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Public health importance and Antibiotic Resistance patterns of *Listeria* species in food and food processing environments in selected Districts of Zambia

A Thesis Submitted in Fulfillment for the Doctor of Philosophy Degree in Public Health

By

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CERTIFICATE OF APPROVAL

This dissertation of Prudence Mpundu fulfills the requirement for the award of a Doctor of Philosophy Degree (Ph.D.) in Public Health by the University of Zambia.

Internal Examiner I

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ABSTRACT

Awareness of food safety risks has increased, given the occurrence and intensity with which zoonotic diseases are reported. Due to the widespread distribution of food products, controlling foodborne pathogens has become increasingly relevant worldwide. Consequently, this present study aimed to evaluate public health importance and antibiotic resistance patterns of *Listeria* species in selected Districts of Zambia across foods and food processing environments.

654 food and environmental samples were collected from poultry abattoirs *n*=150, beef abattoirs *n*=250, fish plants *n*=150, RTE deli meats *n*=52, lettuce *n*=52, and environmental samples *n*=80. Samples were collected randomly, using a cross-sectional study design from March 2020 to August 2021. Samples were first imperiled to pre-enrichment in half-strength Fraser broth, followed by enrichment in full-strength Fraser broth, and subsequent plating on *Listeria* agar. *Listeria* growth characteristics were identified based on their morphological and biochemical reactions. Quantitative survey data were analyzed using STATA Version 15.0 software and WHONET. Further, we used molecular detection to determine genes conferring resistance and genomic relatedness of *Listeria* species. Custom pipeline was used for sequence assembly and analysis.

Of the 654 samples examined, 16.4% (95% CI: 13.5 – 19.2) contained *Listeria* species. When specific allocated sample size for each variable was considered, *Listeria* species were present in 42.5% (95% CI: 33.6 – 51.4), 14.5% (95% CI: 9.6 – 19.4), 23.1% (95% CI: 11.5 – 34.7), 13.5% (95% CI: 4.1 – 22.8) and 10.0% (95% CI: 3.4 – 16.6) of poultry samples, beef samples, RTE deli meats, lettuce, and environmental samples respectively. The proportions of *Listeria* species were *L. innocua* 67.2%, *L. monocytogenes* 21.5%, *L. ivanovii* 5.6%, *L. seeligeri* 3.7%, and *L.*

marthii 1.9%. Considering surface samples, those from poultry had a 60% contamination rate compared to cloacal samples, which was at 25.0%. Among samples from beef, interior samples were more contaminated at 17.0% than exterior surfaces at 12.0%. Isolation of *Listeria* species was not recorded from tilapia fish and environmental swabs from beef abattoirs. Furthermore, *L. seeligeri* was only recorded in lettuce vegetables. At WGS, all 42 (100%) isolates expressed intrinsic resistant genes *fosX* (resistance to fosfomycin) and *lmo0919* (resistance to lincosamides) with only 17.5% expressing the *Tet(M)* (resistance to tetracycline) resistant genes. While only one isolate, 42 (2%) recorded *dfrE* (resistance to trimethoprim), *erm(B)* (macrolide), *lsa(A)* (resistance to Lincosamide, streptogramin), *Tet (L)* (resistance to tetracycline).

Within its limits, this study has elucidated the occurrence of *L. monocytogenes* and other *Listeria* species across different food sources at different processing and value points in Zambia. The isolation of closely related genetic species clustering within and between food sources strongly intimates the potential occurrence of cross-contamination. To a greater extent, these findings strongly suggest the need for policy shift regarding pathogen tracking and monitoring in relation to food safety, food outbreak investigations, and applying transformative food systems.

DEDICATION

I dedicate this work to my late mother, Flavia Nachande, who always inspired me to work hard also I dedicate this work to my husband NATHAN KAZUNGA ZIMBA for understanding and caring. This also includes my children, JOSHUA KAZUNGA ZIMBA, and CONSTANCE JABULANI ZIMBA.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACEIDHA	Africa Center of Excellence for Infectious Diseases of Humans and Animals
ATGC	Adenine, Thymine, Guanine, Cytosine.
Bp	Base pairs
CDC	Center for Disease Control
CI	Confidence Interval
CSO	Central Statistical Office
D.N.A	Deoxyribonucleic acid

dsDNA	double-stranded DNA
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
GMPs	Good Manufacturing Practices
HACCP	Hazard Analysis Critical Point
ISO	International Standard Organization
KDa	1000 Daltons
LB	Luria-Bertani
MALDI-TOF MS	Matrix Assisted Laser Desorption/Ionization-Time of – Fight Mass Spectrometry
MEGA6	Molecular Evolutionary Genetics Analysis version 6.0
MFLCSO	Ministry of Fisheries and Livestock Central Statistical Office
NCBI	National Center for Biotechnology Information
NCC	Namwala Municipal Council
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
Ppm	parts per million
Prs	phosphoribosyl pyrophosphate synthetase
RPM	Revolutions per Minute
rRNA	ribosomal ribonucleic acid
RTE	Ready to Eat
SOSs	Standard Operating Systems
WGS	Whole Genome Sequencing
WHO	World Health Organization

ZHMIS	Zambia Health Management Information Systems

CHAPTER ONE

INTRODUCTION

1.1 Background

Listeriosis is a disease caused by a bacterium of the genus *Listeria*. *Listeria* is an emerging bacterium of public health importance globally, although rare; the mortality rate is about 25% to 30% worldwide, much higher than that of *Campylobacter* or *Salmonella* (de Noordhout et al., 2014b). Listeriosis is one of the significant causes of death from preventable foodborne infections in developed countries (Shoai-Tehrani et al., 2019). Among the over 17 known *Listeria* species, *L. monocytogenes,* and *L. ivanovii* are pathogenic species affecting humans and animals (Troxler et al., 2000). Listeriosis is a potential hazard in food processing and handling environments, particularly in poultry, beef, fish, vegetables, and ready-to-eat foods (RTE) (Berrang et al., 2010, Koskar et al., 2019a). Scrutiny in the food industry is increasing, given the frequency and intensity of zoonotic disease reports. *Listeria* has a high natural distribution, with previous isolation recorded from humans and several animals in the soils, aquatic plants, and assorted types of foods (Orsi et al., 2016).

From the public health point of view, *L. monocytogenes* is the essential *Listeria* species that causes a broad spectrum of clinical syndromes, commonly called listeriosis, with a case fatality of 20 - 30% (Hernandez-Milian and Payeras-Cifre, 2014). The clinical features range from mild influenza-like illness to meningitis, frequently accompanied by blood poisoning and meningoencephalitis (Troxler et al., 2000). *Listeria* is a ubiquitous pathogen, and the common species isolated in food environments are *L. monocytogenes* and *L. innocua* (Kaszoni-Rückerl et al., 2020). *Listeria monocytogenes* are problematic for the food industry as they can persist in

food production and environments when hygienic practices are compromised. It is also known to colonize a wide variety of foods, mostly of animal origin and recently also vegetable foods (Vitas, 2004). Ready to eat meats, fish samples, and cheese with long shelf time are considered high-risk foods for *Listeria* contamination (Thomas et al., 2012, Marian et al., 2012, Liu et al., 2020).

In 2018, the United States of America (USA) experienced listeriosis outbreaks believed to have come from foods considered a moderate risk by existing assessments (Archer, 2018). According to the European Centre for Disease Prevention and Control, many other countries, including Europe, previously had multi-country episodes with 26 cases (case fatality 15.4%) in 2017 (ECDC, 2018). Isolation of *Listeria* in RTE foods of both animal and plant origin (ECDC, 2018). A European Food Safety Authority (EFSA) report in 2013 had 1,763 confirmed cases of listeriosis from 27 member states, resulting in 191 deaths (Hazards et al., 2018, Ricci et al., 2018).

In South Africa (SA), a large listeriosis outbreak occurred in January 2018. The National Institute of Communicable Diseases (NICD, 2018) recorded 1,060 confirmed cases and 216 deaths with a case fatality rate of 21%. The outbreak was recorded as the deadliest since SA began tracking attacks in the 1990s (NICD, 2018). The RTE foods were a significant source implicated in the spells, mainly of animal origin. However, contamination records in raw chicken and meat products were 19.2% and 14.2% respectively (Van et al., 2020, Matle et al., 2019). Studies and active surveillance have been underway to control *L. monocytogenes* since the outbreak occurred in SA (Dara et al., 2016, Van den Honert et al., 2018). South Africa currently exports meat products and other foodstuffs to about fifteen (15) African countries Zambia inclusive (WHO,

2018). So far, only one known study reported the isolation of *L. monocytogenes* from raw vegetables at 20% in Zambia (Nguz et al., 2005).

Accordingly, contamination of processed RTE foods with *L. monocytogenes* occurs primarily after processing through cross-contamination due to the propensity to adhere to food contact surfaces and form biofilms (Da Silva et al., 2013). *Listeria monocytogenes* are challenging to eliminate from the food processing environment even if well-designed sanitation programs are utilized (Kornacki and Gurtler, 2007).

Identifying *L. monocytogenes* and other *Listeria* species involves culture methods founded on selective enrichment and plating. Characterizing specific *Listeria* species includes colony morphology, sugar fermentation, and hemolytic properties (Adzitey and Huda, 2010). Although these methods are the gold standard, their major disadvantage is that they are time-consuming and may not suit foods with short shelf lives. Recently, another culture-based method has been in use Matrix-Assisted Laser Desorption/Ionization-Time of – Fight Mass Spectrometry (MALDI-TOF MS) it is a simple, rapid, and economical technique for the characterization of bacterial isolates (Griffin et al., 2012, Anderson et al., 2012). It can give up to strain level differentiation of bacteria such as *Listeria* species which is crucial in epidemiological investigations of foodborne outbreaks.

However, rapid molecular tests such as Polymerase Chain Reaction (PCR) target specific genes such as the highly conserved housekeeping *prs* gene that encodes phosphoribosyl pyrophosphate synthase are in use (Nightingale et al., 2005). These methods are recently developed (Chen et al., 2007, Kim et al., 2007, Kérouanton et al., 2010), are accurate (Touron et al., 2005, Jadhav et al., 2012), and to a greater extent, can reflect the genetic relationship between isolates (Gasanov et

al., 2005). Although the *prs* gene does not differentiate different types of *Listeria* species, it nevertheless indicates *Listeria* species in each sample of interest (Nightingale et al., 2005, Doumith et al., 2004). Whole Genome Sequencing (WGS) has proven to be an effective method of tracking the onward transmission of bacteria or resistance plasmid transfer between bacteria. Determination and evaluation of an organism's whole DNA (Deoxyribonucleic acid) sequence at low costs in a short period of time. It allows for the identification of AMR and the early detection of outbreaks or their epidemiological investigation (Köser et al., 2012).

Listeria monocytogenes is the etiological proxy of listeriosis in humans and many other vertebrate species, including birds, while *L. ivanovii* only causes infections, mainly in ruminants (Weller et al., 2015). *Listeria monocytogenes* is the third most common cause of foodborne death in humans with impaired immunity (Luque-Sastre et al., 2018b). For approximately a century, antibiotics used to treat infectious diseases such as listeriosis has benefited human and animal health (Morvan et al., 2010). Recently, a recorded increase in the emergence of antibiotic-resistant bacteria due to the overuse of antibiotics in clinical and agriculture activities is typical (Byrne et al., 2016). The commonly used antibiotics are but not limited to penicillin G, ampicillin, gentamicin, erythromycin, vancomycin, chloramphenicol, ciprofloxacin, and tetracycline (Noll et al., 2018). Clinical and Laboratory Standards Institute (CLSI) describes the first-line antibiotics used in treating listeriosis as penicillin G, ampicillin, Trimethoprim/sulfamethoxazole, and imipenem (Sader et al., 2020). Using the same drugs in treating poultry and other animals is common (O'neill, 2015). The disproportionate use of antibiotics in veterinary medicine has contributed significantly to the distribution of antibiotic-resistant pathogens in the environment (Schwartz et al., 2003). Equally, detecting antibiotic-

resistant *Listeria* species in animals, foods, and their environment is familiar (Jamali et al., 2013). Resistant pathogens isolated from food and the environment transmits to humans, and proper measures are needed to prevent their environmental spread.

Infections due to *L. monocytogenes* are becoming increasingly problematic because most generally show low susceptibility. Resistance to numerous antibiotics used in humans and animals is slowing becoming common. *Listeria* species, especially the *L. monocytogenes*, are naturally resistant to cephalosporins, and in recent years, bacterial resistant is becoming one of the significant problems globally (Byrne et al., 2016). Among the common resistance antibiotics in most bacteria, including *Listeria*, is tetracycline, reported in beef and poultry (Khen et al., 2015, Cervantes, 2015). Tetracycline resistance in commensal and pathogenic bacteria is due to acquiring *tet* genes via self-transferable plasmids or conjugative transposons (Charpentier et al., 1995). The common resistant genes associated with *L. monocytogenes* in tetracycline are *tet(K), tet(L),* and *tet(M)* (Escolar et al., 2017). Although in recent years, other pathogens are slowly becoming resistant to antibiotics used in the human and animal treatment (O'Neill, 2014).

1.2 Statement of the Problem

Listeriosis foodborne infection reports in Zambia are periodic (ZHMIS, 2018). It is an uncommon cause of illness in the general population but common in people with impaired immunity systems (Jordan and McAuliffe, 2018). The disease is mainly extra-intestinal and can present itself in varying ways, primarily as abortion in pregnant women. Furthermore, some individuals present with septicemia or central nervous system infections. The disease presents a

high case fatality rate in all these individuals, which may not resemble a typical foodborne-related condition (EFSA, 2015). There is no official data on food contamination caused by *L. monocytogenes* because of lack of screening services, even in vulnerable patients. Moreover, *Listeria* infections mainly occur sporadically, making it difficult to control and diagnose. Therefore, there is inadequate information on *Listeria* species contamination and antibiotic-resistant profiles in various foods and their environments in Zambia.

South Africa, which recently recorded listeriosis outbreaks, is Zambia's second-largest trading partner (https://www.lusakatimes.com/2018/01/24/emmanuel-mwamba-cautions-sa-food-exports-zambia/). Zambia imports various foods from SA. Despite this risk posed by trade, there is a lack of information on the extent of the *Listeria* species situation in Zambia. The lack of active surveillance of *L. monocytogenes* makes evaluating the food safety risk posed by *Listeria* contamination challenging. In the last decade, Zambia has seen increased consumption of food of animal origins, such as beef, fish, and poultry. Zambia has few formal abattoirs to process massive slaughter to meet demand (Haileselassie et al., 2013). These prevailing unhygienic meat slaughter may introduce compromised meat safety. Therefore, fecal spillage on the meat increases, and the likelihood of producing contaminated carcasses may occur throughout the value chain (farm to folk). Therefore, introducing *Listeria* contamination is possible in the food chain (Haileselassie et al., 2013). Globally, statistics on foodborne disease burden are available primarily from developed countries. Despite the numerous reports on listeriosis from other countries, there is a lack of research regarding *L. monocytogenes* contamination and antibiotic resistance in various foods and their environment in Zambia.

Meanwhile, most patients visiting hospitals are from vulnerable groups susceptible to *L. monocytogenes* infections, as recorded (ZHMIS, 2018, Duarte et al., 2019). This study aimed to

answer the critical research question: “The public health importance and antibiotic resistance patterns of *Listeria* species in food and food processing environments in selected Districts of Zambia.

1.3 Rationale of the Study

Listeriosis is a foodborne disease caused by consuming contaminated foods, including RTE foods (Koskar et al., 2019a). However, monitoring food-borne infections requires an effective local, national, and international surveillance system (de Noordhout et al., 2014a). Despite Zambia having a data collection tool that gives various disease burdens affecting the country, this tool lacks information on specific causative agents responsible for causing disease (ZHMIS, 2018). *Listeria* infection occurs sporadically, and compared to other bacterial diseases; it proves difficult to control because of its occurrence. Understanding this is the only way to prevent such outbreaks from the onset and broaden surveillance data from different sources, including food. Most previous studies in Zambia focus on water as the main driving force for bacterial infections (WHO, 2015).

