ASSESSMENT OF MICROBIAL QUALITY AND SAFETY OF GROUND BEEF/PRODUCT SOLD IN DIFFERENT RETAILERS AROUND THOHOYANDOU AREA, VHEMBE DISTRICT, LIMPOPO

BY

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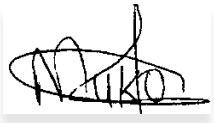
DECLARATION

I, Fulufhelo Valentine Mukosi (Student No:16003676) declare that this dissertation was submitted to the University of Venda for a master's degree in Microbiology in the School of Agriculture, Engineering, and Science is my original work conducted under the supervision of Dr Musie and Prof Traore. This thesis has not been submitted for any degree at any other university or institution. All the citations and sources of information in this dissertation have been duly credited.

Signature: 

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DEDICATION

I dedicate this research to the lion king of Judah (TAU) for his favor and grace manifested in me daily, to my parents for showing me everlasting love and support throughout, to my supervisor for granting me such a great opportunity of working with him on this research project, and to my best friend for supporting and putting me under tremendous pressure.

ABBREVIATIONS

AMP	Ampicillin
C	Chloramphenicol
AMDs	Antimicrobial drugs
°C	Degrees Celsius
%	Percentage
g	Gram
ml	Millilitre
µl	Microlitre
µg	Microgram
mm	Millimetre
rpm	Revolutions per minutes
spp	SPECIES
ADH	Alcohol dehydrogenase
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribose Nucleotide Triphosphate
EAEC	*Entero-aggregative E. coli*
E. coli	*Escherichia coli*
EHEC	*Entero-hemorrhagic E. coli*
EIEC	*Entero-invasive E. coli*
ETEC	*Entero-toxigenic E. coli*
EPEC	Enteropathogenic *E. coli*
ESBLs	Extended-spectrum beta lactamases
Gel	Gelatinase
H2S	Hydrogen sulphite
HUS	Haemolytic uremic syndrome
MAC	MacConkey

MDR	Multidrug resistance
MRSA	Methicillin resistance Staphylococcus Aureus
MHA	Mueller Hinton Agar
MgCl2	Magnesium Chloride
m-PCR	Multiplex-Polymerase Chain Reaction
NTS	Non-Typhoidal-*Salmonella*
PCR	Polymerase Chain Reaction
RVS	Rappaport-Vassiliadis Soya peptone
SMAC	Sorbitol with MacConkey
STEC	*Shiga Toxin Escherichia coli*
Stx 1	Shiga toxin 1
Stx 2	Shiga toxin 2
S. typhi	*Salmonella typhimurium*
S. paratyphi	*Salmonella paratyphimurium*
TDA	Tryptophan deaminase
TSI	Triple sugar iron
XLD	Xylose Lysine Deoxycholate
MSA	Mannitol Salt agar
FOX	Cefoxitin
TE	Tetracycline
S	Streptomycin

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ABSTRACT

Title: Assessment of microbial quality and safety of ground beef/product obtained from selected retailers around Thohoyandou area; Vhembe district; Limpopo.

Background: It has been proven that animal products are easily contaminated with microorganisms, and this supports microbial growth if not properly handled, processed, and preserved. Ground beef and its wholesale products are becoming popular because of the demand for rapid meal preparation and services, especially in the fast-food industry. Despite the control measures in place, foodborne infections continue to be an immense problem, with millions of cases occurring annually worldwide. In South Africa, illnesses and deaths related to food consumption continue to be reported. In addition to the misery caused, the financial loss associated with meat spoilage and illnesses is enormous. Therefore, this study aimed to assess ground beef's microbial quality and safety in different retailers around Thohoyandou area, Vhembe District.

Methodology: A total of 160 ground beef/product samples was randomly purchased from various retailers in Thohoyandou and transported on ice to the University of Venda microbiology laboratory for analysis. The potential microbes were cultured in enrichment media (peptone buffered water) for 5 minutes in room temperature. The culture was then sub-cultured in different plates containing selective media (e.g., EMB for *E. coli*, SS for *salmonella and Shigella*, MSA for *Staphylococcus spp*.) using the spread plate technique. Isolates were then identified by the Gram staining technique and biochemical tests such as Catalase, Urease, Citrate, Kligler Iron Agar, and VITEK system. Moreover, the antibiogram activity of isolated pathogens was screened against medically used and commercially available antibiotics. Furthermore, the DNA of the isolates was extracted, and multiplex PCR was conducted to determine different virulence genes and pathotypes. Hemolysin test was done in blood agar plates to identify virulence characteristics of *E. coli* isolates.

Results: Out of 160 samples analyzed*, E. coli* was detected in 80 (50%), *Staphylococcus* spp. in 117 (73.12%), *Salmonella* in 60 (37.5%), and *Shigella* species in 108 (67.5%). Most Enterobacteriaceae (*E. coli, Salmonella* and *Shigella*) isolates were resistant to

Ampicillin and Cefoxitin. *Staphylococcus* isolates showed high resistance to Cefoxitin (93.33%) and Oxacillin (93.33%). Out of 30 *E. coli* isolates subjected to mPCR assay, 23 isolates were of different pathotypes with EPEC (53.33%) being the most prevalent pathotype. Asta with 73.33% was the dominant virulence gene obtained. Thirty (30) *E. coli* isolates were tested for hemolysin activity and Alpha hemolytic activity was observed in 76.6% isolates, while beta hemolytic activity observed in 10% isolates. Some of the isolates presented non-hemolytic strains (13.3%).

Conclusion: It was concluded that ground beef/products from established retailers were contaminated with pathogenic bacteria, and microbial quality was thus inadequate.

Keywords: Antimicrobial activity, Enteric Pathogens, Foodborne infections, Ground beef, Virulence genes.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 BACKGROUND

Foodborne disease is a leading public threat causing morbidity and mortality across the world. Each year, it is predicted that 92 million people in Africa become ill as a result of consuming contaminated foods, resulting in 137 000 deaths (Bisholo et al., 2018). Food safety, on the other hand, does not appear to be a significant concern in many African countries (Bishop et al., 2018). South Africa has been reporting numerous incidents of foodborne diseases. More than 250 foodborne diseases have been identified in studies, and majority of them are infections caused by a variety of bacteria, viruses, and parasites (Newell et al., 2010). Toxins and hazardous chemicals can also contaminate foods, resulting in foodborne illness (Tshabalala, 2011).

Nausea, vomiting, stomach cramps, and diarrhea are some of common foodborne disease symptoms (Jahan, 2012). Foodborne infections are predominant in older people, young children, and people with weak immune systems due to health problems including diabetes, liver and kidney failure, organ transplants, or human immunodeficiency virus, as well as pregnant women (Lund and O'Brien, 2011).

Some common foodborne microorganisms that cause illnesses include *Norovirus*, *Salmonella*, *Clostridium Perfringes, Campylobacter*, *Staphylococcus aureus, listeria*, *Vibrio*, and *E. coli* (Scallan et al., 2011). Raw animal foods such as; raw meat, poultry, eggs, and unpasteurized milk have all been linked to foodborne illness. Raw meat is the most prone to contamination. When manure is used as a fertilizer, fruits and vegetables can become contaminated with animal waste. Raw sprouts are particularly concerning due to the conditions under which they are sprouted, which are ideal for microbe growth (Norouzi, 2013).

According to research performed to determine bacteriological safety and quality of food, ground meat is nutrient-rich medium favoring bacterial growth (Erdem et al., 2014). Ground beef is beef that has been finely chopped with a meat grinder or mincing machine. Because of the increased demand for quick meal preparation and services, particularly in the fast-food industry, ground beef and ground beef products are

becoming increasingly popular at the wholesale level (Speer et al., 2015). However, with foodborne diseases incidents linked to ground beef getting media attention in recent time, many consumers are questioning the meat's safety (National Research Council, 2010).

Ground beef often contains a variety of bacteria that can cause illness, and such pathogenic bacteria include *Salmonella*, *Campylobacter jejuni*, *E. coli*, *L. monocytogenes*, *Bacillus species*, *Clostridium perfringes* and *Staphylococcus aureus*. Other microbes cause quality deterioration and are usually harmless; however, they can end up causing food to degrade or lose quality by emitting a foul smell or being sticky outside (Woan-Fei, 2015). Indicator bacteria are commonly used as practice to assess the sanitary and hygienic condition of food for the possible presence of microorganism (Brown et al., 2000).

E. coli 015:H7 bacteria is of particular concern in ground beef (Cleary, 2004). They release huge amounts of toxin which are released in the intestine and cause severe damage to the intestine's lining (Flowers, 2011). These toxins cause hemorrhagic colitis and can cause Hemolytic Uremic Syndrome, especially in infant (Cleary, 2004). Although the precise infective quantity is not understood, most scientists believe that only few *E. coli* strains are required to cause severe illness which can lead to death.

S. aureus is considered one of the most dangerous pathogenic bacteria due to its ability to produce extracellular protein toxins such as; toxic shock syndrome toxin 1, exfoliative toxin, coagulase, and hemolysin (Reischl et al., 2000). The organism is present as a short-term resident, a short-lived contaminant, or a long-term colony-forming organism and is capable of causing a wide range of diseases, including septicemia, sepsis, wound sepsis, septic arthritis, osteomyelitis, food poisoning, and toxic shock syndrome (Todd et al., 2009). It is found in raw animal food and ready-to-eat foods due to contamination during the handling process and, if present in large quantities, can cause food poisoning (Adigun et al., 2020). Toxic syndromes and foodborne diseases in animals and humans are caused by enterotoxins and toxic shock syndrome toxin-1 (Todd et al., 2010).

The most common pathogenic *Salmonella* species that cause infection in humans from various food are *Salmonella enteriditis* and *Salmonella typhimurium* (Kramarenko et al., 2014) in both underdeveloped and industrialized countries. *Salmonella* causes salmonellosis, which is associated with nausea, abdominal pain, diarrhea, and occasionally fever, and it can also result in morbidity and mortality in both animals and humans (Nørrung & Buncic 2008). *Salmonella* pathogenicity is linked to several pathogenic genes found inside *Salmonella* pathogenicity islands (SPIs) in the bacterial chromosome (Nayak et al., 2004). *Salmonella* can invade epithelial cells influenced by genes found in SPI such as invA and hila (Cardona-castro et al., 2002; Nayak et al., 2004). While the plasmid-encoded fimbriae (pefA) gene promotes *salmonella* adhesion to epithelial cells (Murugkar et al., 2003). Another chromosomal gene called stn, which codes for the power generation of enterotoxin, is one of the causative agents of diarrhea (Huehn et al., 2010).

Antibiotic resistance of pathogenic bacteria is among the most significant threats to global human health (winters and Gelband, 2011). Because of the use of antimicrobial drugs in the food production, foods originating from animals are regarded as an important reservoir of antibiotic-resistance microorganism (Founou et al., 2016). Antibiotic-resistance pathogens can enter humans indirectly through food supply and food poisoning (Luo et al., 2015). Antibiotic-resistant food contaminating pathogens poses a significant risk to population health due to the fact that antibiotic-resistance defining factor could be passed on to other harmful pathogens, possibly jeopardizing diagnosis of drastic pathogenic infectious diseases (Adesetan et al., 2013). Furthermore, most developing countries, including South Africa, have given antibiotic resistance a low priority and limited attention (winters and Gelband, 2011). *E. coli* can acquire and transfer antimicrobial resistance genes (Oyeleke and Manga, 2008). *Salmonella spp.* and *S. aureus* multidrug resistance is common and recently grown tremendously (Ngoma et al., 2013).

Distinct molecular assays are utilized to detect pathogenic virulence genes found in ground beef (Fakruddin et al., 2013). Polymerase chain reaction is one of the greatest molecular efficiency and cost effectiveness tools used to duplicate or multiply short fragment of DNA or RNA (Fakruddin et al., 2013). Polymerase chain reaction is now popular and frequently required procedure in biology and medical laboratories for a

wide range of purposes (Joshi and Deshpande, 2010). DNA sequencing, and DNA-based phylogeny are examples of infectious disease diagnosis. Various genres of PCR include Conventional PCR (Qualitative), multiplex PCR, nested PCR, Reverse-transcriptase PCR (RT-PCR), Real PCR and ERIC-PCR (genotyping method).

1.2 STUDY RATIONALE

Despite the control measures implemented, foodborne diseases continue to be a major issue, with millions of incidents occurring globally each year (Newel et al., 2010). The World Health Organization (WHO) estimated that on a global scale each year, enteric pathogen-contaminated food cause more than 580 million illnesses and 351,000 deaths (Tesson et al., 2020). Meat and meat-based products are thought to be one of the primary carriers of these pathogens among foods.

Food-related illnesses and deaths continue to be reported in South Africa (Rani et al., 2017). Aside from the accompanying deaths, the financial accident linked to meat putrefaction and illnesses is extensive (Muchenje et al., 2018). Food poisoning grow into a medically worth reporting circumstance in 1990. Nonetheless, because of the absence of active and incorporated vigilance system for detecting foodborne illness, it is less likely for the situation to be published (Smith et al., 2019). Internationally, the CDC's (Centers for Disease Control) National Notifiable Diseases Surveillance System (NNDSS) employs a multifaceted Public Health disease surveillance system that provides public health officials with advanced applications to keep track of disease incidence and transmission. Sadly, South Africa appears to deficit aforesaid structural system, which is desperately needed, because this structure is a competent program of public health vigilance which should be initiated at local- and state health departments.

Studies on microbiological quality of mince have revealed that storage temperature and packaging atmosphere have a significant impact (Emswiler et al., 1976). Ground beef should be stored in the refrigerator or freezer immediately after acquisition. This practice conserves the novelty of beef while also significantly slowing the growth of any bacteria present. Many studies recommend warning consumers around the health issues linked with ingestion of undercooked meat as an important preventative

measure and encouraging people to apply food handling guidelines (Abbot et al., 2009; Evans et al., 2020; Medeiros et al., 2001). Good hygiene practices play a huge role in eliminating pathogens capable of prevailing in ground beef (Tshabalala, 2011). The use of various antibiotics on animals should be investigated as it is the source of antibiotic resistance development.

It is critical to detect and identify food-borne infectious agents, together with assessing the overall microbial quantity (Lund et al., 2000). Studies done by Mabasa (2018) and Mukosi (2019) (unpublished data) showed that the microbial quality and safety of ground beef from established retailers in Thohoyandou is inadequate. Since there is a scarcity of publications on the characterization of enteric pathogenic bacteria associated with raw ground beef, this study will provide data to the scientific community. It will also provide awareness on the existence of the low microbial quality meat to avoid foodborne diseases. In this way, authorities will give retailers and butchers recommendations and proper guidelines on good hygiene practices, preparations, and safe ground beef for human consumption. Therefore, this research desired to assess the microbial quality and safety of ground beef and ground beef product (beef patty) sold in different retailers around Thohoyandou area, Vhembe District Limpopo.

1.3 OBJECTIVES OF THE STUDY

1.3.1 MAIN OBJECTIVE

To assess the safety and microbial quality of ground beef and ground beef products obtained in different retailers around Thohoyandou area, Vhembe District.

1.3.2 SPECIFIC OBJECTIVES

- To determine the prevalence of selected enteric pathogenic bacteria from ground beef by culture methods

-To determine the antibiotic susceptibility profile of bacteria from ground beef using the Kirby Bauer Disc Diffusion method

-To determine the molecular characteristics of isolates (pathotype and virulence genes) using multiplex PCR.

-To determine virulence characteristics of *E. coli* isolates by hemolysin production test.

1.4 HYPOTHESIS

Ground beef from established retailers may be contaminated with pathogenic bacteria

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Food derived from animals can be contaminated mostly on farm, which can be exacerbated if such food is not handled properly during wholesale slaughter and processing, allowing harmful bacteria to increase (Tanih et al., 2015). These foods' microbiological quality is called into question due to the conditions in which they are handled (Gwin et al., 2012). Even though several studies have revealed the health risks linked with consumption of animal origin food, there is a scarcity of research focusing on the microbiological quality and safety of cattle and pigs in Vhembe District (Limpopo) abattoirs (Madoroba et al., 2016).

Meat has been part of human diets for most of humanity's existence. Beef is a high protein source that is widely consumed worldwide, which contains all the essential amino acids and iron (Marangomi et al., 2015). Meat consumption in Southern Africa is four times higher than in any other region of Africa (Brand et al., 2017). South Africa contribute a significant role in agricultural livestock production and meat supply (Soji et al., 2015). South Africa is said to consume 41 kg of meat per year, ranking second only to Ghana in Africa (Jaja et al.,2020).

2.2 LIVESTOCK (CATTLE) IN FARMS

Manufacturers and government agencies have increased measures to promote regulation of food safety hazards in recent years, coinciding with increased media coverage of the human health impacts of zoonotic pathogenic microbes and drug resistant strains. During the same timeframe, consumer preference and accessibility for "organic" and "natural" food, such as beef products increased (Anders and Moeser, 2008). This consumers demand might be coincident or as a reaction to rising attention from the media (Morgan, 2000; Meyer and Abrams, 2010).

Grass-fed and organic beef products are gaining market share in most developed countries as well as developing countries such as South Africa (Mapiye et al., 2007). Most cattle's lives change dramatically after a year, depending on whether they are conventionally raised or 100% grass-fed cows (Gwin et al., 2009). According to research, there are three major motivators that influence purchases of organic or natural products by consumers**.** These factors include perceived differences in product safety and quality, as well as ethical concern regarding production methods (Bernues et al., 2003).

2.3 GROUND BEEF

Ground beef is an animal-derived raw food that accounts for a significant portion of the Turkish population's diet (Siriken, 2004). Ground beef makes up more than half of the beef consumed globally. Ground beef is a common staple of beef product served everywhere from fast-food restaurants to school cafeterias and homes across the country, from burgers to burritos (Watson, 2008). Nonetheless, with foodborne illness outbreaks linked to ground beef getting publicity in recent years, many consumers are questioning the meat's safety (DeWaal et al., 2006). Undercooked ground beef is often recognized as a vehicle for *E. coli* O157:H7 infection because it is a suitable medium for the rapid microbial growth (Siriken, 2004). The public concern about the safety of most beef products has grown as a result of infections caused by *Salmonella* spp. linked to beef products, especially ground beef (Angulo et al., 2006). When handled and cooked correctly, consumers expect meat products to be safe to consume. Nevertheless, ground beef can be contaminated during production, processing, storage, and marketing with microbial pathogens that are harmful to human health (Sofos, 2008).

2.4 POSSIBLE SOURCES OF CONTAMINATION

According to the literature, a healthy living animal's muscle tissue is microorganism-free, and the underside of an animal carcass becomes septic instantly after slaughtering (Gill, 2015). Therefore, raw meat contamination can occur as a result of slaughtering of stressed animals, gastrointestinal and respiratory tracts, and other

various surrounding environmental threats (Shilenge, 2017). Contamination occurs in the abattoir when microorganisms are introduced into meat during operations such as offloading, weighing, processing, cutting, and storage, as well as at points of sale and distribution (Nørrung and Buncic 2008; Rani, 2015). According to the study by Bas et al (2006) pathogenic microorganisms can also be transmitted passively by a contaminated source like raw meat to prepared meals which is consumed when is cold. Contamination of food or ground beef has been previously reported and associated with infections of the consumers (Dechet et al., 2006). Such contamination may occur in different ways and influenced by different factors, including slaughtering, processing factor, intrinsic and extrinsic factors, transportation, food handlers, equipment and utensils (Troy and Kerry., 2010).

2.4.1 SLAUGHTERING

The significance of contamination during slaughtering is determined by the cleanliness of the stock prior to slaughter, the quantity of bacteria introduced, as well as the extent to which they are eliminated by the slaughtered animal's defence mechanisms (Pointon et al., 2012). This source of contamination also shouldn't pose a challenge if reasonable hygiene precautions are applied. Color, appearance, and presentation are the most important factors influencing the sale of meat from refrigerated display cases. Whereas animal stress, display period, meat temperature can all have an impact on the fresh meat's appearance and shelf life (Nortje, 1990).

