Molecular characterisation of extended spectrum beta-lactamases producing *Enterobacteriaceae*

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DECLARATION

I, Montso Kotsoana Peter, declare that the dissertation entitled "**Molecular characterisation of extended spectrum beta-lactamases producing *Enterobacteriaceae***", hereby submitted for the degree of Master of Science in Biology (Molecular Microbiology), has not previously been submitted by me for a degree at this or any other university. I further declare that this is my work in design and execution and that all materials contained herein have been duly acknowledged.

Signed……this the…….day of……2015

DEDICATION

I dedicate this study to my father, Mr Elia Mokhosi Montso (RIP) and my mother, Mrs Mary Mamojabeng Montso for nurturing and taking care of me.

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ABSTRACT

Extended Spectrum beta-lactamase producing *Enterobacteriaceae* have been responsible for numerous outbreaks of infections worldwide. *Escherichia coli* and *Klebsiella pneumoniae* species are the main producers of ESBLs, causing hospital and community acquired infections. The aim of this study was to isolate and identify ESBL producing *Enterobacteriaceae* from cattle faeces and raw beef samples and determine the presence of ESBL determinants using specific PCR assays. A total of 151 samples were analysed and 259 presumptive isolates screened for characteristics of *Enterobacteriaceae* using biochemical tests and 16S rRNA universal gene. A total of 196 isolates were confirmed as *E. coli* and *K. pneumoniae* through amplification of *uidA*, *uspA* and *ntrA* genes fragments respectively. Antimicrobial susceptibility test was performed to determine antibiotic resistance profiles of the isolates. Large proportions (66.7-100%) of isolates were resistant to Amoxicillin, Aztreonam, Ceftazidime, Cefotaxime and Piperacillin.

Correlations between antibiotic resistant isolates from the various sources was determined using the Pearson's product of moment and scored as significant if $P \leq 0.05$. Phenotypic characterisation by cluster analysis of antibiotic inhibition zone diameter data used to determine the commonness of isolates and resolve the differences of isolates from various sources and/or locations revealed a close association between isolates derived from cattle faeces and beef samples. This implies that the isolates may have originated from the common progeny.

A total of 196 isolates were screened for the presence of ESBL determinants. Among all the isolates analysed, the prevalence of ESBL was 53.1%. The *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$ genes were detected in 85.5%, 69.6% and 58% of the isolates respectively and this was most prevalent among *E. coli* isolates. Similarly, the *bla*$_{CTX-M}$ gene was the most frequently detected in *K. pneumoniae* isolates. On the contrary, *bla*$_{OXA}$ gene was highly prevalent in *K. pneumoniae*. The genetic relatedness of the isolates was determined using ERIC PCR technique. The DNA fragments showed great similarities in the banding patterns among *E. coli* and *K. pneumoniae* isolates. From the findings of this study, there is a need for continuous monitoring of ESBL producing strains in food products and food producing animals.

TABLE OF CONTENTS