Midlands State University

Established 2000

DETERMINATION OF PREVALENCE, ANTIMICROBIAL RESISTANCE ASSESSMENT AND SCREENING OF VIRULENT GENES OF *ESCHERICHIA COLI* IN READY-MADE MEAT PRODUCTS AND TRADITIONALLY FERMENTED MILK FROM THE FORMAL AND INFORMAL MARKETS IN HARARE.

By

WARDNER MUNASHE GWESHE

A dissertation submitted in partial fulfilment of the requirements for the

Bachelor of Science Honours Degree in Applied Biosciences and Biotechnology

DEPARTMENT OF BIOSCIENCES AND BIOTECHNOLOGY

FACULTY OF SCIENCE AND TECHNOLOGY

June 2018

APPROVAL FORM

This is to certify that the dissertation entitled "Determination of prevalence, antimicrobial resistance assessment and screening of virulent genes of *Escherichia coli* in ready-made meat products and traditionally fermented milk from the formal and informal markets in Harare", submitted in quasar fulfilment of the requirements for Bachelor of Science Honours Degree in Applied Biosciences and Biotechnology at Midlands State University, is a record of a research carried out with fidelity by WARDNER MUNASHE GWESHE R114344H under my supervision and no part of the dissertation has been submitted for any other degree or diploma.

Name of supervisor:…….....

Signature:……

Chairperson signature:……

ABSTRACT

Escherichia coli is a common gastrointestinal enterobacteriaceae that contaminates food resulting in food poisoning. Due to prolonged exposure to antibiotic therapy in their livestock hosts, *E. coli* continue to develop antimicrobial resistance (AMR). Most *E. coli* strains are commensal but may attain virulence genes through horizontal gene transfer. A cross sectional observational study was carried out to determine the total *E. coli* count in polony, beef burgers and traditionally fermented cow milk from the formal and informal markets. The *E. coli* isolates were tested for antimicrobial resistance against eight commonly used antibiotics. A multiplex PCR was developed for the rapid detection of genes encoding Shiga toxins 1 and 2 (*stx1* and *stx2*), intimin (*eae A*), and enterohemolysin A (*hly A*) under UV-Transillumination. From the 36 samples examined, 14 (39%) samples were *E. coli* positive. *Escherichia coli* was higher in the informal sector with 44% (8 of 18) prevalence than in the formal sector with 16% (3 of 18) prevalence. Polony had the highest total *E. coli* counts (TEC) ($1.2 \times 10^2 \leq TEC \leq 1 \times 10^3$) followed by burgers ($9 \times 10^1 \leq TEC \leq 3 \times 10^2$) and only a single sample of milk was *E. coli* positive with a TEC of 30. Intimin (*eae A*) was detected from 7% (1 of 14) isolates, two variants of *stx* gene (shorter with 406 bp and a longer one with 779 bp) were detected and *hly* was not detected. The shorter *stx* PCR band size was detected from 50% (6 of 14) isolates while *stx* 2 was detected in 7% (1 of 14) isolates. The absence of *E. coli* in fermented milk indicated the inability of *E. coli* to survive in fermented milk most probably due to the production of bacteriocin-like inhibitory substance with a broad

spectrum of antimicrobial activity directed against pathogenic indicator organisms by the lactose fermenting bacteria found in milk. Antimicrobial resistance was 0% for Cephalexin, and relatively low for Trimethoprim/Sulfamethoxazole (21.4%), Ceftriaxone (28.6%) and Tetracycline (35.7%). A total of 6 (42.9%) isolates were extended-spectrum β-lactamase

producers, which may have provided them with important resistance. About 49% (6 of 14) isolates were shiga-toxin producing and are potential causal agents of gastroenteritis, urinary tract infections and *E. coli*-associated neonatal meningitis. The shorter *stx2* gene originates from *Aeromonas* spp. suggesting a high degree of *Aeromonas* spp. - *E. coli* horizontal gene transfer. The findings of this study indicate more violation of food handling standards in the informal sector which requires effective implementation and enforcement of food safety regulation in the food industry. The public is discouraged from buying ready-prepared meat products from the informal sector. The health delivery sector is also encouraged to diligently prescribe antibiotics with prudence as well as promote the use of herbal alternatives if rate of AMR is to be reduced.

Key words: *Escherichia coli*, prevalence, total *E. coli* count, antibiotic, antimicrobial resistance, virulence and food.

ACKNOWLEDGEMENTS

Allahu Akbar (Allah is greater). All praise belong to Allah who is The Almighty God from Whom my sustenance come from due to His continued grace and mercy, I extend my deepest gratitude whole-heartedly to my supervisor Dr. T. Muteveri who did not tire to provided me with the critical guidance during this study and writing of this paper with the best of my ability. I also greatly appreciate the provisions that I was given by the Central Veterinary Laboratory and personnel with specific mention to Gufe C. and Mutengwa I., for bringing ease to my study throughout the process. Over and above all, I want to thank the Almighty God who bestowed for me my brothers in Islam and a family who remained the 'steel' I held on to.

DEDICATIONS

“Waddie, mwana wangu”!!! You exclaimed of my little brother on our last conversation when you were instructing us on the power of family in the year 2009. Your lessons remain a source of inspiration. Your struggle for our success remains our fuel and your dreams for us MOTHER, shall be fulfilled. This paper is the drive towards saving and serving the world. I dedicate this paper to you and your grandson – my fast growing nephew Damian who had a nickname whilst still in the womb. We named him Cue for Cucumber. The affinity for this fruit was never ending for his mother. To you “ya habiba”, I say, “You have been the horizon that made me keep the good hope”.

Contents

LIST OF FIGURES **PAGE**

LIST OF TABLES **PAGE**

LIST OF APPENDICIES **PAGE**

CHAPTER 1: INTRODUCTION

1.1 Background

Escherichia coli is a Gram-negative, rod-shaped bacterium in the Enterobacteriaceae family. It is among the most prevalent pathogenic agents of foodborne diseases, which are a critical health hazard with growing concern as shown by escalating incidence of infectious diarrhoea from epidemiological data (Osservasalute, 2009). *E. coli* is known as one of the most important pathogenic agents causing food poisoning resulting in gastrointestinal infections in fowls and mammals, humans included (Barnes *et al*, 2008). Commensal *E. coli* strains may attain genes of virulence through horizontal gene transfer such as prophages from lysogenic bacteriophage infections with genes that facilitate the damaging of host tissues causing diseases (Van der Westhuizen and Bragg, 2012). Pathogenicity of several *E. coli* strains is generally based on their virulence properties such as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC) and shiga-like toxins producing *E. coli* (STEC) (Barnes *et al*, 2003).

A large proportion of resistant and pathogenic *E. coli* causing human infections are derived from meat and milk products (Collignon *et al*, 2009). The CDC MMWR (55;1-2) state that, *E. coli* O157:H7 and several other *E. coli* including shiga toxin-producing *E. coli* (STEC) strains (serogroups O26, O45, O103, O111, O121, O145 etc.) are important foodborne pathogens that cause significant losses among the human population in both the developed and developing world (FAO/WHO, 2006). The infection with such pathogens, frequently associated with haemorrhagic colitis (HC) is associated with haemolytic uremic syndrome and renal failure (Paton and Paton, 1998a, Paton and Paton, 1998b). Fresh produce can be a source of pathogenic *E. coli*, as demonstrated by a 2006 outbreak of the 0157 strain involving raw spinach, with 199 illnesses, 102 hospitalizations, 31 haemolytic uremic syndrome (HUS), a severe kidney

condition, and three deaths in the US due to contamination from irrigation water (Centres for Diseases Control, 2003).

Escherichia coli is generally a commensal bacteria harbouring in the intestines of ruminants though it can also survive on other environments outside the ruminant host. Transmission of the pathogenic *E. coli* serotypes is via faecal-oral route, due to improperly washed hands or following ingestion of contaminated foods mostly from animal origin harbouring the organism, especially ground meat and the ready-to-eat meat products as well as milk and dairy products which are not treated well by heat (Dilielo, 1982; Soomro *et al*, 2002). For example, *E. coli* can be homogenised with the meat during processing of meat into ready-to-eat products such as sausage, polony as well as burger patties if contamination occurs during slaughtering or from equipment used. In Zimbabwe with the current economic slump, most people with very little knowledge on food safety measures have become vendors trading in areas where lack of sound infrastructure to control temperature and lack of requisite services such as water on the consumer focal points such as bus terminus may result in contamination of the food.

The pathogenicity of *E. coli* is mostly attributed to the ability of the microorganism to produce the shiga toxins (*stx1* and *stx2*) and a characteristic of shiga toxins-producing *E. coli* (STEC) and the presence of the intimin (*eae*), which is essential for adherence of the organism to the intestinal epithelium (attaching and effacing mechanism) and is characteristic of the enteroaggregative *E. coli* (EAEC) (Vallance and Finlay, 2000). Haemolysins (*hly*) are an important virulence factor as they can induce extraintestinal lesions (Bhakdi *et al*, 1990) and have the ability to affect several cells, such as lymphocytes, granulocytes, erythrocytes, and renal cells causing severe effect. Several studies had been carried out to determine the *E. coli* as a shiga toxigenic cause of diarrhoea.

Virulence in *E. coli* results from virulence genes in the genome as well as on plasmids (Dozois *et al*, 2003). The genes occur individually or polygenically in clinical isolates with varying frequencies (Delicato *et al*, 2003). In a study carried out on Zimbabwean and South African isolates (Van der Westhuizen and Bragg, 2012) 18 virulence genes associated with avian colibacillosis were identified. As stated by Westhuisen and Bragg (2012), other virulent genes found in some *E. coli* strains included *sitA* and *sitD* of the *sitABCD* system, the *frz* operon, type 1 fimbrial adhesion gene *fimH* and *pstB*, being part of the *pstSCAB* operon present.

