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, either in part or in its entirety, for the award of any degree.

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Date: _____10 April 2010_____

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DEDICATION

This Thesis is

Dedicated to my parents: Mr. and Mrs. C. N. Osode

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Mary Chukwudumebi Udeh and

Brothers: Mr. Peter Ikechukwu Osode, Mr. Victor Chukwudi Osode,

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LIST OF ABBREVIATIONS

AE	–	Adhesion-effacing
ATCC	–	American Type Culture Collection,
Bp	–	Base pair
cAMP	–	Cyclic adenosine monophosphate
CFU	–	Colony forming units
DAEC	–	Diffusively adherent *E. Coli*
dATP	–	deoxyadenosine triphosphates
dCTP	–	deoxycytidine triphosphates
dGTP	–	deoxyguanosine triphosphates
dTTP	–	deoxythymidine triphosphates
DNA	–	Deoxyribonucleic acid
DWAF	–	Department of Water and Forestry
e.g.	–	*exempli gratia*, for example
eae	–	Attaching and effacing gene
EAEC	–	Enteroaggregative *Escherichia coli*
EC	–	Eastern Cape
EDTA	–	Ethylenediaminetetraacetic acid

EHEC	–	Enterohaemorrhagic *Escherichia coli*
EIEC	–	Enteroinvasive *Escherichia coli*
EMBA	–	Eosin Methylene Blue Agar
EPA	–	Environmental Protection Agency
EPEC	–	Enteropathogenic *E. coli*
et al	–	(*et alii*) and others
EtBr	–	Ethidium bromide
ETEC	–	Enterotoxigenic *E. coli*
fliC	–	Flagellin gene
g	–	Gram
h	–	Hour
HUS	–	Hemolytic-uremic syndrome
LT	–	Heat-labile enterotoxin
$MgCl_2$	–	Magnesium Chloride
mg	–	Milligram
MHA	–	Mueller-Hinton Agar
min	–	Minute
ml	–	Millilitre
mM	–	Millimole
NB	–	Nutrient Broth
NA	–	Nutrient Agar
NaCl	–	Sodium Chloride
NCCLS	–	National Committee for Clinical Laboratory Standards

№	–	Number
O	–	Somatic antigen
H	–	Flagella antigen
K	–	Capsular antigen
^{o}C	–	Degrees Celsius
PCR	–	Polymerase Chain Reaction
rfb	–	Gene cluster encoding biosynthesis of the O-antigen (pO157)
SA	–	South Africa
SAS	–	Statistical Analysis System
SLT	–	Shiga-like toxin
ST	–	Heat-stable enterotoxin
STEC	–	Shiga Toxigenic *Escherichia coli*
Stx	–	Shiga toxin
TAE	–	Tris-acetate
Taq	–	*Thermus aquaticus*
Tris-HCl	–	Trishydroxymethylaminomethane-Hydrochloric acid
UK	–	United Kingdom
UNDP	–	United Nations Development Programme
US	–	United States
USA	–	United States of America
USEPA	–	United States Environmental Protection Agency
UV	–	Ultra Violet

V	–	Voltage
WHO	–	World Health Organization
µg	–	Microgram
µm	–	Micrometre
µℓ	–	Microlitre

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GENERAL ABSTRACT

General Abstract

Escherichia coli (*E. coli*) is a common inhabitant of surface waters in the developed and developing worlds. The majority of *E. coli* cells present in water are not particularly pathogenic to humans; however, there are some present in small proportion that possess virulence genes that allow them to colonize the digestive tract. Pathogenic *E. coli* causes acute and chronic diarrheal diseases, especially among children in developing countries and in travelers in these locales.

The present study, conducted between August 2007 and July 2008, investigated the prevalence and distribution of virulent *E. coli* strains as either free or attached cells in the final effluents of three wastewater treatment plants located in the Eastern Cape Province of South Africa and its impact on the physico-chemical quality of the receiving water body. The wastewater treatment plants are located in urban (East Bank Reclamation Works, East London), peri-urban (Dimbaza Sewage Treatment Works) and in rural area (Alice Sewage Treatment Works).