2.4.2 FOOD HANDLERS AND RELATED HYGIENE PRACTICES

Meat cuttings are essential in the meat production industry because boneless carcasses are cut into relatively small and preferable pieces by hand equipment and machines (de Medeiros Esper et al., 2021). As a result, the threat of meat contamination is primarily determined by the food handlers' health, sanitary conditions, as well as their knowledge and awareness of food hygiene (Ehuwa et al., 2021). According to Ali et al., (2008), unsanitary handling practices may result in the transmission of bacteria from workers and working environment to the meat. Therefore, the handling of meat raises the potential of bacterial contamination. Few studies have also revealed that foodborne illnesses are caused by improper food handling in households (Van Tonder 2004; Griffith 2006; Sanlier and Konaklioglu, 2012). Food handlers may serve as sources of contamination on occasion, particularly

if they have gastrointestinal illnesses or are in the recuperation stage when their signs and symptom have subsided (Shilenge et al., 2017).

2.4.3 PROCESSING FACTOR

As the product progresses through the grinding process, the contamination of beef trimmings rises (Wallace et al., 2018) as a result of a variety of factors which includes enhanced product temperature and product homogenization (Cabrera-Diaz et al., 2013). The ultimate bacterial population of such product is linked to the initial contamination on the raw materials. Moreover, a solitary source of substantial contamination can cross-contaminate multiple end-product bundle of ground beef. Incompetent meat grinder cleaning and sterilization may also result in recurring of ground beef microbial contamination over production period (Siriken, 2004).

2.4.4 TRANSPORTATION

By properly maintaining the cold chain, good quality meat with a long storage period can be guaranteed (Kuo and Chen, 2010). As a result, South African Regulation 962 of November 23, 2012, framed under the Foodstuffs, Cosmetics, and Disinfectants Act, 1972 (Act no. 54 of 1972) and the Meat Safety Act 2000 (Act no. 40 of 2000), echo and mandate that all food described by the regulation and act be retained at a cold temperature (below 4°C) during storage, transportation, and display (Rani, 2015). Furthermore, no food transportation at the same time with a person or other items (Foulds et al., 2014). Thus, as described in South African Regulation 962 of 23 November 2012 issued by the Department of Health, inspections of incoming meat, as well as temperature checks of meat and the transport are of primary importance (DOH) (Shilenge et al., 2017). As a result, adequate transportation of carcasses and raw meat products, as well as preserving refrigerator temperatures, will limit the possibility of contamination (Rani, 2015).

2.4.5 EQUIPMENT AND UTENSILS

During food processing, biological contaminants and other substances may contaminate equipment and appliances, even if they are designed with hygienic factors (Masotti et al., 2019). Numerous epidemic foodborne diseases are linked to inadequately sterilized equipment and utensils. *Listeria monocytogenes* is a bacteria found in an environment, it can survive and multiply in meat processing industrial

machines such as slicers, dicers, and packaging equipment that has not been thoroughly cleaned and sanitized (Lunden et al., 2005).

2.4.6 INTRINSIC AND EXTRINSIC FACTORS

The contamination of ground beef can stem from either intrinsic or extrinsic factors. Intrinsic factors are endogenous to the food, such as pH, water activity, substrate type, and availability (Jay et al., 2008). Extrinsic factors include humidity, atmosphere, and temperature. Storage and processing can influence both intrinsic and extrinsic factors (Majumdar et al., 2018). The circumstance of the animal slaughter, the spread of contaminant during slaughtering and processing, temperature, time, and other storage and distribution conditions are the crucial factors determining the bacteriological meat quality marketed by the abattoirs (Troy and Kerry, 2010).

Bacteria and other microorganisms are commonly found in both raw and processed meat. The majority of them are harmless, but some of them can be threatening to food safety because they are food poisoners. In South Africa, as reported by Powell et al (2011) and Halliday et al. (2012), it remains a challenge to enforce regulations in some sectors due to the lack of surveillance data which results from a lack of outbreaks data recorded. This is because of the absence of a CDC system to record the data of outbreaks of foodborne diseases between 1999 and 2010. Moreover, as Sofos (2008) reported, the 1999 estimates cannot be compared with the current ones for purposes of trend analysis because different diagnostic methods evolve all the time. Furthermore, the epidemiological data of foodborne illness and surveillance estimated by the United States (CDC 2011) such as Food Net and the pathogenic tracking and DNA fingerprinting program (PlusNet) indicated that approximately 60–70% of outbreaks and 40–50% of foodborne illness cases reported remain unresolved as well as the etiologic agent unknowns. Principal pathogens of concern are *Staphylococcus aureus, Escherichia coli 015:H7, Salmonella spp., Listeria monocytogenes, Campylobacter jejuni and Yersinia enterocolitica* (Shilenge et al., 2017).

2.5 *STAPHYLOCOCCUS AUREUS*

2.5.1 HISTORICAL BACKGROUND OF THE SPECIES

Staphylococcus is a genus that contains over 30 species, and in addition, the species with the most significant impact on human health is *Staphylococcus aureus* (Bhunia,

2018). While *S. aureus* is a natural inhabitant (commensal) of human and animal skin, nasal passages, respiratory, and genital tracts, it has the potential to cause invasive and fatal infections in a variety of organs as an opportunistic pathogen (Brown et al., 2014). It can also produce enterotoxins, which occur in food during processing and production, and can contaminate food leading to food poisoning to public health (Centers for Disease Control and Prevention 2011; Gosbell & Van Hal 2013). Although heating contaminated food items may destroy the bacteria, the toxins they produce are heat resistant (Centers for Disease Control and Prevention 2011). There seem to be numerous anti-staphylococcal agents available; but nevertheless, the pathogen has developed specific mechanisms to counteract them, including methicillin resistance mechanism (Vuong et al., 2016). Methicillin-resistant *S. aureus* (MRSA) is becoming a more common cause of health care-associated (HA-MRSA) (Stefani et al., 2012), community-associated (CA-MRSA) (Mediavilla et al., 2012), and livestock-associated (LA-MRSA) infections globally (Wendlandt et al., 2013).

2.5.2 MORPHOLOGICAL CHARACTERISTICS

Staphylococcus aureus is a Gram-negative anaerobic coccus that commonly causes foodborne infections (Bhunia, 2018). The organism *S. aureus* generate various extracellular harmful toxins and proteins, which most likely contribute to the organism's virulence. However, the precise nature of the potential virulence determinants in various types of infections is unknown (Peacock et al., 2002). The high-level expression of extracellular SpA, secreted locally by *S. aureus* isolates colonize the gastrointestinal tract of patients (El-Jakee et al., 2008). The SpA protein can bind to Von Willebrand factor, which is a protein that plays an important role in hemostasis and thrombogenesis (Alfeo et al., 2021). Severe *Staphylococcal* human and animal infections are usually caused by methicillin resistant (MRSA) strains.

2.5.3 LABORATORY DIAGNOSIS AND IDENTIFICATION

Diagnosis

Collection and transport of the specimen: This is dependent on the part of the human and animal body affected. A swab is used to collect samples from the skin, throat, nostrils and wound infections (Albrich and Harbarth, 2008). Those suffering from a urinary tract infection must supply a sample of urine, while those suffering from

blood infection must provide blood samples (Graham and Galloway,2001). To prevent contamination, the samples must be collected in specific sterilized sampling containers while adhering to all necessary precautions and operating under strict aseptic conditions (Sanders, 2012). All specimens are immediately transported to the laboratory and processed to avoid any positive or negative false results.

Identification: Direct microscopy is an option. Gram staining is the most common. Assume the specimen comprise *Staphylococcus spp*. It appears as purple/blue colored (gram-positive) round bacteria in clusters that resemble grapes (Elliott et al., 2012). Since *S. aureus* can be usually located on mucous membranes and the skin, this test is not always conclusive (Moller, 2016). Agglutination tests can be used to determine *Staphylococcus aureus* toxins such as enterotoxins A–D and TSST-1 (Fueyo et al., 2005). The clumping of latex particles by the toxins present in a sample is used to determine the results of the tests.

Biochemical tests: There are several biochemical tests for the *staphylococcus* spp. such as Catalase Test (Patel et al., 2018). The coagulase test is also used to distinguish *S. aureus* from other *Staphylococci*, which is crucial for distinguishing among pathogenic and non-pathogenic *Staphylococci* strains *(*Szafraniec et al., 2020). There is also Mannitol fermentation test and *S. aureus* is the only *Staphylococcus* that ferments the Mannitol (Moller, 2003). Rapid diagnostic tests aid in the bacterial detection in real time. Techniques such as Real-time PCR and Quantitative PCR, are progressively being used in diagnostic laboratory (Forootan et al., 2017).

2.5.4 PATHOGENESIS

S. aureus species are the main etiological agents involved in a variety of human infections, such as urinary tract infections, infective endocarditis, skin and soft tissue infections, meningitis, pulmonary infections, osteomyelitis gastroenteritis, toxic shock syndrome, and septic arthritis (Dayan et al., 2016). The pathophysiology differs significantly depending on the type of *S. aureus* infection (Taylor, 2017). The formation of an antiphagocytic capsule, and inhibition of leukocytes chemotaxis, biofilm formation, isolation of host antibodies, and survival within the cell are all mechanisms **(Figure 2.1)** for invading the host immune response (Pandey et al., 2020). Because of its potential to produce a broad scope of pathogenic agents

implicated in pathogenesis, *S. aureus* contamination of food is a significant public health problem. Enterotoxins, adhesion proteins, toxic shock syndrome toxin (TSST), exfoliative toxin (ETA, ETB), pore-forming haemolysins, ADP-ribosylating toxin, and proteases are among these factors (Pérez et al., 2020). Furthermore, staphylococcal enterotoxins (SEs) play a role in staphylococcal food-borne infection, that occurs as a result of consuming contaminated food with one or more staphylococcal enterotoxins produced by enterotoxigenic *S. aureus* strains (Le Loir et al. 2003). Staphylococcal enterotoxins are resistant to proteolytic enzymes such as trypsin and pepsin, so they remain active in the digestive tract after digestion (Le Loir, 2003).

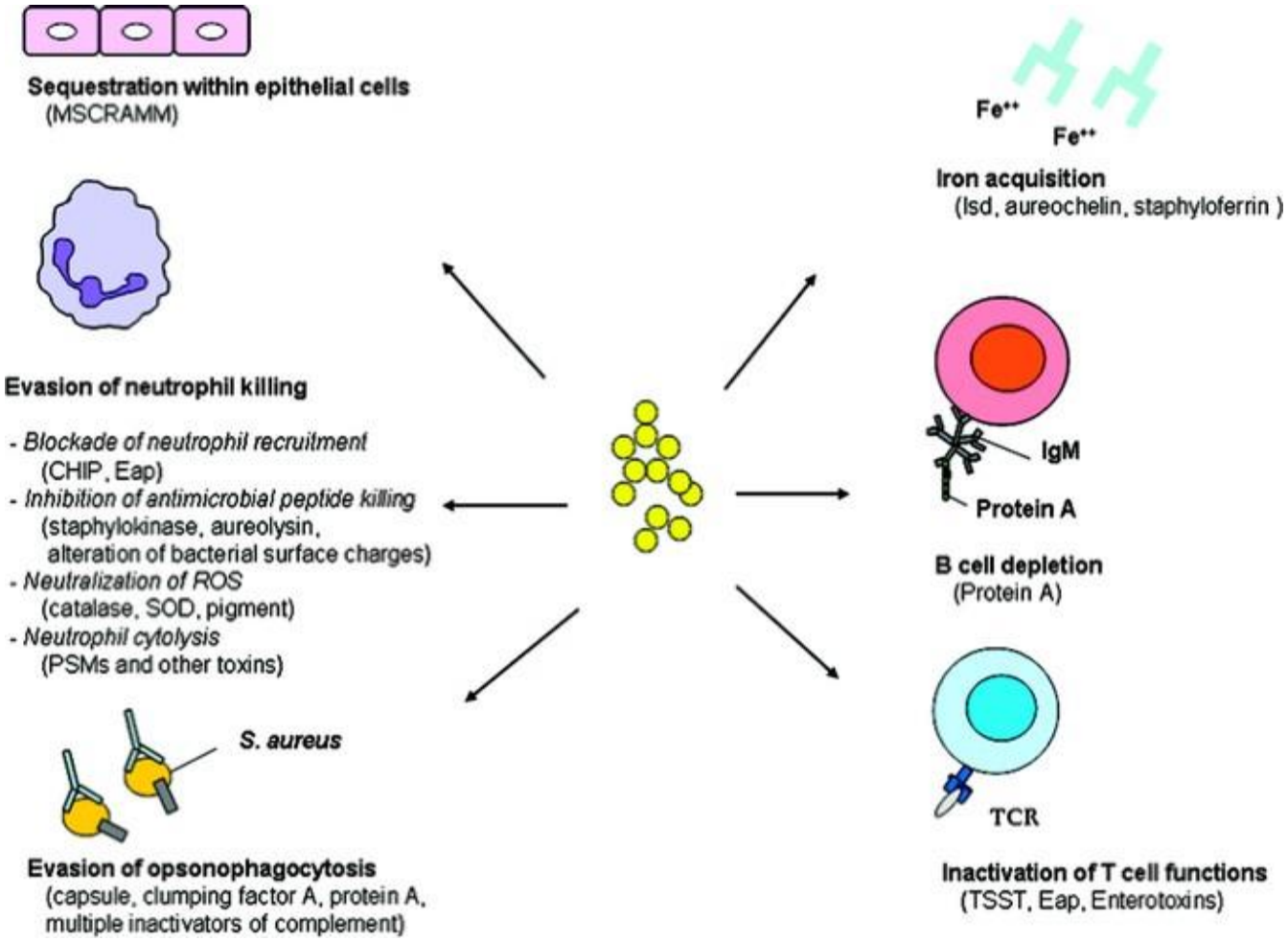

Figure 2.1: A figure showing *S. aureus* survival strategies during infection. MSCRAMM, Microbial surface components recognizing adhesive matrix molecules.

Surface proteins on *S. aureus* cells promote attachment to host proteins like laminin and fibronectin, which are extracellular matrix components. Fibronectin is a component of blood clots and is found on the surfaces of epithelial and endothelial cells (Chagnot et al., 2013). Additionally, the vast majority of strains produce fibrinogen and fibrin binding protein that influence attachment to traumatized tissue and blood clots. The receptor that promotes collagen attachment has been linked to strains that cause osteomyelitis and septic arthritis (Foster and Geoghegan, 2015). Bacterial attachment to injured cells that has been revealed to the underlying layers may also be promoted by collagen interaction (Foster et al., 1996).

2.5.5 EPIDEMIOLOGY

Pathogens that cause food poisoning have been widely implicated as widespread cases of human infections worldwide, with animal derived foods serving as crucial source (Akoachere et al., 2009). In industrialized countries, microbiological food-borne illnesses have been reported in up to 30% of the population (Ayral et al., 2015). Asymptomatic carriers of livestock-associated MRSA (LA-MRSA) include cattle, pigs, and poultry (Anjum et al., 2015). According to O'Donoghue, and Boost (2014), poor handling of raw meat can present a threat of *S. aureus* nasal colonization in food handlers. Furthermore, MRSA carriage in meat-producing animals and MRSA contamination of retail meat have raised concerns that food could be used to transmit MRSA to humans (Vanderhaeghen et al., 2010). Govender et al (2019) in one of KwaZulu-Natal (South Africa) hospital discovered that 21 percent of tuberculosis patients were nasal carriers of MRSA, and 90 percent of these cultures were found in patients who also had HIV.

2.5.6 TRANSMISSION

S. aureus grows best in a variety of environments, including food. SFD has been linked to meat and meat products, poultry and egg products, milk and dairy products (Kadariya et al., 2014). Foods implicated in SFD differ by country due to differences in consumption and food habits. Poor handling practices in the food retail industry are believed to be a contributing factor to the high number of FBD outbreaks (Zanin et al., 2017). It was discovered that after heat treatment, the numbers of *S. aureus* increase.

Pathogenic microbes are capable of adhering to the surface of retail food employees' gloves, posing a risk of cross-contamination if they are not changed on a regular basis (Kadariya et al., 2014). When gloves are used, hand washing is frequently neglected, promoting accelerated microbial growth on the hands because gloves foster bacterial growth by providing a warm, moist environment (Todd et al., 2010).

2.5.7 PREVENTION AND CONTROL

Although there's no 100% prevention for a *Staphylococcus* infection, there are several simple steps that can be taken to reduce the risk of a minor infection (Wilson,2019). Consumers must be aware of the possibility of food contamination in the kitchen while preparing food. Cooking food meticulously is crucial but trying to avert contamination and cross-contamination is the most efficient ways to avoid Staphylococcal foodborne disease (SFD). Because research findings and outbreak investigations indicate that SFD is primarily caused by improper food handling practices, food industry workers' skills and knowledge are considered necessary (Kadariya et al., 2014). Nonetheless, public health interventions to prevent *S. aureus* from pre- and post-slaughter in meat processing facilities should be developed (Thapaliya et al., 2017). Sustaining the cold chain is critical for preventing *S. aureus* growth in food products (Shih, 2016). Other preventive measures need to be implemented, including controlling raw ingredients, proper handling and processing, adequate cleaning and disinfection of food processing and preparation equipment (Marriott et al., 2006). Strict adherence to microbiological guidelines proposed by the WHO and the Food and Drug Administration of the United States, such as Hazard Analysis and Critical Control Points (HACCP), Good Manufacturing Practice (GMPs), and Good Hygienic Practices (GHPs), can aid in the prevention of *S. aureus* contamination (Fraqueza and Barreto, 2014).

2.5.8 ANTIMICROBIAL RESISTANCE

MRSA has been a major public health problem around the world. MRSA with livestock origin has been found to spread widely along the farm-to-fork food chain (Fetsch and Johler, 2018). Livestock-associated (LA) MRSA evolved independently of typical hospital- or community-associated MRSA found in humans, and it primarily belongs to the *S. aureus* clonal complex CC398 and associated with spa types t011 and t034 (Fetsch et al., 2021). However, numerous different CCs, including CC1, CC5, CC97,

and CC130, are found in livestock all over the world (Fetsch and Johler, 2018). LA-MRSA appeared among humans soon after its discovery, implying the transmission of zoonotic diseases from animals to humans (Thwala et al., 2021). As a result, it's vital to track MRSA from farm to fork and begin trying to compare isolates from farm animals' food to human isolates. Further to that, according to EFSA, antimicrobial susceptibility data on MRSA isolates could provide essential epidemiological data mostly on spread of isolates among humans and livestock, especially when researched in line with molecular typing data (Allende et al., 2021).

2.6 *SALMONELLA*

2.6.1 BACKGROUND ON *SALMONELLA SPP*.

Salmonellosis is a leading cause of bacterial enteric disease in both humans and animals. *Salmonella* causes losses in the livestock and poultry industries through death, abortion, lower milk, meat, and egg production, testing, and control programs (Gelaw et al., 2018). Salmonellosis can be caused by any food that becomes contaminated with the organism and stored at temperatures that promote bacterial growth (Podojak et al., 2010). Infections in humans typically manifest as diarrhea. Invasive NTS disease, on the other hand, is prevalent in Africa and primarily affect infants, the elderly, and people with immunocompromised systems (Delahoy et al., 2018).