1.2 Problem Statement

Food safety is a problem of public health concern in Zimbabwe as indicated by recurrent outbreaks of food related diseases (WHO, 2018). About 85% of Traveller's Diarrhoea cases are caused by bacteria and *E. coli* is responsible for the majority of the cases in Zimbabwe (IAMAT, 2018). Food control is the responsibility of various government ministries and local authorities. However, the Food Control administration is weak due to fragmentation, inadequate resources and limited skills for food inspection. The prevailing economic challenges are forcing the majority of people to resort to informal businesses characterized by a low capital outlay, high level of mobility and poor knowledge of, or regard to food safety issues. The existing food control system was not designed to service such informal business and therefore cannot cope with the demands for infrastructure support, training and health education.

Due to limited knowledge of food safety and handling skills meat products and milk vendors and other stake holders in the industry do not observe food safety regulations leading to high chances of food contamination. Zimbabwe has very little up to date data on current pathogens causing infections in human health, making it difficult to inform the EML and STG (GARP, 2017). In Zimbabwe, bacterial infections are treated without establishment of an Antibiogram and/or identifying virulence of the strains *(*Saidi *et al,* 2013). Environmental contamination

leads to perpetuation of pathogenic *E. coli*, acquiring of virulence and AMR genes by non-pathogenic *E. coli.* Lack of information on antibiotic resistance and prevalent virulent gene combination profiles of pathogenic microbes renders the use of antibiotics irrational.

1.3 Justification

Identification and characterisation of virulence genes linked to disease prevalence is essential for the implementation of an efficient disease control and prevention system. For example, specific gene products combinations can thus be designed and used as feasible drug targets for treatment whilst making effort to retard development of antimicrobial resistance to the commercial drugs. The accurate identification of virulent strains of *E. coli* is essential if alternative treatments, such as bacteriophage therapy, are to be developed that target only pathogenic strains of *E. coli*. This study contributes to genetic profiling of *E. coli* virulence genes. The introduction of novel immunopotentiators can be based on the characterisation of pathogenic *E. coli* and its prevalence. The prevailing poor economic conditions of Zimbabwe fostering survival of people on informal trade require great concretisation of the stakeholders of concern with relevant information such that, food safety and health regulations continuously guide their operations to prevent and control EAEC, STEC and EHEC infections. Determination of whether food or other cause of contamination such as water as the cause of the related diseases will provide a basis to advice the Ministry of Health and Child Welfare and all the stakeholders of concern (Regulatory Authorities, NGOs, Humanitarian Support funders, companies etc.) on appropriate ways to prevent disease occurrence and control of any future outbreaks that may arise. The Government of Zimbabwe (GoZ) can be provided with informed advice to improve, maintain and/or ways to effect the current food safety regulation and policy in a one health coordinated approach with participation of all the stakeholders of interest involved taking into consideration the prevailing economic conditions and the trajectory developments.

1.4 Objectives

1.4.1 Main Objective

The main objective was to determine the occurrence of, antimicrobial resistance and virulence of *E. coli* in ready-made meat products and sour milk from the formal and informal market.

1.4.2 Specific objectives

Specific objectives of the study included: to isolate *Escherichia coli* from polony, burgers and traditionally fermented milk, to determine the prevalence of *E. coli* in sliced polony, beef burgers and sour milk from the formal and informal sector, to enumerate *E. coli* from all specimen, to determine TEC, to carry out an antibiotic disk diffusion test on the *E. coli* isolates to determine the AMR and to screen the virulence genes using Multiplex PCR.

CHAPTER 2: LITERATURE REVIEW

2.1 *Escherichia coli* as a human pathogen

Escherichia coli includes not only commensal strains but also pathogenic ones that cause several human diseases resulting in more than 2 million deaths each year worldwide (Kaper *et al*, 2004). There are mainly six well studied strains of *E. coli*, including Shiga toxin producing *E. coli* (STEC) that are either heat liable or heat stable, enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enteroinvasive (EIEC) *E. coli*. To classify these pathotypes, virulence properties and pathogenicity mechanisms causing gastrointestinal diseases such as diarrhoea and UTI infections are used (Kaper *et al*, 2004). The EHEC strain is one type of STEC that can cause severe enteric diseases, such as haemolytic uraemic syndrome (HUS) and haemorrhagic colitis (Kaper *et al*, 2004) leading to acute renal failure and often death. *Escherichia coli* O157:H7 is the EHEC serotype which is known for causing many outbreaks of water and food-borne diseases in many countries. The incidence of non EHEC STEC has been increasing in recent years, including those caused by serotypes O26, O45, O103, O111, O121 and O145 (Farrokh *et al*, 2013). Some *E. coli* strains can also cause extraintestinal diseases, and are called extraintestinal pathogenic *E. coli* (ExPEC). The ExPEC, which were defined by disease association, include uropathogenic *E. coli*, neonatal meningitis-associated *E. coli* and sepsis-causing *E. coli* (Dale and Woodford, 2015).

2.2 Food Contamination by *E. coli*

Pathogenic *E. coli* is believed to mostly live in the intestines of cattle. Certain serotypes of *E. coli*, such as *E. coli* O157:H7 have also been found in the intestines of chickens, deer, sheep, and pigs. These bacteria cause human illness when they are ingested, and can lead to *E. coli* infection through various modes of transmission, including through food and water sources,

animal-to-human contact, and person-to-person contact in public and other unsanitary settings. Throughout the 1990s and into the early 21st century, the majority of foodborne *E. coli* outbreaks were caused by the consumption of contaminated ground beef. In fact, haemolytic uremic syndrome (HUS) secondary to *E. coli* O157:H7 infection was known as "Hamburger Disease." Although *E. coli* is now widely disseminated throughout the food chain, and has even been found in foods such as pizza and cookie dough, the ground beef connection has not gone away. Numerous outbreaks and massive recalls of *E. coli* contaminated ground beef and other meat products continue to plague both the meat industry and the public. Improper sanitation, cross-contamination, and a failure to cook meat to a high enough temperature to kill *E. coli* have all been factors contributing to *E. coli* outbreaks associated with restaurant food and informal market ready-to-eat meat products. Equipment also contributes to contamination of food. Contamination of equipment occurs during production (Marriotti, 1990). Even with hygienic design features, equipment can collect microorganisms and other debris from the air, as well as from employees and materials. A recommended way of reducing contamination of equipment is through improved hygienic design and more effective cleaning (Leach *et al*, 2001).

Though the introduction of pasteurization greatly reduced the number of foodborne illness outbreaks associated with milk and other dairy sources, the consumption of raw milk and unpasteurized cheese remains a risk factor for *E. coli* infection. *Escherichia coli* and other pathogens are shed in the faeces of livestock such as cows and goats and can contaminate milk during the milking process. *E. coli* has been incriminated during the past century together with other pathogens such as *S. aureus, Campylobacter* spp., *Salmonella* spp., *Listeria* spp. and *Yersinia* spp. for causing numerous outbreaks of milk-borne disease in humans, especially since mass production came into effect (Bryan, 1983; Vasavada, 1988). Some lactose fermenters exploited by farmers and traders such as some *Lactobacillus acidophilus* strains

have antimicrobial activity and produce probiotics that retard the growth of pathotypes (Tigu *et al*, 2016).

2.3 Food Safety Violation by Food Handlers

Food handlers are the individuals in the food industry whose hands come in direct contact with food (Little and McLauchlin, 2007). Food handlers can be a source of food contamination and facilitators of cross-contamination, personal hygiene of food handlers is critically important in regard to food safety which is principally associated with cleanliness of hands. Therefore, ways in which food contamination by food handlers occur should be identified and appropriate interventions to reduce or eliminate the risk of food contamination developed (Little and McLauchlin, 2007). Good personal hygiene practice is critical in preventing food safety violation in the food industry and it is documented as one of the most critical element that food handlers must observe in an attempt to ensure safe food supply and preparation (Cruikshank, 1990).

2.4 Food-borne disease surveillance in Zimbabwe

Pathogenic bacteria are the most commonly reported agents of food borne illness, closely followed by viruses (Bean *et al*, 1996). Most reported cases of food borne illness are attributed to poor handling at the home or at retail food establishments rather than failures at the food processing level indicating standardisation of food processing management systems in Zimbabwe from at least as early as 2004 (Anderson *et al*, 2004). It is not possible to determine with certainty the cause of food borne illness in roughly 50% of all food borne illness cases (Bean *et al*, 1996). Most cases of food poisoning go unreported to health agencies. This is partly because, for normal healthy adults, food-borne pathogens only cause mild symptoms and medical health may not be necessary. Food poisoning is much more serious in young children, and in the frail and immune-compromised people (Bean *et al*, 1996). According to (World

Health Organisation, 2017), *E. coli* isolates were found from taped water in Harare during an investigation after a typhoid outbreak around the CBD. This indicated an increase of contamination of food by the ubiquitous *E. coli* from the water and food handlers. This poses danger of development and spread of *E. coli* food-borne illness. Identification of these outbreak strains has suggested that environmental sources, possibly contaminated meat and other foods, may play a role in the local spread of closely related *E. coli* strains (World Health Organisation, 2018). The presence of *E. coli* in food or water is accepted as indicative of recent faecal contamination and the possible presence of other enteric pathogenic bacteria like *Citrobacter*, *Klebsiella* and *Enterobacter* that can also ferment lactose and are similar to *E. coli* in phenotypic characteristics (Feng et *al*, 2002).

2.5 Antimicrobial Resistance in *E. coli*

Escherichia coli is continuously subjected to antibiotic selection pressure in its common host's intestines, leading to AMR against antibiotics consumed by its host (Looft *et al*, 2012). It has been discovered that, specific *E. coli* phylogenetic groups exhibit different resistance levels to antibiotics, regardless of the acquisition of resistance, hence, indicating that the genetic background of *E. coli* also effectively contribute to its antibiotic resistance pattern (Tenaillon *et al*, 2010; Brisse *et al*, 2012). Natural environments, such as water, and soils, and wastewater treatment plants, have been considered to be bacterial genetic reactors, in which active genetic exchanges routinely occur among different bacteria, similar to that which occurs in the host intestine (Baquero *et al*, 2008). Mobile genetic elements such as plasmids and transposons have been frequently associated with genes that encode for AMR and these can be cross exchanged between bacteria belonging to phylogenetic lineages that are varied (Wellington *et al*, 2013). Many previous studies have reported multidrug resistant *E. coli* strains found in the environment, indicating possible public health risks derived from human activities (Dhanji *et al*, 2011; Walsh *et al*, 2011; Jang *et al*, 2013).