Safe food awareness globally has increased significantly with multistate outbreaks meaning contamination from a commercial product imported from another country (Rose et al., 2001). In 2005 WHO, in their joint meeting held in Africa, identified many lapses in African countries concerning food safety (WHO and FAO, 2013). Among the prominent issues were the lack of international standards in food safety legislation and the under-reporting of food-borne illnesses. National foodborne disease burden is vital to establish food safety as a public health priority, rank diseases, and implement interventions (Pires et al., 2021a). Zambia needs to get to the center

stage to improve the reporting system and implementation of legislation, especially in *Listeria* contamination, because this has possible ripple implications on the allocations of interventions for prevention. Subsequently, in addition to being a public health risk, *Listeria* infections can yield economic burdens such as the cost of sample analysis, clientele loss, and recalls of contaminated products (de Noordhout et al., 2014a, Unnevehr, 2015). In current years, there have been significant changes in food production. Cross-contamination in food processing environments from raw to RTE foods has increased exposure to *L. monocytogenes,* especially in commercial foods (Liggans et al., 2019).

Unfortunately, antimicrobial resistance is also an emerging public health threat to food safety. It is associated with using antimicrobials in humans and food animals (Bertsch et al., 2014a). Knowing the profile of food types commonly invaded by *Listeria* species bacteria may help pre-empty surveillance and monitoring foods of high-risk profile to *L. monocytogenes*. Implementing interventions that address the problem may also help improve our data capture, especially in our ZHIMS baseline data reporting tool (ZHMIS, 2018). Furthermore, even possible prevention of sporadic attacks associated with listeriosis yields results, primarily if good surveillance is employed.

1.4 Study Objectives and Research Questions

1.4.1 General Objective

To evaluate public health importance and antibiotic resistance patterns of *Listeria* species in selected Districts of Zambia in food and food processing environments.

1.4.2 Specific Objectives

1. To determine *Listeria* species in selected foods and food processing environments.

2. To determine genetic characteristics of *Listeria* species associated with antibiotic resistance.
3. To assess the genetic relatedness of *Listeria* species across food and food processing environments.
4. To determine genes conferring resistance to *Listeria* species

1.4.3 Research Questions

1. What *Listeria* species are circulating in Zambia's selected foods and food processing environments?
2. What are the genetic characteristics of *Listeria* species associated with antibiotic resistance?
3. Differentiation of *Listeria* species across food and food processing environments in relation to their genes.
4. What are the genes responsible for conferring resistance to *Listeria* species?

CHAPTER TWO

LITERATURE REVIEW

2.1 General overview

Listeria is the leading cause of zoonotic enteric infections worldwide in developed and developing countries (Rodriguez et al., 2011). *Listeria* species are ubiquitous bacteria commonly distributed in the environment and found in various foods and cold storage environments, including water (Tan et al., 2019, Zilelidou and Skandamis, 2018). The widespread distribution of *L. monocytogenes* and other *Listeria* species in natural environments is related to their ability to grow and survive in extreme conditions (Rodriguez et al., 2011, Tan et al., 2019). Among the over 17 species of *Listeria*, only *L. monocytogenes* is commonly pathogenic for humans and can cause serious infections (Zilelidou and Skandamis, 2018). *L. monocytogenes* is an opportunistic human pathogen that leads to listeriosis, often affecting the population with an impaired immune system (Jordan and McAuliffe, 2018, Luque-Sastre et al., 2018a). Listeriosis has increasingly become common in the last 25 years since the first food-borne outbreak records (Aarestrup and Wegener, 1999). Treatment for listeriosis involves supportive therapy and intravenous antibiotics (Rezai et al., 2018). Bacteria resistant to antibiotics have developed over this period, with the first resistant strains of *L. monocytogenes* detected in 1995, and trends are increasing over time (Rodriguez et al., 2011). Treatment of listeriosis is challenging when it involves strains resistant to antibiotics.

Listeria monocytogenes is generally susceptible to a wide range of antibiotics except for cephalosporins and fosfomycin to which the pathogen is naturally resistant (Moreno et al., 2013).

Several studies have indicated that the development of AMR in zoonotic bacteria appears to be associated with primarily antimicrobial use in animals (Aarestrup and Wegener, 1999, Rezai et al., 2018). Veterinary use of antibiotics includes use on pets, farm animals, and fish (Luque-Sastre et al., 2018b). Antibiotic utilization involves human disease therapy, prophylaxis in food-producing animals, and increasing animal growth and feed efficiency (Unnevehr, 2015). Therefore, there is a need to monitor antibiotic use in animals consumed by humans because of the public health impact it may have in treating diseases.

2.2 Epidemiology of the Listeriosis

2.3 Public Health and Economic Significance of Food-Borne Diseases

Worldwide, foodborne illnesses caused by bacteria (226,526,634) are common in relation to those caused by viruses (138,513,782) and parasites (10,284,561) (WHO, 2015). Microbial food safety contaminations are emerging as a global concern because of the effect on consumer health and food losses. Effects such as product recall because of different food contaminates leading to foodborne diseases are common (Unnevehr, 2015). The first estimates of the global and regional disease burden of 31 foodborne hazards were reported (WHO, 2015). The results indicated that each year, 1 out of 10 people get ill from food contaminated with microbial or chemical agents, resulting in 600 million illnesses, 420,000 deaths, and the loss of 33 million health years of life globally (Havelaar et al., 2015). Information on the estimates of the economic impact of foodborne illness is scarce. In the US, the economic impact of foodborne illnesses, although secondary to loss of lives, is driven by medical care, loss of reputation, and loss of customer clientele (Scharff et al., 2016). In 2016, the loss of wages attributed to foodborne in the US was $20 to $43 billion yearly (Scharff et al., 2016). It is also estimated that more than 2 million deaths come from developing countries because of foodborne diseases (Kelly et al., 2014). Africa has

the highest foodborne disease (FBD) burden per population, followed by Southeast Asia and Eastern Mediterranean sub-regions (Pires et al., 2021b).

According to the WHO report of 2015, *Campylobacter* caused the highest number of annual cases (95,613,970) but had a low fatality rate (0.022), followed by *Salmonella* (78,707,591) (WHO, 2015). The report further indicated that the fatality rates due to bacteria were generally low except for *L. monocytogenes,* which had the highest rate (22.4%) (WHO, 2015). *Listeria monocytogenes* accounts for about 2500 cases, 2289 hospitalizations, and 449 deaths yearly in the US (Wesley, 2009, Scallan et al., 2011). The estimated cost of acute illness was $2.3 billion annually (Wesley, 2009). While the costs of listeriosis, including morbidity, mortality, and cost wages, are estimated at $2.8 billion by the Economic Research Service/US Department of Agriculture (ERS/USDA., 2014). Most human listeriosis epidemics were linked to dairy product consumption (Painter et al., 2007). Product recalls, sporadic cases, and outbreaks have incriminated RTE delicatessen items (WHO, 2022). In the last decade, human listeriosis came from consuming contaminated deli meats 54%, dairy products, including cheese 24%, and fresh produce 8.7% (Hoffman et al., 2007). In France, listeriosis outbreaks were traced in pickled pork tongues, and 279 human cases were recorded (Ryser and Marth, 1999).

2.4 Global prevalence of *Listeria monocytogenes*

The epidemiology of listeriosis is complex but occurs after the ingestion of *L. monocytogenes* in food (Moura et al., 2017). *Listeria monocytogenes* are omnipresent in the environment, and the incubation period between consumption of contaminated food and symptoms of severe infection is long and variable (1- >90 days), making it difficult to establish links between cases and specific food exposure (Ferreira et al., 2014a). In the late 1960s and the early 1980s, the

incidence was extremely low in the United Kingdom, with fewer than 100 cases reported annually in England and Wales (McLauchlin et al., 1991). The incidence doubled between 1987 and 1989, with an average of 250 cases reported annually (de W and McClure, 2002). In 1977 and 1989 a near doubling of listeriosis cases in the UK records (McLauchlin et al., 1991). This upsurge consisted of over 355 cases and 94 deaths attributed to strains of *L. monocytogenes.* A sampling of pate in 1989 showed that the product from a Belgian manufacturer was more contaminated with *L. monocytogenes* than similar products from other manufacturers (McLauchlin et al., 1991). The incidence declined following pregnant women's dietary advice and the immune-compromised withdrawal from the retail sale of the implicated brand of pate. More currently, there has been a shift in the epidemiology of listeriosis (Moura et al., 2017). Throughout the 1990s, the incidence was stable, with an average of 110 cases reported annually. However, between 2001 and 2005, the incidence increased to >200 cases annually. The increase was to the vulnerable groups such as patients ≥60 years, under-fives, and pregnant women (Pérez-Trallero et al., 2014). The causes of the increase were not well known, and several sporadic cases have been recorded worldwide (WHO, 2015).

Additionally, notification rates were estimated at 0.44 cases per 100,000 population showing a percentage increase of 8.6% from 2005 to 2010 (WHO, 2015). An average of 99.1% of the cases reported resulted in hospitalizations. Listeriosis cases became the highest number of all zoonotic cases under the European Union (EU) surveillance (EFSA, 2017). In 2016, 28 European countries reported 2536 listeriosis cases among humans, increasing compared to 2008 - 2015, trends to the European Food Safety Authority (EFSA, 2017). Furthermore, from 19 countries, 247 deaths were reported due to invasive listeriosis (EFSA) (EFSA, 2017). In 2018 the reports showed that 12 listeriosis cases were linked to the ingestion of salmon products in three EU

countries; (Denmark (six cases), Germany (five cases), and France (one case). The reports came from the European Centers for Disease Prevention and Control (ECDC, 2018).

Similarly, European researchers reported that *L. monocytogenes* had infected 23,150 people worldwide; 5,463 died from the outbreaks (WHO, 2018). The EU reported increased notifications on listeriosis outbreaks and sporadic cases worldwide, and studies have shown *Listeria* infections in humans and contamination in food products (EFSA, 2015, Nayak et al., 2015). Further, (Barbuddhe et al., 2000) recorded a prevalence of 10.2% from the collected meat samples while, (Akya et al., 2013) reported 12.5% on 530 dairy products, meat, and RTE foods. These different variations could be associated with the sampled locations and sample size. Higher prevalence records in different studies showed up to 28.2% contamination from the total sample (De Cesare et al., 2007). Poland's investigated fish and fish processing plants yielded a 13.2% prevalence of *L. monocytogenes* (Skowron et al., 2019c).

Furthermore, other studies reported an overall prevalence of 17.5% among different vegetables, meat, and seafood samples, including dairy products (Dhanashree et al., 2003). Wu and others reported an overall prevalence of 6.2% from different food samples collected (Wu et al., 2015). The differences between percentage prevalence among studies were food type variations and the number of samples analyzed. In contrast, a lower prevalence of 2.4% in an earlier study conducted on raw milk was recorded (Meyer-Broseta et al., 2003). In a study done in Malaysia on vegetables, soil, water, and fertilizer, *Listeria* species were isolated more in the vegetables from organic farms than from conventional farms (Maurice Bilung et al., 2018). When it came to specific bacterial contamination, *L. monocytogenes* was not isolated. The difference in contamination levels was due to contaminated animal waste (Maurice Bilung et al., 2018). Little

and other co-authors isolated 48% of *L. monocytogenes* contamination in samples of raw vegetable salads in a study done in the UK (Little et al., 2007). While a study done in Brazilian markets, the prevalence attribution in RTE vegetables was 3.3% (Porto et al., 2001). Sanders and others (2012) investigated the diversity of different *Listeria* species in Canada's urban and natural environment (Kalmokoff et al., 2001). The study revealed that *L. monocytogenes* are more associated with urban environments than rural ones. One study reported that urban markets were constantly wet and crowded in their study (Luo et al., 2017). Higher incidences of *L. monocytogenes* may partly be due to unhygienic handling, poor market practices, and ecological niches of the microorganisms.

In China, a study by Chen and others showed a prevalence of 7.9% out of 846 fresh aquatic products sampled (Chen et al., 2018). These results were congruent with other findings in the same country, Beijing (Obaidat et al., 2015). Agricultural activities significantly contribute to water pollution, which can later affect the quality of fish in fresh aquatic bodies (Guzmán et al., 2004). In contrast, several other studies in different cities in China had a lower prevalence ranging from 1.6 to 4.9% (Chen et al., 2014). Differences influencing these variations included sampling size, sample composition of the aquatic products, farming practices, storage, and processing. Centinkaya and others recorded the isolation of *L. monocytogenes* at 8% (Centinkaya et al., 2014) while, (Korsak et al., 2012, Wong et al., 2012) isolated *L. monocytogenes* at 14% and 33.3% of chicken burgers and raw chicken meat. In dissimilarity, high isolation rates in fresh and frozen chicken meat were 60% (Pini and Gilbert, 1988). The differences in the isolation between the studies were sampling and methodological techniques used. Furthermore, variations between foods in the above studies could partly be ascribed to the growth of *L. monocytogenes* during storage following inadequate processing (Pini and Gilbert, 1988).

2.4.1 Prevalence of *Listeria monocytogenes* in Africa

Most African countries have shown serious gaps concerning published data on *Listeria* contamination in food (de Noordhout et al., 2014a). The under-reporting of cases relating to food-borne infections underestimates the magnitude of the actual problem (de Noordhout et al., 2014a). In SA, listeriosis has been around for 40 years, with a case range of 60 to 80 in the previous five years (NICD, 2018). The biggest outbreak occurred from 2017 to 2018, during which 1024 cases of listeriosis, of which 200 were fatal (NICD, 2018). The *Listeria* outbreak strain sequence type 6 (ST6) was isolated from the patients, polonies, and other processing environments. Ten percent of the outbreak victims had at least 18 strains of *Listeria* (NICD, 2018). Further studies in the same country reported *L. monocytogenes* in raw poultry and meat products at 19.2% and 14.2%, respectively (Van Nierop et al., 2005, Matle et al., 2019).

In Zambia, *L. monocytogenes* from vegetables meant for export recorded a 20% prevalence (Nguz et al., 2005). In an earlier study from Nigeria, RTE salads and assorted vegetables investigated, the overall *L. monocytogenes* prevalence was 3.9% (Bello and Kwaga, 2013). While in 2015, a study done in the same country on raw vegetables obtained from traditional markets revealed a much higher overall prevalence of 44% of *Listeria* species compared to a study on RTE foods (Ajayeoba et al., 2016). Another study in Algeria involving heat-treated dairy and meat products recorded a prevalence of 2.6% of *L. monocytogenes*. In Ethiopia, two studies mainly involving food samples from different authors reported *L. monocytogenes* prevalence of 4.8% and 5.4%, respectively (Abebe et al., 2009, Gebretsadik et al., 2014). On the other hand, in a study by (Leong et al., 2016) on raw meat and dairy products, *L .monocytogenes*

accounted for 4.1%, and (Garedew et al., 2015) recorded 6.3% of *L .monocytogenes* from RTE foods of animal origin.

In a study conducted on raw milk and fresh cheese in Morocco, *L .monocytogenes* prevalence was 5.9% (Benouareth et al., 2008). Street vendor RTE foods and *L .monocytogenes* accounted for 14% in Egypt. While in South-Western Nigeria, an investigation of vegetables obtained from traditional markets yielded a low prevalence below the EU limit of 100cfc/g (EU, 2007). This low incidence of *L. monocytogenes* contamination in RTE vegetables across the traditional markets in South-Western Nigeria approximates the EFSA baseline study (EFSA, 2014). Low prevalence rates are due to differences in isolation techniques used from one study to the other (Martins et al., 2018). The EFSA baseline data further indicated that even if the pathogen is isolated from a wide variety of foods, it should be within the minimum acceptable standards of the regulations (EFSA, 2014). On the other hand (Ajayeoba et al., 2015) recorded in their study the incidence and distribution of *L. monocytogenes* in RTE vegetables in South-Western Nigeria. The findings of the above results concerning differences in contamination levels between markets were attributed to the location and overcrowding of some markets.

Adedeji and Ademiluyi indicated that dust, unclean water, improper handling, unhygienic market practices, and poor storage facilities could contaminate various foods (Adedeji et al., 2009). In Southern Gaborone, Botswana, similar results of high prevalence in different locations were congruent with the earlier findings (Adedeji et al., 2009). The prevalence differences of *L. monocytogenes* were attributed to overcrowding and lack of knowledge from the food handlers (Morobe et al., 2009).

