An investigation of hygiene practices in butcheries and the prevalence of antibiotic resistant *Listeria monocytogenes* in Limpopo, South Africa.

By

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A dissertation submitted in fulfillment of the degree of Master of Science in Food Science and Technology in the Department of Food Science and Technology, Faculty of Science, Engineering, and Agriculture

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Abstract

Listeria monocytogenes is a foodborne bacterial pathogen associated with serious public health and food safety problems. It is an intracellular microbe that causes *Listeriosis*, a life-threatening disease that yields to bacteremia, meningitis, and spontaneous miscarriage. This study aimed to conduct the survey and investigate the prevalence and antibiotic susceptibility of *L. monocytogenes* from butcheries (village & commercial) and supermarkets beef plate meat in the Vhembe district. Evaluation of meat safety practices and hygiene among different butcheries and supermarkets was conducted using a questionnaire. The prevalence of *L. monocytogenes* in retail beef plate meat was determined on chromogenic agar and the antibiotics susceptibility was examined from *L. monocytogenes* isolates using the well diffusion method. The results of this study revealed that (i) some supermarkets followed the meat safety practices and hygiene, whereas there was a combination of good and unhygienic meat handling practices identified at commercial and village butcheries (ii) *L. monocytogenes* were identified in high numbers however some other species of *Listeria* such as *L. ivanovii* and *L. innocua* were found in beef plate meat (iii) The *Listeria* isolates were resistant to a wide range of antibiotics used to treat human *listeriosis* such as ampicillin, tetracycline and nalidixic acid in large numbers while a few of the isolates were susceptible to sulphamethoxazole and intermediary responsive to oxytetracycline and chlortetracycline. The findings from this study concluded that some supermarkets follow the safety procedures while commercial and village butcheries do not follow the procedures and regulations, furthermore, the prevalence of the pathogenic *L. monocytogenes* may harm the communities around Vhembe district. Moreover, treatment in case of an outbreak may become burdensome as the pathogen may be resistant to antimicrobial treatment. It is recommended that intervention through training on food safety to improve the hygienic practices of meat handling along the beef supply chain, more especially within commercial and village butcheries be conducted, also *L. monocytogenes* control measures from relevant authorities need to be put in place, meat must be prepared adequately, and antibiotics must be utilized appropriately. In addition, conducting molecular research on *Listeria* species to determine the virulence genes present in *L. monocytogenes* isolated from retail beef, to keep track of the type of strains of *Listeria* that may be circulating in the Vhembe district is recommended.

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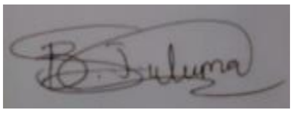
Declaration

I, Siluma Bridget, student number 11617404, hereby declare that the dissertation for the Master of Science degree in Food Science and Technology at the University of Venda, which is hereby submitted by me, has not been submitted previously for a degree at this or any other Institution, that it is my own work in design and execution, and all the references therein have been properly acknowledged.

Signature:  **Date: 28/03/2023**

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List of Acronyms

EU	European Union
US	United States
pH	Potential of Hydrogen
aW	Water Activity
DNA	Deoxyribonucleic acid
HIV/AIDS	Human Immune Virus & Acquired Immune Deficiency Syndrome
SAIMR	South African Institute for Medical Research
NaCl	Sodium chloride
RTE	Ready to Eat
PPE	Personal Protective Equipment
GMP	Good Manufacturing Practices
CFU/g	Colony Forming Units per gram.
EC	European Commission
ICMSF	International Commission on Microbiological Specifications for Food
MDR	Multi-Drug Resistance
NICD	National Institute of communicable diseases
DoH	Department of Health
DAFF	Department of Agriculture, Forestry & Fisheries
FCDA	Foodstuffs, Cosmetics, and Disinfectants Act
SANS	South African National Standards

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CHAPTER 1: INTRODUCTION

1.1. Background to the study

Listeria monocytogenes is a foodborne bacterial pathogen associated with serious public health and food safety problems. It is an intracellular microbe that causes *Listeriosis*, a life-threatening disease that yields to bacteremia, meningitis, and spontaneous miscarriage (Leong *et al*., 2016; Matle *et al*., 2019) and results in 20-30% of mortality (Tchatchouang *et al*., 2020). It can cause serious disease conditions in the elderly, pregnant, newly born, and immunocompromised individuals such as gastroenteritis and septicaemia. *L. monocytogenes* is widely spread in the environment and grows at refrigeration temperatures and harsh conditions of pH (up to 4.7) and salt (up to 10%) (Leong *et al.*, 2016).

Outbreaks of *Listeriosis* are often linked to various foods such as milk, pork, fish, sausage, cheese, ice cream, fruits, and vegetables (Al-mashhadany *et al*., 2016). However, contamination of meat by *L. monocytogenes* has significantly increased and is reported worldwide in various countries such as Canada, the European Union, and the United States (Holley & Cordeiro, 2014, Adikwu *et al.*, 2016). The first reported case of *Listeriosis* outbreak in South Africa was in January 2017, about 945 cases of the disease were confirmed by the South African National Institute of Communicable Diseases (NICD) in February 2018, and 176 of those cases resulted in fatalities (a case fatality rate of 19%) (Ogunbanjo, 2018)

Beef can easily be contaminated by microorganisms owing to its rich nutrient content (Saraiva *et al*., 2018). Moreover, the intrinsic characteristics of beef, such as pH 5.5-6.2 and water activity also account for general susceptibility to spoilage by microorganisms (So-Yeon *et al*., 2018). Furthermore, microbial contamination of beef can occur at any level from slaughtering, packaging, processing, distribution, and storage; therefore, safety measures for beef are of primary concern (So-Yeon *et al.*, 2018). Most literature on the prevalence of *L. monocytogenes* associated with beef meats was on raw and cooked ground beef (Harmayani *et al.*,1993), wine-marinated beef, dry-cured beef, and beef stored at refrigeration temperatures (Saraiva *et al.*, 2016; Gök *et al.*, 2019, Vasilijević *et al.*, 2019.

Antimicrobial resistance profiles of *L. monocytogenes* were conducted by Van Nierop *et al.* (2005), and Maung *et al.* (2019) on chicken meat. Despite all these studies, no study has been done with respect to the prevalence of *L. monocytogenes* on raw beef plate meat, and its antibiotic susceptibility in the Limpopo province. Beef plate meat consumption is increasing due to its high nutritional value, and it is a relatively cheap meat portion.

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Available data on the role of meat as a potential carrier of *Listeriosis* in Africa is scanty (Matle *et al.*, 2020; Dufailu *et al.,* 2021). In addition, the prevalence studies on *L. monocytogenes* have been documented in other regions of the country such as the Gauteng, Mpumalanga, and Eastern Cape provinces (van Nierop *et al.,* 2005; Christison *et al.,* 2008; Odjadjare & Okoh, 2010). Little information is documented on the prevalence, meat safety practices, and antibiotic susceptibility of *L. monocytogenes* in the Vhembe district, Limpopo province.

This study aimed to investigate the hygiene practices in butcheries and the prevalence of antibiotics resistant *L. monocytogenes* in retail beef meat in the Vhembe district of Limpopo province, South Africa.

1.2. Problem statement

In the Vhembe district of Limpopo province of South Africa, retail meat such as raw beef from butcheries serve for nutritional and survival purposes and is widely consumed by the populace. Due to the affordability of the meat from retail butcheries, many people tend to patronize the butcheries. Despite this, butcheries don't always have the right infrastructure, environmental hygiene, and meat safety practises. This often results in cross contamination of meat with foodborne pathogens such as *L. monocytogenes*, that may cause foodborne outbreaks. The recent foodborne outbreak has been reported on ready-to-eat foods such as Polony and Vienna in the province and resulted in a significant mortality rate (Dallagi *et al*., 2023). To safeguard the health of the public, hygiene practises in butcheries need to be emphasized to prevent microbial contamination of beef meat at slaughtering, processing, storage, and distribution level.

1.3. Aim of the study

This research aimed to investigate the hygiene practices in butcheries and the prevalence of antibiotics resistant *L. monocytogenes* in retail beef meat in the Vhembe district of Limpopo province, South Africa.

1.4. Specific Objectives

- To investigate hygiene practices in butcheries
- To determine the prevalence of *L. monocytogenes*
- To determine the antibiotic susceptibility of *L. monocytogenes* meat isolates.

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1.5. Hypotheses

1.5.1 Null hypothesis

- The meat safety practices and hygiene among butcheries and supermarkets in the Vhembe district are good.
- Raw beef plate meat in the Vhembe district does not contain the pathogenic micro-organism *L. monocytogenes* which may not pose a threat to public health.
- Raw beef plate meat in the Vhembe district will not be contaminated with antimicrobial resistant *L. monocytogenes.*

1.6. Dissertation outline

This study consists of six (5) chapters and each of the chapters has been arranged to address each objective of the dissertation, Chapter one (1) gives an introduction, problem statement, objectives, and hypotheses of the study. Chapter two (2) provides an overview of the literature review of *L. monocytogenes,* the genus *Listeria,* the occurrence of *L. monocytogenes* in food, the antibiotic resistance to *L. monocytogenes,* the pathogenesis of *L. monocytogenes* and its diagnosis. The chapter also covers signs and symptoms of infections caused by *L. monocytogenes*, treatment of listeriosis, meat safety practices, and hygiene, regulations relating to *L. monocytogenes,* and the control of *L. monocytogenes*.

Chapter three (3) discusses the survey conducted on meat safety practices and hygiene in various supermarkets and butcheries in the Vhembe district. Chapter four (4) discusses the prevalence and antibiotic susceptibility of *L. monocytogenes* in retail beef meat. Chapter five (5) gives the final conclusion and recommendations of the outcomes of the research for every particular objective researched.

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CHAPTER 2: LITERATURE REVIEW

2.1. Genus *Listeria*

2.1.1 Listeria species

The genus *Listeria* is classified under the family *Listeriaceae* (Orsi & Wiedmann, 2016), found in the phylum *Firmicutes*, class *Bacilli,* and order *Bacillales* (Matle *et al*., 2020). It consists of 17 species such as *L. monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. welshimeri, L. grayi*, and characterized more recently *L. marthii, L. rocourtiae, L. weihenstephanensis and L. fleischmannii, L. floridensis, L. aquatica, L. cornellensis, L. riparia, L. grandensis, L. booriae and L. newyorkensis* (Figure 1). This entails the nine (9) *Listeria* species which was described since 2009 (Orsi & Wiedmann, 2016; Tchatchouang *et al*., 2020), and possesses phenotypic characteristics like the ability to grow at low temperature and flagella motility for *Listeria.*

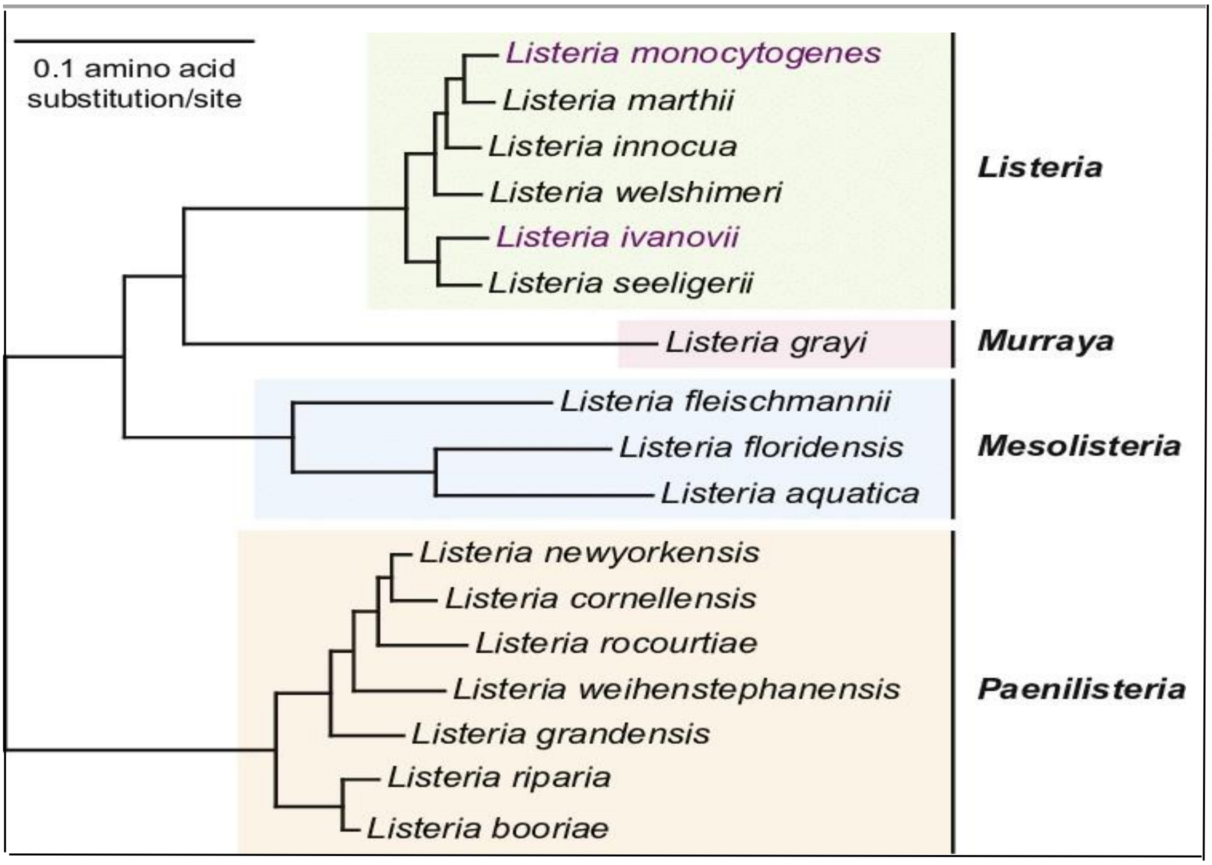

Figure 1: Phylogenetic tree of *Listeria* species (Lebreton *et al*., 2016).

This group is called *Listeria Sensu Strictu* and includes the pathogen *L. monocytogenes* (Orsi & Wiedmann, 2016). The remaining eleven (11) species are divided into three (3) different monophyletic groups and should be considered a separate genus. Apart from L. grayi, these three suggested species are non-motile, nitrate-reducers, and negative for the Voges-Proskauer test. They also do not contain any harmful species. This group is termed *Listeria Sensu Lato* (Orsi & Wiedmann, 2016).

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The sole pathogenic species implicated in several invasive infections in humans and animals, particularly in immunocompromised people, were formerly thought to be *L. monocytogenes* and *L. ivanovii* (Goudar & Prasad, 2020). However, currently, people are getting diagnosed with additional *Listeria* species such as *L. welshimeri* and *L. innocua*. (Okorie-Kano *et al.*, 2020). Although *L. ivanovii* is primarily associated with ruminants and is known to be harmful to animals, sporadic occurrences of Listeriosis in humans have been linked to *L. seeligeri* and *L. welshimeri* (Matle *et al*., 2020).

2.1.2 Morphology of Listeria monocytogenes

Listeria monocytogenes is Gram-positive, rod-shaped, and non-spore-forming with a diameter of 0.5 µm and 1–2 µm in length (Tchatchouang *et al.*, 2020). In the Gram staining of *L*. *monocytogenes,* as the culture ages, it becomes variable, however, in direct smears of Gram staining, the organism may appear coccoid-like and is produced between 20°C and 25°C but not at 37°C. This pathogenic bacterium is catalase-positive and oxidase negative and it produces a b-hemolysin, which is useful in the CAMP diagnostic test (Batt, 2014).

Over 50 different species of mammals, birds, rodents, and fish have all been used as sources for *L. monocytogenes* isolation. Worldwide, *L. monocytogenes* has been detected in farm animal excrement, soil, water, and plants (Gartley *et al*., 2022). Moreover, it has been identified in biofilms attached to the surfaces of machinery and pipes (Mcmullen & Freitag, 2015; Rodriguez-Lopez *et al*., 2018). *L. monocytogenes* outbreaks involving single strains have been documented in food processing facilities. (Smith *et al*., 2019) due to the pathogens capability to infiltrate food supplies before food is processed or during food processing and packaging as indicated in Figure 2 (Mcmullen & Freitag, 2015).

Human illnesses occur from consumption of contaminated food sources, including raw seafood and meat, unpasteurized milk products, and raw vegetables (Zakir *et al*., 2022). Contamination in meat has been suggested to originate from live animals, as they harbour *L. monocytogenes* in animal faeces, tonsils, and hides (Ndahi *et al.*, 2013), moreover the consumption of contaminated silage by ruminants is most likely what causes *Listeriosis* to spread in domesticated mammals like cattle, sheep, and goats (Al-Mashhadany *et al*., 2016).

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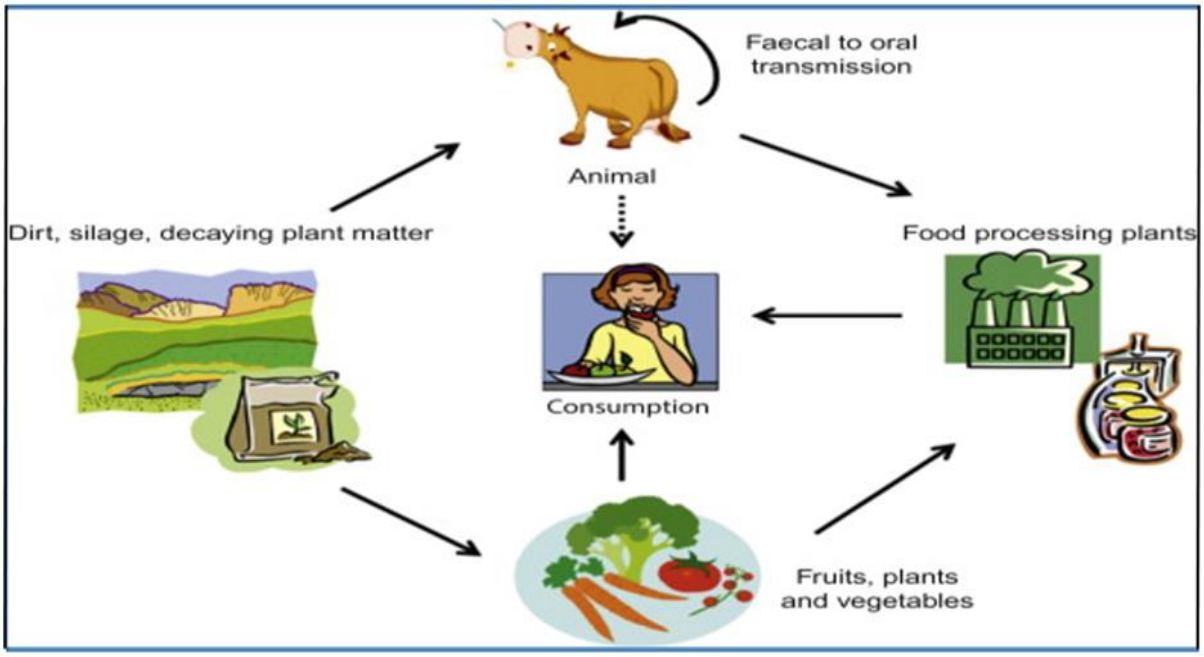

Figure 2: *Listeria monocytogenes* infiltrating food supplies before food is processed or during food processing and packaging (Mcmullen & Freitag, 2015).

2.1.3. Growth and survival characteristics of Listeria monocytogenes

Listeria monocytogenes grow best at temperatures between 30°C and 37°C, with a range of 1.5 to 45°C being optimum*. L. monocytogenes* is considered not heat resistant because it can be removed in milk through pasteurization (Magalhaes *et al.,* 2014). It is psychrotrophic, exhibiting its characteristics as an opportunistic pathogen and a commensal of the stomach at 37°C. The bacteria are resistant to Sodium chloride (NaCl) and capable of thriving in environments with up to 10% salt as indicated in Table 1 below and can endure concentrations of up to 20–30% of salt. It can continue to grow in a pH range from 4.6 to 9.2 since this organism is not inhibited by carbon dioxide, it can withstand numerous processing methods, including freezing and drying (Rees *et al*., 2017). Because of these growth conditions, *L. monocytogenes* is more able to flourish in the harsh environments present in food-processing plants, which poses significant issues for the food industry (Matle *et al*., 2020).

Listeria monocytogenes has a peritrichous flagellum that can move when grown at 20–25°C but not at 37°C (Magalhaes *et al.*, 2014). Most *Listeria* species are identical phenotypically thus biochemical assays, such as the haemolysis test, the formation of acid by D-xylose, mannitol, L-rhamnose, motility, and alpha methyl-D mannoside are utilised to distinguish *Listeria* species (Orsi *et al.* 2011; Carlin *et al.,* 2021). It cannot hydrolyse urea, gelatin, or casein, but it can hydrolyse aesculin and sodium hippurate. When L-rhamnose is introduced to the culture medium, acid is produced; however, not with D-mannitol and D-xylose.