E. coli which is a facultative anaerobic, opportunistic pathogen is known mostly to show antibiotic resistance through expression of an extensive variety of extended-spectrum β-lactamase-producers (ESBL) s and carbapenemases including, KPC, OXA, and several MBLs (van de Bogaard, 2011). *E. coli* have quinolone resistance (*qnr)* genes at over 30% occurrences in isolates (Mead *et al*, 1999). The β-lactams are the most utilised class of antibiotics on the WHO's list of critically important antibiotics to human medicine (Fair and Yitzhak, 2014). There are 28 members, including antibiotic/β-lactamase inhibitor combinations, from three subclasses: penicillins, cephalosporins, and carbapenems that are listed as critically important (Collingnon *et al*, 2009). Their mode of antibacterial activity is by acting as irreversible binding proteins. For example, penicillin binding proteins (PBPs) (transpeptidases) inhibit cell wall biosynthesis, thereby, retarding maintenance of peptidoglycan. Progressive cell stress responses will result in cell lysis (Tomasz, 1979). Many currently used β-lactams have very broad spectrum activity against most aerobic and anaerobic gram-positive and negative bacteria as well as low toxicity profiles making them popular first line antibiotics (Walsh, 2003). Resistance to older members of this class, especially the penicillin subclass, has dramatically proliferated.

ESBL positive antimicrobial resistance occurs via hydrolysis of the β-lactam ring mediated by a wide range of β-lactamases. These enzymes have been divided into four classes by the Ambler classification system: class A - *Klebsiella pneumoniae* Carbapenemase (KPC) s and most ESBLs, class B - metallo beta lactamase MBLs), class C AmpC β-lactamases, and class D- oxacillin hydrolysing capabilities (OXAs). Class A includes many enzymes that can hydrolyse penicillins and cephalosporins as well as some that can hydrolyse monobactams and KPCs that are capable of hydrolysing carbapenems (Young *et al*, 2010). The ESBLs from this class are plasmid mediated which has aided in their intra-species and interspecies diffusion (Fishman, 2006).

CHAPTER 3: MATERIALS AND METHODS

Central Veterinary Laboratory Standard operating procedures (SOPs) were used in carrying out the experimental procedures.

3.1 Sample Collection

Over a period of two days, a total of 36 samples of ready-to-eat meat and milk products were obtained from three informal and three formal traders. Trading entities were randomly selected from both formal and informal sector within the City of Harare CBD. Two samples were collected from each, product from each selected trading entity on each separate day. Samples were collected from beef polony slices, in-use beef burger patties and fermented milk from both the formal shops and informal traders. Altogether, there were six beef polony samples from each of the trading sector/location type, six sour milk samples from each of the trading sector/location type and six beef burgers from each of the trading sector/location type. Samples were immediately placed in a cooler box with ice and transported to CVL and stored at -20 ºC prior to analyses and.

3.2 Isolation and Identification of *E. coli*

A mass of 1g specimens were cut by sterile blades and added into 9 ml of peptone water in universal bottles. The contents were homogenised under sterile conditions, following the CVL Standard Operating Procedure, and incubated at 44 ^{0}C for 24 h. After 24 h a loop full of the culture was directly inoculated on Blood agar and MacConkey agar plates and incubated aerobically at 44ºC for 24 h. Colonies were examined for their cultural characteristics, morphological properties and any changes in the media. Large, red or pink colonies surrounded by a hazy boundary in MacConkey agar indicated *E. coli* isolates. Gram staining, motility test and other various biochemical tests such as the catalase, oxidase, indole, citrate and urease were conducted to confirm whether the bacterial strains were *E. coli* strains.

3.3 *Escherichia coli* enumeration/ Total *E. coli* count

Weight of 1g sample was homogenised and suspended in 9 ml phosphate buffered saline and serial diluted up to 10^{-3}. A volume of 1ml of each was spread plated on Cefixim Tellurite Sorbitol- MacConkey (CT-SMAC) agar and incubated at 44 ºC for 24 h. Suspected colonies of *E. coli* were counted and then confirmed by using gram staining, motility test and other various biochemical tests such as the catalase, oxidase, indole, citrate and urease.

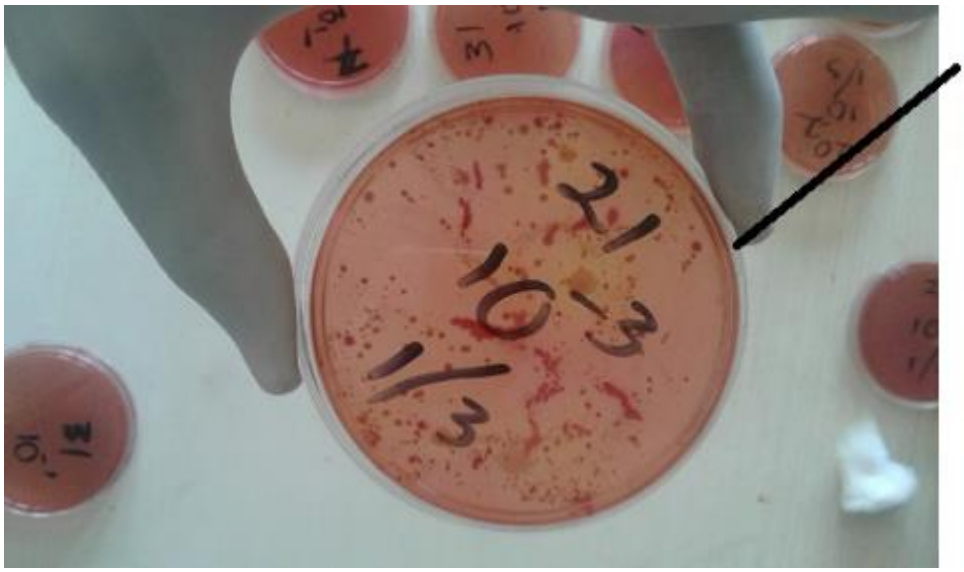

Figure 3.3.1: MacConkey agar plate with *E. coli* forming units for TEC.

3.4 Antimicrobial resistance test (AMR test)

A standardised Disc Diffusion technique was used for antimicrobial resistance testing of the *E. coli* isolates on Mueller Hinton agar (Thermo Fisher Scientific, UK) without any replicates as detected by the Clinical Laboratory Standardisation Institution (CLSI) and European Union Confederation for Antimicrobial Resistance (EUCAST) (Hombach *et al*, 2013).

Figure 3.4.1: A swabbing stick inoculated with an *E. coli* Isolate

The test was done using eight different antibiotic discs, namely, Ampicillin (AMP 10 µg), Cephalexin 30µg (LEX30), Penicillin G 10µg (PEN G10µg), Erythromycin 15µg (ERY15), Tetracycline 30µg (TCY30µg), Neomycin 10 µg (NEO10), Ceftriaxone 30µg (CRO30), and Trimethoprim/Sulfamethoxazole 1.25/23.75µg (SXT 1.25/23.75) (Oxoid, Germany). A microbial suspension of each *E. coli* isolate was swabbed with a sterile cotton swab onto Mueller Hinton agar. Disks of the above named antibiotics were dispensed onto the plates using a disc dispenser.

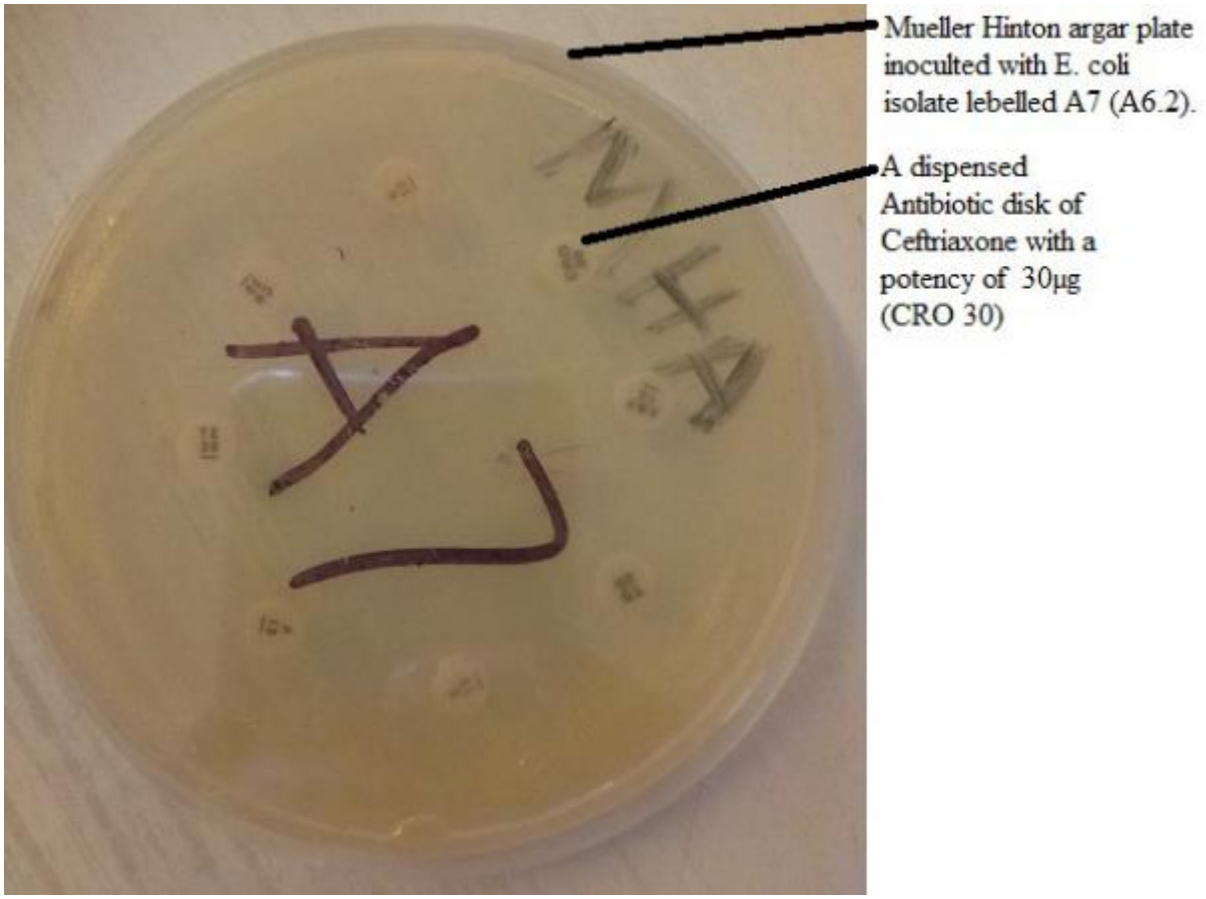

Figure 3.4.2: Disk diffusion test with 8 antibiotic disks to determine *E. coli* AMR.