So far, from literature both globally and in Africa, the problem of listeriosis has been adequately documented in several countries. However, Africa and Zambia generally have inadequate literature regarding the problem of *L. monocytogenes* in foods and their processing environments. Underreporting and lack of operational reporting is the primary cause of the paucity of information on food-borne pathogens such as *Listeria*, especially in Zambia, despite literature showing that Africa is the major contributor to most food-borne infections. Therefore, the prevalence of *Listeria* species must be documented in our setting to subsidize the body of knowledge and ensure decisions made are with full awareness of the problem.

2.5 Etiological agents

The species in the genus *Listeria* are tiny Gram-positive, rod-shaped, facultatively anaerobic, and non-spore-forming bacteria. *Listeria monocytogenes*, *L. ivanovii*, *L. innocua*, *L. seeligeri*, *L. welshimeri, L. grayi,* and *L. marthii,* are typically included in the genus *Listeria* (Farber, 1991, Vázquez-Boland et al., 2001). In addition, records of other new *Listeria*-like species are available; (Leclercq et al., 2010, Halter et al., 2013, den Bakker et al., 2014, Weller et al., 2015, Orsi et al., 2016). Until now, 17 *Listeria* and *Listeria*-like species have been identified (Chapin et al., 2014). As earlier indicated, *L. monocytogenes* and *L. ivanovii* are the only ones recorded to be pathogenic strains of public health concern (Farber, 1991, Leclercq et al., 2010).

2.5.1 *Listeria monocytogenes*

Listeria monocytogenes persist in food-associated environments for months to years (6 - 8) (Ferreira et al., 2014a, Stasiewicz et al., 2015). Mainly persistent strains have links to outbreaks of foodborne disease worldwide (Ferreira et al., 2014b). For example, *L. monocytogenes* was responsible for an outbreak linked to 29 cases and four deaths in the source plant for 12 years

(Ramaswamy et al., 2007). In SA 2017 – 2018 outbreak, mainly involving infants and pregnant women, a 42% fatality rate was recorded (Desai et al., 2019). The bacterium is now a common food contaminant, threatening the food processing industry (Stasiewicz et al., 2015). The persistence of *L. monocytogenes* is by its ability to withstand environmental stress, acidity, cold stress, and oxidative stress (David and Odeyemi, 2007). Depending on the environmental setting, it can switch between saprophytic and virulence (David and Odeyemi, 2007). Further, the pathogen easily forms biofilms and persister cells on surfaces which may not be easy to remove with standard sanitation protocols (Lakicevic and Nastasijevic, 2017).

Listeria monocytogenes is usually a post-contamination pathogen of which contamination can occur at any stage of processing through to the final product (de W and McClure, 2002, Quinlan, 2013, Liggans et al., 2019). *Listeria monocytogenes,* in cold, moist, and dry environments, survive extended storage times. It has also been colonizing specific sites in food manufacturing environments for several years. An example is how *L. monocytogenes* can persistently survive on stainless steel food contact, like on dicing machines, and repeatedly contaminate RTE foods (Lundén et al., 2002). Further, it can quickly multiply in a wide range of settings and food types with high water activity and between a wide temperature range from -2ºC and 45ºC, and pH range 5.5 and pH 8, in 10% sodium Chloride and 200 parts per million (ppm) sodium nitrite (Camejo et al., 2011a). This considerable survival property of *L. monocytogenes* makes it a notorious food pathogen coupled with the difficulty of diagnosis in humans (Camejo et al., 2011b).

2.5.2 Other types of *Listeria* species

The most commonly reported *Listeria* species in food and processing environments is *L. innocua* (Jalali and Abedi, 2008, Vallim et al., 2015). Through independent research, many authors have established a correlation linked to the isolation of *L. innocua* and the specific food types (Chambel et al., 2007, Milillo et al., 2012, Vallim et al., 2015). Furthermore, Vallim and others also established that *L. innocua* was more likely not to be isolated in unprocessed meat products compared to processed (Vallim et al., 2015). They further reported that *L. monocytogenes* had a higher chance of isolation in avian food sources (Vallim et al., 2015); while, Scotter and others (2001) reported that *L. monocytogenes* during stages of selective enrichment could be overshadowed by the growth of *L. innocua* when present in equal or more significant numbers (Scotter et al., 2001). It makes viewing *L. monocytogenes* difficult on the isolation media because of its growth characteristics (Scotter et al., 2001). Most studies have recorded *L. innocua* as the major contaminating *Listeria* species in foods and their environments. In one study done in India, all the sampled foods recorded the highest *L. innocua* contamination of 9.1% shrimp, 10% beef, and 37% from fish (Dhanashree et al., 2003). At the same time, Jalali et al. (2008) reported that 2.6% and 8.3% of beef and shrimp samples were *L. innocua* positive (Jalali and Abedi, 2008). Vitas and Garcia-Jalon (2004) equally reported *L. innocua* as the most frequent species isolated in fresh foods in Spain (Vitas, 2004).

Listeria species are widely present in plants, soil silage, and food processing environment (Farber, 1991, Beresford et al., 2001). *Listeria* may colonize animals due to consuming contaminated food and water (Husu et al., 1990, Fenlon et al., 1996). For this reason, *Listeria* may enter the processing plant via animals harboring *Listeria* in the intestinal tract or as part of pharyngeal microflora. *Listeria ivanovii,* although not common, has been associated with cases of

human listeriosis and is also widely distributed in nature. Primarily found in warm-blooded animals, and has been isolated in fecal specimens from healthy hosts, soil, water, vegetables, processed silage, waste, food, and food establishments (Velge and Roche, 2010). Generally, *L. ivanovii* infects mainly ruminants (Vázquez-Boland et al., 2001). Significant cases of *L. ivanovii* connects to gastroenteritis and bacteremia in humans are also reported (Cummins et al., 1994, Lessing et al., 1994, Snapir et al., 2006, Guillet et al., 2010). Listeriosis caused by *L. ivanovii* is of public health concern in ruminants, as it has caused epidemics of abortion, stillbirths, and encephalitis (Dennis, 1975, Alexander et al., 1992, Chand and Sadana, 1999, Ramage et al., 1999). The other non-pathogenic species, *L. seeligeri*, *L. welshimeri*, *L. grayi,* and *L. marthii* usually detected to exist in lower frequencies (Chapin et al., 2014).

2.6 Listeriosis

Listeriosis is an essential foodborne disease that can be difficult to control and may result in severe clinical presentations (de Noordhout et al., 2014b). The primary source of human listeriosis is consuming contaminated food with *L. monocytogenes* (Koskar et al., 2019b); immune-compromised adults and neonates are particularly susceptible to listeriosis (EFSA, 2015). Signs and symptoms of listeriosis differ depending on the person and part infected (de Noordhout et al., 2014b). Others may get infected with *L. monocytogenes* but rarely become seriously ill (EFSA, 2015). The disease is described in two forms, namely, invasive and intestinal type of illness (Allerberger and Wagner, 2010). Invasive illness occurs when the bacteria spreads beyond the intestines (Goulet et al., 2013). Symptoms of an invasive illness usually start within two weeks after consumption of food contaminated with *L. monocytogenes*. Symptoms atypical to pregnant women are fever, and flu-like symptoms, although others can be mild to no signs in some individuals (EFSA, 2015). Typical signs and symptoms in other people include flu-like and

fever symptoms such as muscle aches and fatigue, headache, stiff neck, confusion, loss of balance and seizures (EFSA, 2015). Symptoms in other individuals can be severe and about one in 20 people with invasive listeriosis die (Allerberger and Wagner, 2010). The other form is intestinal illness, which is rarely diagnosed because laboratories do not regularly test patient stool samples for *L. monocytogenes* (Jacks et al., 2016). Signs and symptoms of intestinal illness may start within 24hrs after consumption of contaminated food with *L. monocytogenes* and usually lasts 1-3 days. Typical symptoms of the intestinal illness include diarrhea and vomiting, which may be mild; however, some people with intestinal illness develop invasive illness which involves stiff neck, confusions, convulsions and loss of balance (Allerberger and Wagner, 2010) .

2.6.1 Pathogenicity

Listeria monocytogenes consist of 13 serotypes with different virulence abilities. Serotyping of *L. monocytogenes* and rib typing of *L. monocytogenes* classification is into three lineages; lineage 1 comprising serotypes 1/2b, 3b, 4b, 4d, and 4e; lineage II comprising serotypes 1/2a, 1/2c, and 3c and lineage III comprises 4a, 4b, and 4c. Pathogenicity records vary at the intra-species level; serotypes 1/2a, 1/2b, 4b, , and account for 95% of all the strains isolated from human listeriosis, as reported by (Barbuddhe et al., 2008, Tamburro et al., 2019). Equally, Wang and others reported that serotype 1/2b was predominant in 64.3% of human listeriosis cases in China (Wang et al., 2015). Accordingly, various studies indicate that the most prevalent serotype isolated from foods is 1/2a, while in humans is 4b (Jacquet et al., 2002, Kathariou, 2002, Raybourne, 2002, Borucki and Call, 2003). (Varma et al., 2007) reported that serotype 4b was the most common serotype in patients associated with pregnancy and 1/2a in immune-compromised patients. Different serotypes were isolated as follows: 1/2a (73.3%) with 1/2b ,1/2c, 3c and 4b recording 6.7% each from frozen broiler carcasses sold in Ankara (Erol et al., 1999). In Brazil Loura and others detected from chicken meat, liver, and surfaces of a processing plant 1/2a,1/2b,1/2c

including 4b serotypes of *L. monocytogenes* (Loura et al., 2005). While in Japan, *L. monocytogenes* from ground chicken samples were isolated, and serotypes 1/2a and 1/2b were identified (Inoue et al., 2000).

Skowron and others (2019) confirmed the isolation of *L. monocytogenes* in the following serogroups in their study on fish and fish processing plants, namely, 1/2a - 3a, 1/2b - 3b, and 4a - 4d - 4e. Most strains belonged to serogroups 1/2a - 3a, followed by group 4b - 4d - 4e, and the other strain belonged to group 1/2b - 3b, respectively (Skowron et al., 2019b). The results agreed with an earlier study by (Jami et al., 2014), which equally did studies on fish and fish products. Information close to these results was reported by (Jamali et al., 2015a). In contrast to the above findings (Montero et al., 2015), serotype 4b was dominant in all food products. However, serogroup 1/2a - 3a was also isolated similarly to the work of (Skowron et al., 2019b). Meanwhile, *L. monocytogenes* had disproportionate distribution in raw foods; the present proportions in raw meat 3.5% and poultry 3.8% were considerably higher than that of raw seafood (1.3%). The *L. monocytogenes* isolates documented came from four serotypes namely; 1/2a, 1/2b, 1/2c, and 4b, with the most prevalent serotype being 1/2a (47.9%) (Zhang et al., 2019).

2.6.2 Transmission

Listeria monocytogenes are transmitted to the consumer mainly through contaminated RTE foods (Allerberger, 2003, Todd and Notermans, 2011). The isolation and potential persistence of *Listeria* species in food processing facilities are by environmental recontamination from the farm to plant level (Gelbíčová and Karpíšková, 2012, Holch et al., 2013). Usually, unraveling the transfer of *L. monocytogenes* between niches and molecular subtyping is essential both in

outbreak notification and in managing contamination events in food business operations (Malley et al., 2013, Stessl et al., 2014). Mostly, source tracking of *L. monocytogenes* remains challenging because of its known ubiquity and adaption to harsh environmental conditions (Lomonaco et al., 2013, Chenal-Francisque et al., 2015). From the time Welshimer and Donker-Voet (Welshimer and Donker-Voet, 1971) and Wesi and Seeliger (Weis and Seeliger, 1975) published work, several authors have hypothesized that the primary habitat of *Listeria* species is soil and decaying vegetation (Vivant et al., 2013).

2.7 Methods of detection for *Listeria* species

Methods for detecting and identifying *Listeria* species have increased significantly in the past decade (Cliver and Riemann, 2002). With the first recognized outbreak of foodborne listeriosis in 1981 came a need to adapt these methods to isolate *Listeria* in foods (Czajka and Batt, 1994).

2.7.1 Bacteriological methods

From time immemorial, it is challenging to isolate *Listeria* from food samples, and this explains why it remained unobserved as a significant food pathogen until recently. In a food industry setting, it is more common to test for *Listeria* species than for *L. monocytogenes* and more common to test environmental samples than food samples (Gebretsadik et al., 2011) . These methods use a selective enrichment in broth followed by isolating colonies on selective agar. *Listeria* in different samples usually requires two enrichment steps (enriched with *Listeria* selective supplements) inoculated on the surface of the *Listeria* selective agar (Holah et al., 2004a, Barocci et al., 2008). *Listeria* can grow in low temperature; this helps to isolate it in clinical samples by incubation for extended periods at 4ºC on agar plates until the formation of visible colonies (Holah et al., 2004a, Barocci et al., 2008). It takes several weeks and usually

does not allow for the isolation of injured *Listeria* cells because of reduced survival due to stress (Gasanov et al., 2005). When testing food or environmental swab samples, methods to determine presence/absence or detection in 25-50g samples (food) or per swab are most employed (Alsheikh et al., 2013). Generally, this sensitivity uses enrichment methods in which the organism grows to detectable levels of ~10^4 10^5 CFC ml^{-1}. *Listeria* cells are slow-growing microorganisms and can be rapidly out-grown by competitors. Hence bacteriostatic agents, such as acriflavine and nalidixic acid, that specifically suppress competing microflora have been introduced into enrichment media or selective agar (Welshimer and identification. Springer Verlag, 1981). These two agents are incorporated into all standard methods to isolate *Listeria* from food and environmental samples. Two of the most widely-used culture reference methods for the detection of *Listeria* in all foods are the Food and Drug Administration (FDA) Bacteriological and Analytical Method (BAM) (Hitchins et al., 2017) and the International Organization of Standards (ISO) 11290 method.

2.7.1.1 Phenotypic characteristics of *Listeria* species

Listeria sensu stricto includes *L. monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. welshimeri,* and *L. innocua,* all described before 1985 and later *L. marthii,* described in 2010 (Weller et al., 2015). *Listeria* sensu stricto species have similar phenotypic characteristics, making this group easily recognizable as members of the *Listeria* genus. The atypical phenotypic characteristics shared by all *Listeria* sensu strictu species include (i) ability to grow at low temperature, (ii) motility (at least 30ºC), (iii) positive catalase reaction, (iv) inability to reduce nitrate to nitrite and (v) positive reaction in the Voges-Proskaner test, indicating the ability to produce acetoin from the fermentation of glucose through the butanediol pathway. Furthermore, all sensu strictu species can ferment D-arabitol, α-methyl D-glucoside cellobiose, D-fructose, D-mannose, D-fructose, D-

mannose, N-actylglucosamine, maltose, and lactose. While none of the species can ferment inositol, L-arabinose and D-mannitol (Bertsch et al., 2014b, Weller et al., 2015). *Listeria monocytogenes*, *L. ivanovii* and *L. seeligeri*, and some *L. innocua* strains show hemolytic capabilities (Johnson et al., 2004). *Listeria ivanovii* is differentiated from *L. monocytogenes* by its unique ability among sensu strictu species to ferment D-ribose (Bertsch et al., 2013, den Bakker et al., 2014). *Listeria welshimeri* can be identified by its ability to ferment D-tagatose, while *L. seeligeri* is the only sensu strictu species capable of fermenting D-xylose, but not capable of fermenting D-xylose and also unable to ferment D-ribose or D-tagatose (Bertsch et al., 2013, Weller et al., 2015). *Listeria marthii* is the only sensu strictu species unable to ferment sucrose, a characteristic that can differentiate strains from other sensu strictu species (den Bakker et al., 2014). While *L. innocua* is typically identified by its inability to cause hemolysis and ferment D-xylose combined with its ability to ferment glycerol (den Bakker et al., 2014, Weller et al., 2015), hemolytic *L. innocua* strains are difficult to differentiate from *L. monocytogenes* and *L. seeligeri* using standard biochemical tests (Johnson et al., 2004). Most importantly, phenotypic characteristics do not always allow for the unambiguous classification of *Listeria* isolates. Phenotypic and genetic data help to describe several atypical *Listeria* isolates.