The effluent quality of the treatment plants were acceptable with respect to pH (6.9-7.8), temperature (13.8-22.0 °C), dissolved oxygen (DO) (4.9-7.8 mg/L), salinity (0.12-0.17 psu), total dissolved solids (TDS) (119-162 mg/ L) and nitrite concentration (0.1-0.4 mg/l). The other

physicochemical parameters that did not comply with regulated standards include the following: phosphate (0.1-4.0 mg/L); chemical oxygen demand (COD) (5-211 mg/L); electrical conductivity (EC) (237-325 µS/cm) and Turbidity (7.7-62.7 NTU). Results suggest that eutrophication is intensified in the vicinity of the effluent discharge points, where phosphate and nitrate were found in high concentrations.

Presumptive *E. coli* was isolated from the effluent samples by culture-based methods and confirmed using Polymerase Chain Reaction (PCR) techniques. Antibiogram assay was also carried out using standard *in vitro* methods on Mueller Hinton agar. The viable counts of presumptive *E. coli* for the effluent samples associated with 180 µm plankton size ranged between 0 – 4.30 × 10^1 cfu/ml in Dimbaza, 0 – 3.88 × 10^1 cfu/ml in Alice and 0 – 8.00 × 10^1 cfu/ml in East London. In the 60 µm plankton size category *E. coli* densities ranged between 0 and 4.2 × 10^1 cfu/ml in Dimbaza, 0 and 2.13 × 10^1 cfu/ml in Alice and 0 and 8.75 × 10^1 cfu/ml in East London. Whereas in the 20 µm plankton size category presumptive *E. coli* density varied from 0 to 5.0 × 10^1 cfu/ml in Dimbaza, 0 to 3.75 × 10^1 cfu/ml in Alice and 0 to 9.0 × 10^1 cfu/ml in East London. The free-living presumptive *E. coli* density ranged between 0 and 3.13 × 10^1 cfu/ml in Dimbaza, between 0 and 8.0 × 10^1 cfu/ml in Alice and between 0 and 9.5 × 10^1 cfu/ml in East London.

Molecular analysis successfully amplified target genes (*fliC*$_{H7}$, *rfbE*$_{O157}$, *ial* and *aap*) which are characteristic of pathogenic *E. coli* strains. The PCR assays using *uidA*-specific primer confirmed that a genetic region homologous in size to the *E. coli uidA* structural gene, including the regulatory region, was present in 3 of the *E. coli* isolates from Alice, 10 from Dimbaza and 8 from East London. Of the 3 *E. coli* isolates from Alice, 1 (33.3%) was positive for the *fliC*$_{H7}$ genes and 3 was positive for *rfbE*$_{O157}$ genes. Out of the 10 isolates from Dimbaza, 4 were

positive for *fliC*$_{H7}$ genes, 6 were positive for the *rfbE*$_{O157}$ genes and 1 was positive for the *aap* genes; and of the 8 isolates from East London, 1 was positive for *fliC*$_{H7}$ genes, 2 were for the *rfbE*$_{O157}$ genes, 6 were positive for the *ial* genes.

Antimicrobial susceptibility profile revealed that all of the *E. coli* strains isolated from the effluent water samples were resistant (R) to linezolid, polymyxin B, penicillin G and sulfamethoxazole. The *E. coli* isolates from Dimbaza (9/10) and East London (8/8) respectively were resistant to erythromycin. All the isolates were found to be susceptible (S) to amikacin, ceftazidime, ciprofloxacin, colistin sulphate, ceftriaxone, cefotaxime, cefuroxime, ertapenem, gatifloxacin, gentamycin, imidazole, kanamycin, meropenem, moxifloxacin, neomycin, netilmicin, norfloxacin and tobramycin.

The findings of this study revealed that the Alice wastewater treatment plant was the most efficient as it produced the final effluent with the least pathogenic *E. coli* followed by the Dimbaza wastewater treatment plant. In addition, the findings showed that the wastewater treatment plant effluents are a veritable source of pathogenic *E. coli* in the Eastern Cape Province watershed. We suggest that to maximize public health protection, treated wastewater effluent quality should be diligently monitored pursuant to ensuring high quality of final effluents.