspensions of confirmed HIV/AIDS positive and non-confirmed HIV/AIDS negative patients with diarrhoea visiting Frere Hospital, East London categorized by race. Patients' category by HIV/AIDS

Status	Race	% prevalence of <i>E. coli</i> O15
HIV + (confirmed)	Blacks	90.5
	Coloured	9.5
	Whites	0.0
	Others	0.0
HIV - (non-confirmed)	Blacks	73.7
	Coloured	12.3
	Whites	12.3
	Others	1.8

Table E1e: Percentage prevalence of presumptive *E. coli* O157 in the stool swab suspensions of confirmed HIV/AIDS positive and non-confirmed HIV/AIDS negative patients with diarrhoea visiting Frere Hospital, East London categorized by residential locations.

Patients category by HIV/AIDS		
Status	Location	% <i>E. coli</i>
HIV + confirmed	Butterworth	2.70
	Duncan Village	2.70
	East London	75.67
	King Williams town	0
	Mdantsane	2.70
	Stutterheim	4.05
	Others	12.6
HIV- non-confirmed	Butterworth	5.26
	Duncan Village	3.51
	East London	73.68
	King Williams town	3.51
	Mdantsane	0
	Stutterheim	3.51
	Others	10.53