2.7.1.2Identification of specific *Listeria* isolates using MALDI-TOF MS

As described above, bacterial identifications in microbiology laboratories are mainly carried out according to phenotype characteristics, including identifications of culture media, colony morphology, gram stain, and various biochemical reactions (Doern et al., 2019). Although all the described methods can achieve high accuracies, it usually takes a minimum of one day or longer to complete the whole identification process. Molecular methods, such as real-time PCR, gene sequencing, and microarray analysis, are quick methods for bacterial identification but are

associated with high costs and require highly-trained technicians. Therefore, molecular methods are not routinely used for bacterial identification. A faster and easier technique for microbial identification would significantly enhance the conventional laboratory in providing more timely feedback. The recent emergence of MALDI-TOF MS, a culture-based technique simple, rapid, and economical technique for the characterization of bacteria, mainly at the species level and sometimes at the strain level, has dramatically revolutionized microbial diagnostics (Böhme et al., 2010, Griffin et al., 2012, Anderson et al., 2012). Specifically, the application informs epidemiological investigations of foodborne outbreaks of robust subtyping techniques to monitor and control the spread of infections. Although MALDI-TOF MS is a rapid and economical technique, it has disadvantages. Distinct from publically available sequence databases such as GenBank, MALDI-TOF MS databases are proprietary. It is sometimes associated with low identification percentages for some microorganisms. Although, this may be improved by adding mass spectral entries of underrepresentation species or strains so that interspecies variability can be covered (Patel, 2015).The earliest application of this technique for bacterial identification dates back to 1975 (Anhalt and Fenselau, 1975).While the first related study was published in 1996, and other studies are progressively coming up (Holland et al., 1996, Seng et al., 2009).

The VITEK 2 Compact® is a microbial identification system fully automated that operates with barcode cards, ensuring complete traceability and a lower risk of transcription errors. Depending on the bacteria metabolism the preparation time and the final result can be obtained in a range of 2 to 18hrs. In the case of *Listeria* species identification, the GP card, used for gram-positive bacteria, is based on biochemical methods that measure carbon utilization, resistance, and enzymatic activity (Crowley et al., 2012, Moehario et al., 2021).

2.7.3 Molecular methods

2.7.3.1 PCR methods

According to Mullis and others, PCR has immensely impacted all molecular applications since its inception (Mullis et al., 1986). Polymerase Chain Reaction is a technique where DNA segment amplification is by heat-stable polymerase and two primers (short DNA sequences specific to a particular gene). The amplification of fragments for detection is later proceeded by using agarose gel electrophoresis (Mullis et al., 1986), which differs from DNA hybridization, where comparatively large amounts of target DNA are required. Polymerase Chain Reaction establishment is a reliable and reproducible technique for identifying *Listeria* species. More importantly, the differentiation of *L. monocytogenes* from *Listeria* species using primers targeting genes of virulence factors or RNA sub-unit such as 16S RNA and 16 – 23S RNA is common (Drebot et al., 1996, O'connor et al., 2000, Koo and Jaykus, 2002, Somer and Kashi, 2003).

Also, targeting specific genes, such as the highly conserved housekeeping *prs* gene found in *Listeria* species, is being utilized (Doumith et al., 2004). The standard microbiological methods routinely used for isolating *Listeria* species, including the *prs,* a general marker gene present in all *Listeria* species (Nightingale et al., 2005). Furthermore, other authors have used *prs* to screen the existence of *Listeria* species because it is known as the housekeeping gene (Nightingale et al., 2005, Graves and Swaminathan, 2001). These methods are new (Chen et al., 2007, Kim et al., 2007), are accurate, and have equal sensitivity with other known PCR methods. Although the *prs* gene does not differentiate different types of species of *Listeria*, it nevertheless acts as an indicator of *Listeria* species in each sample of interest (Nightingale et al., 2005, Doumith et al., 2004).

Detection using PCR is selectively enriching samples for 24 – 48 hrs than direct testing of samples without prior enrichment gives unreliable results (Allerberger, 2003, Aznar and Alarcón, 2003). One major obstacle for PCR in food and environmental samples directly after selective enrichment is that broths and food components contain inhibitors of the PCR, which gives rise to false-negative results (Abu Al-Soud et al., 1998). Several approaches to remove inhibitory factors after selective enrichments, such as sample treatments, are required (Simon et al., 1996, Agersborg et al., 1997). Equally, isolation procedures using magnetic beads, dip sticks, or membranes to remove target DNA from reacting with sample matrices are employed (Li et al., 2000, O'connor et al., 2000).

2.7.3.2 Multi-Locus variable number tandem repeat analysis (MLVA)

Molecular typing methods extant a higher degree of discrimination by providing more strain-level information than culture methods (Allerberger, 2003). Multiple-locus variable number (MLVA) is one of the most widely used molecular strain-typing methods (Lindstedt et al., 2004, Kimura et al., 2008, Chenal-Francisque et al., 2013). The multiple-locus variable number method spots the number of repeat units available at multiple variable number tandem repeats (VNTR) loci that vary depending on the strain and compares them to allow specific strain classification. Variable number tandem repeats loci are placed in the coding regions of the genome. Lately, MLVA was coupled with Sanger sequencing and capillary electrophoresis to determine the fragment size and sequence (Phraephaisarn et al., 2018). However, with the large number of isolates usually collected from food factories, coupling MLVA electrophoresis and DNA sequencing time-consuming, will be laborious and expensive. In addition, coupling MLVA with electrophoresis analysis limits the discriminating power and competence of multiplex PCR because of the determination of amplicon lengths.

2.7.3.2 16S rRNA Next-Generation Sequencing (16SNGS)

The most widely used sequencing method is by Sanger (Sanger et al., 1977). With the availability of Sanger DNA sequencing, bacteria such as *Listeria* identification occurs through nucleotide sequence of the 16S rRNA gene. This short, conserved gene is specific to a bacterial genus which ranges from 87.5% to 96% (Srinivasan et al., 2015). This type of method of bacterial identification is independent of culture because it only requires the DNA of the tested bacteria (Sune et al., 2020). Variable regions of the 16S rRNA gene allow species identification (Chakravorty et al., 2007). In addition to its utility in identifying bacterial pathogens from bio specimens obtained directly from patients, the 16S rRNA gene sequencing-based approach is also helpful, especially in cases of ambiguous culture and biochemical testing results to avoid potential culture-related biases in pathogen identification. The coming of next-generation sequences (NGS) techniques, including 16S rRNA next–generation sequencing (16SNGS), allows further up scaling of sequencing quantity (fragments versus time) even in mixed cultures. A proposal for a possible substitute to replace culture biochemical tests for bacterial identification in diagnostic microbiology laboratories is underway (Srinivasan et al., 2015, Cummings et al., 2016, Sune et al., 2020). However, despite its recorded efficiency, the major disadvantage of 16SNGS is the cost of procuring equipment, timely sustainable maintenance of these platforms, development of specific laboratory infrastructure, and training of technical expertise, all of which are still challenges, especially in middle-income countries (Kwong et al., 2015, Wilson et al., 2018).

2.7.3.3 Multi Locus Sequence Typing

Sequencing of other genes has also been employed, which is the basis for Multi Locus Sequence Typing (MLST). Housekeeping genes or genes coding for virulence factors such as flagellin *(fla)*,

hly, *actA*, *iap*, internalins *(inl)*,the metalloprotease *(mpl)*, *prfA* and other virulence-associated genes (Cai et al., 2002) are PCR amplified to generate internal gene fragments of approximately 450bp, which are then sequenced. Variations within the sequences of these genes are assigned alleles at these loci (Spratt, 1999). The discriminatory power of this approach is extremely high and accurate. Multi Locus Sequence Typing allows unambiguous typing of any strain and comparisons can be made based on sequence databases. The method is for typing of *L. monocytogenes* strain targeting several housekeeping genes (Salcedo et al., 2003, Zhang et al., 2004); however, it cannot apply routinely, and its application is limited to specialized laboratories.

Recently, the WGSs of *L. monocytogenes* strain EGD and *L. innocua* have been determined through collaborative effort (Glaser et al., 2001). Since then, numerous new *L. monocytogenes* genes and their functions have been elucidated, and there are ever-increasing sequence data compiled in databases such as the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) available for comparisons. The information the complete sequence of the *L. monocytogenes* genome provides is invaluable, and such information's impact on all applications is vast.

2.8 Prevention and control

Apart from being difficult to diagnose, the disease is difficult to link to a food source due to the vast and wide incubation period of 1 to more than 90 days (Xu et al., 2019). The broad incubation period makes linking foodborne outbreaks to *Listeria* difficult, affecting foodborne disease investigations associated with this pathogen. The recent sporadic outbreak of *Listeria* in SA caused an awakening from the WHO point of view toward national food safety among African

countries (Zhang et al., 2021). Identification of disease surveillance systems is a prerequisite to preventing sporadic outbreaks. Generally, Africa, Zambia included, has a high degree of under-reporting of foodborne infection despite being the major contributor to foodborne associated infections (WHO, 2014, de Noordhout et al., 2014a, WHO, 2015). Additionally, the absence of awareness or regulation of *L. monocytogenes,* especially in foods, cannot give an accurate picture of our setting. Accordingly, it is essential to profile the types of food commonly colonized by *L. monocytogenes* and possible antibiotic resistance for pre-emptive surveillance and monitoring purposes.

2.9 Antimicrobial Resistance

Antimicrobial resistance is the ability of bacteria to defend themselves from drugs designed to kill them. Resistant infections can be complicated and, many times, impossible to treat (Blair et al., 2015).While multi-drug resistant (MDR) is a type of none response developed by microorganisms to lethal doses of antibiotics in vitro to a minimum of one agent in three or more antimicrobial groups (Magiorakos et al., 2012). Antibiotic resistance is present in hospitals, foods, and environments. Antibiotic resistance may be intrinsic to a microorganism or may be acquired via mutation or adaptation to stressors (Addis, 2015). Livestock and animal products contribute to the spread of antibiotic resistance bacteria and genes to humans (Spellberg et al., 2013). The origin of antibiotic-resistance genes possessed by human pathogens can be traced back to environmental microorganisms (Addis, 2015). The most crucial factor to consider is preventing the entrance of antibiotic-resistant and sensitive pathogenic microorganisms in the food chain and if present inactivating them. Subsequently, a full understanding of the evolution of antibiotic resistance requires the study of natural environment as well as clinical ecosystems.

Unfortunately, most bacteria are slowly becoming resistant including *Listeria* species isolated from food.

2.9.1 Antibiotic Resistance among Food Isolates of *L. monocytogenes*

The acquisition process of antibiotic resistance in the susceptible organism is by the ecology of the species (Baquero et al., 2020). Compared to other relevant foodborne pathogens, *L. monocytogenes* has maintained a high susceptibility rate to antibiotics used for decades to treat human and animal diseases. However, *L. monocytogenes* can acquire antibiotic-resistance genes from other organism's plasmids and conjugative transposons. Comprehensive studies, including reviews of antimicrobial resistance in *L. monocytogenes,* are widely available in the literature (MacGowan et al., 1990, Charpentier et al., 1995, Charpentier and Courvalin, 1999, Poroś-Głuchowska et al., 2003, Morvan et al., 2010, Luque-Sastre et al., 2018a, Yan et al., 2019). Many other publications, mainly from food microbiology in low-middle-income countries, have reported high antibiotic resistance rates in *L. monocytogenes* in recent years (Olaimat et al., 2018). Antibiotic resistance is a problem when treating patients, especially if resistance to the pathogen is unexpected. Regarding *L. monocytogenes,* the standard therapy for severe infections until now is based on amoxillin or ampicillin frequently in combination with gentamicin, penicillin G, Trimethoprim/sulfamethoxazole, and imipenem (Sader et al., 2020).

Antibiotic resistance, predominantly multidrug resistance among food-borne bacteria, including *L. monocytogenes*, has emerged and evolved during the past few decades (Zhang et al., 2019). In the last decade, it has become a burden of public health concern because of the contribution to unsuccessful treatment resulting in increased costs/mortality associated with food-borne diseases

(Olaimat et al., 2018). Antibiotic resistance is mainly observed among vulnerable patients, resulting in prolonged illness and an increased mortality rate (WHO, 2014). The projection is that global deaths from infection caused by antibiotic-resistant pathogens will increase from 700,000 to 10 million annually, and costs will reach 100 trillion US$ by 2050 (O'Neill, 2014).

The first multidrug resistant in chloramphenicol, erythromycin, streptomycin, and tetracycline strain of *L. monocytogenes* was isolated from a patient with meningoencephalitis in France (Poyart-Salmeron et al., 1990). Subsequently, many *L. monocytogenes* strains resist at least one antibiotic from food, the environment, and human clinical samples are equally reported (Málek and Pražák, 2002). The antibiotic resistance among 21 *L. monocytogenes* isolates from water, cabbage, and different environmental samples in Texas (USA), was investigated, and 95% (20) were resistant to at least two or more commonly used antibiotics (Málek and Pražák, 2002). Furthermore, among the 20 multidrug-resistant isolates, 17 were resistant to penicillin, and one was resistant to gentamicin (Málek and Pražák, 2002). In Italy, Aureli and others reported that all *L. monocytogenes* strains isolated from different food products were resistant to fosfomycin, lincomycin, and flumequine (Aureli et al., 2003). Yucel and colleagues indicated that all *L. monocytogenes* isolates from raw or cooked meat product samples in Turkey were resistant to cephalothin and nalidixic acid, and 66% of isolates were resistant to sulfamethoxazole, ampicillin, and trimethoprim (Yücel et al., 2005). In China, 73% of 167 *L. monocytogenes* isolated from retail food products were resistant to sulfonamide, 8.4% were resistant to tetracycline, and 1.8% were resistant to ciprofloxacin (Zhang et al., 2019). The antibiotic susceptibility of 13 strains of *L. monocytogenes* isolated from homemade white cheeses was examined by (Arslan and Özdemir, 2008). Three were resistant to clarithromycin, while one isolate was resistant to each of ampicillin, penicillin, and tetracycline (Arslan and Özdemir,

2008). The assessment of 30 *L. monocytogenes* isolates from a dairy product in Iran on ten antibiotics recorded at least resistance to one antibiotic (Rahimi et al., 2010). The highest frequency of resistance was against oxacillin (approximately 93% followed by penicillin 90% and ampicillin 60% (Rahimi et al., 2010).

An evaluation of resistance of 120 *L. monocytogenes* strains isolated from foods, food handling, and processing environments on 19 antibiotics was conducted 11.7% exhibited resistance to at least one antibiotic (Conter et al., 2009). The isolates also displayed maximum resistance to clindamycin, followed by linezolid, ciprofloxacin ampicillin, rifampicin, trimethoprim-sulfamethoxazole, and the least resistance was recorded in vancomycin and tetracycline (Conter et al., 2009). Rahimi and others reported that 74.3% of *L. monocytogenes* isolates from dairy products resisted at least one antibiotic; however, multidrug resistance was only in two isolates (Rahimi et al., 2010). The percentage of resistance for ampicillin and penicillin among isolates was 26.3% and 31.6%, respectively (Rahimi et al., 2010). In a study on antibiotic resistance of *L. monocytogenes* in chicken slaughter-houses in Northern Greece, 55% of *L. monocytogenes* isolates showed resistance to nalidixic acid and oxalinic acid, whereas 83.6% were resistant to clindamycin (Sakaridis et al., 2011). On the other hand, all isolates were sensitive to ampicillin, cephalothin, amoxicillin, ciprofloxacin, erythromycin, kanamycin, neomycin, vancomycin, streptomycin and sulfamethoxazole-trimethoprim (Sakaridis et al., 2011). Another study detected 100% sensitivity on various antibiotics in chicken carcasses from 35 *L. monocytogenes*, except for clindamycin which accounted for 5% (Oliveira et al., 2018). Similarly (Fallah et al., 2012) in their study of antibiotic resistance out of 17 *L. monocytogenes* isolates from raw and RTE chicken, three isolates were resistant to trimazosin, and two showed resistance to tetracycline. However, only one isolate showed resistant to both trimazosin and tetracycline. However, all

tested isolates were sensitive to enrofloxacin, doxycycline, chloramphenicol, amoxicillin, or trimethoprim and 94.1% were sensitive to erythromycin and gentamicin (Fallah et al., 2012). Concurrently, (Terzi et al., 2015) observed in their study that *L. monocytogenes* isolates from RTE products were resistant to tetracycline, and one isolate was resistant to vancomycin. On the other hand (Garedew et al., 2015) reported 66.7%, 50%, 37.5%, and 16.6% of the 24 isolates of *L. monocytogenes* from RTE foods of animal origin which exhibited resistance to penicillin, nalidixic acid, tetracycline, and chloramphenicol respectively. Additionally, 16.7% of isolates were multidrug-resistant. However, all 24 isolates were sensitive to amoxicillin, sulfamethoxazole-trimethoprim, cephalothin, vancomycin, gentamicin, and cloxacillin (Zelalem et al., 2019). G'omez and others tested the antibiotic resistance of *L. monocytogenes* isolates from RTE meat products and meat-processing environments (Gómez et al., 2014). Resistance to either one or two antimicrobials was observed in 34.5% of *L. monocytogenes* isolates, although multidrug resistance was identified in 2.9% of the organisms (Gómez et al., 2014) . All isolates showed resistance to oxacillin which represented 2.9% of the organisms and only 0.5% were resistant to tetracycline (Gómez et al., 2014).