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Through examination of group-specific surface proteins, such as somatic (O) and flagellar (H) antigens, at least 13 serotypes were discovered. These include serotypes such as 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7 of *L. monocytogenes* (Magalhaes *et al.*, 2014). Most human cases of *Listeriosis* are associated with serotype 4b, 1/2a and 1/2b (McMullen & Freitag, 2015).

Table 1: Limits for growth of *L. monocytogenes* under ideal laboratory conditions

Parameter	**Minimum**	**Optimum**	**Maximum**
Temperature (ºC)	-1.5	30- 37	45
pH	4.0	6.0- 8.0	9.6
Sodium chloride (%)	_	_	10
$a_{w/}$	0.90	0.97	_

(Magalhaes *et al.,* 2014)

2.2. Occurrence of *Listeria monocytogenes* in food

In numerous clinical occurrences of listeriosis in humans, food of animal origin has been implicated. Milk, pork, fish, and sausage have all been reported to have *L. monocytogenes* (Maktabi *et al*., 2015; Teixeira *et al.*, 2019). This bacterium has also been discovered in various processed foods, including cheese and ice cream, as well as processed meats such as polony due to post-processing contamination (Al–Mashhadany *et al.*, 2016). In addition, these bacteria are sometimes isolated from fresh fruits and vegetables (Ajayeoba *et al.*, 2015; Zhu *et al.*, 2017).

The source of contamination in meat has been suggested to be live animals, as they harbour *L. monocytogenes* in animal faeces, tonsils, and hides (Ndahi *et al.*, 2013), however, they acquire contamination through the slaughterhouse environment and procedures used (Maktabi *et al*., 2015). The consumption of contaminated silage by ruminants is most likely what causes *Listeriosis* to spread in domesticated mammals like cattle, sheep, and goats. It has been reported that the contamination of meat carcasses originates from the slaughterhouse due to unhygienic conditions (Al–mashhadany *et al.*, 2016). Sometimes contamination is due to items being displayed uncovered and furthermore subjected to handling by customers. Products purchased in markets, especially meat, may reflect human and environmental contamination at the time of sale (Ndahi *et al.*, 2013, Ohue *et al.*, 2015).

The distribution of *L. monocytogenes* from different retail meats is not uniform. In a study conducted by Barros *et al.*, (2007), the prevalence reported was 12.6% of *L. monocytogenes* in beef meat in Brazil. On the other hand, 22.9% was reported on red meat in Yemen (Al-mashhadany *et al.*, 2016). In addition, Zadernowska *et al.* (2017) reported a 17% prevalence of *L. monocytogenes* on raw meat (beef, duck, chicken, turkey, pork) in Poland and, In

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Brazil, Teixeira *et al.*, (2019) reported a 12% prevalence of *L. monocytogenes* in export-approved beef, whereas Chuku *et al.* (2019) reported 58.2% prevalence of beef in north-central Nigeria. With these reports alone, the theoretical view that retail beef meat is one of the most prominent vehicles of *L. monocytogenes* is clearly indisputable.

2.3. Antibiotic resistance of *Listeria monocytogenes*

Because *Listeria* species were sensitive to a wide range of antibiotics, the infections they produced were thought to be manageable. However, due to antimicrobial resistance, particularly multidrug resistance, treating these infections has recently grown more challenging (Okorie-Kanu *et al.*, 2020). Regardless of the administration of appropriate antimicrobial agents, *L. monocytogenes* still results in a death rate of 20-30% (Obaidat *et al*., 2020) which in comparison is higher than other food-borne pathogens (Hernandez-Milian & Payeras-Cifre, 2014). Bacterial resistance to antibiotics has evolved due to the overuse of drugs in animal husbandry and the overprescription of antibiotics in clinical settings (Fair & Tor 2014; Van *et al.,* 2020). Microbial agents are used in veterinary fields for fish, farm animals, and pets. They are also utilised in food-producing animals to treat illnesses, prevent them from occurring, and promote the animals' growth and feeding effectiveness (Economou & Gousia, 2015).

The literature demonstrates that several antibiotics, including nalidixic acid, oxacillin, tetracycline, gentamicin, penicillin, ampicillin, streptomycin, erythromycin, kanamycin, sulphonamide, trimethoprim, and rifampicin, are resistant to some strains of *L. monocytogenes* (Terzi *et al.*, 2015). Resistance to tetracyclines is one of the most regularly seen resistance traits in *L. monocytogenes* isolates. (Sanlibaba *et al*., 2018). Tetracyclines are widely utilized in animal diets and are noted as the second-most used antibiotics worldwide (Granados-Chinchilla & Rodriguez, 2017). In the gastrointestinal system of domestic animals, where both species coexist and if sub-inhibitory doses of tetracycline are anticipated, *L. monocytogenes* can transmit resistance to one another. (Morobe *et al.*, 2012). Escalating antibiotic resistance has necessitated the need for antibiogram studies of *L. monocytogenes* isolates with an aim to keep track of any emerging resistant strains (Kayode & Okoh, 2022).

In some of the studies conducted on retail meat, the antibiotic resistance of *Listeria* from raw meat (Pork, beef, & chicken) in Nigeria was reported by Okorie-kanu *et al.*, (2020) that more than 65% of the isolates were resistant to penicillin, rifampicin, ciprofloxacin, sulfamethoxazole-trimethoprim, and cephalothin. Some of the isolates from the meats were resistant to equal to or more than three classes of antibiotics (multi-drug resistance). Adikwu

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et al. (2016) reported that all isolates of *L. monocytogenes* isolated from beef, pork, and chicken were susceptible to erythromycin, gentamycin, cotrimoxazole, and chloramphenicol but resistant to augmentin, amoxicillin, tetracycline, and cloxacillin in Makurdi Metropolis, Nigeria. Sanlibaba *et al.* (2018) reported resistance to ready-to-eat (RTE) foods. Each isolate exhibited at least three different antibiotic classes of resistance. An elevated *L. monocytogenes* prevalence in the examined RTE foods poses a serious concern to the public's health. Antibiotic resistance may result in difficulties in treatment especially for the high-risk group patients (Shamloo *et al*., 2019).

Over the last decade, an increasing number of *L. monocytogenes* isolates that are antimicrobial resistant have been reported globally, demonstrating that *L. monocytogenes* rapidly acquire a variety of antibiotic resistance genes (Aras & Ardic, 2015). According to Aras & Ardic (2015), mobilizable plasmids, conjugative transposons, and self-transferable plasmids of other bacterial species, such as *Staphylococcus* and *Enterococcus* species, are the main causes of antimicrobial resistance in *L. monocytogenes*. Moreno *et al*. (2014), report on *L. monocytogenes* which developed resistance through spontaneous mutations become more prevalent. Siu *et al*. (2003) reported that the overexpression of endogenous genes, such as those that encode the lactamase AmpC gene, is a potential consequence of mutations that occur at the promoter and operator regions (Matle *et al*., 2020). Antimicrobial target sites that are resistant to antimicrobials are caused by point mutations in the genes encoding those antimicrobial target regions. This mutation has also been seen in the gyrase gene which results in the development of a fluoroquinolone-resistant gyrase enzyme (Hopkins *et al.,* 2007; Saleh *et al*., 2022).

Olaimat *et al.* (2018) concluded that the most probable route of transmission of antibiotic-resistant strains from animals to humans is through the food chain. During the food production process, *L. monocytogenes* frequently meets low concentrations of antibiotics along with other antimicrobials. This pre-exposure adaptation enables *L. monocytogenes* to withstand larger concentrations of antimicrobial agents. Antibiotic-resistant pathogens thereby spread to other bacteria of human clinical significance, making meat contaminated with these organisms a serious threat to both public health and the economy (Gómez *et al.*, 2014; Matle *et al.*, 2019).

2.4. Pathogenesis of *Listeria monocytogenes*

The pathogenicity of *L. monocytogenes* is dependent on the expression of various genes, which oversee the organism's ability to enter, multiply, and spread across cells (Tchatchouang *et al*., 2020). Actin, which is encoded by the actA gene, internalin, which is

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encoded by the inlA, inlB, inlC, and inlJ genes, invasion-associated protein, encoded by the iap gene, listeriolysin O, encoded by the hlyA gene, phosphatidylinositol phospholipase C, encoded by plcA gene, and virulence regulator, encoded by the prfA gene are all different determinants of Listeria virulence that are reported to be factors involved in the pathogenicity of *L. monocytogenes* (Jamali *et al.*, 2015; Owusu-Kwarteng *et al.*, 2018).

L. monocytogenes produces hemolysin, which is a key component in the activation of human neutrophils. In addition, it also produces the exotoxin listeriolysin (LLO), which is essential for the intracellular growth of the organism and T-cell recognition (Sun & Liu, 2013). *L. monocytogenes* produces phosphatidylinositol-specific phospholipase C (PI-PLC) and phosphatidylcholine-specific phospholipase C (PC-PLC), which together make up two hemolysins. Unlike the LLO, which lyses host cells by pore creation, these two virulence factors work by disrupting the lipids in the membrane. The bacterium also produces a zinc-dependent protease that functions as an exotoxin (Dawlat, 2016). The infection by listeria monocytogenes is summarised in figure 3 below.

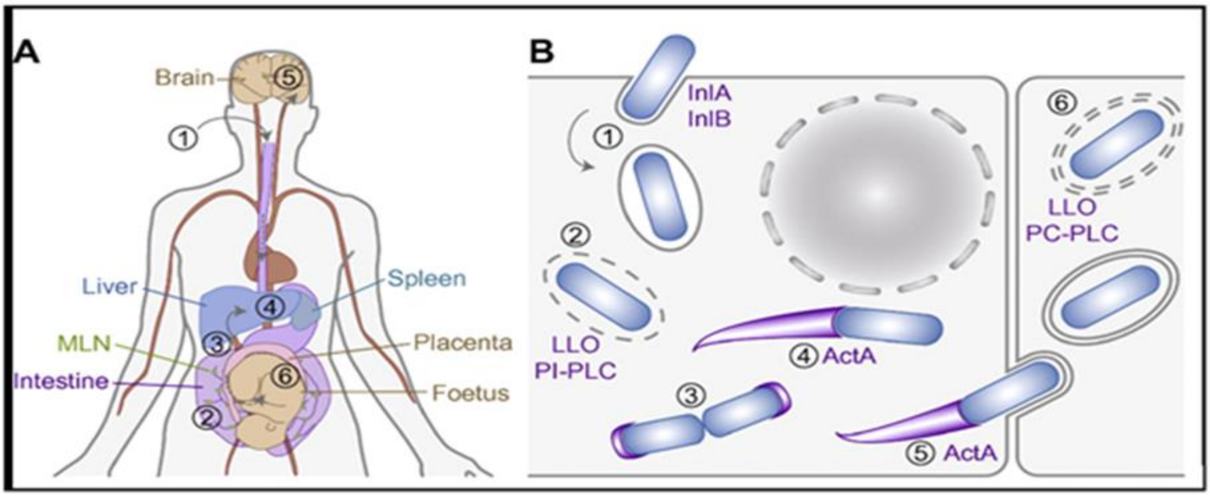

Figure 3: Diagrammatic presentation of Infection by *L. monocytogenes* (Cossart & Lebreton, 2014).

The estimated infectious dosage required for listeriosis to develop in susceptible individuals is 0.1 to 10 million colony-forming units (cfu), while in healthy individuals it is 10 to 100 million cfu (Matle *et al*., 2020). In figure (A), the infection process is described. Following consumption of food that has been contaminated, (1) bacteria colonise the gastrointestinal tract by overcoming the intestinal barrier, and (2) pass via the mesenteric lymph nodes (MLN) to enter the systemic circulation. (3) They predominantly target organs in the systemic circulation, such as the liver and spleen (4), which serve as reservoirs for bacterial persistence if immune responses are unable to control the infection. Bacteria enter the bloodstream and yield to septicaemia. In some instances, *L. monocytogenes* penetrates the blood-brain barrier and accesses the brain (5), resulting in meningitis or encephalitis. It

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passes the placental barrier in pregnant women (6) and can result in abortion or new-born infection (Cossart & Lebreton, 2014).

B) Provides *L. monocytogenes'* intracellular life cycle. (1) *Listeria* penetrates host cells through a zipper mechanism that depends on surface InlA and InlB interacting with the appropriate cell surface receptors E-cadherin and Met (2) The pore-forming toxin Listeriolysin O (LLO) and phosphatidylinositide phospholipase C (PI-PLC), which are secreted effectors, cause the endocytic vacuole to burst. (3) The bacteria then replicate in the cytosol, benefiting from the cytosol's resources. By recruiting the Arp2/3 complex, the bacterial surface protein ActA induces the polymerization of cellular actin. Actin comet tails are created as a result, allowing the bacteria to move inside cells (4) and (5) and spread from cell to cell. (6) LLO and phosphatidylcholine-specific phospholipase C (PC-PLC) are the key mediators of the two-membrane vacuole's rupture (Amenu, 2013; Cossart & Lebreton, 2014).

2.5. Diagnosis of *Listeria monocytogenes*

The diagnosis of a *Listeria* infection might be challenging at times; however, the most crucial aspect of diagnosing *L. monocytogenes* is to have a suspicion of the bacteria's presence and to take that into consideration (Hernandez-Milian & Payeras-Cifre, 2014). It is a normal human intestinal tract inhabitant and research conducted on faecal samples reported that 5-10% of the general population are carriers of *L. monocytogenes*. Therefore, healthy people frequently have antibodies to *Listeria* species (Hernandez-Milian & Payeras-Cifre, 2014). Therefore, the presence of *L. monocytogenes* in faecal samples is not an indicator of infection due to the high rate of clinically healthy carriers. *Listeriosis* is classified as pathogenic if the organism is isolated from the placenta, blood, cerebrospinal fluid, and foetus in cases of abortion (Morobe *et al*., 2012; Wang *et al*., 2021) It is stated that the prevention of illness and management of *L. monocytogenes*, isolation, and identification is crucial. The method for the detection of *L. monocytogenes* has evolved from the cold enrichment technique to conventional and molecular techniques over time (Matle *et al*., 2020).

Välimaa *et al*. (2015) reported that the traditional approach of isolating *L. monocytogenes* entails antibody-based tests, enzyme-linked immunosorbent assays, culture-based procedures, and immune-capture techniques. Among these, culture-based tests are typically favoured over other methods because they are sensitive, affordable, and still the "gold standards" when compared to other methods that have been established (Barajas *et al*., 2019). The disadvantages of culture-based methods are the low resolution while separating bacterial strains and that the procedures are also time-consuming (Leong *et al*., 2014). Using

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culture-based techniques, *L. monocytogenes* can be isolated and identified using selecting agents and an enrichment method. While the enrichment process enables the growth of *L. monocytogenes* to detectable levels and the repair of injured or stressed cells, the selective agents inhibit other competing microflora (Law *et al*., 2015; Chen *et al*., 2017).

Due to international regulations and rules, there are three regularly utilized culture-based methods for isolating L. monocytogenes in foods. This includes The United States Department of Agriculture (USDA), the International Standard (ISO), and the One-Broth *Listeria* technique (Law *et al*., 2015; Matle *et al*., 2020).

2.6. Signs and symptoms of infections caused by *Listeria monocytogenes.*

Consumption of food contaminated by *L. monocytogenes* often results in a life-threatening disease, *Listeriosis*. The manifestation of *Listeriosis* differs from person to person depending on the state of health of the individual at the point of infection (Goudar & Prasad, 2020). In healthy individuals, it is asymptomatic or may cause febrile diarrhoea. However, in immunocompromised individuals, the micro-organism causes invasive *Listeriosis* which manifests in three major platforms: bloodstream infection, the central nervous system, and foetal *Listeriosis* (Hernandez-Milian & Payeras-Cifre, 2014) as displayed in Figure 4.

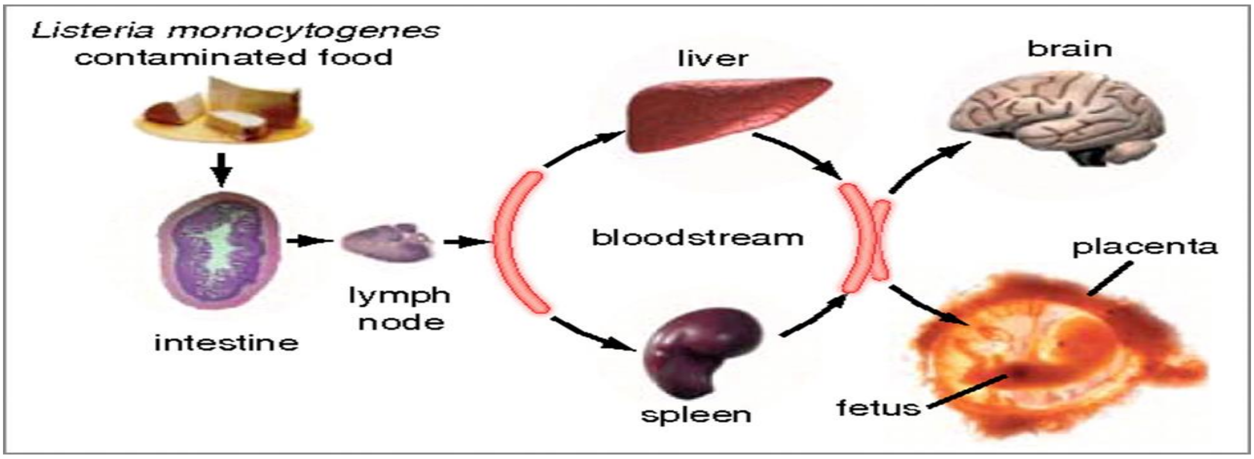

Figure 4: Three major platforms of invasive *Listeriosis*: bloodstream infections, infection of the central nervous system, and maternal foetal *Listeriosis* (Cossart, 2011).

The onset of *Listeriosis* begins with mild, influenza-like symptoms such as persistent fever, nausea, vomiting, and diarrhoea (Morobe *et al*., 2012). However, invasive *Listeriosis* displays additional characteristics such as headaches, stiff neck, confusion, and loss of balance and may result in death for the high-risk group population (Goudar & Prasad, 2020). The duration for severe forms of *Listeriosis* is a few days to 3 weeks, whereas the period to develop gastrointestinal symptoms is only greater than 12 hours. Severe *Listeriosis* results in a high mortality rate of 30% (Morobe *et al*., 2012).

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The infective dose required for listeriosis to develop in susceptible individuals is 0.1 to 10 million colony-forming units (cfu), while in healthy individuals it is 10 to 100 million cfu (Matle *et al*., 2020). In a case where *Listeriosis* is acquired in early pregnancy, abortion, stillbirth, or premature delivery of the newborn may result. When acquired in late pregnancy, it is trans-placentally transmitted and causes neonatal *Listeriosis* (Morobe *et al*., 2012). They yield a variety of diseases such as meningitis, septicaemia, and encephalitis. Individuals at elevated risk are those who are particularly immunosuppressed such as HIV/AIDS & cancer patients, newborn babies, low stomach acidity patients, those who abuse drugs, the elderly, and patience placed on corticosteroid therapy (Batt, 2014; Tchatchouang *et al*., 2020).

2.7. Treatment of *Listeriosis*

Antibiotics are natural or synthetic materials used to treat or prevent infections and can inhibit the growth of microorganisms(bacteriostatic) or kill bacterial cells (bactericidal) (Founou *et al*., 2016). They inhibit cell walls, proteins, or DNA synthesis and are classified into diverse groups based on the chemical structure or mechanism of action (Olaimat *et al.*, 2018). A β-lactam antibiotic which is ampicillin or penicillin is the treatment of choice for *Listeriosis.* In a case of an immunocompromised patient, it is combined with an aminoglycoside like gentamycin. For patients who are allergic to penicillin or ampicillin, a second choice of therapy is administered which is a combination of trimethoprim and a sulphonamide such as Sulphamethoxazole (Ohue *et al.*, 2015). Pregnant women are preferably treated using erythromycin. However, rifampicin, tetracycline, chloramphenicol, and fluoroquinolones can also be used to treat *Listeriosis* (Olaimat *et al.*, 2018). The benefit of starting treatment early for pregnant patients treated with antibiotics for *L. monocytogenes* is that it can prevent infection to the foetus or new-born (Janakiram, 2008; Wang *et al*., 2021).

Apart from tetracycline, erythromycin, streptomycin, cephalosporin, and fosfomycin, isolates of *L. monocytogenes* and other strains of *Listeria* species are often responsive to a broad spectrum of antibiotics (Morobe *et al.*, 2012; Ohue *et al.*, 2015; Noll *et al.,* 2018). This might be because of *L. monocytogenes'* inherent resistance to these drugs, and a lack of affinity for the enzyme catalysing the last step of the bacterium's cell wall synthesis (Matle *et al*., 2020). Due to a lengthy incubation period of *L. monocytogenes*, which causes treatment to differ depending on the stage of the illness and the duration of treatment, listeriosis treatment is frequently ineffective (Buchanan *et al*., 2017; Matle *et al*., 2020), and since there is currently no commercially available vaccine to prevent *Listeriosis*, early detection is

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a prerequisite for the effectiveness of antibiotic therapy, especially for those in high-risk groups (Olaimat *et al.*, 2018).