To the nearest millimetre (mm), the inhibition zones were established by measuring the diameter of the colony free area including the antibiotic disk diameter using an electronic zone of inhibition meter after a 24 h incubation period at 37 ºC. The Clinical Laboratory Standard Institute, (2011); O'Brien and Stelling (2013) were used to interpret results as resistant, intermediate or susceptible (APPENDIX 3)

3.5 Screening for virulence genes

3.5.1 DNA extraction

Total genomic DNA was extracted from 14 different *E. coli* isolates using Zymo Research Quick-gDNA ™ MiniPrep kit (Zymo Research Corporation, South Africa). A volume of 200 µl of each *E. coli* suspension in peptone water were drawn and placed into collection tubes and

400 µl of genomic lysis buffer was added to each volume of sample. The contents were mixed briefly by vortexing and then let to stand at room temperature for 5 min. The mixtures were then transferred by pouring into a Zymo-Spin ™ Column in collection tubes and centrifuged for one minute at 10000 x g (8000 rpm). The collection tubes with the flow through were discarded. The zymo-spin ™ columns were then transferred to new collection tubes and centrifuged for one minute at 10000 x g after adding 200 µl of DNA Pre-Wash Buffer. A volume of 500 µl g-DNA Wash Buffer was added to each spin column and centrifuged at 10000 x g for one minute. Each spin column was transferred to clean micro-centrifuge tubes in which 50 µl DNA Elution Buffer were added and incubated for 5 min at room temperature. The spin columns were then centrifuged at a top speed of 14000 x g for 30 s to elute the DNA. The eluted DNA was then stored overnight at -20 ºC to prevent DNA degradation as time was limiting.

3.5.2 Multiplex PCR

A master mix with a reaction volume of 25 µl per sample DNA template was prepared consisting of all the requisite components for DNA amplification. The master mix consisted of reverse and forward primers (Inqaba, South Africa) for 4 virulence genes which are *stx1*and *2*, *eaeA* and *hly A*. Constitutes of the master mix were added in 0.2 ml PCR tubes in the order as shown in (Table 1) starting with the RNA free water. After dissolving the primers together with the master mix into the RNA free water, the content was homogenised by gentle forward and reverse pipetting. A volume of 20 µl of the master mix was added to new 0.2 ml PCR tubes for each sample and another for the negative control. Five microliters of the samples DNA templates were mixed with all the necessary components for amplification in the 0.2 ml PCR tube (Perkin- Elmer, USA) to form a 25 µl reaction volume for each sample which was then run on a PCR thermo-cycler (Gene PCR System 24, Perkin Elmer, USA).

Table 3.5.1: PCR Master-mix

Reagents	1 Reaction (µl)	X (16) Samples
RNA free dH2O	4.25	68
Master Mix	12.75	204
EHEC hly F-Primer	0.5	8
EHEC hly R-Primer	0.5	8
stx 1 F-Primer	0.5	8
stx 1 R-Primer	0.5	8
stx 2 F-Primer	0.5	8
stx 2 R-Primer	0.5	8
eae A F-Primer	0.5	8
eae A R-Primer	0.5	8
DNA Template	5	
Total	26	336

The components of the reaction included a commercial master mix kit (2x concentration) (Fermentas) (containing- PCR buffer, deoxynucleotide triphosphate (dNTP) mix, $MgCl_2$ and Taq DNA Polymerase) and primers dissolved in nuclease free water (Table 3.5.1) The primer sequences of virulent genes used in Multiplex PCR amplifications were as obtained from literature (Table 3.5.2). The thermo-cycler reaction cycles were set up as shown (Table 3.5.3)

Table 3.5.2: Primer sequences and predicted lengths of the amplicons.

Primer	Direction	Primer Sequence (5′-3′)	Band Size (bases)	Reference
hly A	Forward	ACGATGTGGTTTATTCTGGA	165	Fratamico *et al*, 1995
	Reverse	CTTCACGTGACCATACATAT		Fratamico *et al*, 1995
*stx*1	Forward	ACACTGGATGATCTCAGTGG	614	Gannon *et al*, 1992
	Reverse	CTGAATCCCCCTCCATTATG		Gannon *et a*l, 1992
*stx*2	Forward	CCATGACAACGGACAGCAGTT	779	Gannon *et al*, 1992;
	Reverse	CCTGTCAACTGAGCAGCACTTTG		Gannon *et a*l 1992
*eae*A	Forward	GTGGCGAATACTGGCGAGACT	890	Gannon *et al* 1997
	Reverse	CCCCATTCTTTTTCACCGTCG		Gannon *et al*, 1997

Table 3.5.3: PCR Conditions.

Stage	Temperature/ ^{0}C	Time
Initial Denaturation	94	3 min
Denaturation	94	1 min
Annealing	56	50 sec
Elongation	72	1 min
Final Elongation	72	1 min

N/B Number of Cycle: 35

3.5.3 Gel Casting, Electrophoresis and Imaging

A mass of 2 g of agarose was mixed with 100 ml of 1x TBE buffer in a Schotts bottle and swirled. The dissolved agarose was microwaved for 2 min, allowed to cool down for 5 min to about 60 ^{0}C and 5µl of ethidium bromide (10mg/ml) was added and mixed by swirling. On levelled gel casting apparatus the gel was poured into a gel casting tray which consisted an inserted comb and left to set for 30 minutes. The gel was submerged to 4mm depth of 0.5xTBE buffer in a gel electrophoresis tank and the comb was gently removed leaving the wells open. A 5 µl volume of PCR products (Amplicons) and negative control were mixed with 2 µl of the loading/tracking dye and load into the wells on the gel. Hyperladder IV 100bp DNA (0.1 µg/µl, 50 µg) (Bioline) was employed as a molecular weight marker or ladder. Electrophoresis was run for 1 hour at 120V on a BRL Horizontal Gel electrophoresis Apparatus (Horizon 11.14 Life Technologies Gibco, USA) and all the gel results were viewed and photographed using the Gel Logic 100 Imaging System (Kodak, EEC) under UV trans-illumination. Analysis of the

gel picture was done on the computer to generate the DNA profiles and draw conclusions about the sample by estimating the band size from the DNA ladder/maker.

3.6 Data Analysis

Two way anova was used to compare the influence of trading sector on Total *E. coli* count. The AMR test results analysis was carried out using the WHONET (build number 8.0.1.0.24222) Antimicrobial Resistance test analysis software package as per WHO guidelines.

CHAPTER 4: RESULTS

4.1 The effect of trading sector and food type on the Total *E. coli* count

Generally, *E. coli* occurred more in meat products from the informal sector with a prevalence of 44% (8 of 18) than the 16% (3 of 18) prevalence in the formal sector. Polony was the highest with 50% (6 of 12) prevalence and burgers had 33% (4 of 12) *E. coli* prevalence which was lower than in to that of polony. *Escherichia coli* prevalence was very low in the formal sector with a prevalence of 16% (3 of 18). Total *E. coli* in the informal sector ($1.2 \text{ X } 10^2 \leq \text{TEC} \leq 1 \text{ X } 10^3$) than the formal sector ($9 \text{ X } 10^1 \leq \text{TEC} \leq 3 \text{ X } 10^2$). No *E. coli* was isolated from all the milk samples from the informal sector and from only a single sample of milk from the formal sector was *E. coli* isolated and enumerated. Polony from the informal sector showed a higher number of samples containing *E. coli* with a higher TEC (Table 4.1)

Table 4.1: Total *E. coli* count in the samples.

	Trading Sector	
Product/food type	Informal	Formal
Polony	0	0
	4×10^2	0
	1.8×10^2	0
	3×10^2	0
	8×10^2	0
	1×10^3	1.2×10^2
Sour milk	0	0
	0	0
	0	0
	0	0
	0	3×10^1
	0	0
Burger	0	0
	1.6×10^2	0
	3×10^2	0
	0	9×10^1
	2×10^2	0
	0	0

Food type and trading sector did not influence each other in determining the TEC enumerated (ANOVA: $p>0.05$; APPENDIX 1). The trading sector/location type and product/food type has an effect in influencing the TEC enumerated (ANOVA: $p<0.05$; APPENDIX 1). Total *E. coli* counts from different product/food type were significantly different. The TEC is highest in polony, followed by burgers and the least is from fermented/sour milk (MULTIPLE COMPARISON: $p \geq 0.05$; APPENDIX 2)

4.2 Antimicrobial resistance test

The clear zone or zone of inhibition was interpreted into resistant (R), Intermediate (I) and susceptible (S). The possible ESBL producers were recorded as positive (+) and non-ESBL producing isolates were recorded negative (-).