Al-Maliki and others (2010) found that all 22 *L. monocytogenes* isolates from raw milk and traditional dairy products in Iran were resistant to tetracycline, penicillin, chloramphenicol, and amoxicillin or clavulanic acid (Al-Maliki, 2010). Another study (Kevenk and Terzi Gulel, 2016) found that 15.3% of *L. monocytogenes* isolates from raw milk and dairy products were resistant to at least one antibiotic, and 36.5% were multidrug-resistant. The most common antibiotic resistance encountered was tetracycline 34.6%, followed by chloramphenicol 25% and penicillin G 23% (Kevenk and Terzi Gulel, 2016).

In another study, resistance to oxacillin and clindamycin was recorded at 56.4% (Osaili et al., 2012). Whilst, in the same study other isolates showed sensitivity, intermediate susceptibility and multidrug resistance to gentamicin, imipenem, teicoplanin, rifampicin, linezolid, ciprofloxacin, vancomycin, trimethoprim-sulfamethoxazole, benzyl-penicillin, erythromycin, and tetracycline (Osaili et al., 2012). Jamali and others recorded resistance among *L. monocytogenes* isolated from fish products of about 20.9% to 27.9% from tetracycline and ampicillin (Jamali et al., 2015a). At the same time, 14.0% and 16.3% were recorded from cephalothin and penicillin G, respectively (Jamali et al., 2015b). Furthermore, streptomycin, rifampicin, and chloramphenicol accounted for 2.3% (Jamali et al., 2015a). In India, various resistant levels came from different antibiotics tested from seafood products from 37 *L. monocytogenes* isolated. The distribution was as follows; nalidixic acid, streptomycin, gentamicin, and kanamycin 100%, 78.4%, 75.7%, and 73.0%, respectively; meanwhile, all isolates were sensitive to amoxicillin (Jeyasanta and Patterson, 2016). Wieczorek and Osek found that 57.9% of *L. monocytogenes* strains isolated from fresh and smoked fish showed resistance to oxacillin, 31.6%, and 8.8% were resistant to ceftriaxone or clindamycin (Wieczorek and Osek, 2017).

It is worth knowing that other studies reported even lower prevalence of antibiotic resistance among *L. monocytogenes* strains isolated from food sources. Aarestrup and co-workers found that from 114 *L. monocytogenes,* isolates examined from food product, susceptibility was noted in chloramphenicol, ciprofloxacin, ceftiofur, erythromycin, florfenicol, penicillin, streptomycin, spectinomycin, tetracycline, tiamulin, co-trimoxazole, trimethoprim, antibiotics used except for ceftiofur (Aarestrup et al., 2007). Fallah and others studied the antibiotic resistance from 30 *L. monocytogenes* isolated from food isolates; only tetracycline recorded resistance from all the 16 antibiotics studied (Fallah et al., 2013b). In Poland, from 2004 to 2010, 471 *L. monocytogenes*

cultures isolated from various foods were sensitive to gentamicin, amoxicillin, rifampicin, ampicillin, sulfamethoxazole, trimethoprim, erythromycin, vancomycin, and chloramphenicol (Maćkiw et al., 2016). Only two *L. monocytogenes* strains showed antibiotic resistance to tetracycline and minocycline (Maćkiw et al., 2016). More studies also reported high *L. monocytogenes* susceptibility to antimicrobials: ampicillin, gentamicin, sulfamethoxazole, trimethoprim and erythromycin, and penicillin (Fallah et al., 2013a, Chen et al., 2014, Wieczorek and Osek, 2017, Skowron et al., 2019a). Most studies that recorded high AMR just performed methods that could only observe the phenotypic characteristics, and few did molecular detection methods. From the literature, phenotypic methods performed singularly may be associated with false positives (Allerberger, 2003). Therefore verifications are recommended by using prescribed molecular methods (Olaimat et al., 2018).

So far from the prevalence of antibiotics resistance discussed above, the most common resistance came from penicillin, ampicillin, sulfamethoxazole, trimethoprim, ciprofloxacin, and tetracycline among *L. monocytogenes* isolated from food. Clinical and Laboratory Standards Institute (CLSI) defines the first-line antibiotics used in treating listeriosis as penicillin G, ampicillin, Trimethoprim/sulfamethoxazole, and imipenem (Sader et al., 2020). The use of the same drugs in treating poultry is common even in other animal husbandry such as cattle (O'neill, 2015). Generally, from the prevalence above, the *L. monocytogenes* strains remain susceptible to the first drugs of choice. However, no study from Zambia has reported the current situation regarding antibiotic resistance in *L. monocytogenes* isolated from food samples and processing environments. It will be good to document the current situation in our setting so that the problem is quantified.

2.9.2 Mechanisms of Antibiotic Resistance in *L. monocytogenes*

Acquisition of variable genetic elements, such as self-transferable plasmids, mobilizable plasmids, and conjugative transposons, is the standard device known to account for developing antibiotic resistance in *L. monocytogenes* (Charpentier and Courvalin, 1999).

2.9.3 Antibiotic resistance mediated by conjugation

Listeria monocytogenes uses conjugation as a practical approach to acquire antibiotic resistance (Périchon et al., 2009). *Enterococci* and *Streptococci* characterize the significant reservoirs of resistance genes for *L. monocytogenes* (Bertrand et al., 2005). The bacteria genome comprises the chromosome and accessory movable genetic elements such as transposons and plasmids (Périchon et al., 2009). Conjugation is into three stages: direct cell-to-cell contact, mating pair formation, and transfer of plasmid DNA through a conjugative pilus (Christie, 2004). Studies conducted on conjugation indicated that two types of movable genetic elements plasmids and transposons, in *enterococci* and *streptococci* as earlier indicated, were responsible for the materialization of antibiotic resistance in *L. monocytogenes* (Charpentier and Courvalin, 1999, White et al., 2002). The acquisition of novel genetic material from the conjugative plasmids or transposons from *Enterococcus* or *Streptococcus* to *L. monocytogenes* originates from the gastrointestinal tract of humans (Doucet-Populaire et al., 1991). *Listeria monocytogenes* isolates from food, including food processing environments, harbored the benzalkonium chloride resistance transposons Tn6188 that encodes the tolerance to quaternary ammonium compounds in *Staphylococcus aureus* and other Firmicutes (Müller et al., 2013).

Tetracycline resistance in humans is the most frequent peculiarity in *L. monocytogenes* (Charpentier and Courvalin, 1999). Tetracycline resistance genes are in six classes with the

Gram-positive *tet* (*K*), *tet* (*L*), *tet* (*M*), *tet* (*O*), *tet* (*P*), and *tet* (*S*) (Schnappinger and Hillen, 1996). However, the resistance genes associated with *L. monocytogenes* are recorded only in *tet* (*K*), *tet* (*L*), and *tet* (*M*) (Li et al., 2007). A common determinant for tetracycline resistance, *tet* (*M*), has been found in Tn916 and Tn1545, like conjugative transposons first discovered in *E. faecalis* (Wilcks et al., 2005). Reports from 19 out of 38 *L. monocytogenes* isolates 50% from dairy farms harbored more than one antibiotic resistance gene sequence (Olaimat et al., 2018). For instance, *floR* gene was 66%, *penA* 37%, *strA* 34%, *tet* (*A*) 32%, and *sull* 16% all these isolates were *L. monocytogenes* (Olaimat et al., 2018). Furthermore, Li and others reported that 12 of 78 *L. monocytogenes* isolates, 15.4% came from a pork processing plant in China that carried *tet* (*M*) gene (Li et al., 2016). Similarly, the genes *ermB*, *tet* (*M*), and *dfrD* in the *L. monocytogenes* strain were isolated from France's food and environmental samples from 1996 to 2006; but the *tet* (*S*), *tet* (*K*), and *tet* (*L*) genes were not detected (Granier et al., 2011). The antibiotic resistance genes *tet* (*M*) and *ermB* were from *L. monocytogenes* isolated from freshly mixed sausage and chicken slaughterhouse, respectively (Haubert et al., 2016). Two *L. monocytogenes* isolates from fried fish and salads carried five resistant genes *tet* (*A*), *lmrB*, *mecC*, *msrA* and *fosX* that confer resistance to tetracycline, lincomycin, beta-lactam, erythromycin, and fosfomycin respectively (Wilson et al., 2018). Equally, in the same study *ermB* gene was detected in *L. monocytogenes* isolate that came from different types of foods (Wilson et al., 2018).

The materialization of tetracycline resistance in *L. monocytogenes* necessitates conjugative plasmids and transposons, carried by other antibiotic resistance to *L. monocytogenes* from *Enterococcus* or *Streptococcus* (Poyart-Salmeron et al., 1990). The conjugative transfer of plasmids and transposons carried other antibiotic resistance to *L. monocytogenes* from *Enterococcus*, *Streptococcus,* or other *Listeria* species (Charpentier and Courvalin, 1999).

2.9.4 Antibiotic resistance mediated by efflux pumps

A primary cellular resistance mechanism involves active antibiotic transport from the bacterial cell through efflux pumps. *Listeria monocytogenes* have three efflux pumps; the first operates to extrude antibiotics, heavy metals, and ethidium bromide (Mata et al., 2000). The second pump is associated with the resistance of *L. monocytogenes* to acridine orange and ethidium bromide (Godreuil et al., 2003). The third pump is the genetic basis of fluoroquinolone resistance in *L. monocytogenes* linked to overexpression of the genes *Ide*, encoding an efflux pump of the primary facilitator superfamily and *fepA,* the multidrug toxic compound extrusion (MATE) family, which are in *L. monocytogenes*. It is known to include five families in drug efflux systems: the primary facilitator being the major super-family (MFS), the resistance nodulation-cell division (Ma, 2010).

2.10 Knowledge Gap in Zambia

Presently, the circulating *Listeria* species in various foods and food processing environments lack documentation in Zambia. There was a need to describe the *Listeria* species circulating in food and food environments and further determine antibiotic resistance and genes that confer resistance to help enhance better understanding and implementation of prevention and control measures. Consequently, this study aimed to fill this knowledge gap by determination of public health importance and antibiotic resistance patterns of *Listeria* species in Zambia's food and food processing environments.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

We conducted a cross-sectional study design in Zambia's Namwala, Lusaka, and Siavonga districts from March 2020 to August 2021. The cross-sectional study was suitable to answer the research question because it allows the measurement of multiple variables at a one-time point.

3.2 Study Sites

The study was across three strategically identified districts in Zambia. These included Namwala 16° 41’ south of the equator, Lusaka 15° 25’ south of the equator and 28° 17’ east of Greenwich, and 27° 26’ east of Greenwich and Siavonga districts with coordinates 16° 32’ south of the equator and 28° 44’ East Greenwich.

Namwala District had six beef processing abattoirs during the study (NMC, 2019). All were incorporated in this study except for one waiting for the opening. The district was selected because it has the most significant number of abattoirs supplying beef both on small scale and commercial bases throughout the country (Munyeme et al., 2010). Furthermore, Southern Province reports contributing the highest number of cattle compared to other Provinces, particularly Namwala district (M.F.L, 2019). We identified the abattoirs as one, two, three, four, and five.

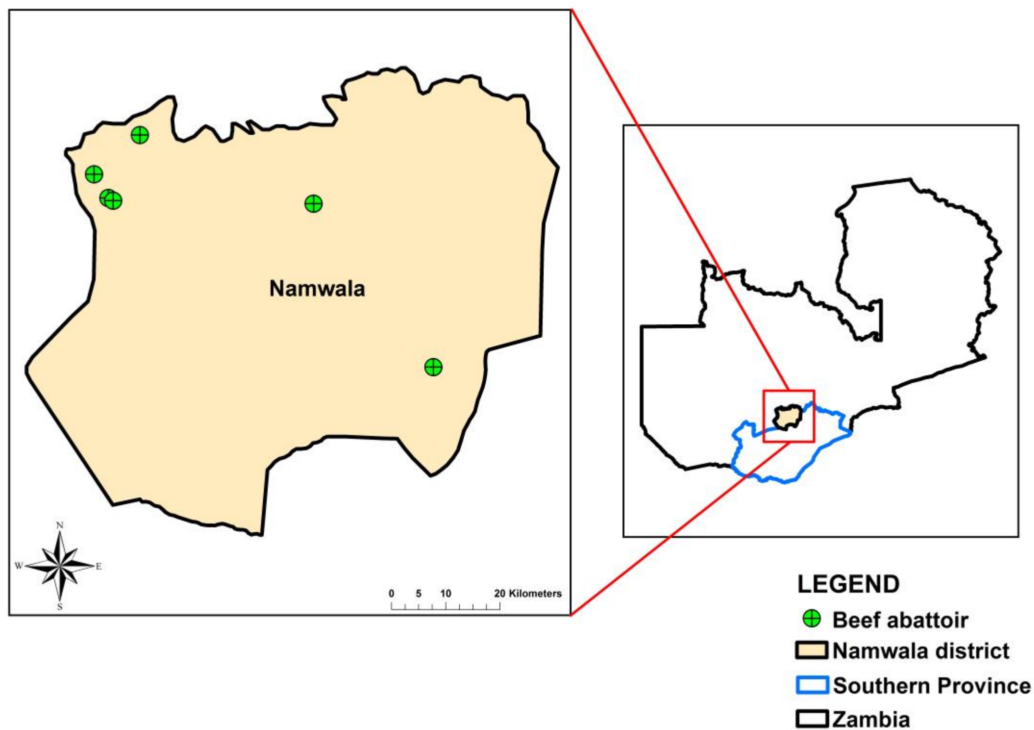


Fig. 3.1: Map of Namwala District showing the sampled areas

During the study period, we counted 30 retail markets in Lusaka processing RTE deli meats, including vegetables like lettuce (LCC, 2019). The designation of retail markets is A, B, C, and D by the Lusaka City Council. Accordingly, we adopted this and randomly selected one retail market from each zone. Accordingly, we used the same zone letters for retail markets to identify sites A, B, C, and D.

Further, we also sampled poultry abattoirs in Lusaka apart from the retail markets. During the sampling period, Lusaka had four poultry abattoirs, and only three enrolled because the other abattoir was closed for renovation. Lusaka province has the most significant number of poultry abattoirs because of the recent rapid demand for chicken meat. Also, the growing population in the capital city has contributed significantly to the speed of transformation seen in this sector to meet production demand. Besides, the city attracts several production experts, such as training

personnel. The convenience of using antibiotics used for the treatment of poultry is among the few things helping this industry thrive. The three selected abattoirs coding was with identification letters as Abattoir A, B, and C. This cohort sampled only included dressed chickens slaughtered at these abattoirs during the study period.