2.6.2 MORPHOLOGICAL CHARACTERISTICS

Salmonella is a Gram-negative bacillus with a size of 1–3 m (Wang et al., 2020). The presence of peritrichous flagella indicates that they are motile. *Salmonella* do not produce spores or capsules, but some strains may produce fimbriae (Berne et al., 2015). They are aerobic and facultative anaerobes. They grow on a various non-selective and selective media at an optimum temperature of 37ºC and pH of 6–8. After 18–24 hours of incubation in nonselective solid media, *Salmonella spp*. produce grey, moist white colonies with a smooth convex surface (Takaya et al., 2003). Rough strains produce opaque and granular colonies with an irregular surface**.** XLD (xylose,

lysine deoxycholate agar) is another selective medium used to isolate *Salmonella spp*. There is a growth of pink colonies with black centers due to H_2S production, confirming positive culture for *Salmonella*. In addition, HS-negative *Salmonella* serotypes form red colonies with no black centers (Subhas, 2012).

2.6.3 LABORATORY DIAGNOSIS

Diagnostic tests are required to identify invasive *Salmonella* infections, detect convalescent and chronic typhoidal *Salmonella* fecal carriage, and estimate disease burden for a public health assessment (Radhakrishnan et al., 2018). For each situation, different tests and biological samples may be required. Since *Salmonella* Typhi and *Salmonella* Paratyphi A infections cannot be distinguished clinically, it may be necessary to detect both (Cheng et al., 2019). The cornerstone of diagnosis is the microbial culture. Despite the limitation of immunoglobulin and toxins identification, as well as nucleic acid amplification assay, they are useful in diagnosing *Salmonella* infections (Chidzwondo and Mutapi, 2021).

2.6.4 PATHOGENESIS

The magnitude of *Salmonella* infections in humans differs according to the serotype implicated and the human host's health status. (Cheng et al., 2019). *Salmonella* infection is more susceptible to children under the age of five, older adults, and immunocompromised patients than in healthy individuals (Wen et al., 2017). Approximately all *Salmonella* strains are harmful to humans since they can invade, replicate, and survive in living host cells, causing possibly deadly disease (Wang et al., 2020). *Salmonella* exhibits an unusual trait while invading non-phagocytic living host cell. To gain access to the host cell, they induce phagocytosis **(figure 2.2)** (Hansen-Wester et al. 2002). *Salmonella* pathogenicity islands (SPIs), gene clusters situated at the substantial chromosomal DNA region and encoding for the components necessary for invasion process, are the spectacular genetics influencing this ingenious strategy (Grassl & Finlay 2008; Babar et al., 2018).

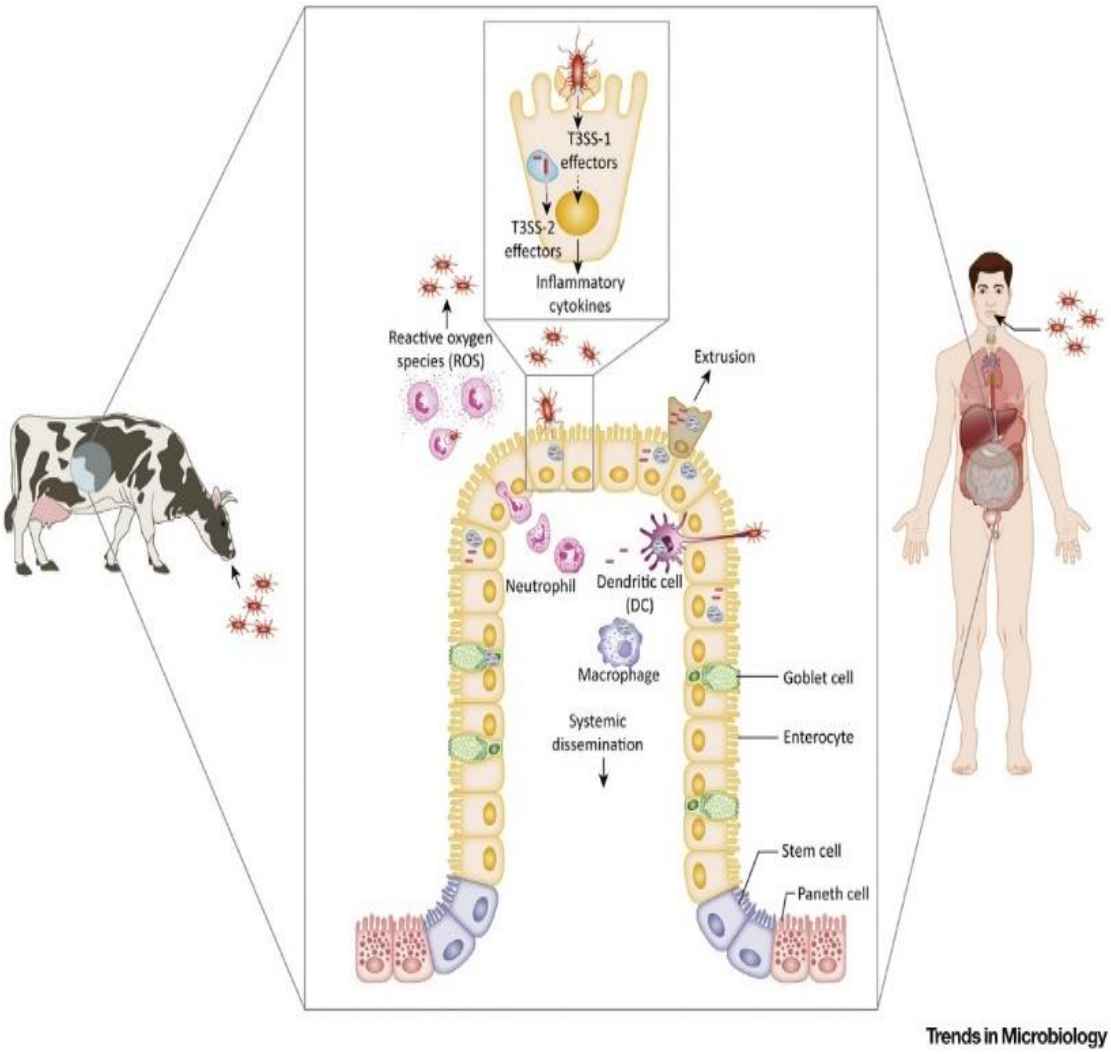

Figure 2.2: A figure showing pathogenic *salmonellae* ingested from food surviving through the gastric acid barrier and invading the mucosa of the small and large intestine and produce toxins

https://r.search.yahoo.com/_ylt=AwrExdmw5aBhjGcAl62jzbkF;_ylu=c2VjA2ZwLWF0dHJpYgRzbGsDcnVybA/RV=2/RE=1637963312/RO=11/RU=https%3a%2f%2fchungcutrungvan1.blogspot.com%2f2021%2f01%2fsalmonella-salmonella (2002).

Under normal conditions, the presence of a bacterial foreign substance would elicit an immune response in the host cell, resulting in lysosome fusion and the secretion of digesting enzymes to degrade the intracellular bacteria (Günther and Seyfert, 2018). *Salmonella*, on the other hand, uses the type III secretion system to inject other effector proteins into the vacuole, causing the compartment structure to change (de Souza Santos and Orth, 2019). The remodeled vacuole prevents lysosome fusion,

allowing bacteria to survive and replicate intracellularly within host cells (Sachdeva and Sundaramurthy, 2020). *Salmonella spp*. can be transported through the reticuloendothelial system because they can survive inside macrophages (Monack et al. 2004).

2.6.5 EPIDEMIOLOGY

Salmonella spp. infections are associated with a high fatality rate in both developing and developed (industrialized) world and high mortality, primarily in the poorest country (Naidoo et al., 2021). *Salmonella* spp. epidemics are thought to have had a significant impact on human history; even today, the impact of *Salmonella* infections on the entire communities' results in financial burden for developing and industrialized nations (Rohr et al., 2019). The National *Salmonella* Surveillance System provides insights on the prevalence of human salmonellosis in the USA, as well as patterns in particular serotypes, for the past twenty years (Harvey et al., 2017). *Salmonella* is estimated to cause 1.4 million illnesses and 600 deaths in the United States annually (Monteiro et al., 2018). Salmonellosis is also estimated to affect 3 people per 1,000 in the Netherlands each year (Pijnacker et al., 2019). Despite the fact that foodborne disease outbreaks are prevalent in South Africa, there is a scarcity of literature on the subject (Kara et al., 2015; Niehaus et al., 2011). According to recent South African surveillance reports, the two most commonly isolated *Salmonella* serotypes are Typhimurium and Enteritidis (Eng et al., 2015). The reports, however, do not differentiate between isolates collected from individual patients and those who have been linked to pandemics.

2.6.6 TRANSMISSION

Microorganisms' ability to survive and adapt to desiccation pressure aids their persistence in these foods and processing environments, which enhances pathogen transmission to humans through the food chain, resulting in frequent outbreaks of foodborne illnesses (Esbelin et al., 2018). Human and animal infections can be caused by *Salmonella* enterica subspecies Typhimurium serovar. The majority of human cases are foodborne; however, nonfoodborne *Salmonella* infection can spread through animals, contaminated water, or the environment (Simpson, 2019). *Salmonella* contamination can occur at any stage of food animal production, from healthy animal to external factors (Park et al., 2009). Cattle hides may be

compromised to *salmonella* spp. at the farm due to contact with contaminated feces, feed, or the environment, posing a threat to food safety if these organisms are transmitted to the carcass during slaughter (Madoroba et al., 2016; Rostagno, 2009). Evisceration and splitting are two procedures that may play a role to carcass contamination (Hui 2015). This is aggravated by some cattle's asymptomatic carrier status, which may present a hazard throughout the food chain (Narvaez-Bravo et al., 2013). As a result, some sources of *salmonella* contamination are available well before the animals are presented for slaughter; therefore, strict hygienic processes during slaughtering are critical in order to minimize the possibility of meat contamination (Madoroba et al., 2016).

2.6.7 PREVENTION AND CONTROL

In Latin America, the incidence of *Salmonella* infections has decreased in tandem due to implementation of hygiene precautions (Crump et al., 2011). The use of typhoid vaccine together with access to safe water and food, and proper sanitation are currently primary preventive measures for enteric fever (O'Reilly et al., 2020). The primary goal of eliminating potential typhoid *Salmonella* and NTS transmission routes is to ensure the safety of drinking water. This critical metric has been successfully implemented in industrialized countries such as Europe and the United States, and not in underdeveloped and developing countries (Clasen et al., 2007).

Beside water, *Salmonella* spp. is also found in a wide range of foods, primarily meat products. To eliminate bacterial contamination of food, proper food handling and cooking are proposed measures (Ncube et al., 2020). Due of its efficacy in preventing the risk of food contamination, food irradiation has been highly promoted in many countries. Given the risk of radioactivity, food irradiation technology, which has been authorized by several public health agencies, such WHO and CDC, however it is only used partly in some areas of Europe and the United States (Eng et al., 2015).

Inactivated parenteral and oral live attenuated vaccines are the two different types of vaccines widely prescribed to prevent enteric fever infections (Milligan et al., 2018). Such certified vaccines are only for infants and are ineffective in preventing *S.*

paratyphi and NTS infections (Lin et al., 2001). Limiting the use of antibiotics in livestock and their feed is one effective NTS measure (Angulo et al., 2006).

2.6.8 ANTIMICROBIAL RESISTANCE

The first case of *Salmonella* resistance to a single antimicrobial drug, chloramphenicol, was recognized in the early 1960s (Montville & Matthews, 2008). The number of *Salmonella* strains isolated with resistance to one or more antibiotics has increased in various countries, including the United States, the United Kingdom, South Africa, and Saudi Arabia (Parry, 2003). Traditional first-line treatments for *Salmonella* infections include the use of antibiotics such as ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. *Salmonella spp*. resistant to all these agents is known as multi-drug resistant (MDR) (Wani et al., 2019). Resistance to extended-spectrum cephalosporins among Enterobacteriaceae members has become an increasing worldwide problem since the emergence of extended-spectrum beta-lactamases (ESBLs) and AmpC-type beta-lactamases (AmpC-type beta-lactamases) (Saravanan et al., 2018). Even though reports of ESBLs associated with *Salmonella spp*. are uncommon in contrast to other Enterobacteriaceae family species, the number of incidents in this organism has indeed been rising in recent years (Bush and Bradford,2020).

Salmonella have been found to express various ESBL types, including TEM, SHV, PER, OXA, and CTX-M enzymes (Ghiglione et al., 2021). *Salmonella* strains that produce plasmid-mediated AmpC-type beta-lactamases have also been identified (Saravanan et al., 2018). According to research, *Salmonella* serotypes with the MDR phenotype can produce a variety of hybrid plasmids (Mangat et al., 2019). The significant proportion of the gene cassettes found in these plasmids are resistance genes that confer the serotypes' antimicrobial resistance against traditional antibiotics. (Guerra et al., 2002).

2.7 E. COLI

2.7.1 BACKGRROUND IN *E. COLI SPP*.

Escherichia coli is one of the most prevalent bacteria found to degrade meat quality. Strains of *E. coli* have been identified as significant zoonotic foodborne pathogens

(Smith and Fratamico, 2018). They've evolved into a substantial public health threat known for their ability to cause numerous sporadic cases and foodborne disease outbreaks in humans. Dr. Theodor Escherich discovered Bacterium coli commune in 1885 while researching bacteria in enteritis-infected infants' stools (Bell, 2002). Since its discovery, it has been recognized as a major causative agent of food-borne illness. The majority of these flagellated gut florae are found in the colon (Walters and Sperandio, 2006).

E. coli is a commensal organism of the human and warm-blooded animal digestive tracts (Rostagno, 2009). Its presence in raw foods is thought to indicate direct or indirect fecal contamination (Juneja et al., 2015). As a result, it acts as an indicator for the existence of enteric pathogens in food and water. The presence of *E. coli* may indicator the source of microbial contamination and may contaminate foods in a variety of ways (Moller, 2016*).* Furthermore, some *E. coli* strains are harmful to humans and animals. Enteric and diarrheal diseases, urinary tract infections, sepsis, and meningitis are all caused by these bacteria in humans. They are also known to cause disease if the immune system is weakened, or they can cause diseases as a result of environmental exposure (Haimanol, 2010).

2.7.2 MORPHOLOGICAL CHARACTERISTICS

Escherichia coli is a member of the genus Escherichia, which is in the tribe Escherichiae, under the family Enterobacteriaceae (Janda and Abbott, 2021). The huge amount of *E. coli* serotypes does not cause disease in humans or other warm-blooded animals. There are, however, some serotypes that, if present in the body, can cause health problems (Jang et al., 2017). Therefore, distinguishing between different *E. coli* serotypes is clinically important. Antibodies define bacterial serotypes in patients' or animals' serum by identifying the specific type of antigen introduced by the bacteria (Gomes et al., 2016). Pathogenic strains are distinguished from commensal strains based on virulence characteristics, pathogenic mechanisms, clinical syndromes, and serotyping of distinct "O" (somatic), "H" (flagella), and "K" (capsule) antigens (Gebisa et al., 2019). Enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), diffuse

adhering *E. coli* (DAEC), and verocytotoxin producing *E. coli* (VTEC), also known as Shiga toxin-producing *E. coli* (STEC) (Bell, 2002).

2.7.3 LABORATORY DIAGNOSIS

Samples can be sent to the lab for the initial rapid and highly sensitive detection of pathogenic *E. coli* (pathotyping) using an enrichment multiplex PCR tailored to the specific animal species, most notably pigs, cattle, dogs and cats, rabbits, and humans (Jaros, 2014). Positive samples could be further analyzed for pathogenic *E. coli* isolation and detailed overview (virotyping) for a broad range of up to 100 or more virulence genes using multiplex PCR and/or colony hybridization, enabling isolates' pathogenic potential to be confirmed (Zhao et al., 2001). In order to assist in the characterization of pathogenic isolates, O serotyping and H typing may be performed. The isolates' antibiotic resistance profile can also be determined. Furthermore, molecular characterization by Pulse-field gel electrophoresis (pulsetyping) can be used to identify emerging virotypes and clones, as well as to track the epidemiology of these and other established pathogenic *E. coli (*Caprioli et al., 2005).

2.7.4 PATHOGENESIS

Cattle and other ruminants consume potentially pathogenic bacteria, which colonize the gastrointestinal tract but cause no disease in these animals (Fairbrother and Nadeau, 2006). These bacteria primarily colonize the large intestine in humans. Bacteria produce their own receptor, which would be injected into the host epithelial cell **(Figure 2.3)** using a bacterial apparatus resembling a syringe (Gonzalez et al., 2008).

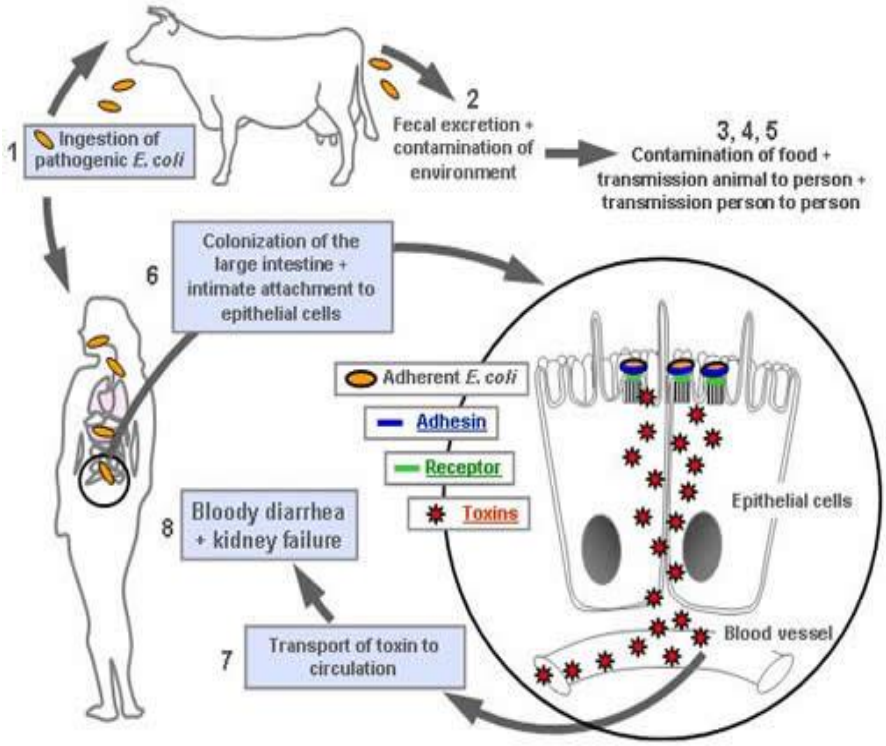

Figure 2.3: "The pathogenicity of *E. coli* O157:H7 is primarily attributed to the microorganism's ability to produce shiga toxins (stx1 and stx2), as well as the presence of the intimin (eae) gene, which is required for the organism's adherence to the intestinal epithelium (attaching and effacing mechanism)" (Vallance and Finlay, 2000).

Bacterial adhesins then facilitate the microbes extremely intimate attachment to the cell receptors, and bacterial signals enhance effacement of the microvilli, or brush border, and cytoskeleton reorganization (Hurwitz et al., 2015). The adherent bacteria produce a toxin, which is transported to the circulatory system via the epithelial cells (Tran et al., 2014). This toxin affects blood vessel endothelial cells, as a result of which there is non-bloody to bloody diarrhea and abdominal cramps (Gonzalez et al., 2008). A complication of hemolytic uremic syndrome may result in acute kidney failure, particularly in children (Scheiring et al., 2008).