The ability of a microbe to endure an antibiotic concentration used in medical care and alter its reaction to the antibiotic is known as antibiotic resistance. One of the main risks to food development, food security, and global public health is this, because it makes it harder to treat diseases (Olaimat *et al.*, 2018). Antibiotic resistance complicates treatment and lengthens the illness (Adikwu *et al.*, 2016). The response of microorganisms to antibiotics varies due to inherent differences or the development of resistance by genetic exchange or adaptation (Reygaert, 2018).

2.8. Meat hygiene practises at butcheries

2.8.1. Meat handlers' hygiene practices

Meat handlers' poor personal hygiene often operates as a vector for the spread of microbes from their hands, wounds, lips, skins, and hairs to meat and butchery surfaces (Wambui *et al*., 2017; Ebuete *et al.,* 2020). Approximately 97% of foodborne-disease outbreaks among consumers are caused by inappropriate food handling and worker personal hygiene, which has resulted in death in some circumstances (Nyamakwere *et al*., 2017). If good sanitation and hygiene measures including hand washing, wearing protective clothes, cleaning, and sanitizing butchery equipment and utensils are not practiced, microbial contamination can occur which will lead to meat quality deterioration, and post-harvest meat losses (Chepkemoi *et al*., 2015; Siluma *et al.,* 2023).

Contaminated tables, hooks, logs, meat chopping boards, weighing scales, knives, and other equipment and utensils can contribute to the contamination of meat by food handlers, in addition, contact with insects, polluted air, and other butchery workers increase the level of contamination (Ntanga *et al*., 2014). Meat handlers must aim to reduce microbial contamination by improving hygienic practices during preparation, distribution, storage, and retail sales (Gutema *et al*., 2021). For health and safety reasons, it is essential to always wear protective gear and wash hands before and after handling meat (Ntanga *et al*., 2014). The wearing an apron or gown during meat handling is an important practice that can prevent the contamination of meat by pathogens (Nel *et al*., 2004; Sulleyman *et al*., 2018; Siluma *et al*., 2023).

Hand washing has been regarded as the main method of preventing the spread of pathogens, and if done correctly it can decrease outbreaks of foodborne illnesses (Assefa *et al*., 2015). According to a study done by Tegegne & Phyo (2017) on the food safety knowledge, attitude, and practices of meat handlers in abattoirs and retail meat shops of

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Jigjiga town in Ethiopia, respondents correctly answered questions on hand washing, however, they did not strictly adhere to food hygiene practices. Also, in a study conducted by Kunyanga *et al*. (2021) in Kenya to determine the meat quality status and postharvest handling practices along the meat value chain, infrequent washing of hands was reported and identified as one of the unhygienic practices in retail butcheries. It was recommended that meat handlers should get ongoing education to improve their meat hygiene practices thereby gaining a deeper understanding and a positive attitude towards meat hygiene practices (Tegegne & Phyo, 2017).

A similar study was conducted by Chepkemoi *et al*. (2015) In Isiolo and Nairobi Counties, respectively, 60% and 34% of operators cleaned utensils by wiping them with a reused cloth, Tegegne & Phyo(2017) also reported that 53% of the workers thought that the same towel can be used to clean many places, these practices could result in cross-contamination in the working environment and therefore pose a risk to the consumer. Bersisa *et al*. (2019) reported that 64.5% of workers wore jewelry during working hours. Wearing jewelry should be discouraged among meat handlers. Microorganisms and dirt can accumulate around such items and easily fall into the meat, posing a risk of body contamination to meat (Bersisa *et al.,* 2019). The skin beneath the jewellery provides a favourable environment for contaminated microbes to flourish, jewellery is a possible source of germs (Upadhayaya & Ghimire, 2018).

Low levels of hygienic knowledge, improper handling of paper cash, and poor butcher shop hygiene are key contributors to the contamination of meat (Atlabachew & Mamo, 2021). Balcha & Gebretinsae (2013) reported that workers handled money while simultaneously handling food in butcheries located in Mekelle City, Ethiopia. Cash is commonly used to exchange products and services in countries all around the world because these notes are exchanged often and in many types of business, cash provides a vast surface area for pathogens to thrive (Bersisa *et al.,* 2019).

2.8.2. The cleanliness of protective clothes at butcheries

The practice of wearing protective clothing is important to protect both the meat handler and the meat from cross-contamination by foodborne pathogens (Nel *et al*., 2004). According to a study by Mirembe *et al*. (2015), In the butcheries, workers wore their own personal clothes. This practice raises the risk of meat contamination as meat handlers have been identified as possible sources of contamination. In a study conducted by Kyayesimira *et al*. (2012), in Uganda on microbial quality of beef and hygiene practices in small and medium slaughterhouses and butcheries, observations revealed that protective clothing used by various individuals were filthy, despite that the carcass comes into contact with them during

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meat transportation. This is a hazard risk as microorganisms thrive in filthy protective garments and cause microbial contamination to meat.

Similarly, a study conducted in Chitwan, Nepal by Khanal & Poudel (2017) on factors associated with meat safety knowledge and practices among butchers of Ratnanagar Municipality reported that 98 butchers wore an apron but in 51 cases it was dirty. In addition, Gutema *et al*. (2021) assessed hygienic practices in beef cattle slaughterhouses and retail shops in Bishoftu, Ethiopia, and reported that 85% of workers use the same coat for the entire day. These are sub-standard practices of handling meat and can lead to contamination and cross-contamination of meat resulting in food-borne outbreaks.

To achieve the aim of protecting the public from hazards of foodborne infections, butcher shops must educate and promote proper sanitation and meat handling procedures (Haileselassie *et al*., 2013), Person's handling meat should be trained and instructed to at least a minimum degree of knowledge and skills for hygienic meat practices (Oyeleke, 2015).

2.8.3. Maintaining hygiene at butcheries

The reality is that surfaces in butcher shops, such as chopping boards, are never cleaned other than scraping off dirt with a knife, which acts as a micro-habitat for the microorganism (Kyayesimira *et al.,* 2012). Meat contamination may be avoided if the disinfection of butchery facilities could be done on a regular basis (Mirembe *et al*., 2015) and hygiene and sanitation could be enhanced by implementing proper cleaning processes that include the use of detergents (Kyayesimira *et al.,* 2012). Meat contamination may be prevented if proper cleaning, sanitation, and maintenance could be done both during and between operational hours. Also, separate chambers should be created for different tasks in slaughterhouses, such as cleaning alimentary tracts, storing hide and skin, dressing, and chilling carcasses. Furthermore, providing the required materials for handwashing, cleaning, and sanitation might help to maintain hygiene and sanitation (Oyeleke, 2015).

2.8.4. The displaying of meat at butcheries

Meat for human consumption in developing countries is accepted based on visual inspection, rather than systematic microbiological testing (Gutema *et al*., 2021). Khanal & Poudel (2017) reported that meat was exhibited openly in all retail stores, with no cooling or cover, exposing it to dust particles and household flies. This practice can result in cross-contamination and result in pathogenic diseases transmitted through meat.

2.8.5. Facilities in butcheries

Butchers serve a critical role in ensuring that meat is available for purchase by consumers of all economic classes and budgets**.** They are vital to public health because of the significant

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role they play in assuring meat safety, in addition, butcheries are a source of employment and money (Asuming-bediako *et al*., 2018). Butchers sell their products in specialized stores called butchery. The butcher may perform primary slaughter of meat and secondary slaughter to prepare fresh cuts of meat for sale. Butcheries may also sell related products, such as food preparation supplies, baked goods, and grocery items (Masanja, 2014; Siluma *et al*., 2023).

Due to a lack of adequate sanitation, infrastructure, and a lack of safe water in many parts of the developing country, accomplishing basic meat hygiene is challenging (Mirembe *et al*., 2015).To combat these challenges and guarantee that workers adhere to personal hygiene requirements, the environment in which they work must be of high quality in terms of facilities and equipment, and the workers themselves must be of good health and have good hygiene and sanitation habits (Nel *et al*., 2004; Jonathan, 2016).

2.8.6. Challenges facing the butcheries in maintaining meat safety.

Food safety refers to conditions and practices that prevent bacteria from contaminating foods and is still a serious public health problem across the globe (Tegegne & Phyo, 2017). Challenges facing butcheries include a hindrance to the use of proper sanitary procedures in the selling of meat because of the lack of infrastructure in butcheries. Lack of regular cleanliness, lack of quality beef production training and workshops for butchers, and a scarcity of modern processing equipment (Oyeleke, 2015). Low levels of hygienic knowledge, improper handling of paper cash, and poor butcher shop hygiene were all also key contributors to the contamination of meat and challenges facing butcheries (Atlabachew & Mamo, 2021).

2.8.7. Training of meat handlers on meat safety and hygiene practices

Identifying and gathering information on butchers' knowledge and existing preventive actions for meat safety is essential for the appropriate authorities to safeguard both the butcher and consumer from meat-borne diseases (khanal & Poudel, 2017). All participants in the food chain share responsibility for the nutritional value of meat. For the purpose of correcting the errors from farm to fork, abattoir workers, butchers, meat producers, suppliers, handlers, and the general public all need to be educated and trained in the prevention of foodborne illnesses (Ebuete *et al*., 2020).

Health extension workers and stakeholders should offer training or workshops for butchers on proper handling and hygiene procedures. As this would help improve the quality of beef produced and the training should be done regularly (Oyeleke, 2015). Aburi (2012) reported that almost 75% of butchers said they were willing to attend any meat hygiene training during the survey conducted in Juba town on hygiene practices used by small butchers and

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slaughter slabs in the beef value chain. In the report of Vaz *et al*. (2005), there were substantial differences immediately after the training course compared to before in a study conducted on a training course for butchers where both microbiological investigations and checklists were used. This result supports the theory that training was successful in enhancing overall food safety. To protect the population from food-borne bacterial diseases, it is necessary to educate and campaign for proper sanitation and meat handling practices in abattoirs and butcher shops, as well as a reduction in the number of backyard slaughters (Haileselassie *et al*., 2013).

2.9. Regulations relating to Listeria monocytogenes.

Listeria monocytogenes continues to pose a significant risk to human health and a hindrance to socioeconomic growth globally (Matle *et al*., 2020). In addition to foodborne pathogens, the food industry has other difficulties including food fraud and a lack of enforcement of laws, which are frequently brought on by the government's incapacity to adequately regulate the food sector. As a result, the risks to food safety keep growing (Boatemaa *et al.*, 2019). The food industry is protected against threats posed by foodborne pathogens by stringent laws that are enforced internationally in accordance with a set of microbiological standards. (Strydom 2015). Based on the bacterial load of a particular microorganism in the food, Microbiological Standards criteria are used to determine if a food product is safe for ingestion by the public. Microbiological criteria vary in each country; however, they are influenced by international standard commissions such as Codex Alimentarius Commission (CAC) and International Commission on Microbiological Specifications for Food (ICMSF) (Rip 2011; Matle *et al*., 2020).

In the United States (US), 0 cfu/g of *L. monocytogenes* is accepted for every 25 g of a food sample. However, according to the European Commission (EC), *L. monocytogenes* in Ready-to-eat (RTE) goods should not exceed 100 cfu/g at the time of consumption (Leong *et al*., 2016 Sanlibaba *et al*., 2018). The microbe per 25 g of the sample of RTE foods must not be present prior to the food leaving the food industry, and its population cap must be 100 cfu/g during the product's shelf life. This is necessary to control the foods that promote the growth of *L. monocytogenes* (Leong *et al*., 2016; Sanlibaba *et al.*, 2018).

The *Listeriosis* outbreak that recently occurred in South Africa brought to attention food safety concerns in terms of policy and practice (Boatemaa *et al.*, 2019). Food safety laws and regulations in South Africa are stringent and compliant with global standards. However, the outbreak demonstrated that the food safety policy can have certain limitations. As a result, officials in the South African Department of Health reviewed the list of diseases that must be reported and added *Listeriosis* (Tchatchouang *et al.*, 2020).

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Two major government departments, namely the Department of Agriculture, Forestry & Fisheries (DAFF) and the Department of Health (DoH), are jointly in charge of assuring meat safety in South Africa. The Animal Diseases Act, Act 35 of 1984, and the Meat Safety Act, Act 40 of 2000, are administered by the DAFF, which has authority over farms, feedlots, and abattoirs (Magwedere *et al*., 2015). The DoH, which is charged with enforcing the Foodstuffs, Cosmetics, and Disinfectants Act (FCDA), Act 54 of 1972, takes control of distribution, retail, and marketing as soon as the meat leaves the abattoir (Matle *et al*., 2020).

According to the Meat Safety Act number 40 of 2000, a registered meat slaughterhouse owner is required by South African law to oversee and ensure that meat is produced in a safe manner. The slaughterhouse's owner must provide documented management plans to prevent, eliminate, or decrease identified risks to allowable levels and make sure that risks are controlled inside the abattoir (Matle *et al*., 2019). Act 54 of 1972 prohibits the handling of meat from animals butchered in violation of the Meat Safety Act by anyone working at a food establishment authorized under the act. According to the regulations, it is unlawful to sell, manufacture, or import for sale any food that is polluted, tainted, or decayed and is considered harmful to human health (Magwedere *et al*., 2015; Matle *et al*., 2020).The South African National Standard (SANS): 885:2011 is the sole microbiological standard available for testing meat in South Africa; it is optional and permits a limit of 100 cfu/g for *L. monocytogenes* in RTE processed meat products (Magwedere *et al*., 2015).

2.10. Control of Listeria monocytogenes

For food safety, *L. monocytogenes* must be kept under control in the processing environment. The key difficulty for the food manufacturer is to focus efforts on preventing *L. monocytogenes* from entering and establishing themselves in the processing environment (Morobe *et al*., 2012). They can be controlled during different phases of contamination. Control can be implemented at the manufacturing facility, retail level, customer residences, and ultimately in the host after consuming *L. monocytogenes-containing* meals (Ncube, 2020).

Listeria monocytogenes is widespread in nature, and it is difficult to control (Buchanan *et al*., 2017). Hygiene precautions are frequently used to control its incidence. These days, *L. monocytogenes* is also controlled using innovative techniques such as Bacteriocins and Bacteriophages (Leong *et al*., 2016). Manipulating the intrinsic and extrinsic characteristics of food can be used to control *L. monocytogenes*. The development is slowed at pH values of 4.4 and below, acidic additions might cause the pH to decrease, which will stop the growth

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of the bacteria (Ncube, 2022). A low pH environment can also be maintained in food storage by using preservatives such as fumaric, acetic, and benzoic acid (Novais *et al*., 2022).

Listeria monocytogenes has a comparatively high-temperature tolerance, which increases its chances of overcoming obstacles linked to temperature (Bucur *et al*., 2018). The nutritional and sensory qualities of food can be impacted by exposure to extremely elevated temperatures and chemical preservatives (Amit *et al*., 2017).

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CHAPTER 3: INVESTIGATION OF THE MEAT HYGIENE PRACTICES IN VARIOUS BUTCHERIES IN THE VHEMBE DISTRICT LIMPOPO PROVINCE, SOUTH AFRICA(Published)

Abstract

Good hygienic practices are required to reduce the risk of microbial contamination during meat processing. Good hygiene and meat safety practices were evaluated among six (6) different village butcheries, eight (8) commercial butcheries, and eighteen supermarket butcheries through direct personal observations. The employees at supermarkets and commercial butcheries wore personal protective equipment and used proper waste disposal procedures. Moreover, there were pest control devices, a safe water supply, and staff handling money away from meat. At village butcheries, wearing hairnets and aprons, and the display of raw meat being separate from offal were identified as good practices. The irregular washing of hands (67%), less use of gloves (83%), wearing of open sandals (67%) and jewellery (33%), use of the same coat for different activities (100%), lack of paper towels (100%) and pest control devices (67%) and mismanagement of waste (33%) were practices that led to unsafe meat handling. Our study identified good meat safety practices at supermarkets. A combination of good and unhygienic meat handling practices were identified at commercial and village butcheries. These findings suggest a need for intervention through training on food safety to improve the hygienic practices of meat handling along the beef supply chain, more especially in commercial and village butcheries.

Keywords: Meat safety practices, hygiene, butcheries, supermarkets, South Africa

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3.1. Introduction

The increased demand for foods of animal origin is often linked to the world's growing human population (Gutema *et al*., 2021). Consequently, meat producers, processors, and consumers give higher importance to meat safety (Shilenge *et al*., 2019). The main source of protein, vitamins, and nutrients for the development and functioning of body cells is meat (Zerabruk *et al*., 2019). Worldwide, foodborne diseases are associated with the consumption of contaminated foods, which may occur during processing, among which meat processing has been attributed as a primary source of illness when contaminated (Atlabachew & Mamo, 2021). Foodborne illnesses are prevalent in some developing countries due to poor food hygiene practices, insufficient laws for food hygiene, weak regulatory systems, lack of funding for the purchase of the necessary equipment, and a lack of food safety education (Abdullahi *et al*., 2016; Nyamakwere *et al*., 2017).

The main source of foodborne diseases is through ingestion of meat contaminated by pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella* species, *Listeria monocytogenes*, *Escherichia coli 0157:H7* and *Campylobacter* species (Bersisa *et al*., 2019). Meat that is not handled hygienically may be meat contaminated by pathogenic bacteria and can constitute to health hazards for the consumer (Haileselassie *et al*., 2013). Practices and maintenance of proper hygiene during meat handling are necessary for the provision of healthy and fresh meat for human consumption (Khanal & Poudel, 2017). Meat handlers' poor personal hygiene often operates as a vector for the spread of microbes through their hands, wounds, lips, skins, and hair to meat (Wambui *et al*., 2017; Ebuete *et al*., 2020). If proper sanitation and hygiene procedures, such as washing hands, wearing protective clothes, cleaning and sanitizing butchery equipment and utensils, are not followed, bacterial contamination, meat loss, and post-harvest meat shortages arise (Chepkemoi *et al*., 2015).

According to the findings of the study conducted in Tanzania by Ntanga *et al*. (2014) and Birhanu *et al*. (2017) in Ethiopia, the bacterial load in the meat, meat contact surfaces, and utensils from the butcheries taken through swabs was higher than what was considered acceptable. The wholesomeness of meat is a shared responsibility of all individuals in the food chain. To correct the errors from farm to fork, there is a deep need for education and training in the prevention of foodborne diseases among abattoir workers, butchery, meat producers, suppliers, handlers, and the public (Ebuete *et al*., 2020). According to the World Health Organization, foodborne illnesses are estimated to have caused 600 million cases, 420,000 deaths, and approximately 33 million years of life of impairment worldwide in 2010, with Africa facing the greatest burden of mortality (Havelaar *et al*., 2015; Gutema *et al*., 2021).

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To reduce microbial contamination, hygienic handling techniques during preparation, distribution, storage, and retail sales must be improved (Gutema *et al*., 2021). Several studies investigated meat safety knowledge and practices (Haileselassie *et al*., 2013, Khanal & Poudel 2017, Al Banna *et al*., 2021), while others determined the handling of meat practices along the beef supply chain (Chepkemoi *et al*., 2015; Sulleyman *et al*., 2018) and bacteriological quality of meat from the abattoir and butcher shops (Aburi, 2012; Sulleyman *et al*., 2018) in different countries. There is a critical need in the literature to investigate the practices of food handlers in their everyday activities of employment and the potential sources of microbiological contaminants that can impair the quality of meat products (Shilenge *et al*., 2019).

The presence of hygiene measures has an impact on hygiene, however, developed countries with excellent levels of hygiene also have foodborne illnesses (Scallan *et al*., 2011). In South Africa, studies on meat safety practices and hygiene were done among slaughterhouse workers (Nyamakwere *et al*., 2017), as well as studies on game meat production for animal class and health compliance (van der Merwe *et al*., 2013), on the management of meat safety in abattoirs (Govender *et al*., 2013) and on the traditional slaughter of goats (Qekwana *et al.,* 2017). To protect the population from food-borne bacterial diseases, it is necessary to educate and campaign for proper sanitation and meat-handling practices in abattoirs and butcher shops (Haileselassie *et al*., 2013, Smigic *et al*., 2016; Gutema *et al*., 2021).

No documentation was available regarding meat safety practices and hygiene among butcheries and supermarkets in the Vhembe district, Limpopo province, South Africa. Thus, the objective of this study was to evaluate meat safety practices and hygiene among different butcheries and retail supermarkets in the Vhembe district. The results of this study may provide information on whether good manufacturing practices of meat are being fully followed at the supermarket and butchery levels, and whether they pose a threat to the health of the public.