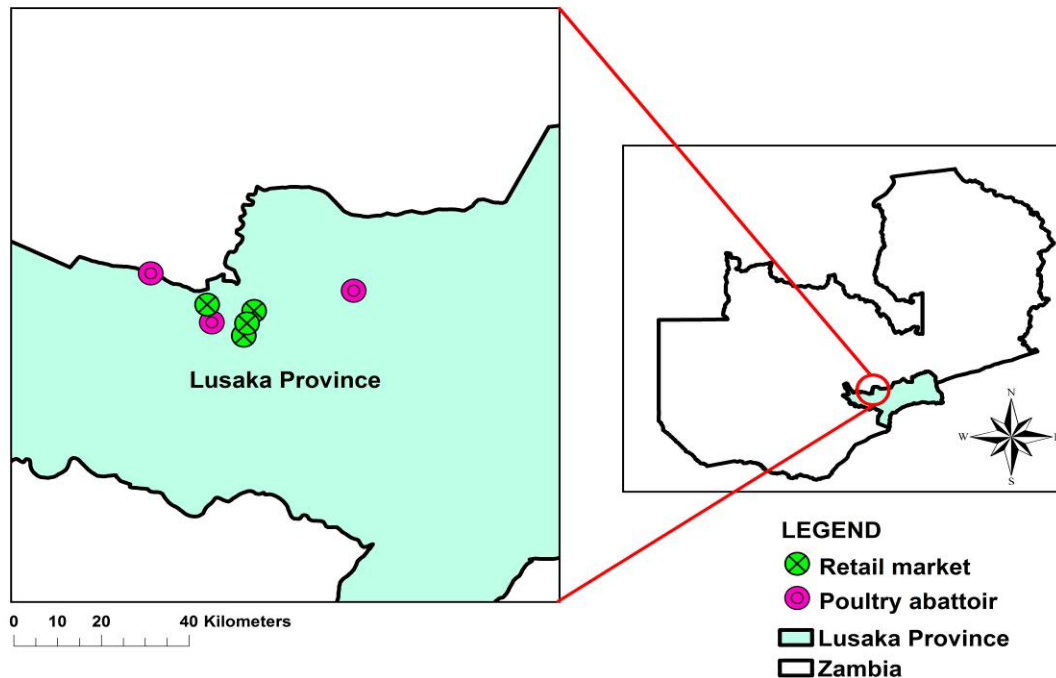


Fig. 3.2: Map of Lusaka Province showing the sampled areas

Tilapia (*Oreochromis niloticus* the most common tilapia in cages) fish swabs were collected from capture fisheries and aquaculture cage farms (SMC, 2019). Most fish comes from aquaculture cage farms (Hasimuna et al., 2019). Compared to the capture fisheries, poor documentation makes effective management impossible. However, efforts were made to capture as many as possible at captured fisheries. Sampling on capture fisheries was on three of the six recorded major sites along Lake Kariba in the Siavonga district. The selection of the three sites was influenced by the number of fisheries and availability of tilapia fish at the time of sampling, and

these were coded as sites one, two, and three. While about 15 aquaculture cage farms were recorded by the Siavonga Town Council, of which three were sampled due to accessibility and availability of fish during the study period, and these were coded as four, five, and six. From each fish, swab samples were from the exterior surface and the gills. We considered the Siavonga district because it has several aquacultures and hosts the highest number of tilapia fish, the country's most consumed fish species, compared to other places (Nyikahadzoi et al., 2017). Equally, the lake has increasingly received many fishers from other fisheries, where fish catches have significantly declined (Hasimuna et al., 2019).

Additionally, we collected environmental swabs from poultry and beef abattoirs from the cold room facilities where the sampled foods were stored. However, no environmental swabs were collected from retail markets and aquaculture cage farms.

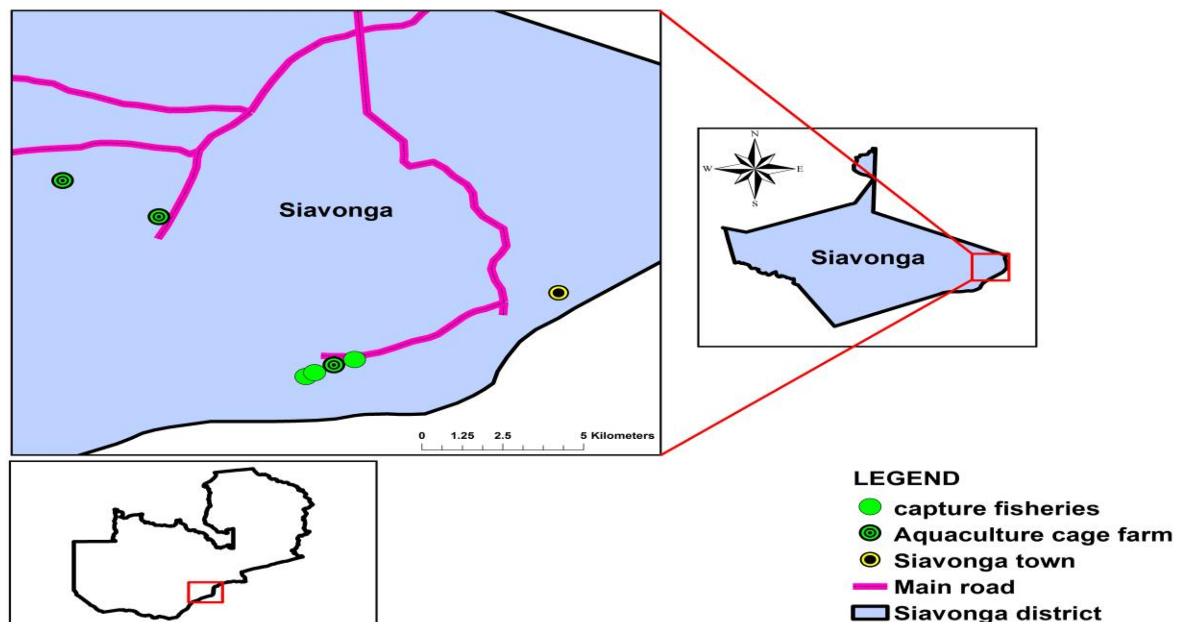


Fig. 3.3: Map of Siavonga District showing the sampled areas

3.3 Sampling procedure

We collected food samples/swabs from RTE deli meat, lettuce vegetables, white meats (poultry and fish products), red meat (beef), and environmental samples.

3.3.1 Inclusion Criteria

All the retail markets selling RTE deli meats (sliced and repackaged at the retail market or sold as packaged from the source of production) and lettuce foods produced locally or imported. Abattoirs dealing with beef and poultry, including aquaculture cage farms, capture fisheries in the study areas.

3.3.2 Exclusion Criteria

Foods that cannot stay at extended refrigeration temperatures, including vegetables that are not RTE and not considered high risk of *Listeria* contamination. For instance, retail markets that were not selling RTE deli meats or lettuce and stores with no refrigeration facilities.

3.4 Sample Size and Sampling

We stratified 654 individual samples according to different sources we sampled using a known prevalence rate based on previous studies of 27.5% (Derra et al., 2013) at 80% power and 5% significance level. The distribution figure was calculated from the total maximum sample size of 654 and proportionately allocated according to the finite population size.

Five beef abattoirs collected 200 hundred carcass swabs and 50 environmental swabs. The 200 hundred carcass swabs were collected as 100 for the exterior and 100 for the interior. All swabs collection was immediately after evisceration and hide removal. We equally allocated the stated

sample size to the abattoirs, and 40 carcass swabs and ten environmental swabs per site were collected. The abattoirs, having the same maximum capacity throughput, slaughtered an average of 30 carcasses per day, with only one recording slaughters of 90 per day as a maximum. Simple random sampling was the technique we used to pick the carcass for swabbing through shuffling before we picked the next one. We selected 200 carcasses in the five abattoirs at the end of the sampling period for bacteriological examination of *Listeria* and 50 environmental swabs.

We used a circular systematic random sampling method to pick a chicken for swabbing (Mpundu et al., 2019). The total sample size for poultry was 150, 60 cloacal and 60 exterior surface swabs, and 30 environmental samples. The abattoir throughputs ranged from 8,000 to 20,000 birds per day. The total sample size we allocated equally regardless of the throughput. Of which a total of 50 samples we collected from each poultry abattoir. We sampled five dressed chickens per batch each day of sampling from each abattoir. At the three abattoirs, we defined a batch as chickens from the same flock with homogenous characteristics (i.e., same source, same farm). We collected a total number of 120 carcass swabs and 30 environmental swabs from the poultry abattoirs. One hundred twenty carcasses were randomly selected using the circular systematic random sampling system for each batch by selecting five dressed chickens. We selected 120 carcasses in the three abattoirs at the end of the sampling period for bacteriological examination of *Listeria* and 30 environmental swabs of different batches.

We used simple random sampling to pick a fish from each capture fisheries and aquaculture cage farm by shuffling before the next pick. We collected 150 fish swabs divided equally between the two sources from the following points: surface skin (n=75) and gills (n=75).

52 RTE deli slices of meat and 52 lettuce vegetables we randomly sampled from the four retail markets located in the Lusaka district. We selected the four retail markets by writing numbers 1 to 30, comprising the total retail markets from all four identified zones. The numbers we placed in the box we shuffled at every next pick of the number until four retail markets. The 52 RTE deli slices of meat were divided into two groups (26 were sliced and repackaged at the retail shop, and 26 were sold as packaged by the manufacturers). The sampling was divided equally across the selected retail markets, and at the end of the sampling period, we sampled 13 RTE deli slices of meat in four days of sampling, like lettuce vegetables. The sampling period was four days in the retail markets, where we sampled at least three samples of RTE deli meats and lettuce from each retail market. We selected 52 RTE deli slices of meat and 52 lettuces in the four retail markets at the end of the sampling period for bacteriological examination of *Listeria* species.

3.5 Sample collection techniques and tools

Sampling was done in a phased manner. Phase one: beef samples in Namwala from abattoirs and their storage environments **Fig. 3.1**. Phase two: poultry samples and their storage environments **Fig. 3.2**. Phase three: tilapia fish samples from capture fisheries and aquaculture in Siavonga district **Fig. 3.3**. Phase four RTE/vegetables (RTE deli meats and lettuce vegetables) samples from retail shops from Lusaka district **Fig. 3.2**. All collected samples were processed and stored at -80 ^{0}C at UNZA veterinary lab before the next sampling was conducted. An in-house data collection procedure was used as follows; a sterile metal template outlined a 5cm^2 x 5cm^2 area parts marked for swabbing (Holah et al., 2004b).

3.5.1. Primary Production Sites Sampling

At the beef abattoir, only surface swabs we collected on the exterior and interior parts. The parts swabbed for dressed chicken carcasses at the abattoir were the cloaca region and the exterior surface of the chicken. The fish samples were collected from (exterior surfaces and gills) (Holah et al., 2004b). Furthermore, RTE deli meats and the lettuce vegetables, more than 30g, sample of each was put in a sterile sample bag.

3.5.2. Secondary Production Sites Sampling

We used standard bacteriological sample collection for *Listeria* contamination on all samples collected (Alsheikh et al., 2013). A sterile metal template outlined a $5cm^2$ x $5cm^2$ area of parts marked for swabbing (Holah et al., 2004a). We used the areas outlined by the metal template to denote the parts swabbed using a sterile moist cotton gauze wrapped around the end of a flat swab stick. We put swabbed samples in screw-cap tubes containing 9ml Fraser broth (Oxoid) (enriched with *Listeria* selective supplement). More than 30g of a representative portion from each sample was collected and put in a sterile sample bag for the RTE foods and the raw lettuce. We recorded the samples we identified according to date, ingredient samples (e.g., poultry carcass), site name and batch code including comments specific to the sample (e.g., interior or exterior). We identified samples by the laboratory code, sampling site/product, ingredient type, date, and site. All samples collected were transported in cooler boxes containing iceboxes to the Microbiology laboratory at the School of the Veterinary Medicine University of Zambia within 3 hours of sampling. While samples from Namwala and Siavonga were at -4ºC before being transported within 72 hours. Upon arrival, samples were immediately transferred in 5ml of

Listeria selective broth and incubated at 37° C for 48 hours. Upon receipt, samples were assigned laboratory codes and processed (Holah et al., 2004a).

3.6 Culture, Isolation, and Identification *of Listeria* species

We tested the poultry, beef, and fish swabs, including RTE deli meats and lettuce vegetables, for the presence of *Listeria* species using Standard international methods recommended by the International Organization for Standardization (ISO g11290 -1: 1996, 2004) (Alsheikh et al., 2013). Procedure First, a 1g of the sample representative portion from each was inoculated in 9ml of pre-enriched broth and incubated at 37° C for 24hrs, then 1ml of pre-enriched broth was transferred into 9ml of Fraser broth (Oxoid) (enriched with *Listeria* selective supplement) and centrifuged vortexed for 1min at 260 pm, followed by incubation at 37°C for 48 hours. For the RTE foods and the raw lettuce vegetables, 25g of a representative portion from each sample was collected and homogenized in a sterile blender. The homogenate was introduced aseptically into a sterile stomacher bag containing 225ml of Fraser broth (Oxoid) (enriched with *Listeria* selective supplement) and at 37°C for 24 hours. A loop-full of enrichment pre-enrichment broth (Oxoid) culture was inoculated on *Listeria* selective agar (Oxoid), incubated at 37°C for 48hrs, and observed for colonies showing growth classic greenish sheen morphology or green-blue colonies colour of *Listeria*. The suspected colonies were then subcultured onto Nutrient Agar (Oxoid) immediately incubated at 37°C for 24 hrs to obtain pure colonies. We performed standard biochemical tests on the purified cultures, namely, Gram's staining, catalase, Sulphur indole motility (SIM), oxidase, urease, and Voges Proskauer (VP), and methyl-red tests (MR), to obtain a presumptive diagnosis of *Listeria* (Alsheikh et al., 2013).

Fig. 3.4.a: Suspected colonies on *Listeria* selective agar

Fig. 3.4b: Suspected colonies on *Listeria* selective agar

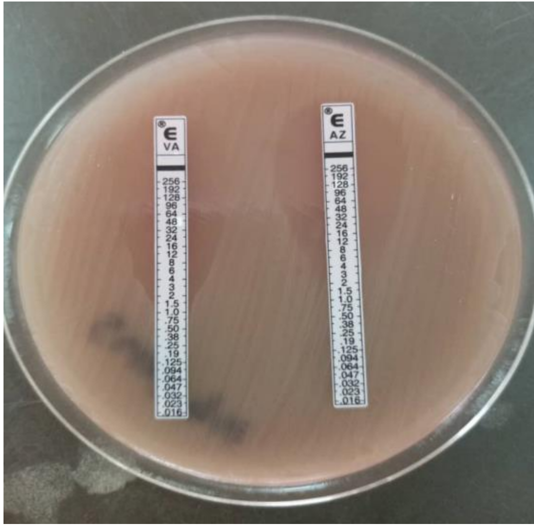
Fig. 3.4: Suspected culture colonies of *Listeria* on Oxoid selective agar

3.6.1 DNA extraction and PCR Identification of *Listeria*

Following biochemical tests and PCR assays, we confirmed the presumptive isolates of *Listeria* species D.N.A. we extracted from the pure culture of the suspected isolates, grown on nutrient agar using a commercial genomic D.N.A. extraction kit (ZYMO Research quick D.N.A. miniprep kit) as per the manufacturer's instructions. The primer pairs are *prs.* -F (5'- GCT GAA GAG ATT GCG AAA GAA G – 3') and *prs* -R (5'-CAA AGA AAC CTT GGA TTT GCG G- 3') were used to amplify a 370bp fragment of the *Listeria prs* gene (Doumith et al., 2004). A 50μl PCR master mixture consisting of 5 μl of 10x PCR buffer, 1.5 μl of 0.5 μl of Taq D.N.A. polymerase, 1 μl of 10mM dNTP5 mix (10Mm 1 μl), 100 ng of template and Nuclease. The thermal cycler conditions were initial denaturation at 94ºC for 2 minutes followed by 35 cycles, denaturation at 94ºC for 45seconds, annealing at 53ºC for 45seconds, and extension at 72ºC for 2 minutes with a final extension at 72ºC for 7 minutes. The amplified PCR products were visualized on 1% agarose gel coated with ethidium bromide.

3.7 Phenotypic detection of antimicrobial resistance in *Listeria* species isolates

We used the Kirby-Bauer disc diffusion method on Mueller Hinton agar with five-per-cent sheep blood (Sader et al., 2020) on 107 isolates of *Listeria* species (Oxoid, Basingstoke, U.K.). Penicillin G (10 unit), gentamicin (30μg), Levofloxacin (30μg), erythromycin (15μg), clindamycin (30μg), trimethoprim-sulfamethoxazole (1.25/23.75μg), (30μg), chloramphenicol (30μg) and tetracycline (30μg) were applied as antibiotic agents. Clinical Laboratory Standards Institute breakpoints for *Listeria* species only include a few antimicrobial agents such as trimethoprim-sulfamethoxazole, ampicillin, imipenem, and penicillin; therefore, we used CLSI breakpoints for Streptococcus pneumoniae for other antimicrobial agents, and this was because the CLSI guidelines indicated that this was the organism used to interpret breakpoints for *L. monocytogenes* (Sader et al., 2020). The identical 107 isolates had their antimicrobial susceptibility tested using the Etest (Biomerieux) with vancomycin and imipenem because no interpretation was provided under disc diffusion in the CLSI (Sader et al., 2020). The quality control strains included were *L. monocytogenes* ATCC19118, *Streptococcus pneumoniae* ATCC 49619, and *Staphylococcus aureus* ATCC 25923. After 24hrs of incubation, the inhibition zones we measured in millimeters, and the strains were classified as susceptibility, intermediate, or resistant in both methods (Lombard and Assurance, 2012).