Hemolysin was first described as virulence factor of p0157 (Scott, 2000). Hemolysins (hly) are a virulence factor because they can cause extraintestinal lesions and have the capacity to impact numerous cells, including lymphocytes, granulocytes, erythrocytes, and renal cells, causing drastic effects (Schwidder et al., 2019). The ability to produce enterohemolysin is considered to be a major virulence factor for

enterohemorrhagic Escherichia coli (EHEC) and is commonly linked to severe human diseases such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Schwidder et al., 2019). The responsible toxin, also known as EHEC-hemolysin (EHEC-Hly, syn. Ehx), causes incomplete turbid lysis zones on blood agar plates containing defibrinated sheep erythrocytes. Aside from the expression of Shiga toxins (Stx) and the locus of enterocyte effacement (LEE), EHEC-Hly is a widely used marker for detecting potentially pathogenic *E. coli* strains (Vallance and Finlay, 2000).

The primary distinction among alpha beta and gamma hemolysis is that alpha hemolysis results in the partial destruction of red blood cells, whereas beta hemolysis results in the complete destruction of red blood cells in the blood (Dhaliwal et al., 2004). There is no breakdown of red blood cells in gamma hemolysis. Haemoglobin is an iron containing metalloprotein found in red blood cells and serves as the primary molecule for oxygen transportation (Glenn and Armstrong, 2019). When red blood cells are destroyed, haemoglobin is released from the cells into the blood plasma. This is referred to as hemolysis (Thiagarajan et al., 2021). The decomposition of red blood cells is catalyzed by a bacterial enzyme called hemolysin. (Sohail, 2021).

2.7.5 EPIDEMIOLOGY

For the first time, *E. coli* O157:H7 was attributed to human outbreaks in 1982, when two outbreaks of gastrointestinal infection caused by undercooked meat were revealed (Saxena et al., 2015). Since then, this bacterium has been detected causing outbreaks globally. Most of the reports on this bacterium are associated with community-acquired infections and its transmission by foodborne and waterborne routes (Munns et al*.,* 2015). In the last two decades, Escherichia coli 0157:H7 has been one of the most significant foodborne pathogens, causing major human population losses (Abebe et al., 2018). Every year, more than 75,000 foodborne illness cases are linked to *E. coli* O157:H7 (Perna et al., 2001). Several studies in South Africa had been conducted to determine the ability of shiga toxigenic *E. coli* to which cause diarrhea. However, there are insufficient reports of *E. coli* O157:H7 food contamination (Lupindua, 2018).

2.7.6 SOURCES AND TRANSMISSION

Cattle are the most essential DEC reservoirs, with carcass contamination occurring primarily during the hide removal and evisceration processes (Bonardi et al., 2001). Foods of bovine origin, particularly ground beef products, have been recognized as main transmission vehicles in the most of outbreaks (Abebe et al., 2020*). E. coli* O157:H7 is mainly transmitted to humans through consuming contaminated food such as raw or undercooked ground meat products (Kiranmayi et al., 2010). There has been evidence of an asymptomatic carrier state in which individuals exhibit no clinical symptoms of disease but can spread to others (Kiranmayi et al., 2010). STEC excretion lasts about a week or less in adults, but it can last longer in children (Haus-Cheymol et al., 2006). Visiting farms and other places where the general public may directly interact with farm animals has been recognized as a primary risk factor for STEC infection (Caprioli et al., 2005).

2.7.7 PREVENTION AND CONTROL

Infection prevention is necessarily required at all stages of the food chain, from agricultural manufacturing, processing to food preparation in commercial restaurants and home kitchens. (Uçar et al., 2016). Numerous minced beef disease alleviation strategies such as, pre-slaughter screening to minimize the introduction of massive quantities of pathogenic organisms into the slaughterhouse surroundings may decrease the number of disease cases in the industry (Lejeune and Wetzel, 2007). Although good hygienic slaughtering practices minimize feces contamination of carcasses, they do not guarantee the absence of STEC in products (Ramoneda et al., 2013).

Knowledge in good hygiene handling of foods for workers at farms, abattoirs and those involved in food production is crucial to keep microbiological contamination to a minimal level (Schlundt et al., 2004). The only way to eliminate STEC from food is by using a bactericidal care like heating or irradiation (Das et al., 2009). Preventive measures for *E. coli* O157:H7 infection in households are the same to those proposed for other foodborne diseases. Basic good food hygiene practices, as described by WHO "Five keys to safer food," can inhibit the transmission of pathogens

responsible for several foodborne diseases and protect against foodborne diseases caused by STEC (Schlundt et al., 2004).

2.7.8 ANTIMICROBIAL RESISTANCE

One of the most serious issues associated with *E. coli* O157:H7 infection is the risk of treating such patients with antibiotics (Wong et al., 2000; Okoli, 2005). Wong et al. (2005) warn that treating *E. coli* O157:H7 infections may result in the release of Shiga toxins into infected individuals' bloodstreams. The release of such toxins is thought to affect the kidneys, resulting in a condition known as hemolytic uremic syndrome (Wong et al., 2000). As a result, the treatment approach to be used in the case of *E. coli* O157:H7 infections poses a significant challenge.

2.8 SHIGELLA

2.8.1. BACKGROUND ON *SHIGELLA* SPP.

Shigella species have been linked to foodborne illnesses in humans, including shigellosis, which is characterized by epithelial destruction in the colon and normally results in an inflammatory response (Makabanyane et al., 2015). The above microorganism is among the significant foodborne pathogens known today, and they are commonly isolated from infected patients (Acheson and Hohmann, 2001). Shigellosis is an acute intestinal infection characterized by severe abdominal cramps, fever, and blood and mucus in the stools (Kotloff et al., 2018). In addition, several patients may experience neurologic symptoms including severe headache, lethargy, confusion, and convulsions (Lan and Reeves, 2002). The disease is usually self-limiting, but it can be fatal if patients are immunocompromised or do not have access to adequate medical care.

2.8.2 MORPHOLOGICAL CHARACTERISTICS

Shigella species are Gram-negative, non-spore-forming, non-motile, non-lactose fermenting, facultative anaerobic bacteria that reside in the gastrointestinal tracts of humans and warm-blooded animals (Makabanyane et al., 2015). *Shigella* is classified into four groups, notably *Shigella* boydii, *Shigella dysenteriae*, *Shigella flexneri*, and *Shigella sonnei*. However, these bacteria are so similar to each other and to *E. coli* strains that they all belong to the same species, *E. coli* (Lan and Reeves, 2002). *S.*

dysenteriae, S. flexineri, and *S. boydii* are all physiologically comparable, but metabolic assays can distinguish *S. sonnei* (Elmanama and Abdelateef, 2012).

2.8.3 LABORATORY DIAGNOSIS

Shigella spp. are traditionally diagnosed through culture isolation, standard biochemical testing, and serotyping via serum agglutination (Hosangadi et al., 2019). Antibiotics are recommended for treatment of moderate to severe infections. Nevertheless, it is critical to remember *Shigella's* growing resistance to antibiotics. Blood cultures should be obtained in patients with acute febrile gastroenteritis to detect shigellemia (Lima et al., 2015).

Although clinical signs may raise the possibility of shigellosis, diagnosis is dependent on *Shigella* isolation and identification from feces (Puzari et al., 2018). *Shigella* isolation in the clinical laboratory typically begins with streaking for isolation on differential/selective media followed by aerobic incubation to prevent the growth of the normal anaerobic flora (Niyogi, 2005).

DNA probes that hybridize with widely known virulence plasmid genes or DNA primers that enhance plasmid genes by polymerase chain reaction (PCR) are used to identify EIEC and *Shigella* species in a sensitive and rapid manner (Gómez-Duarte et al., 2009). An enzyme-linked immunosorbent assay (ELISA) with antiserum or monoclonal antibody recognizing Ipa proteins could be used to screen stools for Enteroinvasive pathogens (Pasetti et al., 2006).

2.8.4 PATHOGENESIS

Shigella is an extremely contagious microorganism because it can be caused by as few as 10–100 bacteria (Sur et al., 2004). *Shigella* species enter the host cell cytoplasm **(Figure 2.4)** and spread intercellularly to infect the colonic and rectal epithelium of primates and humans, causing the acute mucosal inflammation characteristic of shigellosis (Kotloff et al., 2018). This behavior, combined with the host's subsequent inflammatory response, disrupts the colonic epithelial layer, resulting in shigellosis clinical symptoms (Peeng et al., 2009).

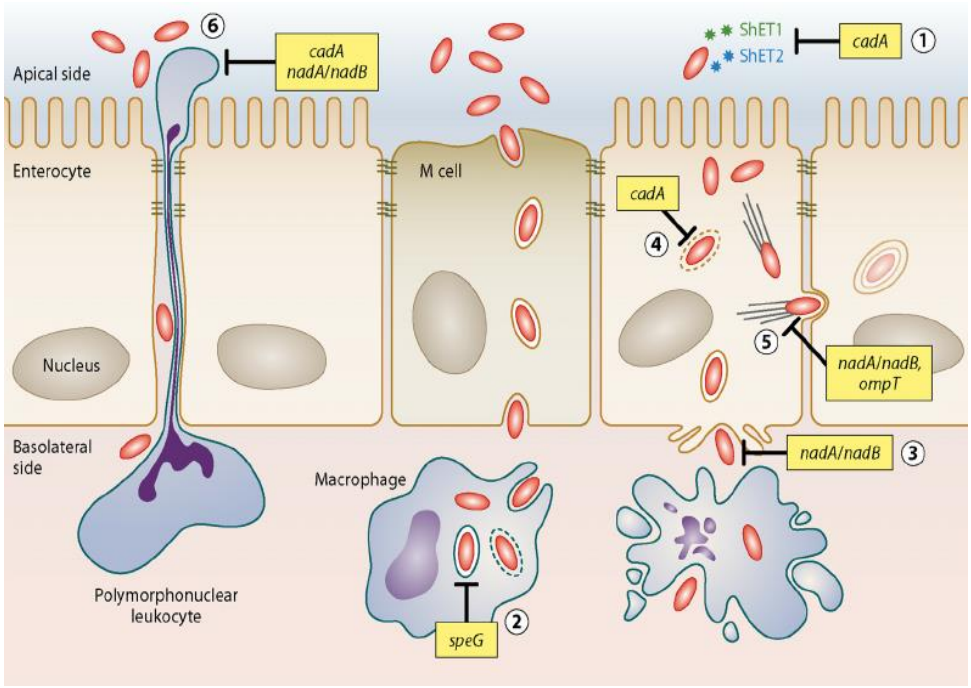

Figure 2.4: A picture showing an inhibition of pathogenesis in *shigella* obtained from an article titled, Antivirulence Genes: Insights into Pathogen Evolution through Gene Loss by Bliven and Maurelli (2012).

Two loci dominate pathogenesis on the virulence plasmid (VP): the mxi-spa locus, which encodes a type III secretion system (TTSS), and the ipa locus, which encodes invasion plasmid antigens (Wu, 2011). Key regulatory genes are also found on the plasmid, and additional regulatory genes can be found on the chromosome. *Shigella* type III effectors stimulate complex signaling pathways that cause localized membrane ruffling, resulting in *Shigella* invasion (Hansen-Wester and Hensel, 2001).

2.8.5 EPIDEMIOLOGY

Shigella-related foodborne infection is a common international health threat. In the vast majority of cases in which foodborne infections and outbreaks occur in humans, meat and meat products are outlined as the contamination sources (Wu et al., 2000). Shigellosis is common, and the majority of cases are likely the result of person-to-person transmission. Nevertheless, foodborne transmission is estimated to cause approximately 130,000 cases in the United States annually (Nygren and Bowen, 2013). Despite the scarcity of surveillance data in the developing world, occurrences of foodborne shigellosis have been documented in several studies in India, South

Africa, and other developed countries as a result of food imported from less-developed countries (Nygren and Bowen, 2013).

Bacillary dysentery, which is prevalent across the world, is one of the infections caused by *Shigella* species. It is responsible for at least 165 million cases per year, with 163 million occurring in developing countries and 1.5 million occurring in developed countries (Cheng et al., 2005). In addition, in South Africa, more than million Journals of Food and Nutrition Research cases of both invasive and non-invasive shigellosis have been noted. The prevalence was highest among children under the age of five (Makabanyane et al., 2015).

2.8.6 TRANSMISSION

Shigella species are most commonly transmitted to humans through consuming contaminated food and water. Unsatisfactory hygiene by infected food handlers, improper food storage temperatures, and poor sanitation at food production facilities are all aspects that commonly contribute to transmission (Michaels et al., 2004). *Shigella* species are mainly spread via the fecal-oral route (Nygren and Bowen, 2013). A common housefly called Musca domestica acts as a vector for pathogen transmission (Khamesipour et al., 2018).

2.8.7 PREVENTION AND CONTROL

Shigella and other several enteric pathogens will be less likely to spread through food if policies and procedures ensuring proper sanitation and hygiene during food harvesting, production, distribution, and preparation are implemented (Nygren and Bowen, 2013). Given *Shigella*'s clinical and epidemiological implications, proper precautions must be taken to prevent the pathogen's transmission. Personal hygiene and sanitation must be maintained. High-quality disease surveillance systems are required to assess the effectiveness of any control measure (Michaels et al., 2004). Efforts to strengthen surveillance capacity in developing countries and to assist foodborne disease surveillance and epidemiology programs in developed countries will increase knowledge of *Shigella* foodborne transmission and aid prevention efforts (Schlundt, 2002). Antibiotic resistance in bacteria should be made more widely known, and effective control measures should be developed. A *Shigella* vaccine that is safe, cheap, and effective has yet to be developed, which is critical because it would be a

long-term strategy against shigellosis (Puzari et al., 2018; Mani et al., 2016). The only licensed vaccine is a bivalent *S. flexneri* 2a-*S. sonnei* vaccine, which is used in China (Taneja and Mewara, 2016).

2.8.8 ANTIMICROBIAL RESISTANCE

Antibiotics must be used with caution to control infections. One such issue with antibiotic use is the use of antibiotics in diseases where their use will not improve the patient's condition (Aly and Albutti, A., 2014). Once more, a lack of access to appropriate antibiotics has the potential to be lethal to humans. Proper regulations and oversight are required (Sartelli et al., 2020). It is critical to report antibiotic susceptibility after analysis on a regular basis in order to guide antibiotic treatment (Puzari et al., 2018).

2.9 ANTIBIOTICS SUPPLEMENTATION IN CATTLE BREEDING

According to South African studies, a substantial percentage of the population depend heavily on pork and beef meat as protein resource (Cawthorn et al., 2013; Delport et al., 2017) which, if contaminated, could expose them to infection. Researchers have also demonstrated that food infections are caused by antibiotic-resistant bacteria in foods, making it an excellent vehicle for disease transmission (Capita and Alonso-calleja, 2013). In the United States, a wide range of antimicrobial drugs (AMDs) are approved for the treatment of sick cattle or for metaphylactic use, including florfenicol, cephalosporins, macrolides, fluoroquinolones, penicillin, sulfonamides, and tetracycline compounds (Morley et al., 2011). Other medications, such as tylosin and tetracycline compounds, are frequently used to minimize the incidence and severity of liver abscessation. Ionophores such as monensin are usually used to enhance growth efficiency (Capita and Alonso-calleja, 2013), as feeding such compounds changes the ecology of microflora in the rumen, promoting more efficient metabolism. According to studies, the long-term antimicrobial agent uses and misuse in agriculture, livestock farming, and the treatment of human diseases has resulted in the rapid resistance of several bacteria to antibiotics of various classes (Landers et al., 2012; Wellington et al., 2013; Shea, 2003).

2.10 ANTIMICROBIAL RESISTANCE

Pathogens isolated from food are increasingly showing signs of resistance (Schroeder et al., 2002). As a result, antibiotic-resistant bacteria in food pose a significant risk to public health because the antibiotic resistance determinants could be transmitted to other bacteria of human clinical importance (Serwecinska, 2020). Furthermore, the transfer of these resistant bacteria to humans has serious public health consequences, as it increases the number of food-borne illnesses and possible treatment failures (Christou et al., 2017). Food of animal origin can become contaminated on the farm, which can be exacerbated if the food is not handled correctly during slaughter and processing, allowing pathogens to grow rapidly (Tanih et al., 2015). The handling conditions of these foods generate concern about their microbiological quality.

2.11. ANTIBIOTIC SUSCEPTIBILITY PROFILE TESTING OF PATHOGENIC BACTERIA FOUND IN GROUND BEEF.

2.11.1ANTIBIOTIC SUSCEPTIBILITY TESTS METHODS

Antimicrobial susceptibility testing in clinical microbiology is critical for confirming susceptibility to empirical antimicrobial agents or detecting resistance in specific bacterial isolates (Behera et al., 2019). Because resistance mechanisms have not been observed, empirical therapy remains effective for some bacterial pathogens (Sjoberg et al., 2007). Commonly used antibiotic susceptibility testing methods includes Broth Dilution test, disk diffusion test, Automated vitek system

2.11.2 BROTH DILUTION TESTS.

The macro-broth tube-dilution method was one of the first antimicrobial susceptibility testing methods (Ericsson and Sherris, 1971). Preparing two-fold dilutions of 63 antibiotics in a liquid growth medium dispensed in test tubes was part of this procedure (Ericsson and Sherris, 1971; Jorgensen and Turnidge, 2007). After that, the antibiotic-containing tubes are inoculated with a standardized bacterial suspension containing 1– CFU/mL. Following 24 hours of incubation at 37°C, the tubes are then analyzed for visible bacterial growth as measured by turbidity.

2.11.3 DISK DIFFUSION TEST.

The disk diffusion susceptibility method is practical and straightforward, and it has been well standardized. According to Wheat (2001), the test is carried out by

applying a bacterial suspension to the surface of a Mueller-Hinton agar containing plate. On the inoculated agar surface, up to 12 commercially prepared, fixed concentration paper antibiotic disks are positioned. Before recording results, plates are incubated at 35°C for 16–24 hours. Each antibiotic disk's growth inhibition zones are measured to the nearest millimetre. The zone's diameter is proportional to the isolate's susceptibility and the rate of drug diffusion through the agar medium. The zone diameters result of each drug are interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS, 2009).

2.11.4 AUTOMATED INSTRUMENT SYSTEMS

Because sensitive optical detection systems detect subtle changes in bacterial growth, the use of instrumentation can standardize endpoint reading and mostly produce susceptibility test results in a shorter timeframe than manual readings (Benkova et al., 2020). The FDA has currently approved four automated instruments for use in the United States. Three of these can produce rapid susceptibility test results (3.5–16 h), whereas the fourth is an overnight system (Clinical and Laboratory Standards Institute, 2009).

2.12 MOLECULAR CHARACTERIZATION: METHODS USED TO CHARACTERIZE PATHOGENIC BACTERIA DETECTED IN GROUND BEEF

2.12.1 MOLECULAR METHODS USED TO CHARACTERIZE MICROORGANISMS

Molecular biology methods have advanced, allowing for considerable speed increases and the identification of microorganisms based on particular genetic makeup encoded in genomic DNA (Horokova et al., 2008). Diagnosis is advised in occasion of prolonged diarrhoea, children with drastic diarrhoea who are non - responsive to treatment, immunocompromised patients with acute diarrhoea, and gastroenteritis outbreaks (Vidal et al., 2005).

Molecular technique has been extensively used in foodborne pathogen vigilance, mutation, and other genetic analysis to improve awareness of the fundamental origin of foodborne pathogens, the cause of infection, and genetic diversity (Aurora et al.,

2009). These techniques have the benefit of being faster, less arduous, more responsive, definite, and productive than traditional methods (Magistrado et al., 2001: Keramas et al., 2004). However, specific food elements, including fats, lipids, salts, enrichment media, or DNA extraction solution could reduce the responsiveness of PCR-based methods (Rossen et al., 1992; Wilson, 1997; Adzitey et al., 2013).