3.2. Materials and Methods

3.2.1. Study settings.

The study was conducted from October to November 2021 at thirty-two (32) butcheries and found in the Vhembe district, including eight (8) commercial butcheries situated in Region 1, Region 2, Region 3, and Region 4, six (6) village butcheries situated in Village 1, Village 2, Village 3, Village 4, Village 5 and Village 6 and eighteen (18) supermarket butcheries situated in Region 1 ,Region 5, Region 6, Region 7, Region 8 Region 9, Region 10 and Region 11, within Vhembe district. The Vhembe district is in Limpopo province, which is in the northern part of South Africa. Village butcheries operated as independent retail

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establishments and each village butchery had about two (2) to three (3) employed workers; commercial butcheries also operated as independent retail establishments and had about three (3) to seven (7) employed workers on site. In supermarkets, butcheries were situated within stores that sell various products such as groceries, food supplies, and baked goods. Supermarket butcheries had five (5) to eight (8) workers on-site at the time of the study.

3.2.2. Study design and data collection.

The study design is the cross-sectional survey design. Systematic random sampling was used for the butcheries visited. Data were collected through face-to-face interview and observation using a structured questionnaire checklist (Table 3.1). The data collection instruments were adapted and modified from questionnaires and survey checklists in similar previous studies (Garedew *et al*., 2016; Smigic *et al*., 2016; Nyamakwere *et al*., 2017; Gutema *et al*., 2021) The questionnaire had the following themes: (i) socio-demographic characteristics of the participants; (ii) hygiene practise of meat handlers; (iii) cleanliness of working clothes; (iv) infrastructure, and maintenance of hygiene in supermarket/butchery; (v) the display of meat in butchery/supermarket. The survey checklist was administered for each retail shop (*n* = 32) being assessed and was based on the South African regulations of the Department of Health on the General Hygiene Requirements for Food Premises, Food Transport, and Related Matters R 638 (Act 54 of 1972): Foodstuffs, cosmetics & disinfectants Act, 1972 (Act 54 of 1972).

Table 3.1. Survey checklist to assess meat hygiene practices in various butcheries.

Checklist	Yes	No
Hygiene of meat handlers		
Meat handlers wash hands before commencing work/ prior to handling meat		
Use of gloves when meat is handled		
Hair is tied back, and hair net/cap is used		
Use of Apron/gown/coat		
Use of waterproof boots for footwear		
Protective clothes are long-sleeved and completely cover personal clothes		
Staff wears watch/ jewellery while meat is handled		
Same Apron is used for different activities in the butchery		
Persons handling meat also handle money		
Staff preparing and handling raw meat is separate from staff preparing and handling ready to eat meats		
Cleanliness of working clothes		
Recent dirt on working clothes		
Ingrained dirt on working clothes		
Infrastructure and maintenance of hygiene in Supermarket/butchery		

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Structure of shop/butchery including walls, floors, ceilings, and fixtures are in good condition and will not yield cross contamination
Butchery/shop floor appears clean
Counter and hooks of butchery/shop are clean
Cutting tables contain non-harmful materials (rust, mold)
Disposable paper towels are available
There is a safe water supply to the butchery/shop
Clean equipment such as weighing scales, mincers and slicers are separately used for raw meat and ready to eat meats
Chopping boards, knives, tongs, and other utensils are separated for raw meat and ready to eat meats
Waste is confined, managed, and properly disposed
Cleaning cloths and detergents are stored in sight
Pest control devices are available
Display of meat
The meat of different species are physically separated and are in the same window display
Meat appears red in color and has no unpleasant odor
Meat appears dark brown/ discolored and has a strong odor

3.2.3. Data management and analysis

The collected data were entered into a Microsoft Excel spreadsheet (Microsoft, Redmond, WA, USA) and analysed using IBM SPSS statistics for Windows, version 28 (IBM Corp., Armonk, NY, USA). The data were summarised using descriptive statistics, including frequency and percentage. A one-way analysis of variance (ANOVA) was used to assess the difference between the various butcheries. A *p*-value of less than 0.05 was set as a significance level. The meat safety practices at various butcheries were described descriptively.

3.3. Results

3.3.1. Sociodemographic characteristics of meat handlers

Table 3.2 summarises the sociodemographic characteristics of the butchery workers from various butcheries in the Vhembe district. Workers (n = 177) were both males and females ranging from 18 to 54 years. Most (68%) participants at the supermarket butcheries studied up to secondary school and only a few (6%) from the village butcheries obtained primary education. Most of the butchery workers earned between R3000 to R5000 per month, which means that they belonged to the poor and lower middle-class income brackets in South Africa.

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Table 3.2. Sociodemographic characteristics of workers at various butcheries in Vhembe district, Limpopo province South Africa.

Variables		Number (%) of respondents (n=177)
Gender	Male	94 (53)
	Female	83 (47)
Age	18-24	29 (16)
	25-49	130 (73)
	50>	18 (10)
Education level	Primary education (1-8)	11 (6)
	Secondary education (9-12)	121 (68)
	Tertiary education	45 (25)
Marital status	Married	75 (42)
	Single	102 (58)
Income	R1000 & below	5 (3)
	R1001- R3000	52 (29)
	R3001- R5000	120 (68)
Location	Market/butchery area	62 (35)
	Residential area	115 (64)
Level of experience	<5 years	95(53)
	>5 years	82 (46)

Note: R = Rand

3.3.2. Meat hygiene practises of meat handlers at butcheries

The majority (72%) of supermarket butcheries required workers to wash their hands prior to work and many (89%) used protective gloves before handling meat. All supermarket butcheries workers wore personal protective equipment such as an apron or coat, protective boots, and a hairnet while handling meat. Among the retail shops, 67% wore long protective clothes. However, they did not completely cover personal clothes. Seventeen percent (17%) of the retailers had staff workers wearing jewellery (watches, bracelets, and rings) while handling meat.

Among the commercial butcheries, 50% washed their hands. The majority (75%) wore gloves prior to meat handling, while 25% handled meat with either bare hands or a plastic bag before distributing it to the consumer. Aprons or coats were worn by all commercial butchery workers; while 62% wore the same protective clothes when conducting other activities in the butchery throughout the day, 75% wore hairnets. In all commercial butcheries, staff workers managing money were separate from those handling meat. Wearing of jewellery was not observed in 87% of the commercial butcheries.

At the village butcheries, 67% washed their hands prior to meat handling, however, this was with water placed in a plastic basin/bucket. The water utilised had been previously used to wash hands and contained a dish cloth used to wipe the hands and counters of the butchery. Most of the time, the water contained dishwashing liquid or soap. Protective gloves were

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worn in only 17% of the village butcheries most of the butcheries (83%) handled meat with bare hands or a plastic bag. All village butchery staff wore either an apron or gown; 33% wore protective boots, and 67% wore open sandals or shoes. All village butchery workers wore aprons or coats while conducting other activities besides meat handling. At the village butcheries, all workers handling money were also handling meat. In Approximately 33% of the village butcheries, workers were observed to be wearing jewellery. Table 3.3 summarises the observational assessments on meat safety practices and hygiene at supermarkets, as well as commercial and village butcheries.

Table 3.3. Meat hygiene practices at supermarkets, commercial and informal butcheries in Vhembe district, Limpopo province, South Africa.

Themes	Supermarket Butcheries		Commercial butcheries		Informal Butcheries	
Hygiene of meat handlers at butcheries						
Meat handlers wash hands prior to handling meat	Yes	13 (72%)	Yes	4 (50%)	Yes	4 (67%)
	No	5 (28%)	No	4 (50%)	No	2 (33%)
Use of gloves when meat is handled	Yes	16 (89%)	Yes	6 (75%)	Yes	1 (17%)
	No	2 (11%)	No	2 (25%)	No	5 (83%)
Hair is tied back, and hair net/cap is used	Yes	18 (100%)	Yes	6 (75%)	Yes	4 (67%)
	No	0 (0%)	No	2 (25%)	No	2 (33%)
Use of waterproof boots	Yes	18(100%)	Yes	6(75%)	Yes	2 (33%)
	No	0(0%)	No	2(25%)	No	4 (67%)
Protective clothes are long-sleeved and completely cover personal clothes	Yes	6 (33%)	Yes	3 (38%)	Yes	4 (67%)
	No	12(67%)	No	5 (62%)	No	2 (33%)
Staff wears watch/ jewellery while meat is handled	Yes	3 (17%)	Yes	1 (13%)	Yes	2 (33%)
	No	15(83%)	No	7 (87%)	No	4 (67%)
Same Apron is used for different activities in the butchery	Yes	16 (89%)	Yes	5 (62%)	Yes	6 (100%)
	No	2 (11%)	No	3 (38%)	No	0 (0%)
Persons handling meat also handle money	Yes	0 (0%)	Yes	0 (0%)	Yes	5 (83%)
	No	18 (100%)	No	8 (100%)	No	1 (17%)
Staff preparing and handling raw meat is separate from staff preparing and handling ready to eat meats	Yes	16 (89%)	Yes	5 (62%)	Yes	2 (33%)
	No	2 (11%)	No	3 (38%)	No	4 (67%)
Cleanliness of working clothes						
Recent dirt on working clothes	Yes	12 (67)	Yes	3 (38)	Yes	6 (100)
	No	6 (33)	No	5 (62)	No	0 (0)
Ingrained dirt on working clothes	Yes	3 (38)	Yes	2 (25)	Yes	2 (33)
	No	15 (83)	No	6 (75)	No	4 (67)
Maintenance of hygiene and infrastructure in the butcheries						

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Structure of butchery including walls, floors, ceilings, and fixtures are in good condition	Yes	18 (100)	Yes	7 (87)	Yes	3(50)
	No	0(0)	No	1 (13)	No	3(50)
Butchery/shop floor appears clean	Yes	7 (39)	Yes	5 (62)	Yes	5 (83)
	No	11 (61)	No	3 (38)	No	1 (17)
Counter and hooks of butchery/shop are clean	Yes	12 (67)	Yes	3 (38)	Yes	4 (67)
	No	6 (33)	No	5 (62)	No	2 (33)
Cutting tables contain non-harmful materials (rust, mold)	Yes	13 (72)	Yes	5 (62)	Yes	5 (83)
	No	5 (28)	No	3 (38)	No	1 (17)
Disposable paper towels are available	Yes	9 (50)	Yes	0 (0)	Yes	0 (0)
	No	9 (50)	No	8 (100)	No	6 (100)
There is a safe water supply to the butchery/shop	Yes	18 (100)	Yes	8 (100)	Yes	6 (100)
	No	0 (0)	No	0 (0)	No	0 (0)
Clean equipment such as weighing scales, mincers and slicers are separately used for raw meat and ready to eat meats	Yes	17 (94)	Yes	6 (75)	Yes	3 (50)
	No	1(6)	No	2 (25)	No	3 (50)
Waste is confined, managed, and properly disposed	Yes	5 (28)	Yes	6 (75)	Yes	4 (67)
	No	13 (72)	No	2 (25)	No	2 (33)
Cleaning cloths and detergents are stored in sight	Yes	15 (83)	Yes	5 (62)	Yes	4 (67)
	No	3 (17)	No	3 (38)	No	2 (33)
Pest control devices are available	Yes	18 (100)	Yes	6 (75)	Yes	2 (33)
	No	0 (0)	No	2 (25)	No	4 (67)
Display of meat						
The meat of different species are physically separated and are in the same window display	Yes	15 (83)	Yes	7 (87)	Yes	4 (67)
	No	3 (17)	No	1 (13)	No	2 (23)
Meat appears red in color and has no unpleasant odour	Yes	18 (100)	Yes	5 (62)	Yes	5 (83)
	No	0 (0)	No	3 (38)	No	1 (17)
Meat appears dark brown/ discoloured and has a strong odour	Yes	0 (0)	Yes	3 (38)	Yes	1 (17)
	No	18 (100)	No	5 (62)	No	5 (83)

3.3.3. Hygiene of working clothes at various butcheries

The majority (67%) of supermarket butchery workers had recent dirt (fresh particles of meat or blood) on work clothes and 17% of butcheries had ingrained dirt (old particles of meat and blood stains) on work clothes. At commercial butcheries, 38% of the butcheries had recent dirt covering working clothes, and 22% had ingrained dirt on their work clothes. Many

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(100%) of the village butcheries had recent dirt covering their work clothes and 33% had ingrained dirt on them.

3.3.4. Infrastructure and maintenance of hygiene at butcheries

All supermarket butcheries had walls, floors, and ceilings in good condition. All the retail shops had a safe water supply and pest control devices. However, 61% had dirty floors. Fifty-eight percent had clean counters and hooks; moreover, seventy-two percent had clean cutting tables for meat and a waste management system. Among the commercial butcheries, 87% had good structures. However, 13% had cracked walls and floors and 38% contained dirty floors. Sixty-two percent of the butcheries had cleaning cloths and detergents and seventy-five percent of them had pest control devices. None of the commercial butcheries utilised paper towels. However, 75% percent of them properly confined and dispose of waste.

At the village butcheries, 50% of the structures had walls, floors, and ceilings in good condition, while 50% had ceilings and walls that were, tearing down and where we observed cracked tiles on the floor. Seventy-five percent of the village butcheries had a tap water supply, while twenty-five percent had water supplied from water reservoir tanks instead of directly from the tap. Many of the village butcheries (83%) confined, as well as effectively managed and disposed of waste. However, 17% lacked a dustbin and disposed of waste on the dumpster site outside of the building of the butchery. Approximately 67% of the village butcheries had cleaning cloths, detergents, and pest control devices.

3.3.5. Display of meat at butcheries

Among the supermarket butcheries, 83% displayed meat of distinct species separate from offal on a meat display fridge. In 89% of the supermarket butcheries, meat appeared red in colour without an unpleasant odour, and in 17% of the supermarket, butcheries meat appeared dark brown. The majority (87%) of the commercial butcheries displayed meats of distinct species separate from offal in a window display fridge, 62% had meat that appeared red in colour and 38% had meat that appeared dark brown and had an unpleasant odour. At the village butcheries, 67% displayed meats of distinct species and offal separately in a window display, 83% of the butcheries had meat that appeared red in colour, while 17% had meat that appeared dark brown with an unpleasant odour. The results for the display of meat in various butcheries are summarised in Table 3.3.

3.3.6. Statistical analysis of variance of hygiene practices in various butcheries and supermarkets

Table 3.4 summarises the mean and standard error of meat safety practices among various butcheries and supermarkets in the Vhembe district, Limpopo, South Africa. The village, commercial, and supermarket butcheries did not significantly differ based on the washing of

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hands, wearing of aprons, gloves, hairnets, jewellery, butchery shop floor cleanliness, cutting tables, water supply, detergents, and pest control devices (ANOVA $p > 0.05$). However, there was a significant difference between the various butcheries in different locations with respect to the use of waterproof boots ($p = 0.01$), disposable paper towel availability ($p = 0.04$), waste management ($p = 0.025$), the condition of the structure of the butchery ($p = 0.05$), staff preparing raw meats being separated from ready-to-eat meats ($p = 0.03$), staff handling money being separated from handling meat ($p = 0.00$) and meat appearing red/brown and having unpleasant odour ($p = 0.029$).

Employees at village butcheries washed hands more than those at commercial butcheries (Mean = 0.67, standard error (SE = 0.211)), however workers at commercial butcheries wore gloves, hairnets, and waterproof boots more than village butcheries (Mean = 0.75, standard error (SE = 0.164)); all butcheries had a water supply, however, some village butcheries had water supplied from reservoir tanks instead of directly from the tap. This water had been collected from the nearest borehole, transferred into the reservoir tanks, and stored for present and future use. Moreover, they wore aprons while handling meat (Mean = 1.00, standard error (SE = 0.00).

Table 3.4. The Mean and standard error of meat hygiene practices in various butcheries in the Vhembe district, Limpopo province, South Africa.

Themes	Informal butcheries	Commercial butcheries	Supermarket butcheries
Washing hands	0.67 ± 0.21	0.50 ± 0.189	0.76 ± 0.106
Gloves	0.17 ± 0.167	0.75 ± 0.164	1.00 ± 0.000
Hairnet	0.67 ± 0.211	0.75 ± 0.164	1.00 ± 0.000
Apron	1.00 ± 0.000	1.00 ± 0.000	1.00 ± 0.000
Waterproof boots	0.33 ± 0.211	0.75 ± 0.164	1.00 ± 0.000
Protective clothes long sleeved	0.67 ± 0.211	0.50 ± 0.189	0.35 ± 0.119
Jewellery	0.33 ± 0.211	0.25 ± 0.164	0.18 ± 0.095
Same apron is worn everywhere	1.00 ± 0.000	0.75 ± 0.164	0.88 ± 0.081
Money & raw meat staff are separate	0.83 ± 0.167	0.13 ± 0.125	0.00 ± 0.000
Staff handling raw meat is similar to RTE meats	0.33 ± 0.211	0.63 ± 0.183	0.88 ± 0.081
Recent dirt on work clothes	1.00 ± 0.000	0.50 ± 0.189	0.65 ± 0.119
Ingrained dirt on work	0.33 ± 0.211	0.38 ± 0.183	0.18 ± 0.095

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clothes			
Structure in good condition	0.50 ± 0.224	0.88 ± 0.125	1.00 ± 0.000
Floor of butchery is clean	0.83 ± 0.167	0.63 ± 0.183	0.35 ± 0.119
Counter & hooks are clean	0.67 ± 0.211	0.50 ± 0.189	0.65 ± 0.119
Cutting tables are non-harmful	0.83 ± 0.167	0.63 ± 0.183	0.76 ± 0.106
Disposable paper towels	0.00 ± 0.000	0.13 ± 0.125	0.47 ± 0.125
Safe water supply	1.00 ± 0.000	1.00 ± 0.000	1.00 ± 0.000
Weighing scales & equipment are clean	0.50 ± 0.224	0.75 ± 0.164	0.94 ± 0.059
Utensils separated for RTE & raw meats	0.67 ± 0.211	0.63 ± 0.183	0.76 ± 0.106
Waste is confined	0.67 ± 0.211	0.75 ± 0.164	0.24 ± 0.106
Pest control devices	0.33 ± 0.211	0.75 ± 0.164	1.00 ± 0.000
Clean cloths & detergents	0.67 ± 0.211	0.75 ± 0.164	0.82 ± 0.095
Meat of different species is separated	0.67 ± 0.211	0.88 ± 0.125	0.82 ± 0.095
Meat is red in colour & has no unpleasant odour	0.83 ± 0.167	0.63 ± 0.183	1.00 ± 0.000
Meat is dark brown in colour and has an unpleasant odour	0.17 ± 0.167	0.38 ± 0.183	0.00 ± 0.000
Total			

Note: RTE- Ready-to-eat

3.4. Discussion

Due to the high likelihood of meat contamination at the butchery level, butcheries play a significant part in the prevention of meat-borne diseases. To provide safe and fresh meat for human consumption, it is vital to practice and maintain good hygiene during meat handling (Khanal & Poudel, 2017). We evaluated meat safety practices and hygiene among supermarkets, commercial and village butcheries in the Vhembe district, Limpopo, South Africa. This study was motivated by the need for information to guide food safety policy development, good manufacturing practices, and training in meat handling and hygiene in butcheries of all levels. The discussion that follows focuses on the primary meat processing techniques and their potential public health implications. Moreover, the practices are discussed considering the demands of the South African proclamations: Foodstuffs, Cosmetics & Disinfectants Act of 1972: General Hygiene Requirements for Food Premises, Food Transport, and Related Matters R638 (Act 54 of 1972). The legislation has been

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designed for the protection of consumers, to secure the production and marketing of safe, nourishing, and high-quality meat and meat products, as well as to provide general guidelines for food hygiene.

3.4.1. Hygiene practices of meat handlers at supermarkets butcheries

In the present study, washing hands, and wearing protective gloves, boots, coats, and hairnets were good practices identified at the supermarket butcheries. Gutema *et al.* (2021) also observed at slaughterhouses in Bishoftu, Ethiopia that the use of aprons, white coats, boots, and hair coverings were good practices at slaughterhouses. According to Nyamakwere *et al.* (2017), these are important practices that protect both the meat handler and the meat from exposure to foodborne pathogens. Although some workers wore protective gloves and clothing, practicing the washing of hands before and after sales in the butchery is necessary for sanitary purposes (Alimi *et al*., 2022) and is key to preventing the spread of infections. If not properly implemented, it can lead to disease outbreaks (Assefa *et al*., 2015). Therefore, the washing of hands by the majority and the wearing of protective clothes in supermarket butcheries were good meat hygiene and safety practices in line with the recommendations of the Department of Health on principles of General Hygiene Requirements for Food Premises, Food Transport, and Related Matters R 638 (Act 54 of 1972) and should be encouraged.

In the present study, half (50%) of the commercial butcheries practiced washing hands, and 75% wore gloves. The present findings revealed a lack of adherence to R 638 (Act 54 of 1972) section 11 (1) (b), which states that food cannot be handled by anyone whose hands have not been completely cleaned with soap and water or another effective cleaning method at the start of each work shift, of each day, or after a break. This, also, indicates a much lower proportion of compliant workers than that reported in the streets and open market meat vendors in Tamale Metropolis, Ghana, as reported by Adzitey *et al.* (2020), Where everyone (99%) of meat handlers knew that washing hands before working minimizes the risk of meat contamination and 96% of the meat sellers washed their hands before handling meat. The lack of adherence by the commercial butchery workers could be due to a lack of frequent training. Hand hygiene is not a new principle in the food industry for preventing microbial contamination of food. Unfortunately, hand hygiene is not always practiced, nor is it practiced properly (Chepkemoi *et al*., 2015).