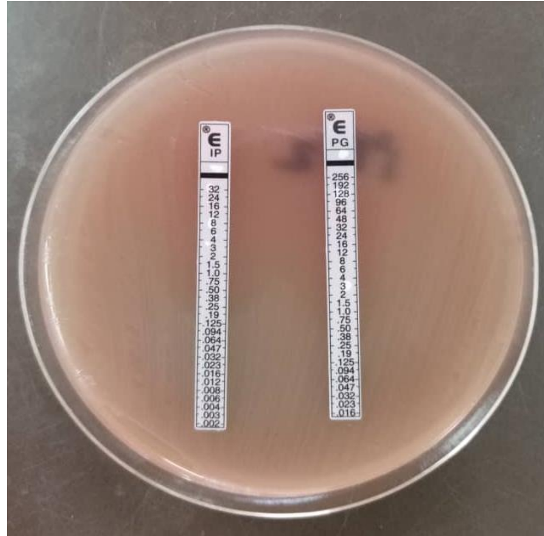
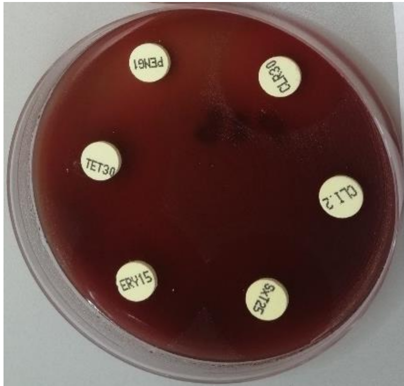
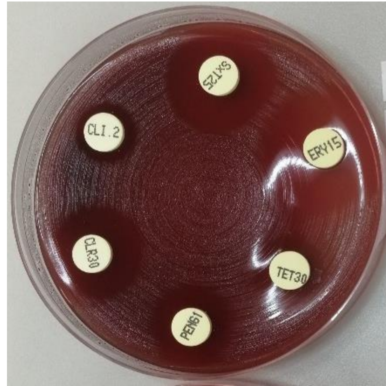


Fig. 3.5a: Etest strip on one of the *Listeria* isolates after 24hrs incubation

Fig. 3.5b: Etest strip on one of the *Listeria* isolates after 24hrs incubation

Fig. 3.5: *Listeria* isolates on blood agar to determine antibiotic resistance using Etest





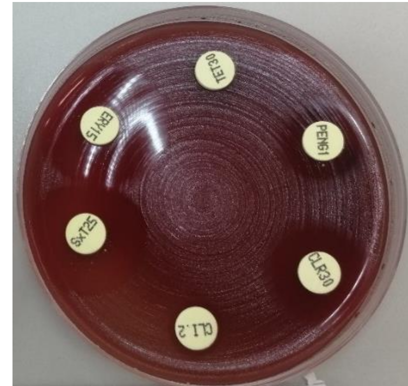


Fig. 3.6a: One of the *Listeria* isolates after 24hrs incubation on blood agar

Fig. 3.6b: One of the *Listeria* isolates after 24hrs incubation on blood agar

Fig. 3.6c: One of the *Listeria* isolates after 24hrs incubation on blood agar

Fig. 3.6: *Listeria* isolates on blood agar to determine antibiotic resistance using disc diffusion

3.8 Identification of specific *Listeria* isolates using MALDI-TOF MS

MALDI-TOF MS identified different specific *Listeria* strains on the isolates that were first confirmed on PCR using the amplification of *prs* gene to ascertain that it was the *Listeria* genus. 10ml of the sample was steak on 5% sheep-blood agar and later incubated at 37ºC and the

cultures were analysed after 24 and 48hours of incubation. Briefly using a loop, we collected a colony of *Listeria* strain from the solid culture medium and spotted it onto a disposable Flexi Mass –DS target plate (Jadhav et al., 2015). Immediately adding 1μL of VITEK MS –CHCA matrix to each target plate spot using different pipette tips was done. For each matrix, each spot was allowed to air dry. *Escherichia coli* (*E. coli*) ATCC8739 was used as control after incubation for 18 to 24 hours at 35ºC on blood agar under aerobic conditions.

The *E. coli* ATCC8739 strain was deposited on positions xA1, xB1 and xC1 depending on the number of the samples to be tested (one carburant per acquisition group of 16 spots). Using a loop, we collected a colony of *E. coli* ATCC8739 onto each acquisition group's calibration spot on the VITEK MS-DS target slide. Immediately we added 1μL of VITEK MS –CHCA matrix to each calibration spot using different pipette tips for each matrix addition. Each spot on the target plate was allowed to air dry completely before taking to the MALDI-TOF machine for the control and the *Listeria* strain. Each target slide runs in the VITEK® MS instrument following the instructions in the VITEK®MS plus workflow user Manual or the VITEK® MS instrument user manual. Target plates were loaded into the Axima performance instrument. Mass spectra were generated by firing a 337nm nitrogen laser on the sample spots in the positive linear mode, baseline corrected, and noise filtered (BIOMERE@2019 BIOMERIEUX SA). We were using the Launchpad software (version 4.16 SARAMIS®). Peak lists containing a peak mass list with the corresponding intensities were generated in the mass range 2-20KDa, which were then exported to SARAMIS for identification. The commercial database has 32,413 Reference Spectra and 4578 Super Spectra for different bacteria and fungi (including thirteen Super spectra representing the genus *Listeria* and twenty representing the reference spectra. Reference spectra are a collection of individual MALDI-TOF MS spectra obtained for different strains, and super spectra

are genus/species-specific spectra that are generated from selected reference spectra already existing in the database. The identification in SARAMIS based on a pattern matching algorithm where the test spectra exported are compared with Super Spectra in the database, resulting in a confidence score ranging from 75% to 99.9%. In the current study, isolates identification at the genus or species level was considered reliable if the confidence score was greater than 90% because the isolates were previously identified by amplification of *prs* gene.

Fig. 3.7a: *Listeria* isolate after 24hrs **Fig. 3.7b:** Disposable Flexi Mass –DS target plate

Fig. 3.7: *Listeria* isolates on blood agar after 24hrs in readiness to be put on Disposable Flexi Mass –DS target plate.

3.9 DNA extraction for Whole-genome Sequencing of *L. monocytogenes* and isolates that recorded antimicrobial resistance

Using a loop, we collected 10μl of *Listeria* strain from the solid culture medium and introduced it into a 20ml cylinder with Luria-Bertani (LB) broth and were left in a shaker incubator at 37°C for 24hrs at a shaking speed of 100-400^{-1} (INFORS AG CH-4103 BOTTMINGEN). Using the Qiagen DNA extraction kit (per the manufacturer's instructions), each sample from the 20ml cylinder filled up the centrifuge tube using a pipette and centrifuged at 13000pm for 1 minute. Later, the sample suspension was removed by pipetting to obtain a pellet in a 180μl enzymatic lysis buffer. Briefly, by pipetting up and down, a homogenized mixture was obtained. Later 10μl

lysozyme (20mg/ml stock solution) was added and mixed. Later incubation followed for 45 minutes at 37°C. After incubation, an additional 200μl buffer AL and 25μl proteinase K were mixed thoroughly and incubated at 56°C for 30 minutes. After incubation, 200μl ethanol was added and mixed, followed by pipetting into a DNeasy Mini spin column in a 2ml collection tube. After that, we centrifuged at ≥6000 x 8000g revolutions per minute (8000rpm) for 1 minute, and the flow-through and collection tubes were discarded. DNeasy Mini spin was later placed in a new 2ml collection tube and 500μl Buffer AW1 and centrifuged for 1 minute at ≥6000 x g; the flow-through and collection tube were discarded. Again the spin column was placed in a new 2ml collection tube, and an additional 500μl Buffer AW2 and centrifuged for 3 minutes at 20,000 x g (14,000 rpm). The flow-through and collection tube was discarded and transferred the spin column to a new 1.5ml or 2ml microcentrifuge tube. We immediately eluted the DNA by adding 100μl buffer AE to the center of the spin column membrane. This was briefly incubated for 1 minute at room temperature (15-25°C) and centrifuged for 1 minute at ≥6000 x g.

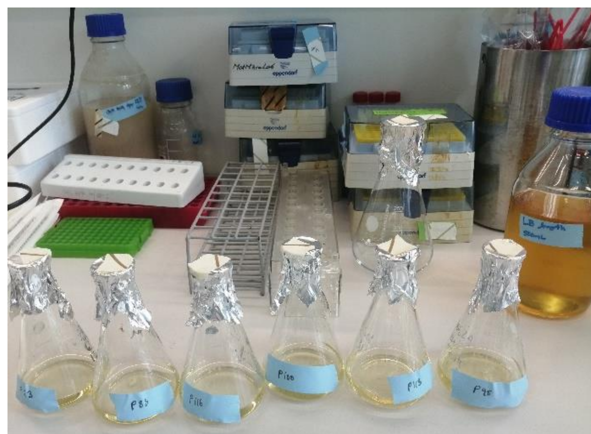


Fig. 3.8a LB broth in 20ml glass cylinders

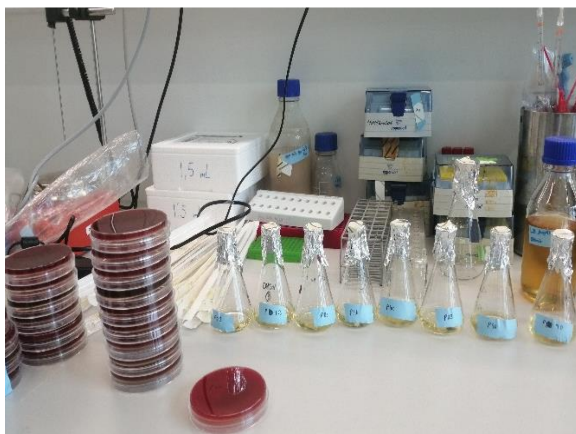


Fig.3.8b *Listeria* on blood agar after 24hrs incubation, ready to be inoculated in LB

Fig. 3.8: *Listeria* isolates on blood agar plates being put in LB for 24hrs incubation in readiness of DNA extraction.

Fig. 3.9 DNA extraction of *Listeria* species incubated for 24hrs in LB broth

3.9.1 DNA Concentration using NanoDrop

The DNA concentration in samples was measured using a (NanoDrop LNS-U-018). Each sample of DNA was placed 1-2ul of mini-prepped DNA on a pedestal. After the lid was closed and click measured, the record of concentration and purity was saved for each measured sample. As part of quality control, the NanoDrop first sample was a blanking with Neasy-free water. After each sample measure, the pedestal was wiped with Neasy-free water to avoid the accumulation of DNA, which may result in a false reading. Standard purity measures under the 260/280 column. A good purity in this study was if the sample was 1.80-2.800.

3.9.2 Qubit Fluorometer 3.0

Double-stranded DNA (dsDNA) was quantified using the Qubit ™ dsDNA HS Assay Kit (Invitrogen, Life technologies; cat # 2383822). Per the manufacturer's instructions, a standard curve was prepared using the 0 and 10 ng/μL Qubit standards provided in the kit. 3μL of the sample was diluted for all dsDNA extraction in 197μL Qubit working solution before measurement. The assay is selective towards dsDNA over RNA and is accurate for initial sample concentrations from 10ng/μL to 100μL.

3.10 Data analysis

3.10.1 Descriptive analysis

All samples collected were entered and established in the excel sheet, where data handling and cleaning were done and imported to STATA version 15.0 (Stata cop, college station, Texas, U.S.A.). Survey summary analysis and descriptive analysis were done. The outcome of interest was *Listeria* species (i.e., *L. innocua*, *L. monocytogenes, L. ivanovii*, *L. seeligeri*, *L. marthii*) from various host samples.

3.10.2 Antibiotic resistance WHONET

All the data that showed resistance from Etest and disc diffusion was entered in the excel sheet and imported to WHONET version 2021 for analysis. The outcome of interest was the resistance of *Listeria* species (i.e., susceptibility, intermediate, or resistance) from various host samples.

3.10.3 Whole-genome Sequencing (WGS).

A custom-made pipeline was used to analyze WGS raw data that was processed using illumina technology (Sam Diego, CA,USA) (Arredondo-Alonso et al., 2018). Briefly, the raw sequence data were first subjected to quality control using FastQC (https://github.com/s-andrews/FastQC/), followed by trimming and barcode removal using SICKLE (https://github.com/najoshi/sickle). This was then followed by de novo assembly using SPADES (Bankevich et al., 2012). Polishing of the assembled contigs was done using PILON (Walker et al., 2014). The quality of the polished contigs was assessed using Quast (Gurevich et al., 2013). Ragtag (Alonge et al., 2021) was used to perform reference-guided assembly of contigs into a final consensus sequence. Variations in specific sequences and housekeeping genes and functionality assessment were achieved using MLST (https://github.com/tseemann/mlst). Identification of AMR and resistant genes, annotation and construction of a dendogram was performed using Abricate

(https://github.com/tseemann/abricate), Prokka (Seemann, 2014) and dRep (https://github.com/MrOlm/drep). In addition, pangenome analysis was performed using Roary (Page et al., 2015).

3.11 Ethical Considerations

Ethical approval for this study was obtained from Excellence Research Ethics and Science Converge (ERES) (2020-Jan -006). Permission to collect samples from the sites was obtained from all local authorities and fish plants. We explained the purpose and importance of the study to the abattoir owners, fish plants and retail market before being invited to participate. Written informed consent was obtained before commencement of the study from all the participants. This study involved the collection of beef swabs and their storage environment, dressed poultry swabs and their storage environment, fish swabs, RTE deli meats and lettuce vegetables. This study had minimal ethical issues to address. The identified ethical issues of concern patterning this study may have involved the following:

Psychological discomfort: The business owners may have felt judged by others that they are selling contaminated foods. We kept confidential all information collected from all sampled facilities to reduce this effect.

Possible economic loss to the business: Abattoir and Fish plant owners, including retail markets, may likely experience economic loss if foodstuffs are contaminated. Anonymity was maintained, and only the researcher knew their location and actual identities; visits were done to the plants/abattoirs outside the study, and gave results and discussed accordingly depending on the outcome and what they needed to do. Assurance that their identities were kept confidential, even to the involved city councils.

CHAPTER FOUR

RESULTS

4.1 Descriptive results

4.1.1 General descriptors

Six hundred fifty-four individual samples were collected across the three main sample sites of Namwala, Lusaka, and Siavonga. The primary sample sources according to study area were as follows: Namwala, Beef (*n*=200); Environmental samples (*n*=50); Lusaka, poultry (*n*=120) and Environmental sample (*n*=30). Siavonga only tilapia fish (*n*=150); additionally, in Lusaka, RTE deli meats (*n*=52 and lettuce vegetables (*n*=52).

Table 4.1 General descriptors of samples collected in different strata

STRATA	Samples	Number of samples	*Listeria*	% contamination	95%CI
Namwala	Beef samples	200	29	14.5	9.6 -19.4
	Environmental samples	50	-	-	-
Lusaka	Poultry samples	120	51	42.5	33.6-51.4
	Environmental samples	30	8	26.7	0.99-43.5
Siavonga	Fish samples	150	-	-	-
Lusaka	RTE deli meats	52	12	23.1	11.5-34.7
	Lettuce	52	7	13.5	4.1-22.8

4.1.2 Bacterial culture and biochemical identification of *Listeria* species

A total of 203 suspect colonies were processed, which came from assorted samples distributed as follows: beef abattoirs (72), poultry abattoirs (90), fish plants (7), and RTE deli meats (19), and lettuce (15). Out of which, 147 presumptive *Listeria* species isolates were obtained. The detected suspected colonies came from Lusaka (70) from poultry and environmental samples), Namwala (46 from beef carcasses), retail markets (16 from deli meats and 12 from lettuce), and Siavonga (3 from tilapia fish samples). Out of the 147 presumptive suspects, only 123 were able to grow on *Listeria* selective media. For all 123 presumptive suspects, we screened them using primary biochemical Gram stain, catalase, SIM, oxidase, urease, and VP and MR. Morphologically, the isolates were Gram-positive, ovoid to rod-shaped isolates. After the screening, only 112 suspects were further run on *prs* gene to confirm if they were *Listeria* species.