2.12.2 POLYMERASE CHAIN REACTION (PCR)

PCR is a simple and inexpensive molecular technique of in-situ DNA replication that allows for the exponential amplification of target DNA in the presence of synthetic oligonucleotide primers and a thermostable DNA polymerase (Wang et al., 200). This assay has long been used to detect, identify, and differentiate foodborne pathogens. PCR combines the characteristics of complementary nucleic acid hybridization with nucleic acid replication, which is repeated over and over (Elijah et al., 2014). DNA template, deoxyribonucleoside triphosphates (dNTPs), PCR buffer, primers (forward and reverse), and Taq polymerase are all components of PCR (Lapa et al., 2018). Kary Mullis invented PCR in 1983. PCR is now an important and frequently required technique in medical and biological research labs for a variety of applications (Joshi and Deshpande, 2010). The presence of PCR inhibitory substances, such as humic substances, is the major impediment to using PCR to detect and identify pathogenic organisms in clinical or environmental water samples (Theron et al., 2001; Omar and Bernard, 2014).

PCR also helps to avoid circumstances in which phenotypic characteristics are ambiguous and incorrectly interpreted (Adzitey and Corry, 2011). Nevertheless, because amplification can be obtained from both viable and non-viable cells, some PCRs may not be appropriate for processed or certain foods (Sails et al., 1998; Wang et al., 2000). Conventional PCR (Qualitative), multiplex PCR, nested PCR, reverse-transcriptase PCR (RT-PCR), real-time PCR, and ERIC-PCR are examples of PCR techniques (genotyping method)

2.12.3 REAL-TIME PCR

Real-time PCR is a polymerase chain reaction procedure that amplifies and quantifies target DNA simultaneously within a reaction (Purcell et al., 2013). To achieve more accurate signals, real-time PCR employs a specific primer set, one or two probes, and

fluorescent dye (Rensen et al., 2006; Dhanasekaran et al., 2010; Shi et al., 2010). Real-time PCR detects nucleic acids by increasing the intensity of a fluorescent signals produced by an intercalating dye or by the breakdown of a dye-labeled probe during amplification of a target sequence (Purcell et al., 2013). Real-time PCR is particularly useful in large commercial laboratories that process a large number of samples of the same type. Bustin et al (2009) propose guidelines for improving the reliability of quantitative PCR data and allowing others to replicate the work. Real-time PCR can quantify gene expression and confirm gene expression differences (Frye et al., 2012).

The ability to measure DNA concentrations over a wide range, sensitivity, the ability to process multiple samples simultaneously, and the ability to provide immediate information are all significant advantages of real-time PCR (Valasek and Repa, 2005; Wong and Medrano, 2005). The machines are more expensive than traditional PCR machines, which is a disadvantage (Shi et al., 2010). When compared to standard PCR, real-time PCR reduces detection time and can evaluate the relative or absolute bacterial load in various samples (Heid et al., 1996; Shi et al., 2010). Moreover, no post-PCR processing of products is performed, resulting in high throughput and a lower risk of amplicon contamination by laboratory environments (Heid et al., 1996; Wong and Medrano 2005; Shi et al., 2010).

2.12.4 MULTIPLEX PCR

Multiplex PCR is a type of a technique that detects multiple pathogens by employing multiple primer sets, each of which targets a different pathogen (Markoulatos et al., 2002). This enables the analysis of multiple targets in a single sample at the same time. Although multiplex PCR reduces costs, limits sample volume, and allows for the rapid detection of multiple bacteria species and strains, primer design is critical in the development of multiplex PCR assay (Shi et al., 2010). All primers must have a similar annealing temperature, the amplicons must be significantly different in size, and multiple primers may interfere with one another during the amplification process (Elnifro et al., 2000; Shi et al., 2010). It reduces PCR reagent consumption while also imposing restrictions on used primers (Naze et al., 2009).

Enough laboratory controls are required in the m-PCR to supervise PCR inhibition. The majority of published research findings mention using the 16s rRNA gene as an internal control to check for false-negative results in m-PCR (Sabat et al., 2000; Grape et al., 2007). However, because 16s rRNA is amplified from *E. coli* DNA, these are insufficient to specifically monitor false-negative results for *E. coli*. It would be impossible to tell whether a lack of PCR amplification of 16s rRNA is due to PCR inhibition in the sample or to the absence of *E. coli* in the sample. According to Janssen et al (2005), the high level of PCR sensitivity increases the risk of false-positive and false-negative results.

2.12.5 SINGLE POLYMERASE CHAIN REACTION

This is a polymerase chain reaction (PCR) in which a single primer set (targeting a specific gene) is used to detect an organism (Joshi and Deshpande, 2010). The primer set can be tailored to a specific species and can detect the target organism even in the presence of other organisms. This type of PCR can be used to detect or identify bacteria in a sample (food, water, clinical, or environmental) quickly and accurately, with or without pre-enrichment. On the other hand, direct detection of foodborne pathogens by PCR assays in the environment or in turbid samples, can detect DNA in dead cells and produce false-negative results (Josefsen et al., 2004; Abulreesh et al., 2006). To address this issue, enrichment prior to PCR detection and the use of fluorescence in situ hybridization (FISH) techniques have been proposed (Lehtola et al., 2005; Abulreesh et al., 2006). Single-step PCR can also be used to confirm bacteria isolates obtained directly from agar plates.

In recent years, PCR with universal or specific primers has been used to first amplify the 16S rRNA genes of bacteria before sequencing to aid in the identification of unknown or novel bacteria species (Magistrado et al., 2001). The benefits of single PCR include more precise, sensitive, and rapid detection of single bacteria or genes (Wang et al., 2000). One of the disadvantages is that it does not produce isolates that can be further characterized. Food components can interfere with PCR performance and produce misleading results. For improved performance, PCR conditions must be optimized (Abulreesh et al., 2006).

2.12.6 NESTED PCR

Nested PCR uses two sets of primers in two consecutive polymerase chain reaction runs, with the first PCR product used as a primer for the second PCR (Olsvik et al., 1991). When compared to conventional PCR, nested PCR improves the sensitivity and specificity of detecting foodborne pathogens. Nevertheless, because the reaction vessel is opened to allow the second primer set to be added, the contamination level is likely to be high (Adzitey et al., 2013).

2.12.7 REVERSE-TRANSCRIPTION PCR

Instead of DNA, RNA is used as the initial template in reverse-transcription PCR. Reverse transcriptase is used to convert the target RNA into its DNA complement (cDNA) and amplify it using polymerase chain reaction (PCR) (Sharma, 2006). Reverse-transcription PCR detects only viable cells of pathogens; however, RNA is unstable, necessitating considerable skill in handling and quantification for pathogen detection (Adzitey et al., 2013; Sharma, 2006; Shi et al., 2010). Only viable pathogen cells can be detected using this technique (Shi et al., 2010).

CHAPTER 3

MATERIALS AND METHOD

3.1 STUDY AREA

This study was conducted in Thohoyandou. Thohoyandou “head of elephant” is a small town located within the Vhembe District (South of Venda) on the main road connecting Louis Trichardt and Kruger National Park in Limpopo Province of South Africa with a population estimate of about 618, 462 people and an estimated annual population growth rate of 0.62 % (Mafune et al., 2016). It is a commercial, legislative, and administrative center for surrounding villages among the communities. University of Venda also situated in these town, in proximity (about 2km) to a shopping complex with established retailers. Thohoyandou has more than 20 established retailers that daily supply different meat accessible to all residents, students, and tourists.

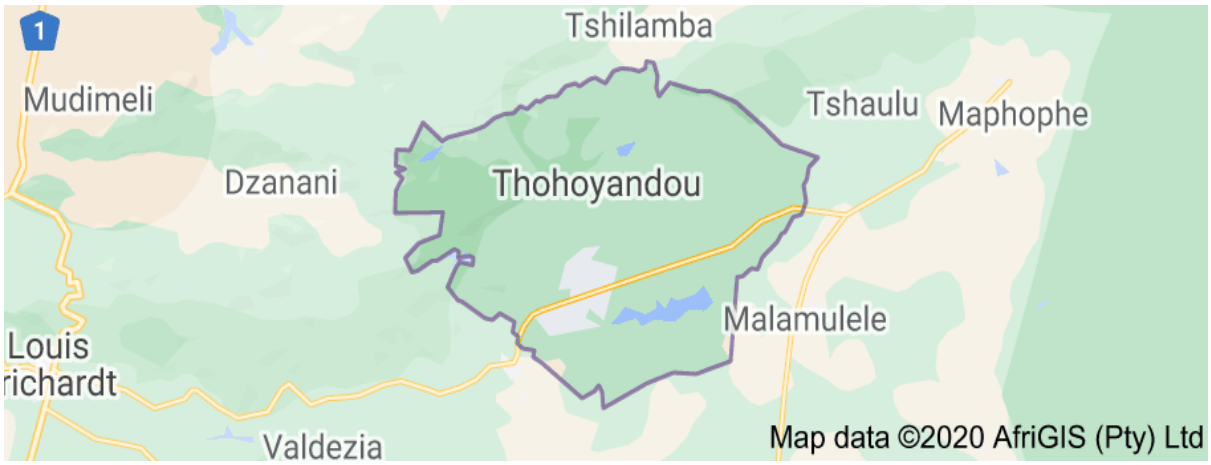

Figure 3.1: A map showing Thohoyandou Town in Limpopo province of South Africa obtained from Map data ©2020 AfrisGIS (22º 57’ S, 30º 29’ E).

3.2 PERMISSION FOR THE STUDY

The study protocol was approved by the Research and Ethics Committee of the University of Venda, South Africa. Ethical clearance with reference number: SEA/21/MBY/12/0707 was obtained from the University of Venda.

3.3 SAMPLE COLLECTION

A total of 160 ground beef and ground beef products (beef patty) categorized as packaged ground beef, measured ground beef, packaged beef patty, and measured beef patty samples were randomly purchased from several local retailers at Thohoyandou Town Limpopo Province. Every sample was collected aseptically, and then sealed into a zip lock bag (Woolworths, Johannesburg, Gauteng, South Africa) marked and then transported to the University of Venda Laboratory of Microbiology, Department of Biochemistry and Microbiology inside an icebox (Shoprite, Cape Town, South Africa. Samples were immediately processed or refrigerated (4-6 ºC) upon arrival for no longer than 24 hours.

3.4 PREPARATION OF SAMPLES

For homogenization, ten grams (10g) from each raw ground beef/product (beef patty) sample were transferred into zip lock bags containing 90 ml of peptone buffered water (Oxoid; Basingstoke, United Kingdom) in the manner described by Ayten et al (2014). The content was then macerated for 2 minutes to aid the recovery of microorganisms. The macerated solution was allowed to stand at room temperature for ten minutes before being cultured in different selective media (Whitehead Scientific, Johannesburg, Gauteng South Africa).

3.4.1 ENRICHMENT OF SAMPLES FOR SALMONELLA ISOLATION

Since it is difficult to isolate *Salmonella*, primary and secondary enrichment were performed in accordance with a procedure reported by Andrews et al (1995). Ziplock bags after maceration were placed into beakers and incubated for twenty-four hours at 37ºC (EcoTherm, Labotec, Johannesburg, Gauteng, South Africa). Secondary enrichment was achieved by inoculation of 1 ml from primary enriched solution in 9 ml of Rappaport-Vasiliadis Soya Peptone Broth (Oxoid) and incubated for forty-eight hours at 42ºC. One ml of macerated solution was then transferred using a sterile pipette into a sterile test tube (Davies diagnosis) that contained nine milliliter (ml) of peptone buffered water (Oxoid) to create a subsequent serial decimal dilution of the sample (Baskaya et al., 2004; Siriken 2004). Therefore, a dilution of 10^{-2} was obtained.

3.5 CULTURING OF ENTERIC BACTERIA

The media was prepared in accordance with the manufacturer's instructions to isolate enteric pathogenic bacteria. For microbiological analysis, Selective agar plates (Davies Diagnosis, Johannesburg, Gauteng, South Africa) such as EMB for *E. coli*, SS for *Salmonella* and *Shigella*, MSA for *Staphylococcus spp*. (Oxoid) were then inoculated with 0.1 ml of the content and cultured using the spread plate technique. Also, 0.1 ml of the serial dilutions were cultured into plate count agar (PCA) (Davies Diagnosis). After that, the plates were incubated overnight at 37°C. Following incubation, the aerobic bacterial count was performed. All presumptive colonies between 15 and 300 were counted and expressed as colony-forming units per grams of meat (cfu/g). For purification, presumptive Isolates were sub-cultured on nutrient agar (Davies Diagnosis) and incubated for twenty-four hours.

3.6 IDENTIFICATION OF ENTERIC BACTERIA

After sub-culturing, the isolates were identified using Gram staining method (Sigma-Aldrich; St Louis, MI, USA) and biochemical testing such as catalase test, urease test, citrate test, kligler iron agar (Sigma-Aldrich) and VITEK system (Bio-merieux, Marcy-l'Étoile, France).

3.6.1 Gram staining

Gram staining was conducted to characterize bacteria according to their gram character (positive and negative) as described by Thairu (2014). Briefly, a smear was prepared on the slide using an inoculating needle, air-dried then heat fixed. Crystal violet (Sigma-Aldrich) solution was added and held for thirty seconds to one minute before rinsing with water. The gram iodine (Sigma-Aldrich) solution washed with water after being flooded for one minute. Then, for about 10-20 seconds, washed with 95 percent alcohol (Sigma-Aldrich) or acetone before rinsing with water. Safranin (Sigma-Aldrich) was added for one minute and rinsed with tap water. Air dry, blot dry and then observed under the microscope.

3.6.2 Catalase Test

The catalase test aids in detecting the catalase enzyme within pathogens that contain the cytochrome system. The catalase enzyme is responsible for neutralizing hydrogen peroxide's bactericidal effects. This test was performed as described by Hendriksen et al (2003). Briefly, on a clean microscope slide (Anatech, Johannesburg, Gauteng, South Africa), an isolated pure colony was placed. A drop of 6% hydrogen peroxide (Merck, Darmstadt, Germany) was introduced to the culture and observed for elaboration of rapid oxygen bubbles.

3.6.4 Citrate test

The Citrate enzyme hydrolyzes citrate to produce acetic and oxaloacetic acid (MacFaddin, 2000). This test is usually used to exemplify an organism's ability to use citrate as its exclusive carbon source for metabolism with resulting alkalinity, as described by MacWilliams (2009). Briefly, Simmons citrate agar (Davies Diagnosis) was prepared as recommended by the manufacturer instruction and then allowed to cool before pouring in sterile test tubes. Test tubes containing melted citrates medium were then tilted to prepare distinct slant and butt. Using sterilized wire, the presumptive pure colonies were inoculated on the slant of the medium. Then the tubes were incubated at 37ºC for 24 hours, and color change was observed in the medium. A color change from green to blue indicated positive results, which were interpreted using standard protocols.

3.6.5 Urease test

Urease catalyzes urea decomposition to form ammonia and carbon dioxide. A urease test was done as described by Benita (2010) to determine whether the bacteria have the ability to produce urease enzyme which break down urea. Briefly, the preparation of the broth (Davies Diagnosis) was carried out in according with the manufacturer's instructions and poured in several sterile tubes. The well-isolated colony was inoculated into the surface of the urea broth. Then the cap was left loosely, and the tube was incubated at 37ºC for 48 hours to 7 days. The results were examined to develop a pink color for as long as 7 days.

3.6.6 Kligler iron agar test

Kligler iron agar was used to differentiate certain Enterobacteriaceae members by demonstrating hydrogen sulphide production and dextrose and lactose fermentation, as Tarh described (2020). Briefly, media (Davies Diagnosis) was prepared following instructions noted on the cover. A well isolated pure colony from solid culture media was transferred with an inoculating needle into the center of the medium in a tube (within 2-5 millimeter from the base). Then inoculated needle was withdrawn and streaked into the slant's surface. After streaking, followed the incubation of the tubes aerobically at 35ºC for 18-48 hours (EcoTherm incubator; Labotec). The results were observed for slant/butt gas acid production and hydrogen sulfide reactions.

3.6.7 VITEK assessment of enteric pathogens

An automated bacterial identification system (VITEK 2; Biomeriux) was used at the Water and Health Research Unit (University of Johannesburg) to detect enteric pathogens. It is an advanced colorimetric system that uses ID cards. The cards were inoculated with standardized bacterial suspension from pure culture, then incubated in the VITEK 2 and read with internal optics.

3.7 ANTIBIOTIC SUSCEPTIBILITY

To assess the antibiotic profile of the isolates, an antibiotic susceptibility test was achieved using the Kirby Bauer Disc Diffusion procedure, which adheres to the Clinical and Laboratory Standards Institute (CLSI) recommended standard as described previously by Bauer et al (1966) and recently by Kassim et al (2016). Briefly, the bacterial inoculum was processed in distilled water, and the turbidity was adjusted to meet McFarland standards of 0.5 (Swenson and Thornsberry, 1984) and equally distributed on Muller Hinton Agar plates. Antimicrobial disks were positioned on the plate containing agar with sterilized forceps, followed by incubation for 24 hours at 37ºC. After incubation, plates were examined for the presence of an inhibition zone. The diameter of the clear zone (no bacterial growth) around the antibiotic disks was used to determine the zone of inhibition. *Salmonella*, *E. coli*, *Shigella*, and *Staphylococcus spp*. sensitivity and resistance to every antibiotic used were analyzed for zone of inhibition and classified into three groups: resistant (R), intermediate (I), and sensitive (S) **(Table 3.1 -3.2**). Different types of antibiotics utilized to assess the

antibiotic susceptibility of selected bacteria are presented below. Antibiotics were selected based on their use in the animal production system, treatment of bacterial infections in humans, and availability (Alban, 2013; Chipangura et al., 2017).

Table 3.1: A table of antibiotics with concentration used to determine the antibiotic susceptibility profile of Enterobacteriaceae using Kirby Bauer Disc Diffusion method following the guideline of the NCLSI (2013).

Antimicrobial agent	Disc Code	Antibiotic Class	Potency	Resistance (mm)	Intermediate (mm)	Susceptible (mm)
Cefoxitin	FOX	Cephalosporins	30 µg	≤14	15-17	≥18
Tetracycline	TE	Tetracycline	30 µg	≤11	12-14	≥15
Trimethoprim-sulfamethoxazole	TS	Sulfonamides	25 µg	≤10	11-15	≥16
Ampicillin	AMP	B-lactams	10 µg	≤11	12-13	≥14
Chloramphenicol	C	Amphenicols	30 µg	≤12	13-17	≥18

*Adapted in part from CLSI document M100 523 (M02 A11). “Disc supplemental tables.” Performance standards for antimicrobial susceptibility testing.

Table 3.2: A table of antibiotics with concentration used to determine the antibiotic susceptibility profile of *Staphylococcus spp*. using Kirby Bauer Disc Diffusion method following the guideline of the NCLSI (2013).

Antimicrobial agent	Disc Code	Antibiotic Class	Potency	Resistance (mm)	Intermediate (mm)	Susceptible (mm)
Cefoxitin	FOX	Cephalosporins	30 µg	≤21	-	≥22
Tetracycline	TE	Tetracycline	30 µg	≤14	15-18	≥19

Trimethoprim-sulfamethoxazole	TS	Sulfonamides	25 µg	≤10	11-15	≥16
Vancomycin	VA	Glycopeptide	30 µg	≤14	15-16	≥17
Ampicillin	AMP	B-lactams	10 µg	≤19	-	≥20
Oxacillin	OX		1 µg	≤10	11-12	≥13
Chloramphenicol	C	Amphenicols	30 µg	≤12	13-17	≥18

*Adapted in part from CLSI document M100 523 (M02 A11). "Disc supplemental tables." Performance standards for antimicrobial susceptibility testing.