In this study, aprons, coats, or gowns were worn by all commercial butchery workers, and 75% of them wore protective boots. This indicates consistency with the Department of Health's regulation (Health Act, 2018), that food handling must be done while wearing protective clothes, including footwear, headwear, and other items of clothing, to prevent food from becoming contaminated. This result indicates a higher proportion of compliant workers

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than reported in butcheries in Kampala district in Uganda, where Mirembe *et al*. (Mirembe *et al.*, 2015) reported that 31.5% wore protective gear during meat handling, as well as a higher proportion in the study conducted in Tamale Metropolis, Ghana by Adzitey *et al*. (2020) wherein half of the meat producers (58%) did not wear an apron while working. This was due to the low level of education that the respondent had. According to Adzitey *et al*. (2020) The low level of education of the meat sellers in Tamale makes it difficult for them to comprehend and adhere to strict sanitation and handling practices required for prevention of meat contamination. To prevent meat-handling staff and the meat from being exposed to infections, practices such as wearing aprons, white coats, boots, and covering one's hair should be utilised (Gutema *et al*., 2021).

In this study, 67% of workers in village butcheries washed their hands; however, this was with water placed in a plastic basin or bucket. The water had been previously used to wash hands, with a dishcloth used to wipe the hands and counters of the butchery. The water contained dishwashing liquid or soap most of the time. This practice was not in line with the requirements of R 638 (Act 54 of 1972), section 5 (c) (i) and 5 (d) (ii), which states that in food establishments, there must be hand-washing facilities with hot water, if appropriate, as well as a supply of soap and clean, disposable hand-drying material or equipment, which must be available for workers to wash and dry their hands. The findings of the present study are like those reported along the beef supply chain in Uganda by Kyayesimira *et al*. (2019), wherein butcheries and hands were mostly washed with cold water and soap (82.6%), but there were some cases where hands were washed using a washcloth (1.4%). In Lahore, Pakistan, Mallhi *et al.* (2019) reported that 27% of workers used a towel for handwashing, and 73% used only water for cleaning the shop, indicating a lack of control over hygiene practices. Food handlers should be equipped with the required knowledge and skills to handle food safely. Consequently, sanitation and hygiene training would be able to modify personal behaviour and attitudes (Adziety *et al*., 2020).

In this study, 17% of the village butcheries wore protective gloves. This is more of a concern because most of the butcheries did not practise hand washing prior to meat handling. This may be due to lack of proper education in terms of meat handling and lack of finance to purchase the necessary equipment and tools to practice safe meat handling. Furthermore, this was not consistent with R 638 which states that a food handler should avoid handling unpackaged food with bare hands unless it is necessary for preparation. This report was closely similar to the report in Chitwan, Nepal by Khanal and Poudel (Khanal & Poudel, 2017) in Uganda by Jeffer *et al.* (2021), and in Western Kenya by Cook *et al.* (2017), in which none of the workers working in the handling of meat made use of gloves.

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In this study, all village butchery workers wore either an apron or gown. This was consistent with R 638, in which no one should handle food unless they are wearing the proper protective gear. This result differed from the report in Bangladesh by Banna *et al.* (2021), where many of the meat handlers rarely used an apron (96.4%) when working. Furthermore, Jeffer *et al*. (2021) indicated that 0% of workers in meat shops wore safety gear such as coats and gloves. According to Mbonabucha & Fweja (2019), if all food workers wore protective clothing to prevent contamination of food equipment and utensils, the contamination of food could be avoided. In this study, 33% of the village butchery workers wore protective boots while 67% wore open sandals or shoes. This proportion is higher than the report in Gondar town, Ethiopia by Birhanu *et al.* (2017) where 34% of the meat handlers wore open sandals while working and is lower than that reported in Uganda by Jeffer *et al*. (2021), where 0% of the workers wore gumboots while working. The Regulation R 638 (Act 54 of 1972) section 9 (1)(2) requires that when handling meat, safety clothing, including protective boots, should always be worn; therefore, this was not consistent.

In this study, all supermarket and commercial butcheries separated staff handling money from those handling meat. Based on the report of Atlabachew & Mamo (2021), this reduces contamination by microorganisms and food-borne pathogens, which can result in health hazards. This situation was different at butcher shops in Gondar town, Ethiopia, where Birhanu *et al.* (2017) reported that 45.5% of respondents handled money concurrently with serving customers. This result was consistent with the regulations of meat safety stipulated in R 638 section 11 (1)(V), in which a person must not handle food after handling money. Cash is commonly used to exchange products and services in countries all around the world and is exchanged often in many types of business and can provide a vast surface area for pathogens to thrive. Therefore, the separation of workers handling money from those that handle meat is a good meat safety practice and should be encouraged (Bersisa *et al*., 2019).

In this study, at all village butcheries, staff handling meat were also handling money. This could be due to the few numbers of workers that are available at the butchery as there were 2 to 3 workers on site and a lack of knowledge on the role that money plays in cross contamination of meat. This result is closely similar to the report by Kyayesimira *et al.* (2019) in Uganda, in which most meat handlers (93.5%) also worked as cashiers, thereby handling both money and meat at the same time. This is also consistent with the report of Mallhi *et al.* (2019) in Lahore, Pakistan, where 71.8% of butchers handled money with their bare hands when handling and cutting meat cuts; this practice could function as a pathogen carrier. During retailing or serving, the individual handling money should not be allowed to handle meat. This is because money is unclean and can contaminate food handlers' hands. According to a study by Simon-oke and Ajileye in Akure, Nigeria, parasites such as

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Enterobius vermicularis, hookworm, *Giardia lamblia*, *Ascaris lumbricoides*, *Hymenolepis nana*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Isospora belli*, *Entamoeba histolytica*, *Balantidium coli,* and flagellates were recovered on currency notes sourced from food vendors and butchers (Simon-oke & Ajileye, 2019).

Ascariasis is a condition brought on by *A. lumbricoides*. Abdominal pain or intestinal obstruction may result from an Ascaris worm infection. Amoebiasis caused by *E. histolytica* is transmitted orally by ingesting the cyst. Amoebic dysentery, liver abscess, and even mortality can be consequences of amoebiasis (Simon-oke & Ajileye, 2019). Furthermore, in a study conducted in Nairobi, Kenya, by Kuria *et al.* (2009), pathogenic bacteria including *E. coli*, *Klebsiella*, *Serratia*, *Enterobacter*, *Salmonella*, *Acinetobacter*, *Enterococci*, *Staphylococcus,* and *Bacillus cereus* were discovered in coin samples obtained from taxi drivers, butchers, food restaurant attendants, grocery store attendants, roast maize sellers, and students (Kuria *et al*., 2009).

Money is handled by a wide range of people, including butchers, who may have contaminated the notes with blood. Blood serves as a good medium for bacterial growth. Moreover, other handlers of the notes such as traders, beggars, and people who conduct other jobs could contaminate hands and result in food cross-contamination (Mbaya *et al*., 2016). According to Chepkemoi *et al.* (2015), meat handlers who proceed to undertake non-food chores such as collecting money from clients are the most essential practices for transmitting foodborne pathogens from contaminated surfaces, resulting in food cross-contamination.

About seventeen (17%) and 13% of supermarket and commercial butcheries had staff workers wearing jewellery (watches, bracelet, and rings) while the meat was being handled, respectively. This was a compromise in relation to the meat safety regulation R638, which stipulates that wearing jewellery, other accessories, or adornments by a person handling non-pre-packaged foods or meat is prohibited unless it is properly covered. This result was a lower proportion than the one reported at abattoirs and butcher shops in Bishoftu, central Ethiopia, where Bersisa *et al*. (2019) reported that 64.5% of workers wore jewellery during working hours; a report conducted in Mekelle City, Ethiopia by Gurmu & Gebretinsae (2013) showed that 66.7 percent of the workers wore jewellery materials while handling meat. Another study in Kebbi state, Nigeria by Ribah *et al*. (2021) reported that rings, watches, and other jewellery were worn by many of the respondents (60%) while working. Because the skin underneath jewellery provides a favourable environment for contaminating microbes, making jewellery a source of germs, the wearing of jewellery should be prohibited (Upadhayaya & Ghimire, 2018; Islam *et al.,* 2022).

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3.4.2. Hygiene status of working clothes at butcheries

Among the supermarkets studied, 67% had recent dirt (fresh particles of meat or blood) on work clothes and 17% were found with ingrained dirt (old particles of meat and blood stains) on work clothes. This indicated some level of adherence to the Department of Health (Health Act, 2018) hygiene regulation that always when handling food, protective clothes must be clean and made of a material and design that cannot contaminate the food. This result differs from the report by Adzitey *et al.* (2020) in the Tamale metropolis, Ghana, in which 39% of the meat sellers appeared to be clean, with only fresh meat particles covering the vendors' garments, while the rest (61%) appeared unclean with clothing ingrained with either fresh or old particles of meat. It also differs from the report by Zerabruk *et al.* (2019) in Addis Ababa, Ethiopia, in which only 37.5% of meat handlers wore clean working clothes. According to Sulleyman *et al.* (2018), meat contamination and subsequent food poisoning can come from poor hygiene and sanitation practices. Since the slaughter process of meat may entail a significant amount of dirty labour, working garments should be cleaned at least once every day (Nyamakwere *et al.,* 2017).

In all commercial butcheries, approximately 38% had recent dirt on their working clothes and 22% had ingrained dirt, and in all village butcheries, recent dirt covered their work clothes and 33% had ingrained dirt. This proportion is lower than that reported in Addis Ababa by Zerabruk *et al.* (2019), in which only 37.5% of meat handlers wore clean working clothes and 62.5% wore dirty clothes. In a study by Nyamakwere *et al.* (2017), most respondents stated that they only washed their protective clothes after three (3) working days. Because the butchering process may involve a lot of dirty labour, working garments should be cleaned at least once a day (Nyamakwere *et al*., 2017). Personal clothes can carry microorganisms into the meat or meat-handling establishment from a range of sources. Protective clothing that has been cleaned thoroughly lessens the build-up of contamination and lowers the contamination risk. Regular cleaning is crucial to preventing contaminants from accumulating (Mbonabucha & Fweja, 2019).

3.4.3. Maintenance of infrastructure at butcheries

During this study, all supermarket butcheries had walls, floors, and ceilings in good condition. Gutema *et al.* (2021) reported related results in a study conducted in slaughterhouses and retail shops in Bishoftu, Ethiopia, in which all retail shops had light bulbs, either concrete or tile floors, and white painted walls and ceilings.

Every retail shop had a tap water supply system. This was consistent with the demands of R 638 (Act 54 of 1972), section 5 (d) (vi), in which a sufficient supply of water must be accessible in food establishments. This result differs from the report of Adeolu *et al.* (2019) in

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Karu abattoir, Abuja, Nigeria, in which more than half of the respondents (53.7%) indicated inadequate water supply in the facility and obtained their water from the tap outside (64.6%).

Waste was not confined, effectively managed, or disposed of in 72% of the supermarket butcheries. This contradicted the Hygiene Requirements of R 638, section 5 (d) (iii), in which a managed waste disposal system is required in all premises. Refuse containers must be regularly cleaned and disinfected. As often as necessary, garbage must be taken out of the area where food is handled or produced, and refuse must be kept or disposed of in a way that does not cause a hazard.

This value was higher than the one in the report of Mirembe *et al*. (2015) in Kampala district Uganda, wherein hardly 19 (26.0%) of the butcheries had waste storage containers and these were mostly sacks and polyethylene bags. Furthermore, in a report by Ribah *et al.* (2021) in Kebbi state, Nigeria, 66.1% of the respondents reported inadequate waste disposal provision in major slaughter slabs of raw meat. In addition. In the report by Mbonabucha & Fweja. (2019) at Rungwe district, Tanzania, more than half of the butchers lacked a dustbin, which suggests improper management of the butcher waste. Although the waste disposal system in this study was not satisfactory, it was better than the waste disposal systems in the previous studies. The supermarket butcheries had refuse containers; however, disposal was not mostly done effectively. Wastes from abattoirs and butcheries must be disposed of carefully since they may contain pathogens that cause food-borne illnesses (Kwaghe *et al*., 2016).

Among commercial butcheries, about (13%) had cracked walls and floors and 38% had dirty floors. According to Mbonabucha & Fweja (2019), the cleaning process is complicated by a rough or cracked wall. A compromised physical state may compromise the effectiveness of cleansing in the butchery. Poor butchery infrastructure is one of the many major obstacles to using appropriate hygienic procedures when selling meat (Aburi, 2012). More focus is required on enhancing the infrastructure of slaughterhouses and retail stores as well as the government regulatory agencies' food-quality-monitoring systems, to ensure sanitary meat production and marketing at all stages (Gutema *et al*., 2021).

The disposable paper towels at commercial butcheries were not available. This practice was not consistent with the requirements for premises in R 638, section 5.3 (d)(iii) (Health Act, 2018). Yousif & Mustafa (2020) reported similar results in Khartoum state, Sudan, in which all (100%) open and closed butcher shops in three regions evaluated indicated poor hygiene procedures in terms of paper towel and soap availability, Worsfold & Griffith (2001) also reported that staff had not been properly taught to dry the equipment with paper towels and that there was a lack of understanding of the significance of cleaning and disinfecting hand

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contact surfaces. The usage of soft, absorbent paper towels is more acceptable to users as it corresponds with compliance with the standards recommended on hand hygiene (Abd-Elaleem *et al.,* 2014).

Most of the commercial butcheries (75%) had pest control devices. This result is similar to the report by Mbonabucha & Fweja (2019) on meat protection, in which many butchers (72.1%) had mesh wire or shutter glass for the control of pests. According to the R 638 (Health Act, 2018), food facilities must be pest-proof using the best techniques presently available, and they must have access to effective prevention measures for flies, cockroaches, and other insects.

Among the village butcheries, 50% had walls, floors, and ceilings in good condition; however, 50% had ceilings, walls that were torn down, and cracked tiles on the floor. This was not in line with R638 section 5 (3)(a), in which food establishments must be made of smooth, toxic-free, cleanable, non-absorbent, and dust- and water-resistant material that has no open joints on the interior surfaces of walls, ceilings, and floors. These findings were consistent with the results reported by Mirembe *et al.* 2015) in Uganda, in which 51 (69.9%) of the butcheries were permanent structures, however inspections of the floor and walls found that 24 (32.9%) had fractured walls and 66 (90.4%) had damaged flooring. According to Yousif and Mustafa (Yousif & Mustafa, 2020), the infrastructure of butcher shops is typically poor. Butcher shops are areas where there is a greater risk of meat contamination (Khanal & Poudel, 2017).

However, 75% of the village butcheries had a tap water supply, and 25% had water supplied from water reservoir tanks instead of directly from the tap; this water had been previously collected from the nearest borehole and stored in reservoir tanks for present and future use. This result differs from the result reported by Mirembe *et al.* (2015) in Uganda, in which there was no running water within the butcher shop and the butcheries' major water source was outside tap water within distance (91.8%), with the rest of the butcheries getting their water from neighbouring protected springs. Similarly, in a report by Adeolu *et al.* (2019), more than half of those surveyed (53.7%) reported that the water supply infrastructure was insufficient and that they get their water from the tap (64.6%). According to R 638, section 5.3 (d)(vi), a sufficient supply of water must be accessible in food establishments. Water used for cleaning and meat processing in butcheries and abattoirs must meet drinking water standards. Therefore, sufficient potable water must be made available to meet operational and clean-up requirements (2019).

Moreover, waste was confined, managed properly, and disposed of properly in 83% of the village butcheries. This was consistent with R 638. However, 17% of the butcheries lacked a

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dustbin and disposed of waste on the dumpster site outside of the building of the butchery. This proportion is lower than the result of Mirembe *et al*. (2015) in Uganda, in which hardly 19 (26.0%) of the butcheries had waste storage containers, which were mostly sacks and polythene bags, and this made it difficult to manage waste, this similar to the report by Mallhi *et al.* (2019), in which most of the shops' garbage was found near butchery businesses and was used to feed mice, dogs, cats, and other animals. To avoid the attraction of deadly diseases and flies, it is advised that butcher shops, like other food operations, be built away from damp garbage and that waste should be placed in durable, clean materials (Mbonabucha & Fweja, 2019).

3.4.4. Display requirements of meat at butcheries

In the present study, 83% of the supermarket butcheries had meats of distinct species physically separated in a meat display fridge. In comparison with the current study, Zerabruk *et al.* (2019) reported that out of all the butcher shops, only one had a refrigerator for meat storage. Most of the beef products in the investigation were kept on hangers or tables for more than five (5) hours. Similarly, Ribah *et al.* (2021) reported that fresh meat was usually sold in open markets on trays or displayed on tables without proper hygiene procedures, as well as left at room temperature. The displaying of meat in non-refrigerated conditions provides an opportunity for microbial growth (Zerabruk *et al*., 2019). The display of meat in this study in refrigerator conditions is a good practice, is consistent with R 638, and should be encouraged.

commercial butcheries (87%) had the physically separated meats of distinct species and placed them in a window display fridge. This was consistent with the demands of R 638 (Health Act, 2018), in which foodstuff displayed or stored must not be in direct contact with the floor, ceiling, wall, or any other surface on the ground and a chilling and freezing facility must be provided for the meat. In comparison to the present study, in the report of Smigic *et al.* (2016), 95% of meat items were in open displays, despite the fact that butcher shops were located on the edge of the road, exposing the meat to dust and smoke from passing automobiles, and just 5% were shown in a clear, closed case. Since meat contains an abundance of nutrients required for the growth of bacteria in adequate quantities, it therefore should be stored and displayed in a refrigerator (Bafanda *et al*., 2017).

The village butcheries (67%) had meats of distinct species physically separated and displayed in the refrigerator. Based on the report of Mbonabucha & Fweja. (2019), to ensure that meat is free of bad odours, butchers must always maintain a clean environment. The display of meat in the village butcheries is contrary to the report by Aburi (2012), in which open shelter butcheries displayed meat by hanging it in the open air most of the time, while kiosk butcheries displayed 20% of their meat in open public tables and 20% within

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refrigerators. This supports the view that many butcheries lack cooling facilities and therefore only stock meat that can be sold within a day (Kyayesimira *et al*., 2019). Maintaining the meat's temperature during transportation, retail display, and handling may prolong the meat's shelf life and preserve its quality (Mbonabucha & Fweja, 2019).

3.5. Conclusion

The present study identified good hygiene practises at supermarkets. A combination of good and unhygienic meat handling practices was identified at commercial and village butcheries. The supermarkets follow the safety procedures in the handling of meat better than all other butcheries. It is recommended that other butcheries (village and commercial) to adhere to meat safety regulations. The findings of this study suggest a need for intervention by the Department of Health (DoH) and Department of Agriculture, Land Reform and Rural Development (DALRRD) through training on food safety to improve the hygienic practices of meat handling along the beef supply chain, more especially within commercial and village butcheries.

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CHAPTER 4: PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY OF LISTERIA MONOCYTOGENES IN RAW RETAIL BEEF IN THE VHEMBE DISTRICT, LIMPOPO PROVINCE, SOUTH AFRICA

Abstract

Listeria monocytogenes is a foodborne bacterial pathogen linked to significant issues with public health and food safety. This study aimed to isolate and identify *L. monocytogenes* from raw retail beef sold in the Vhembe district, Limpopo province, South Africa, and to assess their antimicrobial susceptibility. Fifty-Seven (57) samples of raw beef were purchased from nine (9) supermarkets butcheries, seven (7) commercial butcheries, and three (3) informal butcheries in Vhembe district. The isolation and identification of *L. monocytogenes* was conducted on Harlequin *Listeria* chromogenic agar and analysed for antimicrobial susceptibility using the well diffusion method on Mueller-Hinton agar using the ISO 11290-1:2017 standard method. Results showed that fifty-five (55) (96%) out of fifty-seven (57) beef plate meat samples collected from the supermarkets and butcheries were contaminated with *L. monocytogenes, Listeria ivanovii,* and other species of *Listeria*. The highest antibiotic resistance was observed against nalidixic acid (81%), followed by tetracyclines (73%). Ampicillin, the choice of first-line treatment for *Listeriosis* exhibited a high resistance for most of the isolates (65%). There is a high prevalence of *L. monocytogenes* in beef plate meat from supermarkets and butcheries and the isolates are antibiotic resistant to most of the recommended antibiotics in clinical practice.

Keywords: Prevalence, *Listeria monocytogenes, Listeria* species, antimicrobial susceptibility, retail beef plate meat.