Table 4.2: Antibiotic zone of inhibition on *E. coli* and interpretation

Sample Identity (Plate Number and Product Type)	Trading Sector (Formal / Informal)	Antibiotic	Zone of Inhibition (mm)	Interpretation	ESBL
A1 (Polony)	Informal	AMP10	12	R	-
		CRO30	26	S	
		TE30	17	I	
		TS25	19	S	
		CN30	19	R	
		N10	11	R	
		E15	6	R	
		P10	6	R	
A2 (Polony)	Informal	AMP10	19	S	+
		CRO30	23	I	
		TE30	19	S	
		TS25	19	S	
		CN30	21	R	
		N10	13	I	
		E15	9	R	
		P10	6	R	

A3 (Burger)	Informal	AMP10	18	S	-
		CRO30	30	S	
		TE30	22	S	
		TS25	30	S	
		CN30	19	R	
		N10	11	R	
		E15	6	R	
		P10	10	R	
A4 (Polony)	Formal	AMP10	17	S	-
		CRO30	28	S	
		TE30	8	R	
		TS25	14	S	
		CN30	19	R	
		N10	19		
		E15	7	R	
		P10	6	R	
A5 (Polony)	Informal	AMP10	6	R	+
		CRO30	16	R	
		TE30	17	I	
		TS25	8	R	
		CN30	22	I	
		N10	14	I	
		E15	8	R	
		P10	8	R	
A6.1 (Polony)	Informal	AMP10	15	S	+
		CRO30	27	S	
		TE30	6	R	
		TS25	15	S	
		CN30	19	R	
		N10	12	R	
		E15	6	R	
		P10	6	R	
A6.2 (Polony)	Informal	AMP10	21	S	-
		CRO30	32	S	
		TE30	19	S	
		TS25	25	S	
		CN30	20	R	
		N10	11	R	
		E15	6	R	
		P10	11	R	
A8 (Burger)	Informal	AMP10	6	R	-
		CRO30	27	S	
		TE30	6	R	
		TS25	6	R	

		CN30	20	R	
		N10	6	R	
		E15	6	R	
		P10	6	R	
A9 (Polony)	Informal	AMP10	6	R	+
		CRO30	13	R	
		TE30	13	R	
		TS25	15	S	
		CN30	14	R	
		N10	12	R	
		E15	8	R	
		P10	6	R	
A10.1 (Burger)	Informal	AMP10	16	S	-
		CRO30	29	S	
		TE30	23	S	
		TS25	27	S	
		CN30	20	R	
		N10	12	R	
		E15	6	R	
		P10	6	R	
A10.2 (Burger)	Informal	AMP10	8	R	+
		CRO30	20	R	
		TE30	12	R	
		TS25	14	S	
		CN30	11	R	
		N10	8	R	
		E15	6	R	
		P10	6	R	
A12.1 (Burger)	Formal	AMP10	9	R	-
		CRO30	29	S	
		TE30	26	S	
		TS25	26	S	
		CN30	20	R	
		N10	12	R	
		E15	9	R	
		P10	22	S	
A12.2 (Burger)	Formal	AMP10	8	R	-
		CRO30	28	S	
		TE30	17	I	
		TS25	17	S	
		CN30	20	R	
		N10	14	I	
		E15	6	R	
		P10	6	R	

A14 (Milk)	Formal	AMP10	22	S	+
		CRO30	6	R	
		TE30	22	S	
		TS25	6	R	
		CN30	22	I	
		N10	14	I	
		E15	6	R	
		P10	7	R	

All *E. coli* isolates were susceptible to LEX and 92.5%, 85.7% and 64.3% of the isolates are resistant to PEN G, ERY and NEO, respectively (Table 4.3). About 50% and 35.7% of the isolates showed resistance to AMP and TCY, respectively (Table 4.3). *Escherichia coli* isolates showed a low resistance to CRO and SXT with less than 29% and 22% of the isolates showing resistance respectively. The isolates showed intermediate susceptibility to CRO, NEO, ERY, TCY and CRO, and from the 4 antibiotic that showed intermediate susceptibility CRO had the lowest Intermediate susceptibility at 7.1%. Only 7.1% of the *E. coli* isolates were susceptible to PEN G and NEO. Therapy from SXT, CRO and AMP was effective with 78.6%, 64.3% and 50% isolates susceptibility respectively. About 43% of the isolates were susceptible to TCY and no isolate was fully susceptible to ERY. The zone of inhibition diameter was very large for LEX (Figure 4.2.1), followed by SXT which had 78.6% diameters above the breakpoint and CRO 71.4% (Figure 4.2.2) above the breakpoint (Table 4.3). TCY and AMP had 64.3 and 50% diameters above the breakpoint respectively. LEX, SXT, CRO, AMP and TCY showed overlapping zones of inhibition diameter or synergy. An antimicrobial resistance alert from WHONET (build number 8.0.1.0.24222) indicated that, 6 isolates (42.9%) which include: A2, A5, A6.1, A9, A10.2 and A14 (Table 4.2), are a medium priority, possible ESBL-producing Enterobacteriaceae (AST) case and are an important resistance which calls for an Infection Control Alert.

Table 4.3: Antibiogram for multiple antimicrobial resistance analysis of the 14 *E. coli* isolates (WHONET, build number 8.2.20.24222).

Code	Antibiotic name	Site of infection	Breakpoints	Number	%R	%I	%S	%R 95%C.I.
PEN_ED10	Penicillin G		12-21	14	92.9	0	7.1	64.2 - 99.6
AMP_ED10	Ampicillin		S >= 14	14	50	0	50	24.0 - 76.0
CRO_ED30	Ceftriaxone	Oral	22 - 24	14	28.6	7.1	64.3	9.6 -58.0
LEX_ED30	Cephalexin	UTI	S >= 14	14	0	0	100	0.0 26.8
NEO_ED10	Neomycin		13 - 16	14	64.3	28.6	7.1	35.6 - 86.0
SXT_ED1.2	Trimethoprim / Sulfamethoxazole		11-13	14	21.4	0	78.6	5.7 - 51.2
ERY_ED15	Erythromycin			14	85.7	14.3	0	59.7 - 93.8
TCY_ED30	Tetracycline			14	35.7	21.4	42.9	6.4 - 63.7

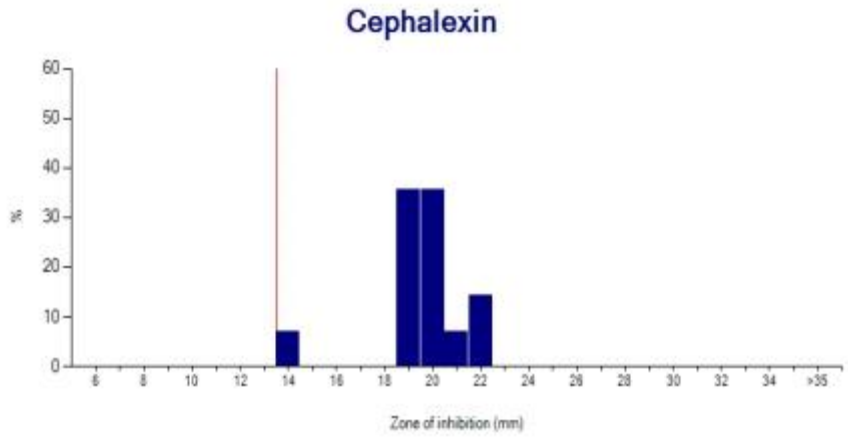

Figure 4.2.1: Comparison of the inhibition zone of LEX against the breakpoints.

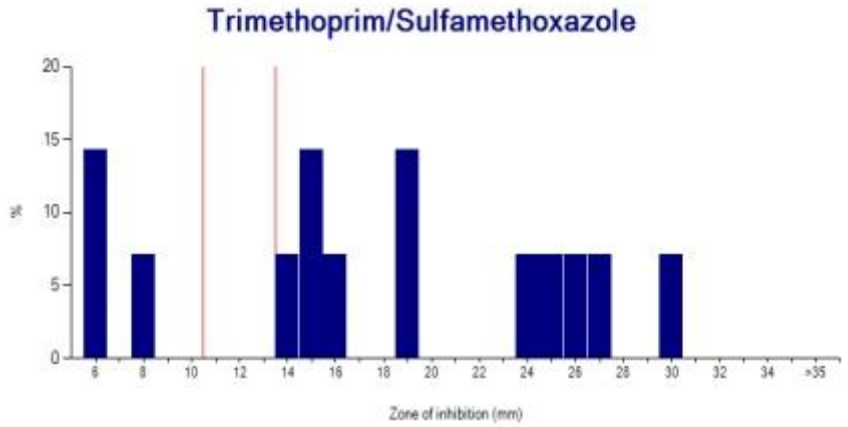

Figure 4.2.2: Comparison of the inhibition zone of SXT against the breakpoints.

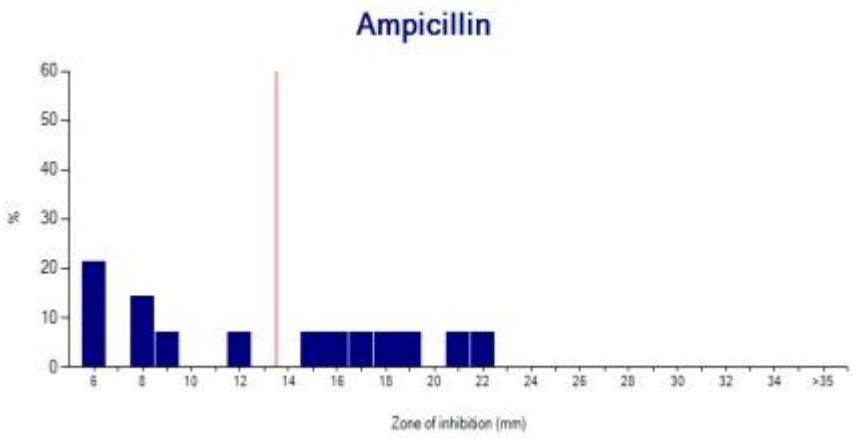

Figure 4.2.3: Comparison of the inhibition zone of AMP against the breakpoints.

4.3 PCR of the Virulent Genes

4.3 PCR of the Virulent Genes

PCR image indicate two different band sizes of the *stx2* gene. Both band 406 (A and 779 indicate different band sizes of the *stx2* gene which is responsible for expression of the shiga-toxin. Band 406 of the *stx2* gene was located in six *E. coli* isolates and band 779 of *stx2* gene was only found in a single *E coli* isolate (A2) simultaneously with *stx2* gene band size 406. The 890 bps *eae A* gene (intimin expression was present only in *E. coli* isolate number A8. The 614 (*stx1*) and 165 (*hly A*) bands were absent, indicating lack of the *hly A* gene in all the 14 *E. coli* isolates. A nonspecific DNA band A was found around the 200 bps region and below 100 bps, respectively.