3.8 MOLECULAR CHARACTERISATION OF SELECTED ENTERIC PATHOGENS

3.8.1 Molecular characterization For *E. coli* isolates

DNA extraction

DNA extraction was done following the protocol explained/reported by Omar and Barnard (2014). 2 mL of the sample was loaded into 2ml plate and centrifuged at 13,000rmp for about two minutes. Deoxyribonucleic acid was extracted from obtained bacterial cells by silica/guanidium thiocyanate procedure described by Boom et al (1990) and spin column adjustments described by Borodina et al (2005). DNA binding to celites was then enhanced by adding 250 microliters of ethanol (100 %) to lysis buffer. DNA binding membrane (Borodina et al., 2003) was used to charge the spin columns with the celite containing the bound DNA. Elution buffer (Southern Cross Biotechnology) was utilized to extract the DNA (Omar et al., 2010). Polymerase Chain reactions used the DNA extracted as a template.

Gene amplification by multiplex PCR

An overall reaction volume of twenty microliter was used in multiplex polymerase chain reaction (m-PCR) on a Biorad MycyclerTM thermal cycler. The m-PCR protocol was carried out using a Qiagen hot start multiplex PCR kit. Multiplex PCR amplifications were conducted in a reaction mixture that contained 1X PCR multiplex mix (including DNA polymerase, buffer and dNTP); 2 µL of the primer mixture (0.1 lM of mdh and lt primers), 0.2 lM of ial, eagg primers, astA primers, bfp primers and gapdh primers (F and R), 0.3 lM of eaeA and stx2 primers (F and R), 0.5 lm of stx1 and st primers (F

and R); 2 µl of sample DNA, 1 µl of gapdh cDNA and 5 µl PCR grade water. The reactions were confined to amplification conditions that included a 5-minutes introductory stimulation step at 95º C, accompanied by 35 cycles of denaturing at 94º C for 45 seconds, annealing at 50º C for 45 seconds, extension at 68º C for 2 minutes, and finally elongation at 72ºC for 5 minutes.

Table 3.3: Primers used in the m-PCR reaction for molecular characterization to determine different strains and pathotypes of *E. coli* (Adapted from Omar et al., 2014)

Pathogen	Primer	Sequence (5'-3')	Size (bp)	Concen. (µm)	Reference
E. coli	*mdh* (F) *mdh*(R)	GGT ATG GAT GCT TCC GAC CT GGC AGA ATG GTA ACA CCA GAG T	304	0.1	Tarr et al (2002)
EIEC	*ial*(F) i*al(*R)	CTG AAC GGC GAT TAC GCG AA CCA GAC GAT ACG ATC CAG	650	0.2	Lopez-Sasucedo et al (2003)
EHEC/Atypical EPEC	*eae*A(F) *eae*A(R)	CTG AAC GGC GAT TAC GCG AA CCA GAC GAT ACG ATC CAG	9s17	0.3	Aranda et al (2004)
Typical EPEC	*bfp*A(F) *bfp*M(R)	AAT GGT GCT TGC GCT TGC TGC TAT TAA CAC CGT AGC CTT TCG CTG AAG TAC CT	410	0.3	Aranda et al (2004)
EAEC	*eagg*(F) *eagg*(R)	AGA CTC TGG CGA AAG ACT GTA TC ATG GCT GTC TGT AAT AGA TGA GAA C	194	0.2	Pass et al (2000)
EHEC	*stx*1(F) *stx*1(R) *stx*2 (F) *stx*2 (R)	ACA CTG GAT GAT CTC AGT GG CTG AAT CCC CCT CCA TTA TG CCA TGA CAA CGG ACA GCA GTT CCT GTC AAC TGA GCA CTT TG	614 779	0.5 0.3	Moses et al (2006) Moses et al (2006)

ETEC	*t*(F) *lt (*R) *st* (F) *st* (R)	GGC GAC AGA TTA TAC CGT GC CGG TCT CTA TAT TCC CTG TT TTT CCC CTC TTT TAG TCA GTC AAC TG GGC AGG ATT ACA ACA AAG TTC ACA	360 160	0.1 0.5	Pass et al (2000) Pass et al (2000)
E. coli toxin	*ast*A (F) *ast*A (R)	GCC ATC AAC ACA GTA TAT CC GAG TGA CGG CTT TGT AGT C	106	0.3	Kimata et al (2005)
External control	*Gapdh* (F) *Gapdh* (R)	GAG TCA ACG GAT TTG GTC GT TTG ATT TTG GAG GGA TCT GC	238	0.3	Mbene et al (2009)

Gel electrophoresis

Under UV light, gel electrophoresis was used to analyze the DNA. The amplified DNA was visualized utilizing a 2.5 % (w/v) agarose gel in TAE buffer (40 mmol l-1 Tris-acetate; 2 mmol l-1 EDTA, pH 8.3) and stained with 0.5 lg ml-1 ethidium bromide for high DNA visibility. Electrophoresis was done for one to two hours in electric field strength of 8 V cm-1 gel and the DNA was viewed with UV light (Syngene, Bengaluru, Karnataka, India). The roughly equivalent measurements of the DNA fragments were determined by differentiating their electrophoretic mobility to that of molecular markers used with the sample, either a 1 kB or 100 bp marker (Fermentas, Waltham, MA, USA).

3.9 HEMOLYSIN PRODUCTION TEST

Hemolysin are lipids and proteins that cause red blood cell lysis by destroying their cell membrane. Hemolysin production test was done using sheep blood agar media to examine the virulence characteristics of bacterial isolates (*E. coli).* Hemolysin production test was done as outlined by Hashemizadeh et al (2017). Briefly, the pure *E. coli* isolates were tested for blood hemolysis by streaking on 5% sheep blood agar plates (ThermoFisher Scientific, Waltham, MA, USA). After that, the cultured plates were then incubated aerobically for 24 hours at 37ºC. The presence of clear zones

surrounding the growth colonies indicates a positive reaction. After incubation, results were recorded in 3 types of hemolysis: designated alpha, beta and gamma.

3.10 DATA ANALYSIS

Excel was used to perform statistical analysis on the results. The data was then interpreted in the form of graphs, charts and tables.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 RESULTS OBTAINED

4.1.1 PREVALENCE OF PATHOGENS IN GROUND BEEF AND GROUND BEEF PRODUCT

The results of microbiological analyses of retail ground beef/product samples relative to the contamination levels of microorganisms are represented in **Table 4.1**. A total of 160 ground beef/ product (Beef burgers patty) samples were obtained from different retailers and then assessed using the culture methods. Out of 160 samples, 80 (50%) were positive for presumptive *E. coli*, 117 (73.12%) were positive for presumptive *Staphylococcus spp.* presumptive *Shigella spp,* showed a prevalence of 108 (67.5%), and 60 (37.5%) were positive for presumptive *Salmonella spp.* Measured samples showed a high prevalence of pathogenic bacteria than pre-packaged samples.

Table 4.1: Prevalence of pathogenic bacteria in ground beef and ground beef products obtained from retailers in Thohoyandou town. *GB- ground beef, BP- beef patty

	Microorganisms			
Sample	***E. coli***	***Staphylococcus spp.***	***Shigella spp***	***Salmonella spp***
Packaged (GB) N= 65	32 **(49.23%)**	44 **(67.69%)**	35 **(53.84%)**	17 **(26.15%)**
Measured (GB) N= 55	27 **(49.09%)**	46 **(83.63%)**	47 **(85.45%)**	28 **(50.90%)**
Packaged (BP) N= 20	10 **(50%)**	13 **(65%)**	11 **(55%)**	7 **(35%)**
Measured (BP) N= 20	11**(55%)**	14 **(70%)**	15 **(75%)**	8 **(40%)**
Total n=160	80 **(50%)**	117 **(73.12%)**	108 **(67.5%)**	60 **(37.5%)**

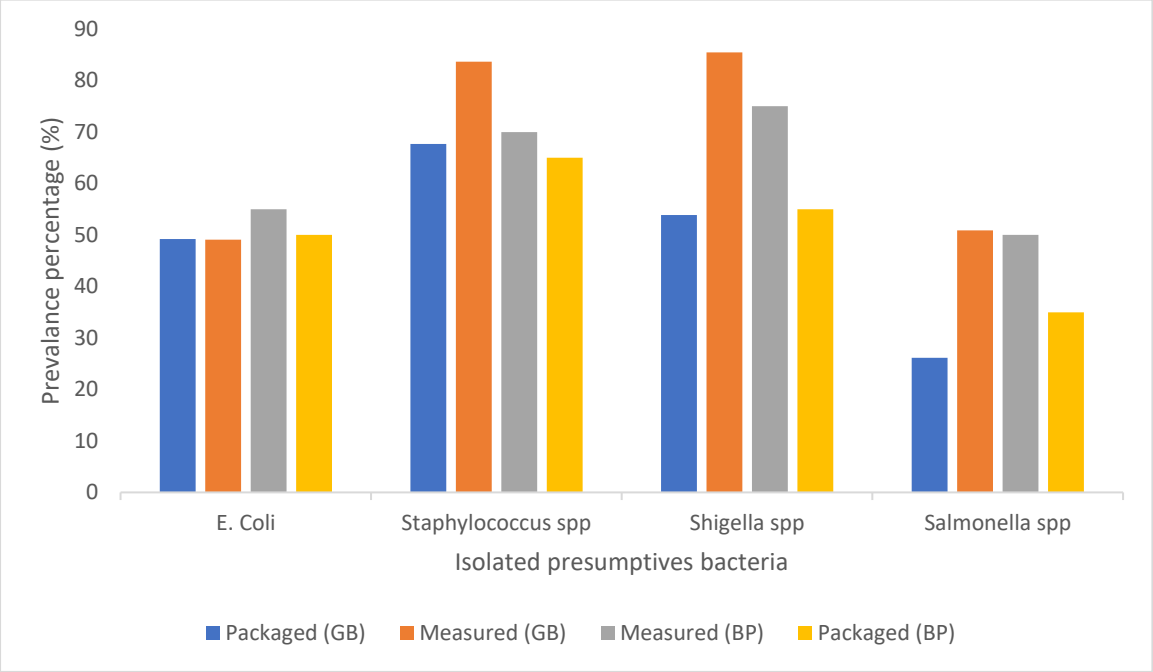

Figure 4.1: Graph showing the prevalence of pathogenic bacteria in ground beef/product from established retailers in Thohoyandou town Limpopo province.

4.1.2. BACTERIAL LOAD

Ground beef samples' microbial quality and safety depend on specific standards such as the number of microorganism present. **Table 4.2** show the bacteriological load of ground beef and ground beef product (beef patty) from established retailers. Codes were used for retailers to ensure confidentiality. None of the samples exceeded the microbial standard of raw mincemeat, which is 10^5 cfu/g.

Table 4.2: Total average of bacteriological load of ground beef/product from established retailers (cfu/g).

Sample	Min	Max	Mean
Packaged (GB) N= 65	6.5×10^3	1.92×10^4	1.26×10^4
Measured (GB) N= 55	6.3×10^3	1.97×10^4	1.23×10^4

Measured (BP) N= 20	6.9 x 10^3	1.99 x 10^4	1.32 x 10^4
Packaged (BP) N= 20	6.1 x 10^3	1.77 x 10^4	1.39 x 10^4
Total N= 160	6.1 x 10^3	1.99 x 10^4	1.2 x 10^4

NB: microbial standard of raw mincemeat 10^5 cfu/g

4.1.3 VITEK RESULTS

An automated bacterial identification system (VITEK 2; Biomeriux) was used at the Water and Health Research Unit (University of Johannesburg) to detect *Staphylococcus* spp. It is an advanced colorimetric system that uses ID cards. The cards were inoculated with standardized bacterial suspension from pure culture, incubated in the VITEK 2 and read with internal optics.

The results showed that out of 13 presumptive *Staphylococcus* isolates only 12 (92.8%) were confirmed to be *Staphylococcus spp* presented in **Table 4.3**. The Most identified *staphylococcus* species were *Staphylococcus saprophyticus* (42.8%), with an excellent identification probability of 97% to 99%.

Table 4.3: VITEK results of presumptive *Staphylococcus spp*. showing organisms detected with their probabilities. (n=13)

Identified organism	Number of *Staphylococcus spp.* Detected
Staphylococcus lentus	1(7.69%)
Staphylococcus scinri	1 (7.69%)
Staphylococcus xylosus	1(7.69%)
Staphylococcus saprophyticus	6 (46.15%)
Staphylococcus warneri	1(7.69%)
Staphylococcus kloosii	1(7.69%)

Staphylococcus equorum	1(7.69%)
Staphylococcus vitulins	1(7.69%)

4.1.4 ANTIBIOTIC SUSCEPTIBILITY TEST

The antimicrobial susceptibility of presumptive *E. coli, Salmonella, Shigella* and *Staphylococcus* isolates were determined. Presumptive isolates were subjected to different antibiotics. The results were interpreted according to the National Committee for Clinical Laboratory Standards guidelines for antimicrobial susceptibility testing (CLSI, 2013). The results are shown in **Table 4.4.1-4.4.4.**

Thirty (30) *E. coli* isolates were tested against 5 different antimicrobial agents and showed sensitivity against 4 agents, with Trimethoprim Sulfamethoxazole showing the highest sensitivity rate of 100% followed by Chloramphenicol 76.60%. The isolates exhibited highest resistance against 3 antimicrobial agents with Cefoxitin displaying a 100% resistance rate and Ampicillin 96.66%.

Table 4.4.1: Antimicrobial susceptibility profiles of *E. coli* to different microbial agents by Kirby Bauer Disc diffusion method

Antibiotic	**Disc content (µg)**	**Resistance (R)**	**Intermediate (I)**	**Susceptible(S)**
Ampicillin (AMP) n= 30	10 (µg)	≤19 29 **(96.66%)**	12-13 0	≥14 1 **(3.33%)**
Cefoxitin (FOX) n= 30	30 (µg)	≤14 30 **(100%)**	15-17 0	≥18 0
Chloramphenicol (C) n= 30	30 (µg)	≤12 6 (20%)	13-17 1 **(3,33%)**	≥ 18 23 **(76.66%)**
Tetracycline (T) n= 30	30 (µg)	≤11 10 **(33.33%)**	12-14 0	≥15 20 **(66.67%)**

Trimethoprim-Sulfamethoxazole (TS) n= 30	25 (µg)	≤ 10 0	11-15 0	≥16 30 **(100%)**

Salmonella spp. isolates were subjected to 5 various antimicrobial agents and Table 4.4.2 presents the findings. The results demonstrated that the isolates were highly susceptible to Trimethoprim Sulfamethoxazole (96.66%) followed by Chloramphenicol (86.66%) and Tetracycline (66.66%). The most resistance percentages recorded was against Cefoxitin (86.66%) followed by Ampicillin (80%).

Table 4.4.2: Antimicrobial susceptibility profiles of *Salmonella* to different microbial agents by Kirby Bauer Disc diffusion method.

Antibiotic	**Disc content (µg)**	**Resistance (R)**	**Intermediate (I)**	**Susceptible(S)**
Ampicillin (AMP) n= 30	10 (µg)	≤19 24 **(80%)**	- 2 **(6.6%)**	≥20 4 **(13.3%)**
Cefoxitin (FOX) n= 30	30 (µg)	≤14 26 **(86.66%)**	15-17 3 **(10%)**	≥18 1 **(3.3%)**
Chloramphenicol (C) n= 30	30 (µg)	≤12 2 **(6.6%)**	13-17 2 **(6.6%)**	≥ 18 26 **(86.6%)**
Tetracycline (T) n= 30	30 (µg)	≤11 9 **(30%)**	12-14 1 **(3.3%)**	≥15 20 **(66.6%)**
Trimethoprim-Sulfamethoxazole (TS) n= 30	25 (µg)	≤ 10 0	11-15 1 **(3.3%)**	≥16 29 **(96.6%)**

The antimicrobial sensitivity of *Shigella* isolates is recorded in Table 4.4.3. Results were interpreted according to the National Committee for Clinical Laboratory Standards guidelines for antimicrobial susceptibility testing (CLSI, 2013). An overall of

30 *Shigella* isolates were subjected to 5 various antimicrobial agents. Among the 5 agents, isolates showed sensitivity against 4 and the highest sensitivity rate was recorded for Trimethoprim Sulfamethoxazole (100%), chloramphenicol (90%) and Tetracycline (63.33%). It was also noted that Ampicillin (96.66%) and cefoxitin (80%) resistant were the most common phenotypes found in *Shigella* isolates.

Table 4.4.3: Antimicrobial susceptibility profiles of *Shigella* to different microbial agents by Kirby Bauer Disc diffusion method.

Antibiotic	**Disc content (µg)**	**Resistance (R)**	**Intermediate (I)**	**Susceptible(S)**
Ampicillin (AMP) n= 30	10 (µg)	≤19 29 **(96.6%)**	- **1 (3.3%)**	≥20 0
Cefoxitin (FOX) n= 30	30 (µg)	≤14 24 **(80%)**	15-17 1 **(3.3%)**	≥18 5 **(16.6%)**
Chloramphenicol (C) n= 30	30 (µg)	≤12 0	13-17 3 **(10%)**	≥ 18 27 **(90%)**
Tetracycline (T) n= 30	30 (µg)	≤11 10 **(33.3%)**	12-14 1 **(3.3%)**	≥15 19 **(63.33%)**
Trimethoprim-Sulfamethoxazole (TS) n= 30	25 (µg)	≤ 10 0	11-15 0	≥16 30 **(100%)**

Table 4.4.4 present the sensitivity of *Staphylococcus* spp. isolates against various antibiotics used. It was observed that the isolates exhibited resistance against 5 antibiotics, with the highest resistance rate recorded for cefoxitin (93.33%) and Oxacillin (93.33%). It was also noted that isolates showed the highest sensitivity rate for Vancomycin (93.33%), Chloramphenicol (90%), Tetracycline (83.33%) and Ampicillin (66.66%).

Table 4.4.4: Antimicrobial susceptibility profiles of *Staphylococcus spp* to different microbial agents by Kirby Bauer Disc diffusion method.

Antibiotic	Disc content (µg)	Resistance (R)	Intermediate (I)	Susceptible(S)
Ampicillin (AMP) n= 30	10 (µg)	≤19 10 **(33.3%)**	- 0	≥20 20 **(66.6%)**
Cefoxitin (FOX) n= 30	30 (µg)	≤14 28 **(93.3%)**	15-17 0	≥18 2 **(6.6%)**
Chloramphenicol (C) n= 30	30 (µg)	≤12 0	13-17 3 **(10%)**	≥ 18 27 **(90%)**
Vancomycin (VA) n= 30	30 (µg)	≤14 1 **(3.3%)**	15-16 1 **(3.33%)**	≥17 28 **(93.3%)**
Oxacillin (OX) n=30	1 (µg)	≤10 28 **(93.3%)**	11-12 1 **(3.33%)**	≥13 1 **(3.3%)**
Tetracycline (T) n= 30	30 (µg)	≤14 4 **(13.3%)**	15-18 1 **(3.3%)**	≥19 25 **(83.3%)**
Trimethoprim-Sulfamethoxazole (TS) n= 30	25 (µg)	≤ 10 0	11-15 0	≥16 30 **(100%)**

4.1.5 MOLECULAR CHARACTERIZATION OF *E. COLI*

E. coli isolates were subjected to molecular characterization to identify different pathotypes, and the results are presented in Table 4.5. Out of 80 positive *E. coli* samples, 30 isolates were moreover analyzed by multiplex polymerase chain reaction assay for gene amplification. Different target genes were used in detecting various *E. coli* pathotypes such as bfp, eae, stx 1, stx 2, ial, it, st, eagg and asta with mdh and gapdh used as positive controls. The results showed that 23 isolates were confirmed

to be *E. coli* strains and 7 isolate was not amplified, possibly due to inhibition or very low concentration of DNA and the results observed are presented in **Table 4.5.**

Table 4.5: Showing selected pathogenic genes and pathotypes.