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4.1. Introduction

The main reason often cited by consumers for consuming meat is that it has a desirable nutritional benefit and supports human health. Meat is recognised as an important source of amino acids (proteins), vitamin B complex, iron, minerals, and other essential nutrient required for the human body (Boler & Woerner, 2017). Despite this highly beneficial nutrient content, meat such as beef may carry bacteria such as *L. monocytogenes* because of its intrinsic characteristics like pH and water activity that supports the growth of the microorganism (Saraiva *et al*., 2018; So-Yeon *et al*., 2018).Moreover, poor handling and hygiene practices along the meat supply chain might result in a significant level of bacterial contamination on the meat and its contact surfaces (Rani *et al*., 2017). Meat that has become contaminated is typically undesirable and unfit for human consumption, leading to significant financial losses.

It is known that a healthy slaughtered animal's internal tissue may be free from bacteria at the time of slaughtering (Jay, 2000), however, it is reported that examined fresh beef meats contain various microorganisms (Demaitre *et al*., 2020; Obaidat, 2020). The biota of meat at the retail level is argued to reflect the slaughtering, processing environments, and other factors like handling, storing environment, non-sterile utensils, improper storage, poor personal hygiene during preparation, extended shelf-lives refrigeration, inadequate cooling and reheating, inadequate food safety laws, weak regulatory systems, and a lack of financial resources (Maktabi *et al*., 2015; Gebremedhin *et al*., 2021). In addition, poor animal management practices may lead to contamination of meat thereby transmitting the pathogens to humans (Jankielsohn, 2015; Matle *et al*., 2020).

Listeria monocytogenes is a foodborne bacterial pathogen linked to critical problems with public health and food safety. According to the World Health Organization (WHO), there are 23,150 cases of listeriosis worldwide, resulting in 172,823 disability-adjusted life years (Dufailu *et al*., 2021). *Listeriosis* is a fatal condition brought on by *L. monocytogenes* and can lead to bacteremia, meningitis, and spontaneous miscarriage resulting in 20-30% of fatalities (Jeffs *et al*., 2020; Tchatchouang *et al.*, 2020; Kundul & Ame *et al.,* 2022). Consuming meat contaminated with pathogenic *L. monocytogenes* results in food-borne *listeriosis*. (Gebremedhin *et al*., 2020). Food regulators around the world have set tolerance limits for the presence of *Listeria monocytogenes* in food items; the United States, the European Union, and Korea have zero-tolerance policies for raw meat products (Jang *et al*., 2021).

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With the recent discovery of numerous additional species, the genus *Listeria* comprises of twenty-Six (26) species: *L. monocytogenes*, *L. ivanovii, L. innocua, L. welshimeri, L. seeligeri*, *L. grayi*, *L. marthii*, *L. costaricensis*, *L. rocourtiae, L. fleischmannii*, *L. newyorkensis*, *L. weihenstephanensis, L. floridensis*, *L. aquatica, L. thailandensis, L. cornellensis*, *L. riparia*, *L. booriae, L. goaensis* and *L. grandensis*, *L. valentina*, *L. farberi*, *L. portnoyi*, *L. cossartiae*, *L. rustica*, and *L. immobilis* (Carlin *et al*., 2021; Barbuddhe *et al*., 2022). Among these, *L. monocytogenes* is known to be pathogenic for both people and animals, but *L. ivanovii* is solely known to be pathogenic for animals. Abortion, newborn *listeriosis*, systemic *listeriosis*, and gastroenteritis all result from infections with *L. monocytogenes* (Arslan & Ozdemir, 2020).

There are several different environmental conditions that *L. monocytogenes* can withstand, including pH (5.4-9.6), a wide range of temperatures (4 to 42 ºC), and salt (Gebremedhin *et al*., 2021). Unique physiological traits in *L. monocytogenes* enable the growth of the micro-organism at refrigeration temperatures, survival, and tolerance of up to 10% salt concentration (Osek *et al*., 2022). Due to its ability to endure preservation treatments*, L. monocytogenes* has emerged as a serious foodborne pathogen (Matle *et al*., 2020) and because of these growth conditions*, L. monocytogenes* is more able to flourish in the harsh environments present in food-processing plants, which poses significant issues for the food industry (Matle *et al*., 2021). In addition, the presence of *L. monocytogenes* and other *Listeria* species in foods often indicates inadequate hygienic conditions (Cufaogle *et al*., 2021).

A major issue for human and animal health at a global level is the emergence of antimicrobial resistance in foodborne pathogens, particularly multidrug resistance (Sanlibaba *et al*., 2020). The first multidrug-resistant strain of *L. monocytogenes* was identified in France in 1988, since then more cases of multi-drug resistance have been isolated globally (Hanes & Huang, 2022). Foodborne pathogens that are resistant to practically all the existing antibiotics are being reported more frequently every day (Cufaogle *et al.,* 2021). The frequent use of antibiotics in food for livestock production, treatment, and growth enhancement has been a critical contributor to the establishment of strains that are resistant to several antibiotics. As a result, treating and managing listeriosis without addressing antibiotic resistance is a challenging and potential risk (Gebremedhin *et al*., 2020).

Antibiotics are antimicrobials used to prevent or cure infections in both humans and animals. They can be either natural, moderately synthetic, or synthetic (Stanton *et al*., 2020; Pancu *et al*., 2021). The current standard antibiotics used for human listeriosis is a β-lactam antibiotic

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such as ampicillin or penicillin combined with an aminoglycoside such as gentamicin. Vancomycin, erythromycin, and trimethoprim-sulfamethoxazole combination are the second-choice prescription for pregnant women or patients who are allergic to β-lactams (Lepe, 2020; Andriyanov *et al*., 2021). Until recently, infections brought on by Listeria species could easily be treated because these microorganisms were thought to be resistant to a wide range of medications. However, recently antibiotic resistance, most notably multidrug resistance, has made treating these infections more challenging (Okorie-Kanu *et al.*, 2020; Mancuso *et al*., 2021).

Because multi-drug resistance could result in increased hospitalization expenses and longer antibiotic administration times, it is typically linked to challenges in treating the diseases they cause, and since multi-drug resistance strains tend to be widespread in the environment, manufacturing workers or incoming raw materials are often reported to introduce them to food processing facilities (Kayode & Okoh, 2022). Regardless of the administration of appropriate antimicrobial agents, *L. monocytogenes* still results in a fatality rate of 20-30% which in comparison is higher than other food-borne pathogens (Shamloo *et al*., 2019; Keet & Rip, 2021). It is critical to verify the efficacy of antimicrobials for listeriosis and keep an eye on the evolution of antimicrobial-resistant *L. monocytogenes* considering the high death rate of listeriosis (20 to 30%) (Heidarzadeh *et al*., 2021).

In South Africa, a *listeriosis* outbreak emerged in January 2017. Approximately 945 confirmed cases of the disease were reported by the South African National Institute of Communicable Diseases (NICD) in February 2018, and 176 of those cases resulted in fatalities (a case fatality rate of 19%) (Ogunbanjo, 2018). Several literatures on the prevalence of *L. monocytogenes* conducted in South Africa were on processed meats (Vorster *et al*., 1993; Thomas *et al*., 2020), ready-to-eat foods (Nyenje *et al*., 2012; Kayode & Okoh, 2022), meat and meat products (Matle *et al*., 2019), food from retail shops and street vendor stalls (Ncube, 2020) and various sources (Meat, meat products, water, and cattle feces) (Iwu & Okoh, 2020; Tchatchouang *et al*., 2022; Thomas *et al*., 2022). Despite all these studies, there is still limited data with respect to the prevalence of *L. monocytogenes* in retail beef meat and its antibiotic susceptibility, especially in the Limpopo province.

This this study aimed to determine the prevalence of *L. monocytogenes* and antimicrobial resistance in retail beef plate meat in the Vhembe district of the Limpopo province.

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4.2. Materials and Method

4.2.1. Study design and sample collection.

A cross-sectional study was conducted at nineteen (19) butcheries in Vhembe district, Limpopo province, South Africa to determine the prevalence of Listeria in beef. Raw Beef samples of short ribs and steak cuts of fifty-seven (57) in total were purchased from nine (9) supermarkets, seven (7) commercial butcheries, and three (3) village butcheries in the Vhembe district. Samples were collected in triplicates and at random from each supermarket and butchery yielding twenty-seven (27) samples for different retail Supermarkets, twenty-one (21) samples each for the commercial butcheries, and nine (9) samples for village butcheries. Beef plate meat samples were placed in separate, sterile specimen bags with suitable labels before being placed in a cooler box with ice packs. When collecting samples from various retail supermarkets and butcheries, gloves were utilized to prevent cross-contamination. Samples were transported to the food microbiology laboratory, Department of Food Science and Technology, University of Venda, and were stored at 4ºC to be processed within 1(one) hour of sampling. The agar media for the isolation, identification, and antibiotic susceptibility were purchased from Lasec (SA).

4.2.2. Isolation and identification of Listeria species

Isolation was done according to ISO 11290: 2017 standard method. To create a 1:10 sample dilution, 25 g of each beef plate was aseptically added to a sterile filter-lined stomacher bag that contained 225 ml of Listeria enrichment broth (Lasec). The samples were homogenized in a stomacher circulator unit for 2 minutes at 225 rpm before being incubated for 48 hours at 30°C. At both 24 and 48 hours of incubation, 0.1 ml of a subsample from the Listeria enrichment broth culture was introduced to 10 ml of Fraser broth supplemented with 0.1 ml of Fraser's broth supplement. The following 48 hours were spent incubating Fraser broth cultures at 35°C.

After 48 hours Fraser broth cultures were spread onto Harlequin chromogenic *Listeria* agar (Lasec) using a sterile cotton swab method. The chromogenic agar was incubated for 24 hours at 35°C and observation was made for suspect colonies after 24 and 48 hours of incubation at 35°C. Colonies that appeared blue/green with an opaque white halo on chromogenic agar as described by Hegde *et al.* (2007) & Hunt *et al*. (2017) were identified as *L. monocytogenes* while other species of *Listeria* appear blue/green without an opaque white halo.

4.2.3. Antibiotic susceptibility testing of the Listeria isolates.

Antibiotic susceptibility of *Listeria* isolates was performed using the Well diffusion method on Mueller-Hinton agar (Oxoid, England), according to Clinical Laboratory Standards Institute

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(CLSI, 2000) guidelines. Implanted Gram-positive antibiotics were sulphamethoxazole (25 µg), Nalidixic acid (30 µg), Tetracycline (30 µg), Oxytetracycline (30 µg), Chlortetracycline (30 µg), Ampicillin (10µg), and Enoxacin (10 µg). The interpretation of the susceptibility of the results was done according to CLSI (2000) guidelines. Colonies of *L. monocytogenes* was transferred singularly to BHI broth (Lasec, SA) and incubated at 37°C for 24 h. An antibiotic was added to the well on the Mueller-Hinton agar surface after a suspension (0.5 McFarland) from the BHI broth had been applied to the agar. For each antibiotic, the diameter of the inhibitory zone was determined following a 24-hour incubation period at 35°C. Following interpretation using the CLSI (2000) guidelines, the isolates were categorized as either resistant, intermediate (with reduced sensitivity), or susceptible.

4.3. Prevalence of *Listeria monocytogenes* and listeria species in raw beef

4.3.1. Prevalence of Listeria monocytogenes and Listeria species from supermarket butcheries

Twenty-six (26) raw beef samples were examined in supermarket butcheries. Presumptive colonies of *L. monocytogenes* and *L. ivanovii* were detected in all twenty-six (26) of the samples examined (100%). Other species of *Listeria* were detected in only one (1) sample from retailer 8 (3%) (Table 4.1).

Table 4.1. Presumptive *Listeria* species from supermarket/retailer butcheries

Retail Butcheries	Sample Number	*L. monocytogenes*	*L. ivanovii*	*Other Listeria species*
Retailer 1	1	+	+	-
	2	+	+	-
	3	+	+	-
Retailer 2	4	+	+	-
	5	+	+	-
	6	+	+	-
Retailer 3	7	+	+	-
	8	+	+	-
	9	+	+	-
Retailer 4	10	+	+	-
	11	+	+	-
	12	+	+	-
Retailer 5	13	+	+	-
	14	+	+	-
	15	+	+	-
Retailer 6	16	+	+	-
	17	+	+	-
	18	+	+	-
Retailer 7	19	+	+	-

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	20	+	+	-
	21	+	+	-
Retailer 8	22	+	+	-
	23	+	+	-
	24	+	+	+
Retailer 9	25	+	+	-
	26	+	-	-

Note: + Listeria species isolated & identified, - Listeria species not isolated & identified

4.3.2. Prevalence of Listeria monocytogenes and Listeria species from commercial butcheries

Approximately twenty-one (21) samples were examined for the presence of *Listeria* species in seven (7) commercial butcheries in Vhembe. Results indicated that all twenty-one (21) samples (100%) contained presumptive *Listeria* species; *L. monocytogenes and L. ivanovii*. Other species of *Listeria* also occurred in 7 (%) of the samples that were tested from commercial 3-7 (Table 4.2).

Table 4.2. Presumptive *Listeria* species from commercial butcheries

	Sample number	*L. monocytogenes*	*L. ivanovii*	Other *Listeria* species
Commercial 1	27	+	-	-
	28	+	+	-
	29	+	+	-
Commercial 2	30	+	+	-
	31	+	+	-
	32	+	+	-
Commercial 3	33	+	+	-
	34	+	+	-
	35	+	+	+
Commercial 4	36	+	+	-
	37	+	+	-
	38	+	+	+
Commercial 5	39	+	+	-
	40	+	+	-
	41	+	+	+
Commercial 6	42	+	-	-

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	43	+	+	+
	44	+	+	+
Commercial 7	45	+	+	-
	46	+	+	+
	47	+	+	+

Note: + Listeria species isolated & identified, - *Listeria* species not isolated & identified

4.3.3. Prevalence of Listeria species from informal butcheries

The results from informal butcheries emanated from nine (9) samples examined from butcheries in butchery 1, butchery 2, and butchery 3. Results revealed that all (100%) samples were contaminated with presumptive *Listeria* species (Table 4.3). presumptive colonies of *L. monocytogenes* and *L. ivanovii* occurred in all the samples. Other species of Listeria were not present (0%).

Table 4.3. Presumptive *Listeria* species in informal butcheries

	Sample number	*L. monocytogenes*	*L. ivanovii*	*Other Listeria species*
Butchery 1	48	+	+	-
	49	+	+	-
	50	+	+	-
Butchery 2	51	+	+	-
	52	+	+	-
	53	+	+	-
Butchery 3	54	+	+	-
	55	+	+	-
	56	+	+	-

Note: + Listeria species isolated & identified, - Listeria species not isolated & identified

Overall, nineteen (19) shops were examined for the presence of *L. monocytogenes* in raw beef samples in the Vhembe district. This included nine (9) supermarkets butcheries, seven (7) commercial butcheries, and three (3) informal butcheries. Results revealed that all supermarkets, commercial butcheries, and informal butcheries are contaminated with presumptive *L. monocytogenes and L. ivanovii* colonies apart from one (1) Supermarket located in region 5 which showed no occurrence of *L. ivanovii* (Table 4.6) (check the supermarket). The occurrence of *Listeria* species (*L. monocytogenes* & *L. ivanovii*) was highest in supermarket samples and lowest in commercial butchery samples.

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Table 4.4. The overall distribution of presumptive *L. monocytogenes*, *L. ivanovii,* and *other Listeria species* from different retail shops in the Vhembe district

Retail shop	**Number of retail shops**	**Number of meat samples**	**Positive culture samples for *L. monocytogenes***	**Positive culture samples for *L. ivanovii***	**Positive culture samples for other species**
Village butcheries	3	9	9	9	0
Commercial butcheries	7	21	21	20	7
Supermarkets	9	27	25	23	9
Total	**19**	**57**	**55**	**52**	**16**

4.3.6. Antibiotic susceptibility test of Listeria monocytogenes isolates in retail beef plate meat in supermarkets and butcheries from Vhembe district.

The results of the antimicrobial susceptibility of *L. monocytogenes* isolates are summarized in Table 4.7. Seven (7) antibiotics were tested against twenty-six (26) *L. monocytogenes* isolates. Sulphamethoxazole, the second choice of treatment used in combination with trimethoprim for severe *listeriosis*, showed considerable resistance to *L. monocytogenes*. Additionally, a large percentage of isolates exhibited resistance and susceptibility to enoxacin.

Table 4.5. Antimicrobial profile of *Listeria monocytogenes* against sulphamethoxazole, Enoxacin, Tetracyclines, Oxytetracycline, Chlortetracycline, nalidixic acid, and Ampicillin

		***Listeria monocytogenes* (*n*=26)**		
Antimicrobial class	**Antimicrobe concentration**	**No. of susceptible (%)**	**No. of Resistant (%)**	**No. of intermediates (%)**
Sulfonamide	Sulphamethoxazole (30 µg)	9 (35)	16 (62)	0
Fluoroquinolones	Enoxacin (25 µg)	7 (27)	18 (69)	1(4)
Tetracyclines	Tetracycline (30 µg)	4 (15)	19 (73)	3 (12)
	Oxytetracycline (30 µg)	2 (8)	19 (73)	5 (19)

	Chlortetracycline (30 μg)	2 (8)	19 (73)	5 (19)
Quinolones	Nalidixic acid (30 μg)	4 (15)	21 (81)	1 (4)
B-Lactams	Ampicillin (10 μg)	5 (19)	17 (65)	4 (15)

Table 4.8. summarises the results of seven (7) antimicrobe(antibiotics) from five (5) different antimicrobial classes tested against 26 *L. monocytogenes* isolates. The highest resistance was observed against nalidixic acid (81%), followed by tetracyclines (73%). The highest susceptibility of the isolates was observed against sulphamethoxazole (35%) and the lowest in oxytetracycline (8%) and chlortetracycline (8%). Intermediate resistance was not observed for sulphamethoxazole but was highest against oxytetracycline (19%) and chlortetracycline (19%) and lowest against nalidixic acid (4%) and enoxacin (4%). Ampicillin, the choice of first-line treatment for *listeriosis* exhibited a high resistance from most of the isolates (65%).

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Table 4.6. Antibiotics used in testing the susceptibility of *Listeria* isolates.

Antibiotics (µg)	Test strains																									
	#A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	**X**	**Y**	**Z**
Sulphamethoxazole (SXT) (25 µg)	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**S**	**R**	**I**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**S**	**S**
Enoxacin (ENO) (30 µg)	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**S**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**S**	**R**
Tetracycline TET (30 µg)	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**R**	**S**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**I**	**R**	**S**
Oxytetracycline (30 µg)	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**S**	**I**	**R**	**I**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**S**	**I**	**R**	**R**
Chlortetracycline (30 µg)	**S**	**R**	**R**	**S**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**I**	**S**	**R**	**S**	**R**	**S**	**R**	**I**	**R**	**S**	**R**	**S**	**I**	**R**	**I**
Nalidixic acid (30 µg)	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**R**	**S**	**S**
Ampicillin AMP (10 µg)	**S**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**I**	**R**	**S**	**R**	**I**	**R**	**S**	**R**	**R**	**I**	**R**	**R**

NOTE: A- retailer 9, B-retailer 14, C-commercial 6, D-commercial 4, E-butchery 5, F-Butchery 3, G-butchery 2, H-commercial 1, **I**-commercial 3, J-commercial 5, K-commercial 2, L-retailer 6, M-retailer 7, N-commercial 6, O-commercial 1, P-commercial 3, Q-retailer 9, R-commercial 2, S-retailer 6, T-retailer 7, U-butchery 2, V-commercial 6, W-butchery 3, X-commercial 1, Y-commercial 3, Z-retailer 6, R-resistant, S-susceptible, **I**-intermediate

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4.4. Discussion

4.4.1. Prevalence of Listeria monocytogenes

L. monocytogenes has been examined in raw beef samples from supermarkets butcheries, commercial butcheries, and village butcheries. The findings of the current study revealed that presumptive colonies of *L. monocytogenes*, *L. ivanovii*, and other species of *Listeria* were found in 55 (96%) of 57 retail beef plate meat samples obtained from the supermarkets and butcheries of the Vhembe region. This occurrence was higher than previously reported in retail beef meat samples in other countries such as Nigeria (33.75%) (Eruteya *et al*., 2014), Dhamar governorate, Yemen (26.01%) (Al-Mashhadany *et al*., 2016), Erbil City, Kurdistan region, Iraq (13.9%) (Al-Mashhadany *et al*., 2019) and Meknes City, Morocco (7.14%) (Boukilli *et al*., 2020).

Based on the report of Korsak & Szuplewska (2016) and Matle *et al.* (2019), this could be due to contamination that mostly occurs during or after processing through cross-contamination. In addition, Cufaogle *et al*. (2021) argued that the presence of *Listeria* species in meat is usually an indicator of inadequate hygienic conditions that the meat was prepared. The presence of *L. monocytogenes* in meat has a detrimental effect on both the economy and public health. According to the report of Matle *et al*. (2019) on meat and meat products, the high prevalence of *L. monocytogenes* in retail beef was critical for South Africa's economy, trade, and public health. Such strains of *L. monocytogenes*, in addition to financial losses, can result in consumers avoiding a product completely.