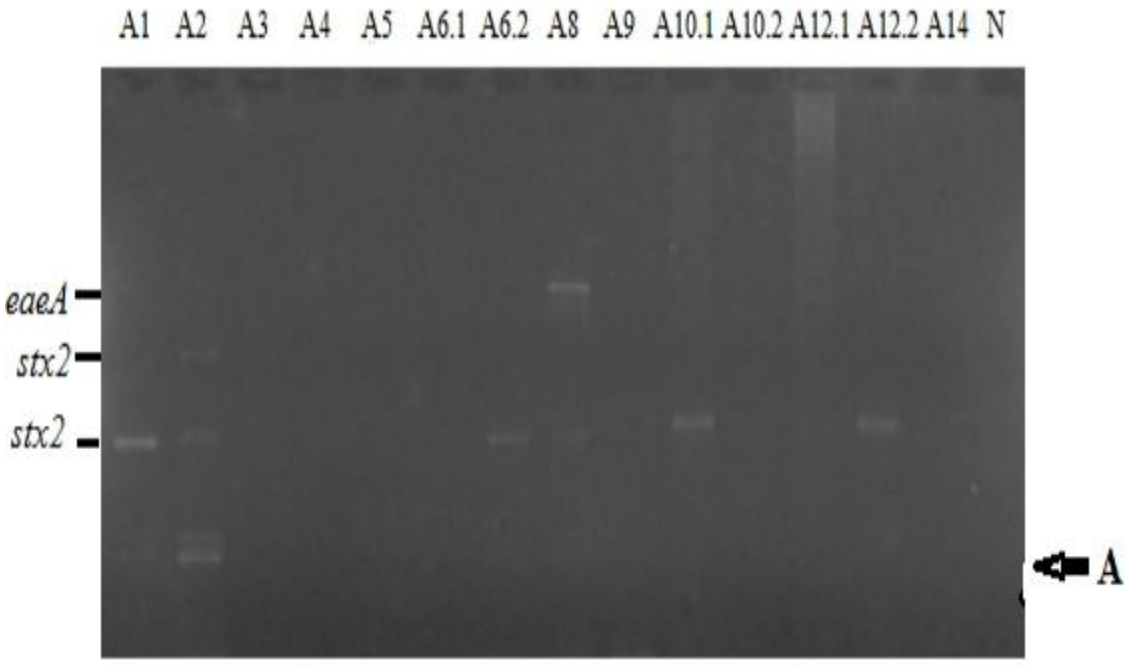

Fig 4.3: Multiplex PCR of *stx 1*, *stx 2, eae A* and *hly A* genes in *E. coli*. Arrow A refers to nonspecific PCR bands (see the text). Lanes 1-14 consist of the 14 *E. coli* isolates. For lanes A6.1 and A6.2, A10.1 and A10.2 and A12.1 and A12.2; each combination refers to two individual *E. coli* isolates as differentiated by colour of colony from the same sample culture plate. Lane N – negative control (DNA free water).

Chapter 5: Discussion

5.1 Prevalence of E. coli and possible sources of contamination.

Many factors may contribute as sources of contamination of meat-products prior to consumption of ready-to-eat meat products and sour milk. Factors of contamination may include the equipment such as machines and cutting tools, unhygienic environment, non-compliance with proper food handling standards and lack of personal hygiene (Jaja *et al.*, 2018). This study indicated that there is higher contamination by *E. coli* ($1.2 \text{ X } 10^2 \leq \text{TEC} \leq 1 \text{ X } 10^3$) in the ready-to-eat meat products sold in the informal sector than in the formal sector especially polony with a prevalence of about 50% (6 of 12). The high prevalence of *E. coli* is alarming as it is indicative of a possible outbreak of *E. coli* associated diseases. This is in line with previous studies, for example, Makwanda and Woyo (2014) found that the informal sector food handling practices are not in accordance with the National Food Laws & Regulations of Zimbabwe (Government of Zimbabwe, 2016) hence, may result in food contamination. Some of the cutlery used such as knives for cutting polony may not be cleaned or cleaned after long intervals resulting in accumulation and persistence of *E. coli*. Contamination of food may have also resulted from poor personal health of both the consumers and the food handlers. The consumers include the commuter omnibus operators who frequently urinate on the vehicles and the surrounding infrastructure resulting in perpetual environmental contamination because *E. coli* can survive outside the usual hosts in other environments such as soil and water (Jang *et al*, 2017). The unhygienic individuals who may directly handle food and the equipment or cutlery used by the food handlers without a hands wash perpetuates the spread of Urinary Tract infection (UTI) causing *E. coli* (Vincent *et al*, 2010). Total *E. coli* count is lower in burgers ($9 \text{ X } 10^1 \leq \text{TEC} \leq 3\ 10^2$) than polony ($1.2 \text{ X } 10^2 \leq \text{TEC} \leq 1 \text{ X } 10^3$) probably due to the

heating of burgers during preparation by the food handlers which contribute towards sterilisation unlike the polony.

In general the TEC for samples from the formal sector was low ($9 \text{ X } 10^1 \leq \text{TEC} \leq 3 \text{ X } 10^2$), probably owing to clean environment and good food handling practices by the food handlers. The formal shops also extended the sanitation consciousness and services to the consumers by provision of sanitary wipes at the entrance and several points within the shops. The formal sector may have small station cleaning intervals which prevent *E. coli* from reaching its log growth phase on the surfaces. Though at lower prevalence, detection of *E. coli* in some of the sample from the formal sector indicate possible faecal and urine contamination from food handlers with gastrointestinal diseases and/or UTIs or from the toilets. The fermented milk samples generally show no contamination by *E. coli* and this is attributed to the gram negative lactose fermenters which produce bacteriocin-like inhibitory substance with a broad spectrum of antimicrobial activity directed against pathogenic indicator organisms suggesting its protective value against enteric pathogens (Kats and Pollan, 2012). However, the single sample of contaminated sour milk was identified from the formal market and request for an Infection Control Alert since the colonising *E. coli* indicated to be an ESBL producing strain.

5.2 Antimicrobial resistance pattern of *E. coli*

Overall, LEX showed 100% antimicrobial therapeutic effect on the *E. coli* isolates and remains a good antibiotic for the treatment of *E. coli* UTI. This suggest that, LEX can be used at low doses since it shows very high potency. The above 35% resistance to TCY, AMP, NEO, ERY and PEN G observed is not surprising. TCY has been widely used in therapy and to promote feed efficiency in animal production systems since its approval in 1948 (Tadesse *et al*, 2012). The prevalent use

of antibiotics is proportional to AMR development (Pitout *et al*, 2005). In recent studies ESBL producing *E. coli* have been isolated from food and UTI patients in another region of Zimbabwe (Mbanga *et al*, 2016). Due to variability of affinity of ESBLs for different substrates the resistance of 42.9% (6 of 42) isolates to ceftriaxone with specific zones of inhibition diameter indicate ESBL production as indicated by WHONET from analysis (Wayne, 2009). Penicillin G, shows a relatively poor antibiotic activity against Gram-negative bacteria as ESBL production confers resistance to all the beta-lactam antibiotics, except for some carbapenems and cephamycins. In addition, ESBL/ampC encoding plasmids may also carry genes which encode resistance to other class of antibiotics such as fluoroquinolones, *aminoglycosides* and *sulphonamides* (Abhilash, 2010), hence the high resistance to PEN G, ERY, NEO, and AMP, as well as resistance for TCY, SXT and CRO in some of the Isolates (Momtaz, 2013). Provisions that were made to decrease the prevalence of ESBL producing organisms by substituting earlier cephalosporins with a fourth-generation cephalosporin or beta-lactam/betalactamase inhibitor combinations seem to be successful as LEX was found to be effective on 100% of the *E. coli* isolates. (Taneja *et al*, 2008). The synergistic effect observed for LEX, SXT, CRO, AMP and TCY also provide an incentive for adoption of combination treatment. Incorporation of plant essential oils in the treatment administration/ regiment together with the conventional antibiotics is required if AMR is to be reduced (van Vuuren, 2011).

5.3 The distribution of virulence genes in *E. coli*

The presence of the *Aeromonas* spp. variant of the *stx2* (shiga toxin expressing gene: 406bp) in *E. coli* suggests gene exchange between *E. coli* and *Aeromonas* spp (Alperi and Figueras, 2010). In several studies *E. coli* and *Aeromonas* spp have been simultaneously detected as part of the coliforms from food, faeces and urine samples of patience with uncomplicated UTI. The STEC

strains usually have an average incubation period of about 3-4 days and patients my recover after about 10 days from the time of first symptoms (WHO, 2018) Shiga toxin-producing *E. coli* causes symptoms that include abdominal cramps and diarrhoea which may in some cases progress to haemorrhagic colitis and fever may develop as well. However, the elderly and children may not recover resulting in a life-threatening disease, such as haemolytic uraemic syndrome which is characterized by acute renal failure, haemolytic anaemia and thrombocytopenia (low blood platelets). Neurological complications such as seizure, stroke and coma may also result and has been noted in 25% of HUS patients and mild chronic renal sequelae, in about 50% of survivors. Despite the presence of *stx* gene, most of the isolates from this study except one (A8), are unlikely to cause any serious infection due to lack of hly and *eae A* as other virulent factors besides *stx* are required to cause serious disease in humans. (Brian *et al*, 1992; Paton *et al*., 1993) suggested that other virulence factors besides *stx* are required to cause serious disease in humans.

The amplification of non-specific band could have resulted from lower annealing temperature, poor quality of the in use dNTPs, however the thermo-cycler was not recalibrated as a result of low and limited resources. However, elimination of non-specific band can be achieved by raising the annealing temperature and appropriate concentration of nucleotides (Ertl, 2018). The nucleotide sequence band below hundred could be half of the haemolysin *hly* (161bp) due to failure of primer annealing and poor primer quality which is a problem faced by many researchers (Protocol Online, 2018).

