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DECLARATION

I declare that this research dissertation for the degree of Masters of Science in Microbiology; Faculty of Science and Agriculture in the Department of Biochemistry and Microbiology, University of Fort Hare hereby submitted, has not been submitted by me or anyone else for a degree at this or any other university. That it is my own work and that materials consulted have been properly acknowledged.

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DEDICATION

I dedicate this dissertation to my uncle Mr T.N Komani who has help me through the years it was hard but with him by my side I have made it this far and also my mother Miss N Nani who as a mother guided me to do well in my studies together with the rest of my family and friends. I also dedicate my work to my father and to my loving God who has been my source of strength of all times.

ABSTRACT

Tuberculosis (TB) is an ancient infectious disease that has been infecting different populations around the globe and it has also been considered as one of the most successful human and animal disease. TB found in animals such as cattle and other known bovids is known as bovine tuberculosis. Bovine tuberculosis (BTB) is an infectious disease found in cattle mainly caused by *Mycobacterium bovis. M. bovis* is a member of the *Mycobacterium tuberculosis* complex (MTC) together with *M. tuberculosis, M. africanum, and M. canetti* where the natural host is humans; whereas *M. caprae, M. microti and M. pinnipedii* usually have animals as their natural host.

In this study the molecular characterization of the MTC from cow milk in the Eastern Cape was investigated. One hundred and twenty samples (40 ml each) were collected from three dairy farms in the Eastern Cape, South Africa. These samples were processed using a modified Petroff decontamination method. Sample processing was followed by DNA isolation using a Zymo Bacterial/Fungal DNA Kit and the amplification and detection of the MTC was done using the Seeplex MTB Nested ACE assay. The drug susceptibility tests were done using GenoTypeMTBDRplus assay which detects mutations and resistance to INH (isoniazid) and RMP (rifampicin). The milk isolates were further analyzed using a spoligotyping method which is based on the PCR amplification of a highly polymorphic direct repeat locus in the *M. tuberculosis* genome which detects and types the MTC.

A percentage of 20.8 % samples were found to be positive for MTC using the Seeplex MTB Nested ACE assay. There were 42.1 % samples that were resistant to both INH and RMP with the rest sensitive to either INH or RMP. The spoligotyping method showed that 78.3 % samples resembled Family 33 strains and the rest (21.7 %) resembled a spoligotyping signature known to be that of *M.africanum.* Both these strains belong to the Ancestral lineage with Indo-Oceanic and West Africa 2 lineage. The outcomes of our study showed that molecular methods for detection of MTC can be applied directly on milk samples without the need for culturing.

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ABBREVIATIONS

AIDS	Immuno Deficiency Syndrome
BCG	Bacillus Calmette-Guerin
BTB	Bovine Tuberculosis
DNA	Deoxyribonucleic Acid
DOTS	Directly Observed Treatment Short Course
DR	Direct Repeat
DVR	Direct Variable Repeat
EAI	East African Indian
HIV	Human Immunodeficiency Virus
IS	Insertion Sequence
LAM	Latin American Mediterranean
MDR	Multidrug Resistant
MIRU	Mycobacterial Interspersed Repetitive Units
MTC	*Mycobacterium tuberculosis* Complex
NTM	Nontuberculous Mycobacteria
PGG	Principal Genetic Groups
PZA	Pyrazinamide
RFLP	Restriction Fragment Length Polymorphism
RD	Region Of Difference

TB	Tuberculosis
TCH	Thiophene-2-Carboxylic Acid Hydrazide
VNTR	Variable Number Of Tandem Repeats
WHO	World Health Organization
XDR	Extensively Drug-Resistant