In the present study samples examined for the presence of *Listeria* species from a supermarket in region 5 indicated that one (50%) of the samples was contaminated with *L. monocytogenes* and the other (50%) presented other species of *Listeria*. The prevalence of *L. monocytogenes* in region 5 was closely similar to that of Eruteya *et al.* (2014), where it was reported a 52.78% prevalence of *L. monocytogenes* in cow flesh in Port Harcourt, Nigeria. Arslan & Baytur. (2018), reported a lower prevalence of 26.6% prevalence of *L. monocytogenes* in ground beef in Bolu, Turkey, and Gebremedhin *et al*. (2021) reported a much lower prevalence of 4.4% of *L. monocytogenes* in meat from abattoirs, butchers, and restaurants in Ambo and Holeta, Ethiopia. Based on the report of Al-mashhadany *et al*. (2019), red meat offered to consumers at the retail level has gone through a number of phases in the slaughterhouse, including slaughter, skinning, evisceration, handling by consumers, and other procedures which may result in cross-contamination.

In South Africa, from 2017 to 2018, a large-scale outbreak caused by the presence of *L. monocytogenes* (1060 laboratory-confirmed cases, 216 fatalities) occurred, which had

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substantial health and economic repercussions (Ogunbanjo, 2018). The source was discovered in one of South Africa's famous brands. A subsidiary meat processing plant with low sanitary standards (Dallagi *et al*. 2023). According to Rodriguez-Lopez *et al*. (2018), Food contamination with L. monocytogenes may have been caused by biofilms on surfaces that encounter food, like cutting boards and slicers, as well as by biofilms on non-food surfaces like floors or sinks. Dallagi *et al*. (2023) argued that removing and preventing biofilms is essential to attaining cleaning objectives because of the difficulties connected with their presence in the food sector. Moreover, Siluma *et al*. (2023) recommended that meat safety regulations must be strictly followed by butcheries as unhygienic methods of handling meat carry a high risk of cross-contamination and could lead to serious public health issues.

During this study, among the nine (9) samples examined for the presence of *Listeria* species in two (2) supermarkets and one (1) commercial butchery in region 6, results indicated that all nine (9) samples (100%) contained presumptive *Listeria* species; *L. monocytogenes and L. ivanovii*. Other species of *Listeria* also occurred in 8 (89%) of the samples that were tested. The occurrence of *Listeria* species was highest in Sibasa supermarkets. This was higher than the prevalence of *Listeria* species reported by Ali *et al*. (2010) of 14 (4%) isolated in raw meat from retail shops in Karachi, Pakistan, and that of Dogruer *et al*. (2015) who reported 9.5% of *Listeria* species with 22.10% being *L. monocytogenes* and 4.21% being *L. ivanovii* in retail meat and meat products.

This may be significant concern, as reported by Cufaogle *et al*. (2021) that among the sensitive populations at risk such as pregnant women, unborn children, and newborns who acquire transmission across the placenta or during delivery, sensitive populations are significantly impacted by food-borne illness, and immunosuppressed adults are particularly vulnerable to such infections. *Listeria* thrives in extremely cold temperatures, therefore keeping contaminated food in the refrigerator could raise the risk of illnesses for the high-risk population. As a consequence, a study by Arslan & Baytur. (2018), recommended that immunocompromised individuals and pregnant women avoid consuming raw or undercooked beef or any other potential *Listeria* sources of food.

In the present study, among the three (3) samples examined from a commercial butchery in region 2 the presence of *Listeria* species. Results showed that all three (3) (100%) samples were contaminated with presumptive *L. monocytogenes* isolates and two (2) (67%) with presumptive *L. ivanovii* and other species of *Listeria*. This was higher than the report of Arslan & Ozdemir (2020) of 19.7% of *Listeria* spp. Including 5% of *L. monocytogenes* in

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Ready-To-Eat foods in Bolu, North-west Turkey, and the report of Adesokan *et al*. (2021) of 65.2% *L. monocytogenes* occurrence in raw meat in formal and informal meat markets in Ibadan, Nigeria.

The high prevalence of presumptive colonies of *L. monocytogenes* in our findings may be due to food handlers' poor hygiene procedures. According to Matle *et al*. (2019), in butcheries, and retail establishments in South Africa, many excellent hygiene measures have been identified as lacking. Furthermore, this could be due to the fact that at the retail level, hygiene regulations are not always strictly implemented. Based on a study by Siluma *et al*. (2023) conducted on meat safety practices and hygiene in supermarkets and various butcheries in the Vhembe district, South Africa, only half (50%) of the commercial butcheries practiced washing of hands and only 75% wore gloves during meat handling. These findings revealed a lack of adherence to meat safety regulations stipulated in the meat safety act of South Africa for retail stores and butcheries.

In this study, among the samples examined from the village butcheries, results indicated that all (100%) samples were contaminated with presumptive *Listeria* species, *L. monocytogenes* and *L. ivanovii* occurred in all the samples. All samples from village butcheries had a high occurrence of presumptive *L. monocytogenes and L. ivanovii and* no occurrence of other species of *Listeria.* This result was slightly higher than the report of Chuku *et al*. (2019) who reported a prevalence of 80.8% *L. monocytogenes* and 23.1% *L. ivanovii* in raw beef collected from North-central, Nigeria. It is also higher than that of Daniel *et al*. (2015) who reported 13.33% of *L. monocytogenes* and 11.76% of *L. ivanovii* in frozen chicken in Makurdi, Nigeria.

The high prevalence of presumptive *L. monocytogenes* and *L. ivanovii* found in the village butcheries could be attributed to inadequate cleaning and sanitization in the retailing environment. According to the findings of Siluma *et al*. (2023) on meat safety practices and hygiene among various butcheries in the Vhembe district, Limpopo province, South Africa, workers at village butcheries washed their hands with water that was placed in a plastic bucket or basin 67% of the time. The water utilised had previously been used to wash hands throughout the day, and it also held a dishcloth for wiping the butcheries' counters and hands. This practice contradicted the requirements of R 638 (Act 54 of 1972), which mandates that hand-washing facilities with hot water, a supply of soap, and clean, disposable hand-drying material or equipment must be available. Furthermore, during the study of Siluma *et al*. (2023), findings revealed that only seventeen (17%) of the village butcheries' employees used protective gloves when handling meat, all of the village butcheries' employees handled money while handling meat and 17% of the butcheries

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lacked a dustbin and dumped garbage outside the butchery's structure at a nearby dumpster site, all of these practices resulted in cross-contamination. Village and commercial retail stores are less likely to have sufficient sanitary materials for staff to utilize, such as gloves, appropriate disinfectants, and cleaning agents, therefore increasing the risk of contamination. Unlike the national retail competitors or supermarkets, the Vhembe district is primarily rural, hence training on the sanitary handling of meat and compliance with national safety regulations is often overlooked.

In the present study, overall, nineteen (19) shops were examined for the presence of *L. monocytogenes* in retail beef plate meat samples in the Vhembe district, including nine (9) supermarkets, seven (7) commercial butcheries, and three (3) village butcheries. Results revealed that all supermarkets, commercial butcheries, and village butcheries have *L. monocytogenes and L. ivanovii* apart from one (1) Supermarket located in region 5 which showed no occurrence of *L. ivanovii*. The occurrence of presumptive *Listeria* species (*L. monocytogenes* & *L. ivanovii*) was highest in supermarket samples and lowest in commercial butchery samples. This may be attributed to the large number of available supermarkets examined in Vhembe than commercial butcheries. These findings of prevalence are higher than the report of Mpundu *et al*. (2022) who reported 26.3% of *L. monocytogenes* and 19% of *Listeria* species in freshly slaughtered carcasses. Despite the fact that their study was conducted in freshly cut carcasses, compared to the present study in meat, their findings presented a much lower prevalence percentage. Besides the similarity in meat source(beef) and similar identification methods in the two studies, the variation in prevalence may be attributed to different sample sources as the samples from the present study were collected from the retail level (supermarkets, commercial and village butcheries) whereas their sample was obtained from freshly cut carcasses at the slaughterhouse.

4.4.2. Antibiotic susceptibility

During this study, approximately Seven (7) antibiotics were tested against twenty-six (26) *L. monocytogenes* isolates. The antimicrobials (antibiotics) used emanated from five (5) different antimicrobial classes. The antibiotic susceptibility tests revealed that the highest resistance of the isolates was against nalidixic acid (81%), followed by tetracyclines (73%). This was closely similar to the report of Gebremedhin *et al*. (2021) whose findings were a 70% resistance of *L. monocytogenes* against nalidixic acid in beef from abattoirs, butchers, and restaurants in Ambo and Holeta, Ethiopia. In a study by Farhoumand *et al*. (2020) on fresh beef and chicken meats marketed in Zanjan, Iran, and in a study by Adesokan *et al.* (2021) on raw meats sold in formal and informal markets in Ibadan, Nigeria, all (100) isolates were resistant to tested antibiotics including tetracycline.

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In the present study the highest susceptibility of the isolates was observed against sulphamethoxazole (35%) and the lowest in oxytetracycline (8%) and chlortetracycline (8%). This was different from the results of Matle *et al.* (2019) who reported resistance of sulphamethoxazole trimethoprim in 56% of *L. monocytogenes* isolated from meat and meat (why is my result different) products in South Africa but was lower than the result of Dogruer *et al*. (2015) who reported that all *L. monocytogenes* isolates (100%) were susceptible to sulphamethoxazole trimethoprim in meat and meat products in Turkey.

Intermediate resistance was not observed for sulphamethoxazole but was highest in oxytetracycline (19%) and chlortetracycline (19%) and lowest against Nalidixic acid (4%) and Enoxacin (4%). Although the resistance to oxytetracycline and chlortetracycline is intermediary in a few of the isolates. The study by Makumbe *et al*. (2021) revealed that such resistance can develop to full resistance and is therefore worth noting with a cautious approach. The danger of antibiotic resistance is that the antibiotic-resistant genes may render the treatment of patients that have contracted the food-borne disease unsuccessful by preventing the work of antibiotics and resulting in fatalities (Nwobodo *et al*., 2022). Therefore, it is of paramount importance to monitor the prevalence of *L. monocytogenes* and *Listeria* spp. in local products and the antimicrobial resistance acquired by *L. monocytogenes* that is found in beef meat circulating in retail shops of all kinds.

In this study, Ampicillin, the choice of first-line treatment for *listeriosis* exhibited a high resistance from most of the isolates (65%). This was higher than the report of Arslan & Baytur (2018) of 1.6% resistance of *L. monocytogenes* isolates in retail meat in Bolu, western Turkey, and lower than the report of Al-mashhadany *et al.* (2019) of 82% of *L. monocytogenes* isolates resistance to ampicillin in retail meat at Erbil city, Kurdistan region, Iraq. The results of this study were different from the report of Matle *et al*. (2019) of 85.6% and 62.59% sensitivity of Ampicillin and Tetracycline in meat and meat products in South Africa. Van *et al*. (2020) argued that this may be related to the frequent use of these antibiotics as growth promoters and supplements in animal feed, and for the treatment of human diseases. According to Larsson & Flach (2022), antibiotic resistance infections can spread to other bacteria of human clinical significance, making contaminated meat a serious threat to both public health and the economy.

Resistance against ampicillin, which is the first line of treatment for Listeriosis indicates a concern to the health of the public and reflects that the pathogen had acquired antimicrobial resistance genes. Such a phenomenon of acquired resistance has been best explained by

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Dutta *et al*. (2019) that the unwarranted application of antibiotics in animal feed as supplements in veterinaries and farms has been the misuse of antibiotics in animals. In addition, the degree of antibiotic resistance that the *L. monocytogenes* isolates in this investigation demonstrated warrants severe public health attention.

4.5. Conclusion

This study revealed a high prevalence of presumptive colonies of *L. monocytogenes*, *L. Ivanovii,* and other *Listeria* species from raw beef samples in various supermarket butcheries, commercial butcheries, and informal butcheries in the Vhembe district, Limpopo province, South Africa. The isolates were also resistant to a wide range of antibiotics used to treat human listeriosis such as ampicillin, tetracycline, and nalidixic acid in large numbers while a few of the isolates were susceptible to sulphamethoxazole and intermediary responsive to oxytetracycline and chlortetracycline. There is a high prevalence of presumptive *L. monocytogenes* isolates in raw beef from butcheries in Vhembe, Limpopo province. It is therefore recommended that *L. monocytogenes* control measures from relevant authorities be put in place. It’s also crucial to prepare meat adequately, train beef handlers more frequently, and to utilise antibiotics appropriately.

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CHAPTER 5: GENERAL CONCLUSION & RECOMMENDATIONS

5.1. Conclusion

Based on the findings of this study, it can be concluded that supermarkets follow the safety procedures in the handling of meat better than all other butcheries, it is very important for other butcheries (village and commercial) to adhere to meat safety regulations. Unhygienic practices of handling meat carry the potential and high possibility of cross-contamination and may result in serious public health problems. furthermore, raw beef sold in various butcheries in Vhembe is contaminated with presumptive colonies *L. monocytogenes* which may be a threat to the health of the Vhembe population, this was attributed to poor hygienic practices in handling meat at these shops. Moreover, most of the isolates are resistant to most used antibiotics such as tetracycline, ampicillin, and nalidixic acid. Treatment in case of a *listeriosis* outbreak may become burdensome as the pathogen may be resistant to antimicrobial treatment.

5.2. Recommendations

- It is recommended that intervention through training on food safety to enhance the hygienic procedures of meat handling along the beef distribution system, more especially within commercial and village butcheries be conducted and *L. monocytogenes* control measures from relevant authorities be put in place.
- Antibiotics must be utilised appropriately and only when prescribed to reduce the rate of antibiotic resistance.
- Additional tests such as biochemical testing, PCR and sequencing need to be conducted to further confirm the identity of the isolates found in raw beef from butcheries in Vhembe.
- Additional molecular research on *Listeria* species to determine the virulence genes present in *L. monocytogenes* isolated from retail beef needs to be conducted, to keep track of the type of strains of Listeria which may be circulating in the Vhembe district.
- Meat must not be undercooked but be prepared adequately in order to kill any pathogenic microbe that may be present in the meat and prevent foodborne outbreaks.

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APPENDIX I Chromogenic agar plate results

Chromogenic agar results from various supermarkets on retail beef plate meat Thohoyandou

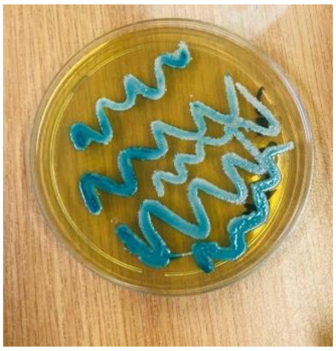

Figure A1: Retailer 1

Listeria monocytogenes: blue colonies with opaque white halo around colonies

Listeria ivanovii: Blue with halo around colonies

Figure A2: Retailer 1

***Listeria monocytogenes*:** green with opaque white halo around colonies

Listeria ivanovii: blue/green with withe halo around colonies

Figure A3: Retailer

Listeria monocytogenes: blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with withe halo around colonies

Figure B1: Retailer 3

***Listeria monocytogenes*:** green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure B2: Retailer 3

***Listeria monocytogenes*:** Blue with opaque white halo around colonies

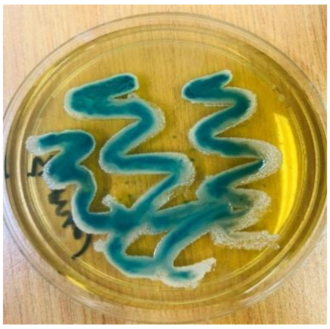

Figure B3: Retailer 3

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

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Figure C1: Retailer 6

***Listeria monocytogenes*:** green with opaque white halo around colonies

Listeria ivanovii: green with white halo around colonies

Figure C2: Retailer 6

***Listeria monocytogenes*:** blue with opaque white halo around colonies

Listeria ivanovii: blue with white halo around colonies

Figure C3: Retailer 6

***Listeria monocytogenes*:** blue with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure D1: Retailer 7

***Listeria monocytogenes*:** Blue with opaque white halo around colonies

Listeria ivanovii: blue with white halo around colonies

Figure D2: Retailer 7

***Listeria monocytogenes*:** Blue with opaque white halo around colonies

Listeria ivanovii: blue with white halo around colonies

Figure D3: Retailer 7

***Listeria monocytogenes*:** green with opaque white halo around colonies

Listeria ivanovii: green with white halo around colonies

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Figure E1: Retailer 2

***Listeria monocytogenes*:** blue green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure E2: Retailer 2

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue with white halo around colonies

Figure E3: Retailer 2

***Listeria monocytogenes*:** blue /green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

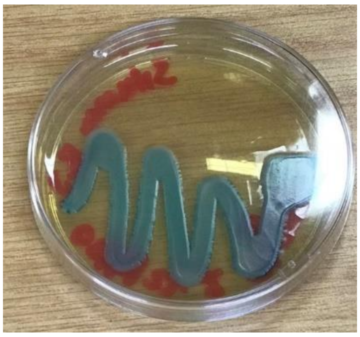

Figure F1: Retailer 4

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure F2: Retailer 4

***Listeria monocytogenes*:** green with opaque white halo around colonies

Listeria ivanovii: green with white halo around colonies

Figure F3: Retailer 4

***Listeria monocytogenes*:** green with opaque white halo around colonies

Listeria ivanovii: green with white halo around colonies

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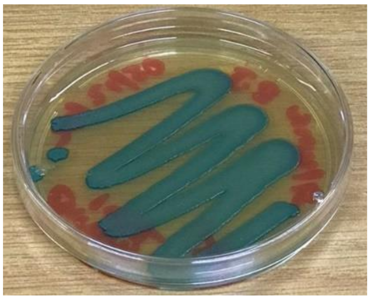

Figure N1: Commercial 1

Listeria monocytogenes**:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure N2: Commercial 1

Listeria monocytogenes**:** blue with opaque white halo around colonies

Listeria ivanovii: blue with white halo around colonies

Figure N3: Commercial 1

Listeria monocytogenes**:** blue with opaque white halo around colonies

Listeria ivanovii: blue with white halo around colonies

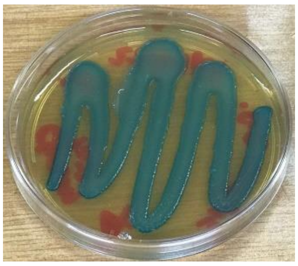

Figure O1: Commercial 3

Listeria monocytogenes**:** green with opaque white halo around colonies

Listeria ivanovii: green with white halo around colonies

Figure O2: Commercial 3

Listeria monocytogenes**:** green with opaque white halo around colonies

Listeria ivanovii: green with white halo around colonies

Figure O3: Commercial 3

Listeria monocytogenes**:** green with opaque white halo around colonies

Listeria ivanovii: green with white halo around colonies

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Figure L1: Commercial 2

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure L2: Commercial 2

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure L3: Commercial 2

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

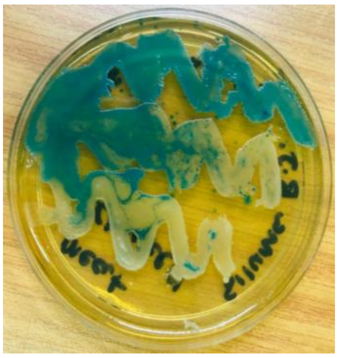

Figure M1: Commercial 5

***Listeria monocytogenes*:** blue with opaque white halo around colonies

Listeria ivanovii: blue with white halo around colonies

Figure M2: Commercial 5

***Listeria monocytogenes*:** blue with opaque white halo around colonies

Listeria ivanovii: blue with white halo around colonies

Figure M3: Commercial 5

***Listeria monocytogenes*:** blue with opaque white halo around colonies

Listeria ivanovii: blue with white halo around colonies

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Figure P1: Commercial 4

***Listeria monocytogenes*: blue** with opaque white halo around colonies

Listeria ivanovii: blue with white halo around colonies

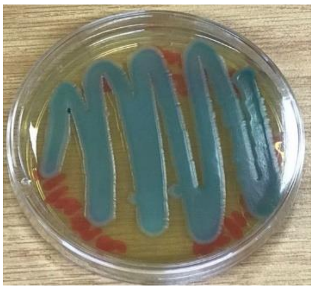

Figure P2: Commercial 4

***Listeria monocytogenes*:** blue with opaque white halo around colonies

Listeria ivanovii: blue with white halo around colonies

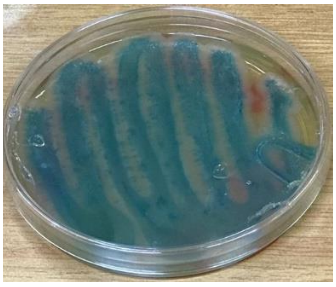

Figure P3: Commercial 4

***Listeria monocytogenes*:** blue with opaque white halo around colonies

Listeria ivanovii: blue with white halo around colonies

Phangami

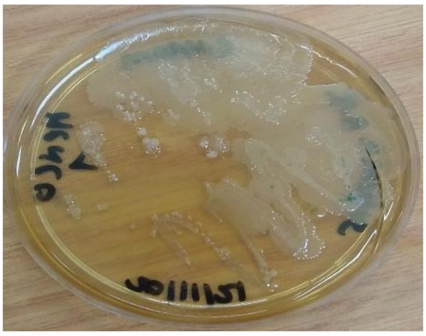

Figure G1: Retailer 11

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure G2: Retailer 11

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

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Sibasa

Figure H1: Retailer 9

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure H2: Retailer 9

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure H3: Retailer 9

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

© University of Venda

Figure I1: Retailer 10

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure I2: Retailer 10

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure I3: Retailer 10

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Shayandima

Figure J1: Commercial 6

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure J2: Commercial 6

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure J3: Commercial 6

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

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Village butcheries

Lwamondo

Figure Q1: butchery 1

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure Q2: butchery 1

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Figure Q3: butchery 1

***Listeria monocytogenes*:** blue/green with opaque white halo around colonies

Listeria ivanovii: blue/green with white halo around colonies

Vuwani

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Figure R1: butchery 2

***Listeria monocytogenes*:** green with opaque white halo around colonies

Listeria ivanovii: green with white halo around colonies

Figure R2: butchery 2

***Listeria monocytogenes*:** green with opaque white halo around colonies

Listeria ivanovii: green with white halo around colonies

Figure R3: butchery 2

***Listeria monocytogenes*:** green with opaque white halo around colonies

Listeria ivanovii: green with white halo around colonies

Tsianda

Figure S1: butchery 3

***Listeria monocytogenes*:** green with opaque white halo around colonies

Listeria ivanovii: green with white halo around colonies

Figure S2: butchery 3

***Listeria monocytogenes*:** green with opaque white halo around colonies

Listeria ivanovii: green with white halo around colonies

Figure S3: butchery 3

***Listeria monocytogenes*:** green with opaque white halo around colonies

Listeria ivanovii: green with white halo around colonies

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APPENDIX II Antibiotic susceptibility plate results