5.4 Recommendations

Consumers are discouraged to buy their meaty foods from the informal sector as the risk of contracting life threatening diseases such as bloody diarrhoea and UTIs is high. It is recommended

that, the participating parties in the ready to eat meat products be educated on good food handling standard practices to maintain food safety. The Government of Zimbabwe as well as other developing countries with similar economic environment should set up food safety systems that are in tandem with their economic environment. Civil societies are encouraged to provide the direly needed knowledge on food safety, sanitation and personal hygiene to the general people and to individuals within the areas of concern by doing major campaigns mainly with the informal sector in support of cleanliness. The local authority or City Council should also make the provision of requisite amenities such as water and infrastructure a priority. Rigorous food safety checks by the food regulatory authorities are also very critical in both sectors so as to encourage them to meet the zero TEC standards in all their food services products. There is need to maintain at low or even reduce AMR by decreasing application of antibiotics to which the enterobacteriaceae have developed resistance and exchanging them with other derivatives of such antibiotics. Use of plant essential oils in synergy with other conventional antibiotics or not can also go a long way in effective treatment and diminishing AMR. The low treatment cost that comes with herbal therapy also decrease the use of the cheaper antibiotics, thereby decreasing their use and reducing chances of AMR development. Medical practitioners should also provide antibiotic treatment to cases which inevitably requires them to reduce casual use of antibiotics and consequential AMR development. Vaccination to curb *E. coli* should also be made a priority since this can provide herd immunity as well as reduce the occurrence of AMR strains. Generally, an effective antimicrobial stewardship program (ASP) should be followed to optimize clinical outcomes while minimizing toxicity associated with antimicrobial use and the emergence of resistance, resulting in a reduction of healthcare costs while maintaining or improving quality of care.

CONCLUSION

A high level of *E. coli* in meat products from the informal sector indicate lower observance of standard food handling practise, lack of personal hygiene and contamination of the environment. Consumers should avoid buying meaty products from the informal sector as this poses a risk of several infections from *E. coli*. Good sanitation is critical in maintaining a healthy environment hence people should be more conscious of good hygienic practices. The study indicates horizontal gene exchange between *E. coli* and other pathogens hence antibiograms of *E. coli* should be known before treatment of *E. coli* infections. Persons who experience bloody diarrhoea or severe abdominal cramps should seek medical care.

REFERENCES

Abhilash A. (2010). Epidemiology and outcome of bacteraemia caused by extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and Klebsiellaspp in a tertiary care teaching hospital in south India. *Journal of Associate Physicians of India*. 58, 7.

Alperi A. and Figueras M. J. (2010). Human isolates of *Aeromonas* spp. possess Shiga toxin genes (*stx1* and *stx2*) highly similar to the most virulent gene variants of *Escherichia coli*. *Clinical Microbiology and Infection*, 16, 1563 – 1567.

Anderson J. B., Levy T. A., Shuster K. E., Hansen A. S. and Volk A. (2004). A camera's view of consumer food-handling behaviours. *Journal of American Dieticians Association* 104, 186 – 191.

Barnes J.H., Vailancourt J., and Gross W.B. (2003). Diseases of poultry (11th ed). *Iowa State University Press*, Ames, I.A. 631-652.

Barnes J.H., Vailancourt J., and Gross W.B. (2008). Diseases of poultry (12th ed). *Iowa State University Press*, Ames, I.A. 691-738.

Baquero F., Martínez J-L. and Cantón R. (2008). Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology*, 19, 260 – 265.

Bean N. J., Goulding C. and Angulo F. (1996). Surveillance for foodborne disease outbreaks. Morbidity and Motility weekly Report 445, 1 – 55.

Beloeil P-A. (2013). Harmonised monitoring and reporting of antimicrobial resistance in food-producing animals and food. European Food Safety Authority. 7th EURL-AMR Workshop, Lyngby, Denmark, 4 April 2013.

Bhakdi S., Muhly M., Korom S. and Schmidt G. (1990). Effects of *Escherichia coli* hemolysin on human monocytes. Cytocidal action and stimulation of interleukin 1 release. *Clinical Investigations*, 85, 1746 – 1753.

Brian M. J., Frosolono M., Murray B. E., Miranda A., Lopez E. L., Gomez H. F. and Cleary T. G. (1992). Polymerase chain reaction for diagnosis of enterohemorrhagic *Escherichia coli* infection and hemolytic uremic syndrome. *Journal of Clinical Microbiology*. 30, 1801 – 1806.

Brisse S., Diancourt L., Laouenan C., Vigan M., Caro V., Arlet G., Drieux L., Leflon-Guibout V., Mentre F., Jarlier V. and Nicolas-Chanoine M-H. (2012). Phylogenetic distribution of CTX-M- and non-extended-spectrum β-lactamase-producing Escherichia coli isolates: group B2 isolates, except clone ST131, rarely produce CTX-M enzymes. *Journal of Clinical Microbiology*, 50, 2974 – 2981.

Bryan, F. L. (1983). Epidemiology of milk-borne diseases. *Journal of Food Protocol*. 46, 637–649.

Byappanahalli M. N. (2002). Lake Michigan Ecological Research Station, Great Lakes Science Center, U.S. Geological Survey Chesterton, USA. Report.

Centres for Diseases Control. (2003). Bacterial Foodborne and Diarrheal Disease National Case Surveillance: *Annual Report. Atlanta Centres for Disease Control and Prevention*, 55, 18 – 21.

Collignon P., Powers J. H., Chiller T. M., Aidara-Kane A., and Aarestrup F. M. (2009). World Health Organization Ranking of Antimicrobials According to their Importance in Human

Medicine: A Critical Step for Developing Risk Management Strategies for the use of Antimicrobials in Food Production Animals. *Clinical Infectious Diseases*, 49, 132 – 41.

Cruikshank, J. G. (1990). Food handler and food poisoning training programmes are best. *British Medical Journals*, 300, 207 - 208

Dale A. P. and Woodford N. (2015). Extra-Intestinal Pathogenic *Escherichia coli* (ExPEC): Disease, Carriage and Clones. *Journal of Infectious Desieses*, 71, 615 - 626.

Delicato E.R., de Brito B.G., Gaziri L.C.J. and Vidotto M.C. (2003). Virulence associated genes in *Escherichia coli* from poultry with colibacillosis. *Veterinary Microbiology*, 94, 97 – 103.

Dhanji H., Murphy N. M., Akhigbe C., Doumith M., Hope R., Livermore D. M.and Woodford N., (2011). Isolation of fluoroquinolone-resistant O25b:H4-ST131 *Escherichia coli* with CTX-M-14 extended-spectrum b-lactamase from UK river water. *Journal of Antimicrobial Chemotherapy*, 66, 512 – 516.

Dilielo L.R., (1982). *Methods in Food and Dairy Microbiology*. AVI publishing Co. Inc. Westport.

Dozois C.M., Daingle F. and Curtiss R III. (2003). Identification of pathogen-specific and conserved genes expressed in vivo by avian pathogenic *Escherichia coli* strain. *National Academic Science. USA*, 100, 247 - 252.

Doyle, M. P. and Buchanan. R.L. (2007). *Food Microbiology: Fundamentals and Frontier*. ASM Press, Washington.

Ertl P. (2018). Working with PCR. Sigma Aldrich: Customer Support Technical Services.

FAO/WHO. (2006). Food safety risk analysis: A guide for national food safety authorities. FAO, Food and Nutrition Paper.

FAO/WHO. (2002). Global Forum of Food Safety Regulators. Marrakech, Morocco, 28 - 30 January 2002

Farrokh C., Jordan K., Auvray F., Glass K., Oppegaard H., Raynaud S., Thevenot D., Condron R., De Reu K., Govaris A., Heggum K., Heyndickx M., Hummerjohann J., Lindsay D., Miszczycha S., Moussiegt S., Verstraete K. and Cerf O. (2013). Review of Shiga-toxin-producing *Escherichia coli* (STEC) and their significance in dairy production. *International Journal of Food Microbiology*, 162, 190 – 212.

Feng P., Weagant S. D., Grant M. A. and Burkhardt W. (2002). Enumeration of *Escherichia coli* and the Coliform Bacteria. *Food Microbiology*, 19, 111 - 115.

Fijan S. (2014). Nicroorganisms with claimed probiotic properties: An overview of recent Literature. *International Journal of Environmental Research and Public Health*,

Fair R. J. and Yitzhak T. (2014). Antibiotics and Bacterial Resistance in the 21st Century. *Perspectives in Medicinal Chemistry*, 6, 25 – 64.

Fishman N. (2006). Antimicrobial Stewardship. *American Journal of Medicine*, 119, 53 – 61.

GOZ (2016). National Food Laws & Regulations. Zimbabwe. Codex Alimentarius.

Hombach M., Zbinden R. and Böttger E. C. (2013). Standardisation of disk diffusion results for antibiotic susceptibility testing using the sir scan automated zone reader. *Biomedical Central Microbiology*, 13, 225.

International Association for Medical Assistance to Travellers (2018). Food and water safety: Overview. Foundation for the Support of International Medical Training (FSIMT).

Jaja I. F., Green E. and Muchenje V. (2018). Aerobic mesophilic coliform, *Escherichia coli*, and *Staphylococcus aureus* counts of raw meat from the formal and informal meat sectors in South Africa. *International Journal of Environmental Research and Public Health*, 15, 819 – 831.

Jang J., Hur H. G., Sadowsky M. J., Byappanahalli M. N., Yan T., Ishii S. (2017). Environmental *Escherichia coli*: ecology and public health implications — a review. *Journal of Applied Microbiology*, 123 (3), 570 – 581.

Jang J., Suh Y.-S., Di D. Y. W., Unno T., Sadowsky M. J. and Hur H.- G. (2013). Pathogenic *Escherichia coli* strains producing extended-spectrum b-lactamases in the Yeongsan River basin of South Korea. *Environmental Science Technology*, 47, 1128 – 1136.

Kaper J. B., Nataro J. P. and Mobley H. L. T. (2004). Pathogenic *Escherichia coli*. *National Review of Microbiology*, 2, 123 - 140.

Katz, S.E. and Pollan M. (2012). The art of fermentation: an in depth exploration of essential concepts and processes from around the world. *Dairy Science*, 120, 12.