Sample	*Asta*	*St*	*Eagg*	*Gapdh*	*Mdh*	*Lt*	*Bfp*	*Stx1*	*Ial*	*Stx2*	*EaeA*	Pathotype
FV1	1				1	1	1		1	1		ET/EP/EI/EH
FV2	1											nothing
FV3	1		1		1				1		1	EA/EI/EP
FV4	1											nothing
FV5	1	1	1		1	1		1		1	1	EA/ET/EH/
FV6	1		1	1		1	1	1		1		EAET/EP/EH
FV7	1	1	1		1							ET
FV8	1	1		1	1		1		1	1		ET/EP/EI/EH
FV9	1						1				1	EP
FV10	1	1	1	1	1		1					ET/EA/EP
FV11	1			1			1		1			EP/EI
FV12	1			1	1		1	1		1		EP/EH
FV13	1		1		1	1			1	1	1	EA/ET/EI/EH
FV14	1			1	1		1	1	1	1	1	EP/EI/EH
FV15	1				1	1					1	ET/EP
FV16	1				1		1		1		1	EP/EI
FV17	1		1	1	1	1					1	EA/ET/EP
FV18	0	0	0	0	0	0	0	0	0	0	0	nothing
FV19	0	0	0	0	0	0	0	0	0	0	0	nothing
FV20			1		1							EA
FV21	0	0	0	0	0	0	0	0	0	0	0	nothing
FV22					1							EC
FV23					1							EC
FV24	0	0	0	0	0	0	0	0	0	0	0	nothing
FV25	0	0	0	0	0	0	0	0	0	0	0	nothing
FV26	1	1			1		1				1	ET/EP
FV27	1		1	1	1		1			1	1	EA/EP/EH
FV28	1				1						1	EP
FV29	1					1		1		1		ET/EH
FV30	1						1			1		EP/EH

The most prevalent virulence genes were Asta (73.33%) followed by Bfp (40%) and Eae (36.66%) and lowest was Stx1 (16.66%) and St (16.66%). About 17/30 (56.66%) carried more than one pathotypes, therefore they were more pathogenic. Isolates showed highest rate of EPEC (53.33%) pathotype and lowest rate of COM (6.66%) pathotype and the results are shown in **Figures 4.5.1 & 4.5.2**.

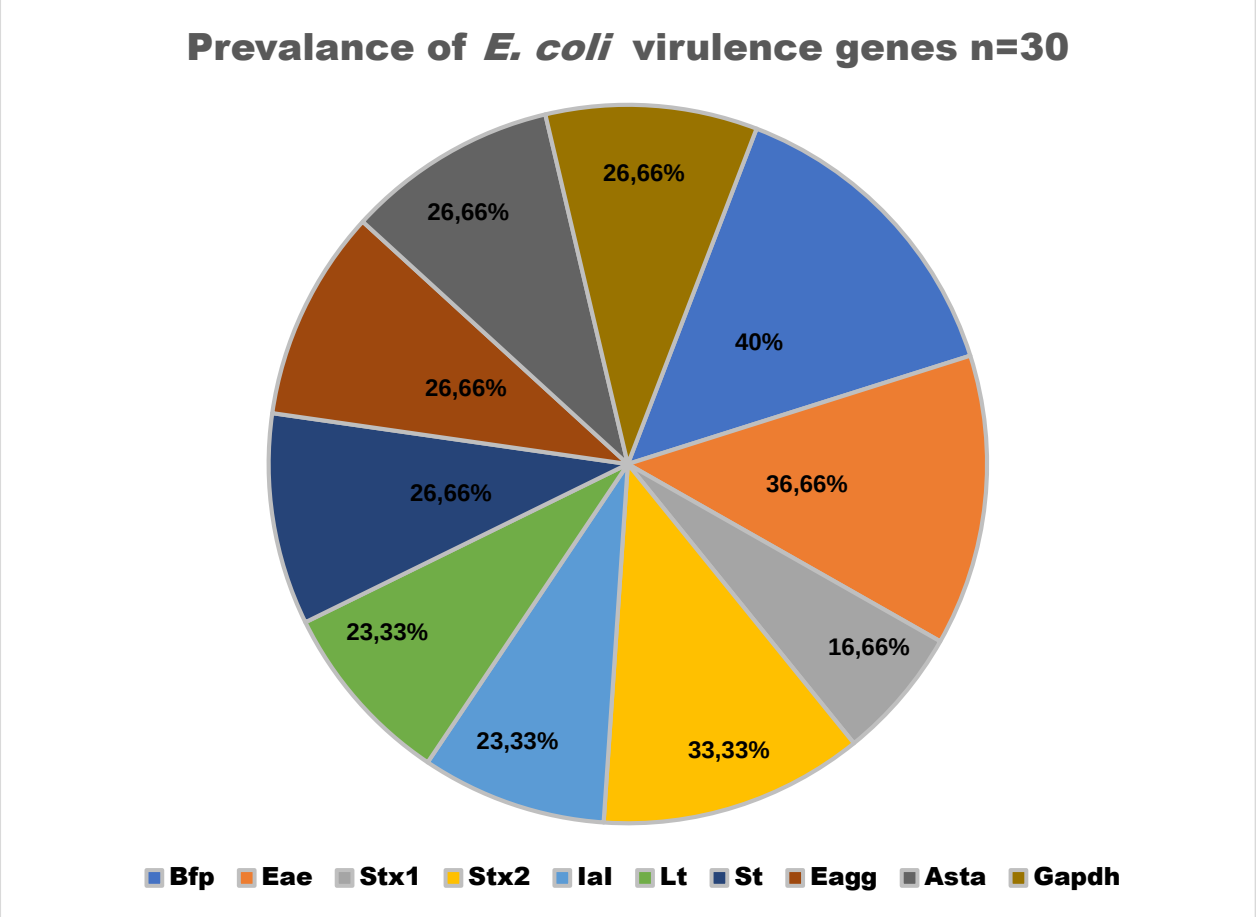

Figure 4.5.1: A pie chart showing the prevalence of virulence genes detected in ***E. coli*** isolates by mPCR with Asta being the most detected gene from ground beef/product

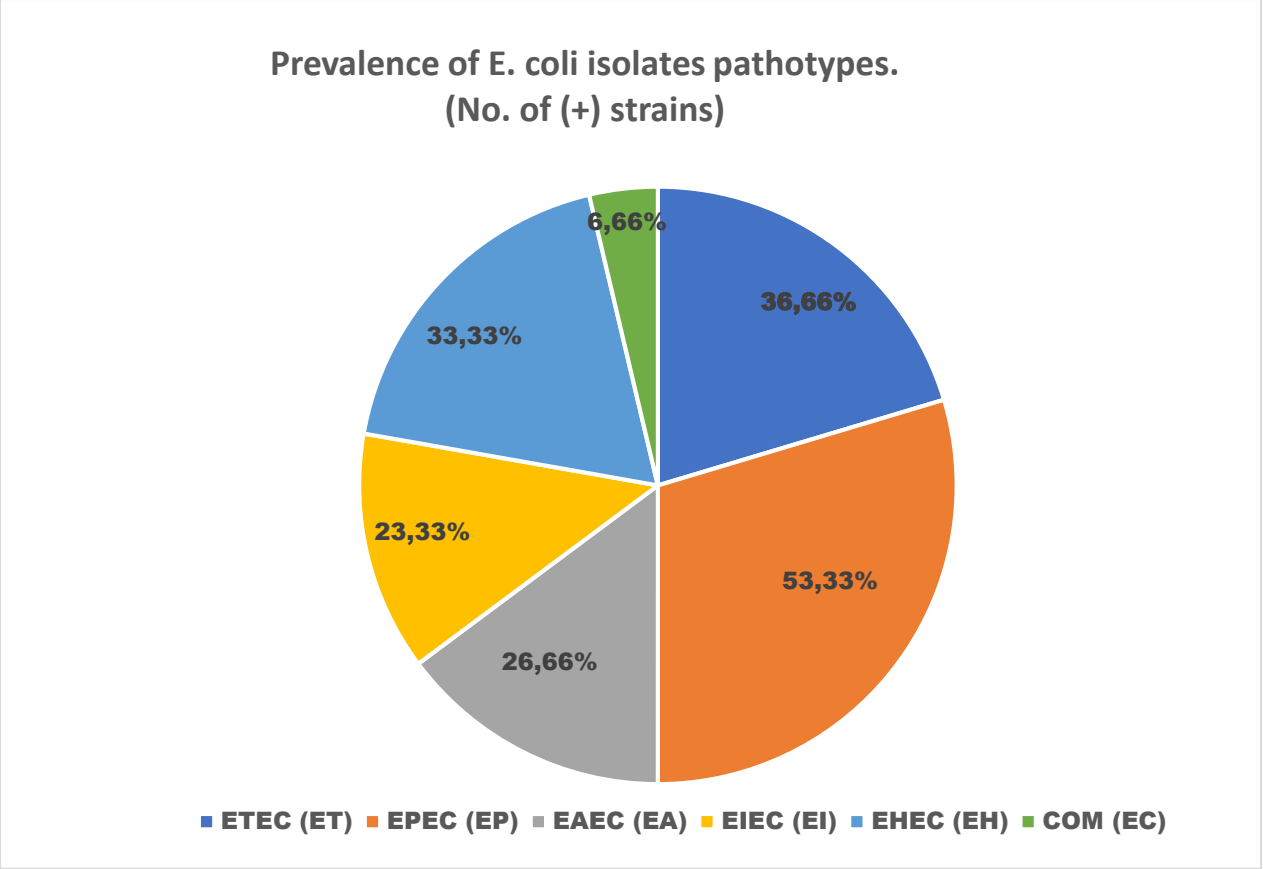

Figure 4.5.2: A Pie chart showing the prevalence *E. coli* pathotypes observed in ground beef/product obtained from established retailers in Thohoyandou Town, Limpopo province. 17 isolates showed more than one pathotypes with EPEC (53.33%) being most prevalent pathotype of them all.

4.2 DISCUSSION

Every year, millions of people become ill as a result of microbiological food-borne diseases, whereas many cases go unreported (Wallace et al., 2018). Ground beef/product is a great medium for the growth of various microorganisms, and this is due to nutrient and water availability as well as an exposed surface area common among retailed ground meat (Erdem et al., 2014; Baskaya et al., 2004). The quality and safety of ground beef is of major interest in meat production industry, with foodborne pathogens as the utmost concern (Eastwood et al., 2018). Packaging and package environment have the greatest influence on a meat product's microbial and oxidation potential. The main objective of this study was to assess the microbial quality and safety of ground beef/product from established retailers in Thohoyandou Town, Vhembe district. The findings of the study demonstrated that out of 160 samples

analyzed, the following pathogenic bacteria were detected *E. coli* (50%), *Staphylococcus spp*. (73.12%), *Salmonella* 60 (37.5%) and *Shigella* species (67.5%). The majority of Enterobacteriaceae (*E. coli, Shigella* and *Salmonella*) isolates were resistant to Ampicillin and Cefoxitin, while *Staphylococcus* isolates exhibiteing resistance to cefoxitin (93.33%) and Oxacillin (93.33%). *E. coli* isolates were subjected to mPCR assay and results showed that isolates were of different pathotypes with EPEC (65%), ETEC (48%), EHEC (44%) being the most prevalent pathotypes detected and the most prevalent virulence genes detected were asta (73.33%), Bfp (40%), Eae (36.66%) and Stx 2 (33.33%).

Many investigations have revealed the prevalence of *E. coli* isolated in ground beef from established retailers. Based on our findings, the contamination rate of *E. coli* (50%) in retail ground beef/product samples is relatively low compared to a report from Voster et al (1994) with a very high level of contamination of meat and meat product with *E. coli* (74.5%) in the study conducted in Pretoria (South Africa). The result of the current study is also in contrast with the survey conducted by Heredia and Garcia (2018), where *E. coli* was detected in 76% of 88 retail ground meat sample in Mexico. In comparison with other studies, our results are higher when compared with a study carried out in Alice, fort Beaufort and Mdantsane (Eastern Cape, South Africa) by Abong'o and Momba in 2009 which reported low prevalence of *E. coli* contamination (2.8%) of the 180 meat and meat product samples.

The dissemination of *E. coli* throughout the mincing procedure is the most likely cause of the huge prevalence of *E. coli* in minced meat. When one carcass is contaminated with *E. coli* from feces, mixing meat from several cows can lead to spread of the pathogen and it contaminates the whole batch of minced meat. The mincing blades may also cause cross-contamination of the minced meat. *E. coli* may be present in raw meat utilized in the production of alternative meat commodity (Flores and Tewart, 2004). Personal hygiene by meat sellers and butchers who work with retail minced meat are often ignored or given little attention. The lack of knowledge based on the significance of disinfecting and sanitizing continuously also plays an important role in the higher contamination level.

Detection and enumeration of *Staphylococcus* spp. allow for the assessment of food risk for consumers, provided that they are the primary species capable of producing the proteinic enterotoxin that causes food poisoning (Hennekinne et al., 2012). The current study showed that 117 (73.12%) of the 160-ground beef/product samples from established retailers around Thohoyandou, were contaminated with *staphylococcus* spp. The results showed the highest prevalence of *Staphylococcus* spp. in measured ground beef samples with 83.63%. This was probably because the measured minced meat sample is stored at a temperature favoring the growth of pathogenic bacteria. This study is in agreement with the research done in Nigeria by Salihu et al (2013) which showed high prevalence of *Staphylococcus spp*. 69.9% in minced beef. Contamination of minced meat by *staphylococci* can be the result of deficient hygiene on the part of workers who have been proven to be carriers of *Staphylococcus spp*. The most dominant *Staphylococcus* species identified was *Staphylococcus saprophyticus. S. saprophyticus* is part of the normal human flora, as well as a common gastrointestinal flora in pigs and cows, and may thus be transmitted to humans through consumption of contaminated pig and cattle.

Staphylococcus aureus was not detected in this study, which contrasts with the findings of Gundogen et al (2005), who isolated *S. aureus* from 14 of 30 ground beef samples in Ankara, Turkey. In the previous study done by Erdem et al (2014) to determine the “microbiological quality of minced meat samples marketed in Instanbul” showed 58 out of 60 minced meat samples contaminated with *S. aureus.* It has been proposed that *S. aureus* in raw meats implies contamination from human nasopharynx (Belhaj et al., 2012). Various researchers studied the microbiological quality of ground beef and ground beef product, and the results showed that measured minced meat poses high risk of danger to one's health.

Salmonellosis is a major health and economic impact on people all over the world (Heredia and Garcia, 2018). Human salmonellosis has been linked to raw and undercooked beef (Mor-Mur and Yuste, 2010). In South Africa, there is also insufficient documented evidence of the presence of *Salmonella spp*. in food products. Although prevalence rates on ground beef/product may vary from one sample to another, the microbiological quality of meat is determined by the regulation measures used during the slaughtering process (Rhoades et al., 2009). In this study, 37.5% of samples tested

positive for *Salmonella* spp. *Salmonella* species were recovered from fresh raw packaged ground beef (26.5%), measured ground beef (50.90%), Measured beef patty (40%) and packaged beef patty (35%) samples. The prevalence of *Salmonella* in retail ground beef/product in this study was greater than previous studies recorded in Vhembe District. A study done by Mabaso (unpublished data), showed lower percentage (21%) of *Salmonella* spp. contaminating ground beef obtained from established retailers in Thohoyandou Town. The findings of the current study are in agreement with studies conducted by Letha et al (2017) which reported around 30.37% prevalence of *Salmonella* in beef and contrary to study done by Maradiga et al (2015) who demonstrated a much lower 10% prevalence of *Salmonella* spp. Although many studies showed lower prevalence of *Salmonella* contamination in beef meat, other studies demonstrated a higher prevalence percentage than the current study such as a study done by Azage and Kibret (2017) which reported 70% prevalence of *Salmonella* in 30 samples. Slaughterhouses and the distribution channels equally share the results of *Salmonella* contaminants through inappropriate handling and houseflies. Furthermore, it has been demonstrated that the serotype of *Salmonella* found in cattle feces and rumen can be observed in beef carcasses and meat from retailers, creating direct threats to consumer health (Ateba and Mochaiwa, 2014). This emphasizes the importance of implementing appropriate hygiene practices throughout the food production processes. Freezing appears to be a hazard influence for *Salmonella* contamination, though this is not convincing because, according to some researchers, contamination tends to increase with outside temperature (Klein and Louwers, 1994). Food handlers are natural reservoirs of *Salmonella typhimurium* (Ehuwa et al., 2021). Similarly, many researchers were unable to detect salmonella in minced beef samples. Fathi and Thabet (2001) concluded that the negative result does not imply the absence of the organism, but that it could be because of the low sensitivity and specificity of the isolation method used.

Shigellosis is prevalent in the majority of developing countries (Niyogi, 2005). Consuming contaminated foods causes a substantial number of Shigellosis outbreaks annually (Ahmed and Shimamoto, 2014). Food contamination is generally caused by an infected individual using inadequate meal preparation procedure (Lampel et al.,

2000). The present study showed that 67.5% of ground beef and ground beef product from established retailers around Thohoyandou were contaminated with *Shigella* spp. Measured ground beef demonstrated the highest prevalence of *Shigella* spp. (85.45%), followed by measured beef patty (75%), then packaged beef patty (55%). This could be due to the storage temperature favoring the growth of organisms isolated. Prepacked meat is sealed which could be the reason of less contamination level since most bacteria require oxygen for survival and growth. The vacuum seal inhibits the oxygen from seeping out. The results of the current study were in contrast with most of the previous studies. There is a scarcity of information on the occurrence of *Shigella* in ground beef meat. Many researchers reported no *Shigella* species' growth. This could be since animals are not thought to be a popular reservoir for *Shigella spp*. *Shigella species* found on the base of food animal tissue are thought to be transmitted to meat surfaces via workers' hands and knives during retail meat processing (Todd et al., 2009).

Total plate count is an accurate indicator of the bacteriological load in ground beef and ground beef products. When the total number of bacteria on fresh meat is among 10,000 (1,0104) and 100,000 (1,0105), critical hygienic dimensions are reached (Matthews et al., 2017; Yilmaz and Velioglu, 2009). The total number, however, doesn't really authorize for any definitive conclusion about the descriptive of the microbe, like whether the microorganism is toxic or non-toxic. One of the microbiological indicators of food quality is the plate count of aerobic mesophilic microorganisms in food. The presence of aerobic organisms indicates the presence of favorable conditions for microorganism multiplication (Nyenje et al., 2012). Coliforms are indicators of food quality, and their presence may indicate an unsanitary condition (Azage and Kibret, 2017). Enterobacteriaceae are common in the environment and are used as an effective indicator of sanitation and post-processing contamination of raw meat. Furthermore, even in low numbers, their count can be used as an indicator of probable contamination of enteric pathogens in the absence of coliforms (da Silva et al., 2016).

The current study reported low bacterial load in measured and packaged ground beef/product samples. The results showed 6.1 x 10^3 cfu/g minimum bacterial count and a maximum of 1.99 x 10^4 cfu/g. The recorded mean of these current study was 1.2 x 10^4 cfu/g. The overall results of these study presented bacterial contamination

profile (mean values) in ground beef/product are as follows: packaged ground beef (1.26×10^4 cfu/g), for measured ground beef (1.26×10^4 cfu/g), measured beef patty (1.32×10^4 cfu/g) and packaged beef patty (1.29×10^4 cfu/g). Measured ground beef product (beef patty) showed higher level of bacterial count (1.99×10^4 cfu/g). This could be caused by the addition of certain additives to meat product which may lead to marked increase in the bacterial population (Abdelrahman et al., 2014). Tremendous amount of total bacterial load in minced beef could be attributed to insufficient sterilization and disinfection, contaminant materials (for example, packaging), poor storage condition, untreated water sources, and a lack of disinfection treatment (Ghougal et al., 2021).