Figure A: Retailer 9

Ampicillin- Resistant

Tetracycline-Intermediate

Oxytetracycline-
Intermediate

Chlortetracycline-
Intermediate

Nalidixic acid-Resistant

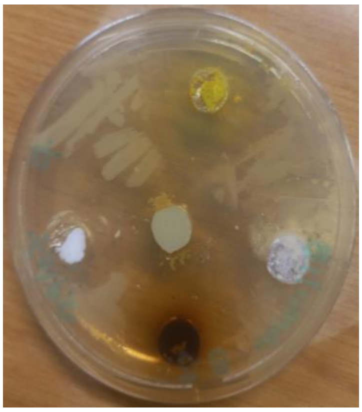

Figure B: Retailer 14

Ampicillin-Resistant

Tetracycline- Sensitive

Oxytetracycline-Sensitive

Chlortetracycline-
Sensitive

Nalidixic acid- Sensitive

Figure C: Commercial 6

Ampicillin-Intermediate

Tetracycline- Sensitive

Oxytetracycline-Sensitive

Chlortetracycline-
Sensitive

Nalidixic acid-
Intermediate

Figure D: Commercial 4

Ampicillin- Resistant

Tetracycline- Resistant

Oxytetracycline-
Resistant

Chlortetracycline-
Resistant

Nalidixic acid- Resistant

Figure E: Butchery 5

Ampicillin- Resistant

Tetracycline-Sensitive

Oxytetracycline-
Sensitive

Chlortetracycline-
Sensitive

Nalidixic acid- Sensitive

Figure F: Butchery 3

Ampicillin- Resistant

Tetracycline-Sensitive

Oxytetracycline-
Sensitive

Chlortetracycline-
Intermediate

Nalidixic acid- Sensitive

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Figure G: Butchery 2

Ampicillin- Resistant

Tetracycline-Intermediate

Oxytetracycline-Sensitive

Chlortetracycline-Sensitive

Nalidixic acid- Sensitive

Figure H: Commercial 1

Ampicillin- Resistant

Tetracycline-Intermediate

Oxytetracycline-Sensitive

Chlortetracycline-Sensitive

Nalidixic acid-Sensitive

Figure I: Commercial 3

Ampicillin-Intermediate

Tetracycline-Sensitive

Oxytetracycline-Sensitive

Chlortetracycline-Sensitive

Nalidixic acid-Sensitive

Figure J: Commercial 5

Ampicillin -Resistant

Tetracycline- Resistant

Oxytetracycline-Resistant

Chlortetracycline-Intermediate

Nalidixic acid-Resistant

Figure K: commercial 2

Ampicillin- Resistant

Tetracycline- Resistant

Oxytetracycline-Intermediate

Chlortetracycline-Intermediate

Nalidixic acid- Resistant

Figure L: Retailer 6

Ampicillin-Resistant

Tetracycline-Intermediate

Oxytetracycline-Sensitive

Chlortetracycline-Sensitive

Nalidixic acid-Resistant

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Figure M: Retailer 7

Ampicillin-Sensitive

Tetracycline-Sensitive

Oxytetracycline-Sensitive

Chlortetracycline-Sensitive

Nalidixic acid- Resistant

Figure N: Commercial

Ampicillin- Resistant

Tetracycline- Resistant

Oxytetracycline-Resistant

Chlortetracycline-Sensitive

Nalidixic acid-Resistant

Figure O: Commercial 1

Ampicillin-Resistant

Tetracycline-Sensitive

Oxytetracycline-Intermediate

Chlortetracycline-Resistant

Nalidixic acid-Resistant

Figure P: Commercial 3

Ampicillin- Resistant

Tetracycline-Resistant

Oxytetracycline-Resistant

Chlortetracycline-Resistant

Nalidixic acid-Resistant

Figure Q: Commercial 5

Ampicillin- Resistant

Tetracycline-Resistant

Oxytetracycline-Resistant

Chlortetracycline-Resistant

Nalidixic acid-Resistant

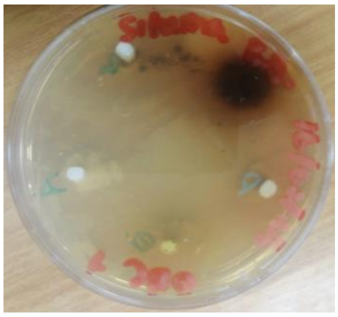

Figure R: Commercial 2

Ampicillin-Intermediate

Tetracycline-Intermediate

Oxytetracycline-Resistant

Chlortetracycline-Resistant

Nalidixic acid-Intermediate

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Figure S: Retailer 6

Ampicillin-Resistant

Tetracycline-Sensitive

Oxytetracycline-Sensitive

Chlortetracycline-Sensitive

Nalidixic acid- Resistant

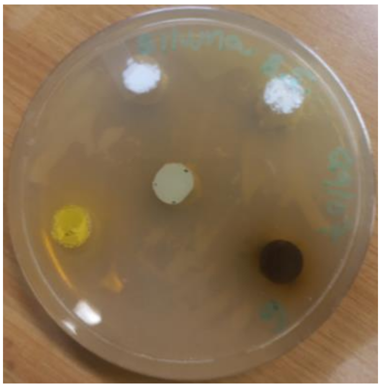

Figure T: Retailer 7

Ampicillin-Resistant

Tetracycline-Intermediate

Oxytetracycline-Intermediate

Chlortetracycline-Sensitive

Nalidixic acid-Resistant

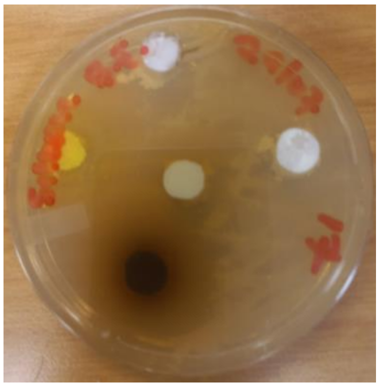

Figure U: Butchery 2

Ampicillin-Resistant

Tetracycline-Resistant

Oxytetracycline-Resistant

Chlortetracycline-Resistant

Nalidixic acid-Resistant

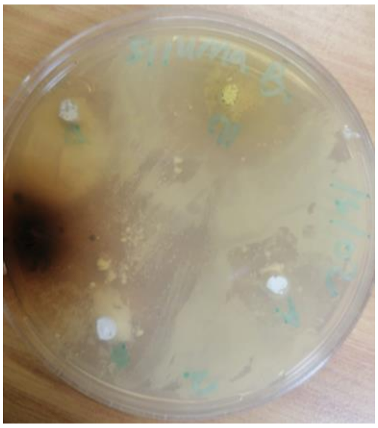

Figure V: commercial 6

Ampicillin- Resistant

Tetracycline- Resistant

Oxytetracycline-Resistant

Chlortetracycline-Resistant

Nalidixic acid-Resistant

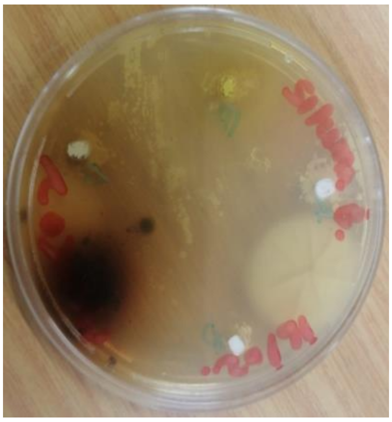

Figure W: Butchery 3

Ampicillin- Resistant

Tetracycline-Resistant

Oxytetracycline-Resistant

Chlortetracycline-Resistant

Nalidixic acid-Resistant

Figure X: Commercial 1

Ampicillin-Resistant

Tetracycline- Resistant

Oxytetracycline-Resistant

Chlortetracycline-Resistant

Nalidixic acid-Resistant

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Figure Y: Commercial 3

Ampicillin-Resistant

Tetracycline-Intermediate

Oxytetracycline-Resistant

Chlortetracycline-Intermediate

Nalidixic acid-Resistant

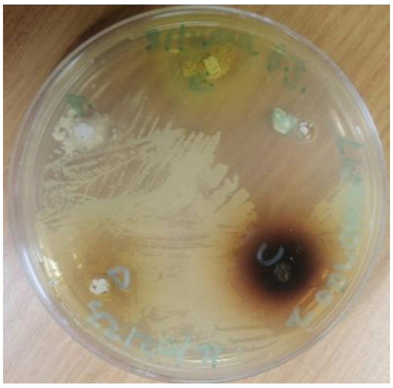

Figure Z: Retailer 6

Ampicillin- Sensitive

Tetracycline- Resistant

Oxytetracycline-Resistant

Chlortetracycline-Resistant

Nalidixic acid-Resistant

Figure a1: Retailer 9

Ampicillin- Sensitive

Tetracycline-Intermediate

Oxytetracycline-Intermediate

Chlortetracycline-Intermediate

Nalidixic acid- Resistant

Figure b1: Retailer

Ampicillin-Sensitive

Tetracycline-Resistant

Oxytetracycline-Resistant

Chlortetracycline-Sensitive

Nalidixic acid-Resistant

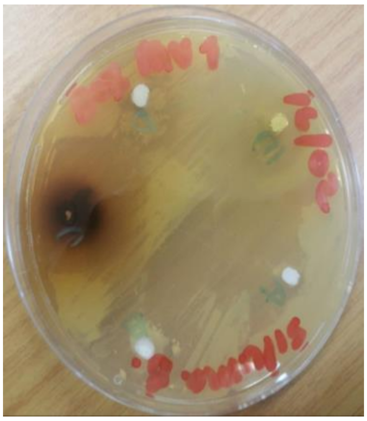

Figure c1: Retailer 14

Ampicillin- Resistant

Tetracycline-Intermediate

Oxytetracycline-Intermediate

Chlortetracycline-Intermediate

Nalidixic acid-Resistant

Figure d1: Control

Ampicillin- Sensitive

Tetracycline-Sensitive

Oxytetracycline-Sensitive

Chlortetracycline-Sensitive

Nalidixic acid-Sensitive

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Figure e1: Butchery

Enoxacin- Intermediate

Sulphamethoxazole-
Resistant

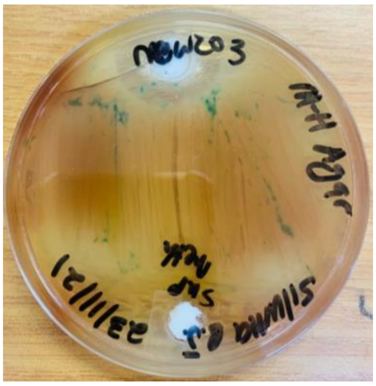

Figure f1: Commercial 6

Enoxacin-Intermediate

Sulphamethoxazole-
Resistant

Figure g1:
Commercial 4

Sulphamethoxazole-
Sensitive

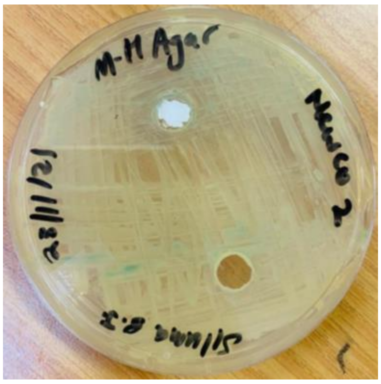

Figure h1:
Commercial 6

Sulphamethoxazole-
Resistant

Figure i1: Retailer 14

Enoxacin- Sensitive

Sulphamethoxazole-
Resistant

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Figure j1: butchery 5

Sulphamethoxazole- Sensitive

Figure k1: Commercial 6

Enoxacin- Sensitive

Sulphamethoxazole- Resistant

Figure l1: Commercial 4

Sulphamethoxazole- Sensitive

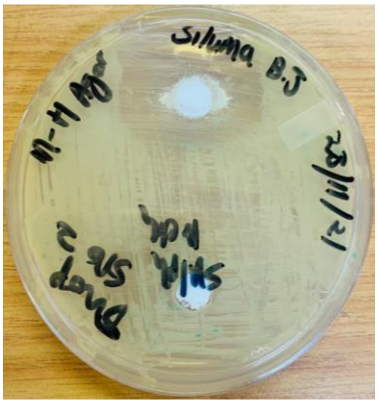

Figure m1: Retailer 9

Enoxacin- Sensitive

Sulphamethoxazole- Resistant

Figure n1: Retailer 9

Enoxacin-Sensitive

Sulphamethoxazole- Resistant

Figure o1: butchery

Sulphamethoxazole- Sensitive

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Figure p1: butchery 3

Sulphamethoxazole- Resistant

Figure q1: Commercial 6

Sulphamethoxazole- Resistant

Figure r1: Retailer 9

Enoxacin- Resistant

Sulphamethoxazole- Resistant

Figure s1: Retailer14

Enoxacin-Resistant

Sulphamethoxazole- Resistant

Figure t1: Retailer 9

Enoxacin- Intermediate

Sulphamethoxazole- Resistant

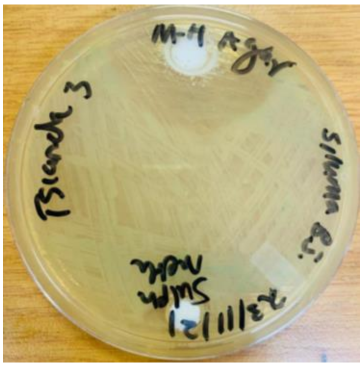

Figure u1: butchery 3

Enoxacin-Sensitive

Sulphamethoxazole- Resistant

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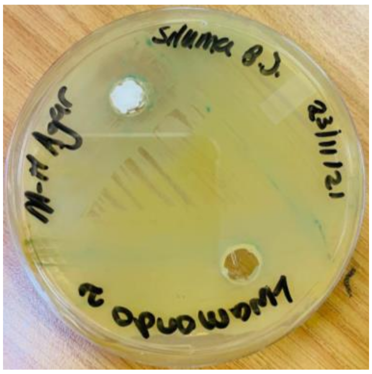

Figure v1: butchery 2

Sulphamethoxazole- Resistant

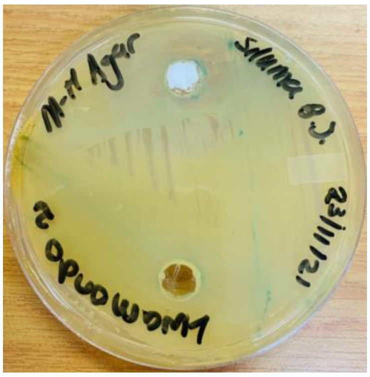

Figure w1: Butchery 2

Sulphamethoxazole- Intermediate

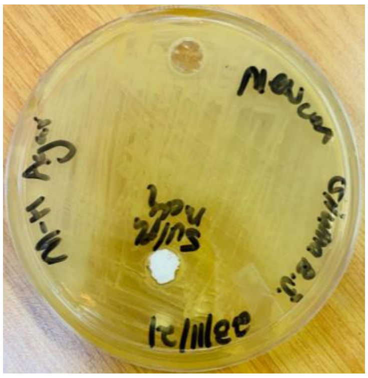

Figure x1: Commercial

Sulphamethoxazole- Resistant

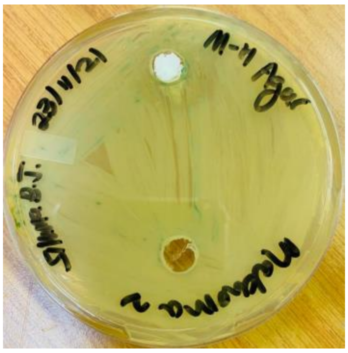

Figure y1: commercial 4

Sulphamethoxazole- Resistant

Figure z1: butchery 2

Sulphamethoxazole- Resistant

Figure a2: butchery 2

Sulphamethoxazole- Resistant

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Figure b2: Retailer 6

Enoxacin- Sensitive

Sulphamethoxazole-
Resistant

Figure c2: butchery 5

Enoxacin- Sensitive

Sulphamethoxazole-
Resistant

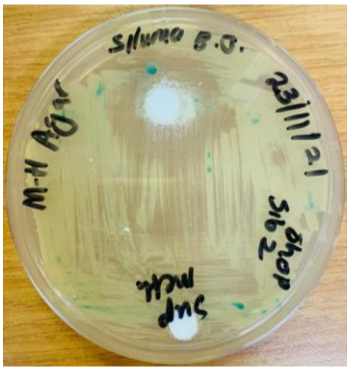

Figure d3: Retailer 9

Enoxacin-
Intermediate

Sulphamethoxazole-
Resistant

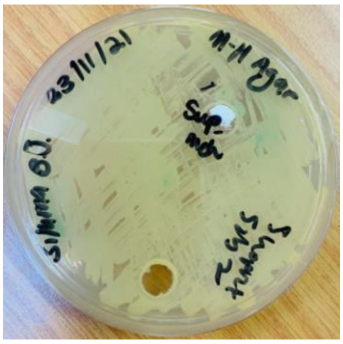

Figure e2: Retailer 6

Sulphamethoxazole-
Resistant

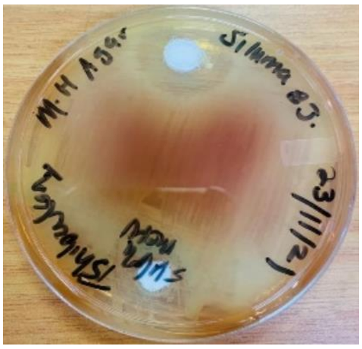

Figure f2: butchery 5

Enoxacin- Intermediate

Sulphamethoxazole-
Resistant

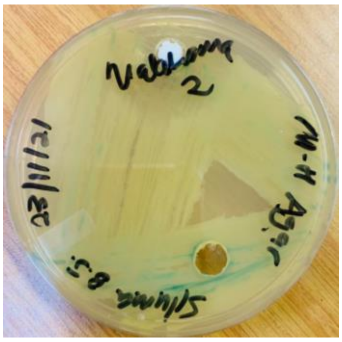

Figure g2: commercial 4

Sulphamethoxazole-
Resistant

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APPENDIX III LIST OF ARTICLES IN PUBLICATION/SUBMITTED MANUSCRIPT

Results from this dissertation have been submitted for publication and one has been published as an original research article.

Journals.

- Siluma, B. J., Kgatla, E. T., Nethathe, B., & Ramashia, S. E. (2023). Evaluation of Meat Safety Practices and Hygiene among Different Butcheries and Supermarkets in Vhembe District, Limpopo Province, South Africa. *International Journal of Environmental Research and Public Health*, *20*(3), 2230.
- Siluma, B. J., Kgatla, E. T., Nethathe, B., & Ramashia, S. E. (2023). Prevalence and antibiotic susceptibility of *Listeria monocytogenes* in retail beef plate meat in the Vhembe district, Limpopo province, South Africa. *Cogent Food & Agriculture journal. Submitted manuscript.*

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