Leach J., Mercer H., Stew G., and Denyer S. (2001). Improving food hygiene standards-a customer focused approach. *British Food Journal*, 103: 238 - 252.

Little, C. & McLauchlin T. (2007). *Food Poisoning and Food Hygiene,* 7th Edition, Hodder Education and Hauchette UK Company. London.

Looft T., Johnson, H.K. and Allen T. A. (2012). "In-feed antibiotic effects on the swine intestinal microbiome". *Proceedings of National Academic Science U S A*, 109, 1691 - 1696.

Louie M., de Azavedo J. C. S., Handelsman M. Y. C., Clark C. G., Ally B., Dytoc M., Sherman P. and Brunton J. (1993). Expression and characterization of the *eaeA* gene product of *Escherichia coli* serotype O157:H7. *Infections Immunology*. 61, 4085 – 4092.

Makwanda P. N. and Woyo E. (2014). Food safety violations by food handlers in the food industry in Zimbabwe. *American Journal of Nutrition and Food Science*, 1, 25 – 31.

Marriott N.G. (1990). *Meat sanitation guide II.* American Association of Meat Processors and Virginia Polytechnic Institute and State University, Blacksburg.

Mbanga J., NcubeV. and Magumura A. (2016). Detection of extended-spectrum β-lactamase producing *Escherichia coli* in retail chicken meat and humans in Bulawayo, Zimbabwe. *American Journal of Research Communication*, 4, 2016: 190 – 207.

Mead P. S., Slutsker L., Dietz V., McCaig L. F., Bresee J. S. and Shapiro C. (1999). Food-related Illness and Death in the United States. *Emergence Infectious Diseases*, 5, 607– 25.

Mhone, T.A., Matope, G. and Saidi, P.T. (2011) Aerobic bacterial, coliform, *Escherichia coli* and *Staphylococcus aureus* counts of raw and processed milk from selected smallholder dairy farms of Zimbabwe. *International Journal of Food Microbiology*, 151, 223 – 228.

Momtaz H. (2013). Serogroups virulence genes and antibiotic resistance in Shiga toxin-producing *Escherichia coli* isolated from diarrheic and non-diarrheic paediatric patients in Iran. *Gut Pathology*, 5, 39.

Morbidity and Mortality Weekly Report (2009). Surveillance for Foodborne Disease Outbreaks, 58, 609 – 615.

O'Brien T. and Stelling J. (2018). WHO collaborative centre for the surveillance of antimicrobial resistance. World Health Organisation's WHONET Build number 8.0.1.0.24222.

Osservasalute M., (2009). Health status and quality of the Italian regions. Report: 175–177.

Paton A. W., Paton J. C., Goldwater P. N. and Manning P. A. (1993). Direct detection of *Escherichia coli* Shiga-like toxin genes in primary faecal cultures by polymerase chain reaction. *Journal of Clinical Microbiology*. 31, 3063 – 3067.

Paton A.W. and Paton J.C. (1998). Detection and characterization of Shiga toxigenic *E. coli* by using multiplex PCR assays for *stxl, stx2, eaeA,* enterohaemorrhagic *E. coli hly A, rfb* O111 and *rfb* O157. *Journal of Clinical Microbiology*, 42, 598 – 602.

Paton J.C. and Paton A.W. (1998). Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Journal of Clinical Microbiology*, 11, 450 – 479.

Pitout J. D. D., Gregson D. B., Church D. L., Elsayed S. and Laupland K. B. (2005). Community-wide outbreaks of clonally related CTX-M-14 beta-lactamase-producing Escherichia coli strains in the Calgary Health Region. Journal of Clinical Microbiology, 43, 2844 – 2849.

Soomro A.H., Arain M.A., Khaskheli M. and Bhutto B. (2002). Isolation of *Escherichia coli* from raw milk and milk products in relation to public health sold under market conditions at Tandojam. *Pak. Journal Nutrition*; 1, 151 – 152.

Saidi B., Mafirakureva P., and Mbang J. (2013). Antimicrobial Resistance of *Escherichia coli* Isolated from Chickens with Colibacillosis in and Around Harare, *American Association of Avian Pathologists*, 57, 152 – 154.

Tadesse D. A., Zhao s., Tong e., Ayers S., Singh A., Bartholomew M. J. and McDermott P. F. (2012). Antimicrobial Drug Resistance in *Escherichia coli* from Humans and Food Animals, United States. *Emergency Infectious Diseases*, 5, 1950 – 2002.

Taneja N., Rao P., Arora J. and Ashok D. A. (2008). Occurrence of ESBL and Amp-C β-lactamases & susceptibility to newer antimicrobial agents in complicated UTI. *India Journal of Medicine Resistance*, 127, 85 - 88.

Tenaillon O., Skumik D., Picard B., and Denamur E. (2010). The population genetics of commensal *Escherichia coli*. *National Review of Microbiology*, 8, 207-217.

The Global Antibiotic Resistance Partnership (GARP), (2017). Situational analysis of antimicrobial use and resistance in humans and animals in Zimbabwe. Government of Zimbabwe. Report.

Tigu F., Assefa F., Mehari T. and Ashenafi M. (2016). Probiotic property of lactic acid bacteria from traditional fermented condiments: Datta and Awaze. *International Food Research Journal*, 23, 770 – 776.

Tomasz A., (1979). The Mechanism of the Irreversible Antimicrobial Effects of Penicillins: How the Beta-lactam Antibiotics Kill and Lyse Bacteria. *Annual Review in Microbiology*, 33, 113 – 37.

Vallance B.A., and Finlay B.B. (2000). Exploitation of host cells by enteropathogenic *Escherichia coli*. *National Academic Sciences. USA*, 97, 8799 - 8806.

van den Bogaard A. E., London N., Driessen C. and Stobberingh E. E. (2001). Antibiotic Resistance of Faecal *Escherichia coli* in Poultry, Poultry Farmers and Poultry Slaughterers. *Journal of Antimicrobial Chemotherapy*, 47, 763 – 71.

van der Westhuizen A.W. and Bragg R.R. (2012). Multiplex polymerase chain reaction for screening avian pathogenic *Escherichia coli* for virulence genes. Avian Pathology, 41(1), 33-40.

van Vuuren S. and Viljoen A. (2011). Plant-based antimicrobial studies–methods and approaches to study the interaction between natural products. *Planta Medicine*, 77, 1168.

Vasavada P. C. (1988). Pathogenic bacteria in milk-a review. *Journal of Dairy science*, 70, 2800-2816.

Vincent C., Boerlin P., Daignault D., Dozois C. M., Dutil L., Galanakis C., Reid-Smith R. J., Tellier P-P., Tellis P. A., Ziebell K. and Mangescorresponding A. R. (2010). Food reservoir for *Escherichia coli* causing urinary tract infections. *Emergence Infectious Diseases*, 16, 88 – 95.

Walsh C. (2003). *Antibiotics: Actions, Origins, Resistance*. American Society for Microbiology (ASM) Press. Washington DC.

Walsh T. R., Weeks J., Livermore, D. M. and Toleman M. A. (2011). Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infectious Diseases*, 11, 355 – 362.

Wayne P. A. (2009). Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing. Nineteenth Informational Supplement. CLSI document M100-S19.

Wellington E. M. H., Boxall A. B. A., Cross P., Feil E. J., Gaze W. H., Hawkey P. M., Johnson-Rollings A. S. and Jones D. L. (2013). The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *Lancet Infectious Diseases*, 13, 155 – 165.

World Health Organisation, (2018). *E. coli*; WHO response, Report.

Yong M. K., Buising K. L., Cheng A. C. and Thursky K. A. (2010). Improved Susceptibility of Gram-negative Bacteria in an Intensive Care Unit Following Implementation of a Computerized Antibiotic Decision Support System. *Journal of Antimicrobial Chemotherapy*, 65, 1062 – 1069.

APPENDICES

APPENDIX 1: *Escherichia coli* identification key

Test	***Escherichia coli***
Gram stain	+ ve
Catalase	- ve
Motility	(+) ve
Oxidase	- ve
Indole	+ ve
Citrate	- ve
Urease	- ve

APPENDIX 2: SPSS output: Between-Subjects effects.

Tests of Between-Subjects Effects

Dependent Variable: Total E. coli Count

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	917555.556[a]	5	183511.111	6.650	.000
Intercept	356011.111	1	356011.111	12.902	.001
Trading_Sector	266944.444	1	266944.444	9.674	.004
Product_Type	344272.222	2	172136.111	6.238	.005
Trading_Sector * Product_Type	306338.889	2	153169.444	5.551	.009
Error	827833.333	30	27594.444		
Total	2101400.000	36			
Corrected Total	1745388.889	35			

a. R Squared = .526 (Adjusted R Squared = .447)

APPENDIX 3: SPSS output, Tukey HSDa,b.

Total E. coli Count

Tukey HSDa,b.

Food	N	Subset	
		1	2
Fermented/Sour Milk	12	2.50	
Beef burger	12	62.50	
Beef polony	12		233.33
Sig.		.654	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 27594.444.

a. Uses Harmonic Mean Sample Size = 12.000.

b. Alpha = .05.

APPENDIXE 4: Guideline for AMR zone of inhibition interpretation WHONET (O'Brien and Stelling, 2018).

				Diameter of Zone of Inhibition to the nearest mm		
Organism	**Site of infection**	**Antibiotic**	**Potency**	**R ≤**	**I**	**S ≥**
Enterobacteriaceae		Ampicillin_EUCST_Disk	10µg	13		14
Enterobacteriaceae	Oral	Ceftriaxone_EUCST_Disk	30µg	21	22-24	25
Enterobacteriaceae	UTI	Cephalexin_EUCST_Disk	30µg	13		14
Escherichia coli		Neomycine_EUCST_Disk	10µg	12	13-16	22
Escherichia coli		Penicillin G_EUCST_Disk	10units	11	Dec-21	22
Enterobacteriaceae		Trimethriprim/Sulfamethoxazole_EUCST_Disk	1.25/23.75µg	10	Nov-13	14
All bacteria		Erythromicin_EUCST_Disk	15µg	13	14-22	23
All bacteria		Tetracyclin_EUCST_Disk	30µg	14	15-18	19