Result of this study agrees with the study done by Abdelrahman et al (2014) which showed the mean value of Enterobacteriaceae count ranging between 6.3×10^4 and 2.8×10^4 cfu/g in fresh ground beef. Gonulalan and Kose (2004) performed a study in Kayseri and reported bacterial contamination profile in minced meat samples of 7.4×10^5 -4.5×10^8, which is higher than the results observed in the current study. In another study, Baskaya et al (2004) examined ground meat samples and discovered that the bacterial load ranged from 3.1 x 104 to 6.3 x 107 cfu/g for total aerobic bacteria. A study titled “microbial contamination of meat samples in Italy” discovered aerobic microorganism average content being greater than 108 cfu/g (Larney et al., 2003). The difference among results of analysis was mostly the methods used, samplings number, different geographical location/climate, and hygiene quality practices. However, the existence of certain pathogenic bacteria in food intended for human consumption is extremely concerning even in extremely low infectivity dose.

Meat is a significant route for transmission of antibiotic resistance from animal to human. Such transfer can happen in three different ways: through the consumption of resistant parts of the original food-borne microorganism, antibiotic residues in food, or the transfer of resistant food-borne pathogens, (Mayrhofer et al., 2004). In addition to antibiotic misuse, the amount of resistance to these antibiotics is high in poor countries, which is believed to be due to their use in gastroenteritis infections, self-medication, and use in animal feed (Adekunle et al., 2009).

The current study showed that *E. coli* isolates demonstrated high levels of resistance against cefoxitin and ampicillin and high levels of susceptibility to trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol. Findings of this study were consistent with findings reported in Egypt by Shaymaa and colleagues (2015) which revealed that 96% of *E. coli* isolates were resistance to ampicillin and reported a lower resistance percentage rate (36%) of *E. coli* against chloramphenicol. Several studies have revealed an increase in *E. coli* antibiotic resistance (Magwira et al, 2005). For instance, research done by Martinez-vazquez et al (2018) recorded *E. coli* isolates in ground beef resistant to ampicillin (86,5%) tetracycline (60,80). The results of the current study are in contrary to results reported by Ramadanet et al (2020) which showed a lower resistance of *E. coli* against ampicillin (37%), cefoxitin (7.4%), and tetracycline (44,4%). Seza and Ayla (2010) also reported that *E. coli* isolates in retail ground beef are resistant to ampicillin (7,7%), tetracycline (38,5%) and chloramphenicol (7,7%).

The antimicrobial resistance pattern of *Salmonella* spp., in this study showed high resistance to cefoxitin and ampicillin. The isolates also showed high sensitivity levels to Trimethoprim sulfamethoxazole, chloramphenicol and tetracycline. Seza and Ayla (2010) found that antimicrobial susceptibility rates against *Salmonella* isolates obtained from retail meat products (ground beef) were similar to those found in the current study. Antimicrobial use in animals is increasingly thought to select for resistance in zoonotic and commensal microorganisms (Norrung and Buncic, 2008). Little et al (2008) discovered that 48.1% of *Salmonella* isolates from red meats in the United Kingdom were resistant to multiple antibiotics. They furthermore reported that the more frequent antimicrobial drug resistance found in *Salmonella* strains was to ampicillin. These findings are consistent with the results of the current study. Another study done by Bosilevac et al (2009), reported resistance rate of *Salmonella* isolated from ground beef against ampicillin, cefoxitin and tetracycline.

In this study, *Shigella* isolates were resistant to more than one or two of antibiotics used, including ampicillin, cefoxitin, and tetracycline. Several isolates were either intermediately or fully sensitive to some antibiotics. The current findings are in agreement with a study performed in Gondar, Northwest Ethiopia, by Garedew et al (2015), which found a higher rate of resistance to ampicillin and amoxicillin, but all

Shigella isolates were susceptible to gentamycin, tetracycline, and trimethoprim-sulfamethoxazole. A study on the "genetic diversity and antibiotic resistance of *Shigella* spp. isolates from food products" by Pakbin et al (2021) reported *Shigella* resistant against several antibiotics including: amoxicillin-clavulanic acid, tetracycline, sulfamethoxazole, streptomycin, ampicillin, chloramphenicol, amoxicillin, and azithromycin while sensitive to gentamycin, cefoxitin, cefepime. Another study by Shahin et al (2019) also reported *Shigella* isolates of food origins demonstrated resistance against streptomycin, tetracycline, amoxicillin, cephalothin and nalidixic acid. In addition, MDR status was assigned to 17 of 19 isolates that showed resistant to more than three different classes of antimicrobial agents. According to Rahimi et al (2017), *Shigella* isolates in meat and meat products showed high levels of antibiotic resistance to gentamicin, tetracycline, and ampicillin.

Thirty (30) *Staphylococcus* species isolates were tested to various antibiotics. The resistant pattern varied among the 5 drugs. The isolates showed resistance to cefoxitin and oxacillin while highly sensitive to vancomycin, chloramphenicol, tetracycline, and ampicillin. A study done by Kelman et al (2011) to determine "antimicrobial susceptibility of *Staphylococcus aureus* from retail ground meats" showed a much lower resistance rate of the isolates against Ampicillin and Oxacillin compared to the current results. Another study conducted in Vhembe district by showed *S. aureus* isolates highly resistant to Oxacillin and support the findings of the current study which demonstrated high resistant rate of Oxacillin (Bessong et al., 2015). The study also reported highest susceptibility to Vancomycin and Ampicillin which is in agreement with the current study. Feben et al (2018) also reported that *S. aureus* isolates in beef are sensitive to chloramphenicol, clindamycin and ampicillin. All isolated strains revealed resistance to neomycin, methicillin and tetracycline. Another study on the "antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat" by Pesavento et al (2007) reported isolates resistant to oxacillin, ampicillin, tetracycline, but sensitive to vancomycin and methicillin.

Healthy dairy and beef cattle serve as a major reservoir for a wide range of STEC that infect humans through food, water, and direct contact. *E. coli* isolates were subjected to molecular characterization in order to identify different pathotypes. Gapdh genes were used as the external control gene, the inclusion of internal and external controls

is necessary to ensure that there are no polymerase chain reaction inhibitors present in the reaction and to verify the PCR's precision in differentiating accurate negative from false negative results. The mdh house-keeping gene was found in 18 of 30 *E. coli* isolates from ground beef/product samples tested positive in this study. The remaining isolates that did not have the mdh gene may have the malic acid dehydrogenase gene instead, also regarded as a house-keeping enzyme of the citric acid cycle (Hsu and Tsen 2001).

The results of the study showed that more than 70% of the isolates had more than one pathotype, it was also confirmed that more than 70% of the isolates harbored *E. coli* toxin Asta gene. Soto et al (2009) reported that the Asta gene is a structural gene that encodes an Enteroaggregative heat stable toxin 1 (EAST-1). They further reported that this gene is confirmed to contribute a significant aspect in the pathogenicity of EAEC and it has also been detected in EIEC, EPEC, atypical EPEC, and ETEC strains (Yatsuyanagi et al., 2003; Omar and Bernard, 2014). In a study done by Shayman and colleagues (2015) reported 28% Asta genes amplified from *E. coli* isolated in ground beef and beef patty which is lower than the prevalence of Asta genes observed in the current study. The previous study also demonstrated 32% of the isolates harboring EaeA genes supporting the findings of the EaeA (36.66%) prevalence in the current study. Liorente et al., 2014, detected and identified 36.1% of the isolates harboring the stx genes.

Diarrhea continues to be an important health issue worldwide (Thakur et al., 2018), particularly in developing countries. Among the bacteria associated with diarrhea there are different *E. coli* pathotypes which colonize the human intestine (Kolenda et al., 2015). The current study identified EPEC (53.33%), ETEC (36.66%), EHEC (33.33%), EAEC (26.66%) and EIEC (23.33%) strains. Enterotoxigenic *E. coli* (ETEC) strains are widely regarded as a pathogenic pathotype. Infections caused by these strains are characterised by watery diarrhoea, usually without blood, mucus, or pus and may also include symptoms like fever and vomiting (Nataro and Kaper., 1998). Enterohemorrhagic *E. coli* (EHEC) is the utmost common pathogenic *Escherichia. coli* bacteria throughout the world (Yoshimura, 2010). Consumption of poorly cooked and contaminated ground beef with EHEC has been a cause of bloody diarrhoea that can evolve into hemolytic-uremic syndrome (HUS) and encephalopathy (Surendran-Nair,

2017). Another *E. coli* strain associated with diarrhoea is enteropathogenic *E. coli* (EPEC), which is a significant class of diarrheagenic *E. coli* associated with infant diarrhoea in the developing countries. In adults, symptoms include severe diarrhoea, abdominal cramps, nausea, headache, vomiting, fever, and chills (Nascimento et al., 2022). In both developed and developing countries, enteroinvasive *E. coli* (EIEC) strains are unusual. The bacteria can take over and damage the colonic epithelium, resulting in an infection identified initially by watery diarrhoea. A small percentage of people suffering from this infection advance to the dysenteric form of the disease, which includes fever, abdominal cramps, and blood and leukocytes in stool specimens (Liu, 2015).

Mohammed (2012) characterized the virulence-associated genes of 32 *E. coli* strains isolated from various meat products and discovered that eleven (37.5%) were possibly diarrheagenic, 15.63% were ETEC, 6.26% were EPEC, and 3.13% was EIEC. *E. coli* 0157: H7 is a serotype of the enterohemorrhagic (EHEC) bacteria group. Cattle have been shown to be a source of *E. coli* 0157:H7. Carcass contaminated while slaughtering and processing of beef and beef products can spread the microorganism to human through food consumption (Elder et al., 2000). Commensal *E. coli* (E. com) strains are critical because they nurture the physiological environment of the gut, aid in digestion, and protect against enteric pathogens (Foster, 2004). The current study showed that two out of 30 (6 .6%) *E. coli* isolates tested negative for Shiga toxin or enterotoxin genes, which are associated with virulence. This most generally implies that the *E. coli* isolates found were part of the normal enteric flora present in animals and frequently detected in food production, processing, and distribution environments (Stanley et al., 2018).

Defining the virulence factors and mechanisms of *E. coli* pathogenesis has been a focus of numerous studies. Hemolysin production test was performed to determine the effect of 30 bacterial isolates on the blood agar solid growth medium that contain red blood cells. In this study, alpha hemolytic activity was observed in 76.6% isolates, while beta hemolytic activity was observed in 10% isolates. In 13.3% of *E. coli* isolates, gamma hemolytic or non-hemolytic strains were found. Alpha-hemolysin is a prevalent exotoxin produced by *E. coli* that increases virulence in a variety of clinical animal infections (Mishra et al., 2018). The alpha-hemolysin has activity against human

lymphocytes and entero-hemolysin typically generated by VTEC group of *E. coli*. In comparison, a previous study done by Aksoy et al (2017) showed that in 52 *E. coli* strains from cattle and sheep, 40% hemolyzed and the type of hemolysis formed were gamma hemolysin (46.2%), alpha hemolysin (19.2%), beta hemolysin (13.5%), and enterohemolysin (7.7%). Another study done by Shekh et al., 2013 in Parbhani city, Maharashtra, Insia on the “isolation of pathogenic *Escherichia coli* from buffalo meat” recorded a total of 3.6% pathogenic *E. coli* isolates by in-vitro pathogenicity testing. Bist et al (2014) conducted a study on the “virulence associated factors and antibiotic sensitivity pattern of *E. coli* isolated from cattle and soil” reported only 4.3% fecal *E. coli* were positive for hemolysis assay. Bashar et al (2011) recorded that among the isolates of *E. coli* isolated from poultry meat, 45% and 14% isolates demonstrated beta and alpha hemolysis, respectively. Gamma hemolytic strains were detected in 41% of 250 isolates. Because of the presence of the hemolysin gene, red blood cells of the host organism are lysed, which aids in spreading of the pathogenic bacteria.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The primary objective of the study was to assess the safety and microbial quality of ground beef and ground beef products obtained in different retailers around Thohoyandou area, Vhembe District. This was done through isolation, culturing of bacteria and a combination of identification tests (citrate, urease, Kligler iron, Gram staining and VITEK 2 system), hemolysin production test, multiplex PCR, antibiotic susceptibility test.

The first secondary objective was to determine the prevalence of selected enteric pathogenic bacteria from ground beef by culture methods and the results obtained are detailed in **Table 4.1 and Figure 4.1.** Out of 160 samples collected from different retailers, measured samples showed a high prevalence of pathogenic bacteria than pre-packaged samples. In summary, 50% were positive for presumptive *E. coli spp*, 73.12% were positive for presumptive *Staphylococcus spp.* Presumptive *Shigella spp,* showed a prevalence of 67.5%, and 37.5% were positive for presumptive *Salmonella spp.*

The second secondary objective was to determine the antibiotic susceptibility profile of bacteria from ground beef using the Kirby Bauer Disc Diffusion method. The antimicrobial susceptibility of presumptive *E. coli, Salmonella, Shigella* and *Staphylococcus* isolates are reported in **Tables 4.4.1 - 4.4.4.** The results were interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards for anti-microbial susceptibility testing (CLSI, 2013). The result showed resistance to more than one antibiotic used, resulting in multidrug resistance profile of some isolates.

The third secondary objective was to determine the molecular characteristics of isolates (pathotype and virulence genes) using multiplex PCR. *E. coli* isolates were subjected to molecular characterization to identify different pathotypes, and the results are presented in **Table 4.5.** The results showed that 23 isolates confirmed to be *E. coli* strains. The data were further analyzed for prevalence of *E. coli* virulence genes and

different pathotypes reported in **Figure 4.5.1 and 4.5.2.** Asta was the most prevalent gene detected from ground beef/product.

The last secondary objective was to determine virulence characteristics of *E. coli* isolates by hemolysin production test. Hemolysin production test was conducted to assess the effect of 30 bacterial (*E. coli*) isolates on the blood agar solid growth medium that contain red blood cells. In this study, alpha hemolytic activity was observed in 76.6% isolates, while beta hemolytic activity was observed in 10% isolates. In 13.3% of *E. coli* isolates, gamma hemolytic or non-hemolytic strains were found.

In conclusion, the current study concluded that the microbial quality and safety of ground beef/product from established retailers is inadequate and therefore not acceptable for safe consumption when undercooked, this is due to ground beef and ground beef product containing various pathogens such as *E. coli*, *Salmonella*, *Shigella* and *Staphylococcus* spp. that may trigger dangerous health issues.

5.2 RECOMMENDATION

It is essential to monitor the presence of pathogens in all stages of meat processing from farm to slaughtering processes as well as monitoring whether meat handlers are taking good hygiene practices into consideration. The study also showed a high level of resistance to a wide range of antimicrobial drugs. Therefore, constant monitoring of antimicrobial susceptibility is recommended and the use of various antibiotics on animal should be investigated as it is the source of antibiotic resistance development. Further studies are needed to identify the potential sources of contamination so that contamination can be reduced to improve the quality and safety of ground beef/product of different retailers. Growing concern about the quality and safety of meat has resulted in numerous advancements in meat preservation.

5. 3 STUDY LIMITATIONS

Biochemical tests were limited and only few isolates of *Staphylococcus, Salmonella* and *Shigella* spp. were subjected to VITEK system for further confirmation due to financial constraints.

CHAPTER 6

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ANNEXURE

ETHICAL CLEARANCE REPORT

ETHICS APPROVAL CERTIFICATE

RESEARCH AND INNOVATION
OFFICE OF THE DIRECTOR

NAME OF RESEARCHER/INVESTIGATOR:
Ms FV Mukosi

STUDENT NO:
16003676

PROJECT TITLE: Assessment of microbial quality and safety of ground beef obtained from retailers around Thohoyandou Vhembe District, Limpopo Province.

PROJECT NO: SEA/21/MBY/12/0707

SUPERVISORS/ CO-RESEARCHERS/ CO-INVESTIGATORS

NAME	INSTITUTION & DEPARTMENT	ROLE
Dr E Musie	University of Venda	Supervisor
Prof AN Traore	University of Venda	Co - Supervisor
Ms FV Mukosi	University of Venda	Investigator – Student

Type: Masters Research
Risk: Minimal risk to humans, animals or environment (Category 2)
Approval Period: July 2021 – July 2023

The Human and Clinical Trails Research Ethics Committee (HCTREC) hereby approves your project as indicated above.

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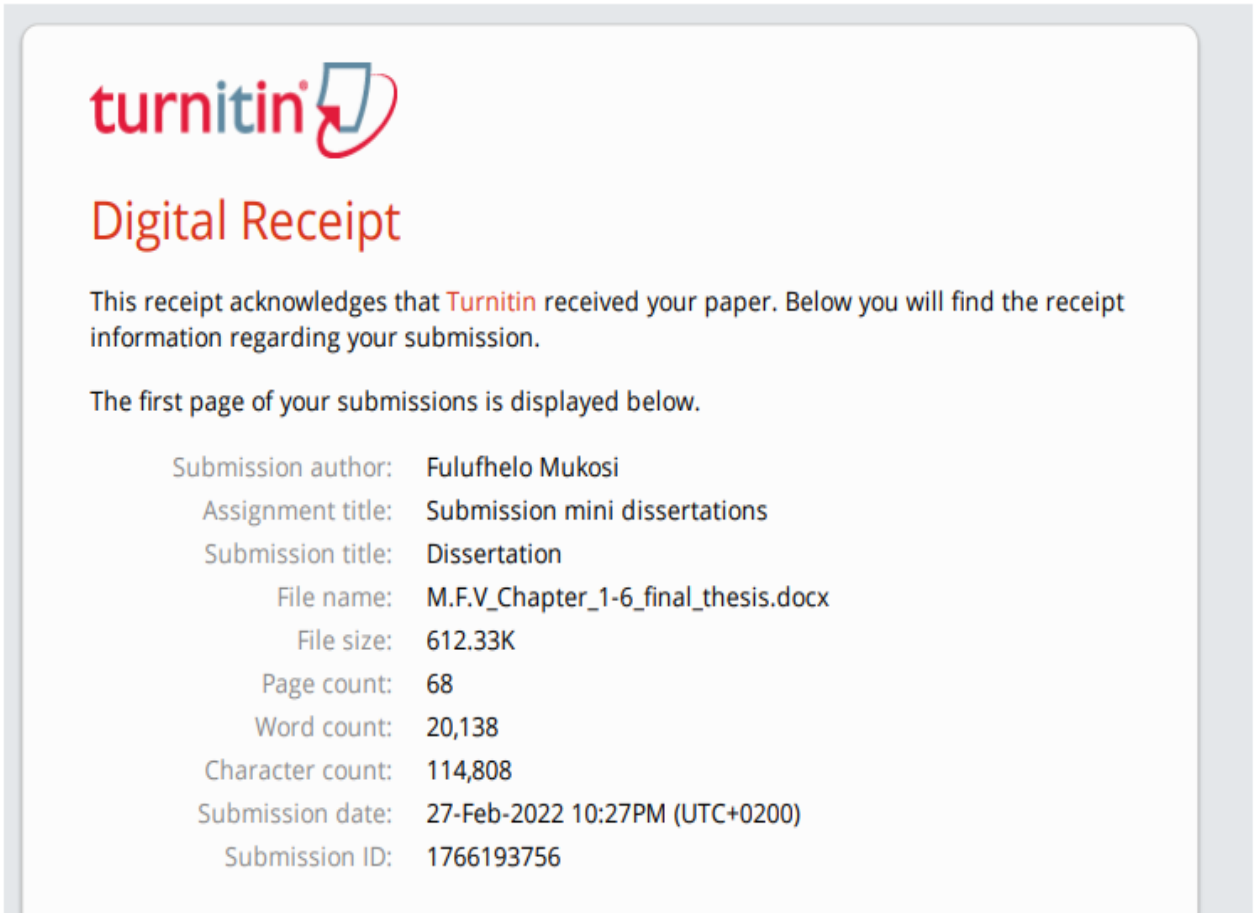

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