4.1.2.1 Identification of specific *Listeria* isolates using MALDI-TOF MS

Different *Listeria* species were identified using MALDI-TOF MS of the total 107 *Listeria* species isolated, (71) were *L. innocua,* (23) *L. monocytogenes*, (6) *L. ivanovii*, (2) *L. marthii*, and (5) *L. seeligeri*. All the identified strains had a similarity score of 99.9 with the existing super spectra in the database. These isolates were distributed as follows: *L. monocytogenes* had at least a representation in all isolated samples, while *L .innocua* was the primary contaminant. *Listeria ivanovii* isolation was in all collected samples except in environmental swabs and lettuce vegetables. *L. marthii* and *L. seeligeri* were only recorded in lettuce vegetables. **(Appendix 14)**.

4.2 Phase One Results: Specific *Listeria* results under Beef Study

4.2.1: General Descriptors of *Listeria* in Beef Abattoirs, Namwala District

Of the 250 environmental and beef carcasses collected, 19 had *Listeria* species, of which 50 came from environmental samples and 200 from carcass swabs. When specific *Listeria* contamination in the abattoirs was considered, *L. innocua* accounted for 14 (73.7 %) and *L. monocytogenes* 5 (26.3 %). Furthermore, when specific establishment contamination was considered abattoir one, it showed the highest contamination of both *L. innocua* 8 (42.1 %) and *L. monocytogenes* 4 (21.1 0%). Meanwhile, no *Listeria* species were isolated from the environmental samples collected in this study. (Table 4.2). Out of the five abattoirs sampled, only abattoir one had the highest contamination.

Table 4.2 Prevalence of isolated *Listeria* species in beef carcasses (*n*=19)

	Overall *Listeria* contamination (%)	**Prevalence of *L. innocua* (%)**	**Prevalence *of L. monocytogenes* (%)**
Abattoir			
1	**12 (63.2)**	**8 (42.1)**	4 (21.1)
2	3 (15.8)	2 (10.5)	1 (5.3)
3	1 (5.3)	1 (5.3)	0 (0.0)
4	0 (0.0)	0 (0.0)	0 (0.0)
5	3 (15.8)	3 (15.8)	0 (0.0)
Totals	19 (100)	14 (73.7)	5 (26.3)

4.2.2 Phylogenetic analysis of *Listeria* species isolated from Beef abattoirs

Phylogenetic analysis of the obtained sequences and the reference sequences showed the presence of three clusters, namely clusters A, B, and C (Fig. 4.1). Cluster A comprised both *L. monocytogenes* and *L. innocua*. The majority of the *Listeria* strains originated from abattoir 1. On the other hand, clusters B and C exclusively comprised of *L. innocua*. In cluster B, sequences

from abattoirs 1, 2, and 3 formed a cluster, while in cluster C, sequences from abattoirs 1 and 5 were present. Sequence LC629081 from this study did not cluster in any of the above clusters; however, it was closely related to sequences from C (Fig. 4.1). Sequences from abattoir five were only present in cluster C.

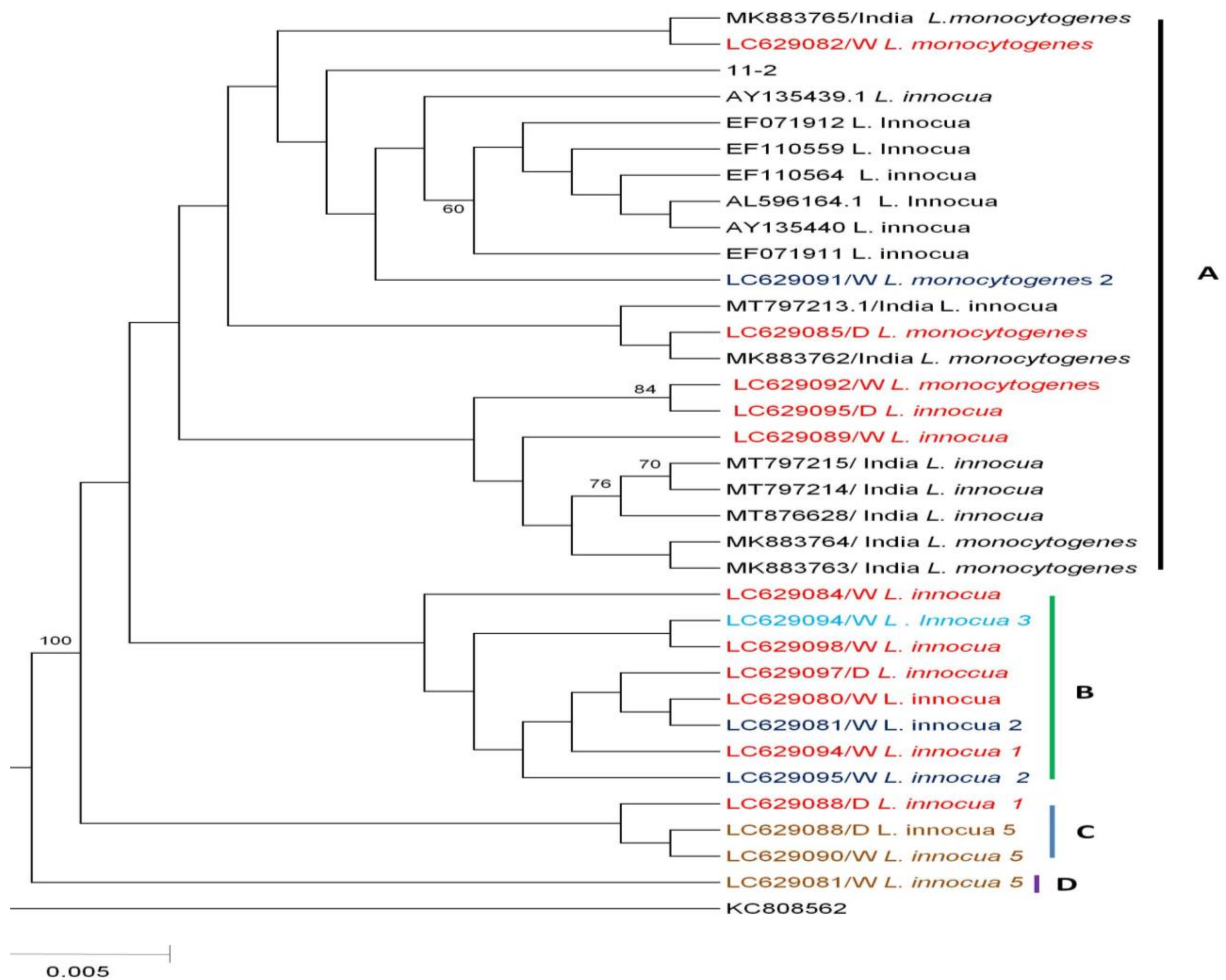


Fig. 4.1: Phylogenetic tree of *prs* gene.
Comprising of 35 sequences (19 obtained in this study and 16 downloaded sequences from (GenBank) based on 370 bp partial nucleotide sequences of the *prs* gene of *Listeria*. The tree was inferred using the Neighbour-joining method with 1000 bootstrap replicates as a confidence interval. The Maximum Composite likelihood method computed the evolutionary distances with all ambiguous positions or each sequence pair removed. The colour codes and number at the end of the study sequences represent the abattoir of origin, and the season of sampling is designated as D, dry season, and W; wet season.

4.3 Phase Two Results: Specific *Listeria* results under Poultry study

4.3.1: General Descriptors of *Listeria* in Poultry Abattoirs, Lusaka Province

Of 150 samples, 30 were from environmental swabs, and 120 were from poultry carcasses. The proportion positivity of *Listeria* species isolation was 15 % (23/150) at 95% CI (10.16 – 22.33%) (**Table 4.3**).

Table 4.3. Prevalence of *Listeria* species in dressed poultry and environmental samples (*n*=150).

Variable	*n*	Frequency	Prevalence %	95% CI	*P*-value
Environmental swabs	30	3	2	0.52 - 6.19	<0.001
Carcass swabs	120	20	13	8.53. – 20.08	<0.001
Overall	150	23	15	10.16 - 22.33	<0.001

n=number of swabs, 95% CI= Confidence Interval, *P*-value = 0.05.

4.3.2 *Listeria* species identification

BLAST analysis was performed based on the similarity scores ranging from 97 to 100% with reference sequences to confirm the type of *Listeria* species isolated. Out of the 23 *Listeria* species sequences, seventeen (17), one (1), and five (5) were identified as *L. monocytogenes*, *L. innocua*, and *L. welshimeri*, respectively (Table 4.4).

Table 4.4. Description and identification of samples collected from poultry abattoirs in Lusaka.

Site of swabbing	Abattoir	*Listeria* spp.	Accession number	Blast analysis similarity score (%)	Reference accession number
Cloacal	A	*L. welshimeri*	LC700404	97	LT906444.1
Cloacal	A	*L. monocytogenes*	LC700405	94	MK883765.1
Cloacal	A	*L. monocytogenes*	LC700406	99.39	MK883765.1
Cloacal	A	*L. monocytogenes*	LC700407	99.39	MK883765.1
Cloacal	A	*L. monocytogenes*	LC700408	99.38	MK883765.1
Exterior surface	A	*L. monocytogenes*	LC700410	99.39	MK883765.1
Exterior surface	A	*L. monocytogenes*	LC700412	99.70	MK883765.1

Exterior surface	A	*L. monocytogenes*	LC700413	98.79	MK883765.1
Exterior surface	A	*L. monocytogenes*	LC700414	100.00	MK883765.1
Exterior surface	A	*L. monocytogenes*	LC700415	98.79	MK883765.1
Exterior surface	A	*L. welshimeri*	LC700411	99.39	CP065605.1
Exterior surface	A	*L. monocytogenes*	LC700416	99.02	CP054846.1
Exterior surface	A	*L. monocytogenes*	LC700418	98.79	MK883765.1
Exterior surface	A	*L. monocytogenes*	LC700420	98.48	CPO54846.1
Exterior surface	A	*L. monocytogenes*	LC700421	99.37	MK883765.1
Exterior surface	A	*L. monocytogenes*	LC700422	100.00	AY1135439.1
Exterior surface	A	*L. monocytogenes*	LC700423	94.24	MK883763.1
Environmental swab	B	*L.innocua*	LC700409	99.70	KC808562.1
Cloacal	B	*L. welshimeri*	LC700417	98.18	CP065605.1
Exterior surface	B	*L. welshimeri*	LC700419	96.54	CPO65605.1
Environmental swab	B	*L. welshimeri*	LC700424	99.39	KC808562
Environmental swab	B	*L. monocytogenes*	LC700425	99.39	CPO65605.1
Exterior surface	B	*L. monocytogenes*	LC700426	98.79	CP065605.1

4.3.3 Distribution of Antimicrobial Resistance *Listeria* species among poultry abattoirs

Among the antimicrobial resistance of *Listeria* species. Clindamycin was the most common resistance phenotype from the poultry abattoirs A and B (*n*=14/23). The distribution based on clindamycin resistance was as follows; *L. monocytogenes* (*n*=10/23) isolated from abattoir A, *L. innocua* (*n*=1/23) isolated from abattoir B, and *L. welshimeri* (*n*=3/23) isolated from abattoir B. Resistance to tetracycline was also recorded in abattoir A (*n*=7/23) of which all isolates were *L. monocytogenes*. In addition, erythromycin resistance was also recorded (*n*=5/23) and distributed as follows; *L. monocytogenes* (*n*=3/23) isolated from abattoir A, and *L. welshimeri* (*n*=2/23) isolated from abattoir B. While vancomycin showed susceptibility on both the disc and Etest

methods, chloramphenicol, gentamicin, and levofloxacin only showed susceptibility on the Etest method Table 4.5.

Table 4.5. Antimicrobial Resistance of *Listeria* species isolated from raw dressed broilers (*n*=23).

	(Disc diffusion) *n*=23			**(Etest) *n*=23**		
Antibiotics	**R (%)**	**I (%)**	**S (%)**	**R (%)**	**I (%)**	**S (%)**
Penicillin G	8 (35)	14 (61)	1 (4)	1 (4)	2 (9)	20 (87)
Imipenem	-	-	-	0.0	14 (61)	9 (39)
Gentamicin	2 (9)	2 (9)	19 (82)	0	0	100
Levofloxacin	3 (13)	-	20 (87)	0	0	100
Trimethoprim/sulfamethoxazole	8 (33)	0	15 (65)	0.0	1 (4)	22 (96)
Clindamycin	13 (57)	2 (9)	8 (35)	14 (61)	0	9 (39)
Erythromycin	5 (22)	2 (9)	16 (70)	5 (22)	10 (42)	9 (38)
Vancomycin	-	-	-	0	0	100
Chloramphenicol	3 (13)	0	20 (87)	0	0	100
Tetracycline	13 (57)	3 (13)	7 (30)	7 (30)	1(4)	15 (65)

***(R) means resistance, (I) means intermediate, and (S) means susceptible**

4.4 Phase Three Results: Specific *Listeria* results under Tilapia Fish study

4.4.1 General Descriptors of *Listeria* in Tilapia fish, Siavonga district

Of the 150 tilapia samples collected from captured fisheries and aquaculture cage farms, 2.0% (3/150) were phenotypically indicative of *Listeria* species contamination. None of the presumptive *Listeria* isolates were *prs* positive. However, the positive control samples gave the expected 370 bp on the agarose gel, which secured reliability. The highest prevalence of presumptive *Listeria* isolates occurrence was found in samples from capture fisheries 2.7% (2/75) (95% CI: -1.048 - 6.453), aquaculture cage farms 1.3 % (1/75) (95% CI: 1. 284 – 3.916) (Table 4.5).

Table 4.6 Presumptive *Listeria* species results in tilapia fish from captured fisheries and aquaculture cage farms from Kariba, a large freshwater lake, Zambia (*n*=150)

Variable	Number of samples	Contaminated	Prevalence	95% CI
Part swabbed				
Gills	75	2	2.66	0.048 - 6.453
Exterior surface	75	1	1.33	0.284 – 3.916
Source of the tilapia				
Capture fisheries	75	2	2.66	0.048 - 6.453
Aquaculture cage-farms	75	1	1.33	0.284 – 3.916

4.5 Phase Four Results: Specific *Listeria* results for RTE, deli meats, and lettuce study

4.5.1 Specific *Listeria* species isolated from RTE foods

Listeria innocua emerged as the significant contaminant together with *L. monocytogenes* at 9.6% (5/52) and 11.5% (6/52) in RTE deli meats, followed by *L. ivanovii* at 7.6% (4/52). While lettuce *L. seeligeri* at 7.7% (4/52), *L. marthii* at 3.8% (2/52), and *L. monocytogenes* at 9% (1/52) (Table 4.7).

Table 4.7: Specific *Listeria* species were isolated from the RTE deli meats and Lettuce from retail markets

Retail markets	*Listeria* species	Number of isolates	Prevalence %
RTE deli meats (*n*=52)			
	L. monocytogenes	5	9.6
	L.innocua	6	11.5
	L. ivanovii	4	7.6
Lettuce (*n*=52)			
	L. marthii	2	3.8
	L.seeligeri	4	7.7
	L. monocytogenes	1	1.9

4.6 Proportionate results of combined samples on *Listeria* species

Combined samples collected from assorted food products yielded an overall positivity rate of *Listeria* species 16.4% (107/654) at 95% CI: 0.135 – 0.192. When specific food proportions of *Listeria* species were considered, the highest contamination was noted in samples from poultry 42.5% (51/150) (95% CI: 33.6 – 51.4), RTE deli meats 23.1% (12/52) (95% CI: 11.5 – 34.7), beef was third at 14.5 % (29/250) (95% CI: 0. 9.6 – 19.4), lettuce 13.5% (7/52) (95% CI: 4.1 – 22.8) and environmental swabs 10.0% (8/80) (95% CI: 3.4 – 16.6). *Listeria* species were not isolated from the fish swabs (**Table 4.8**).

Table 4.8. Proportionate *Listeria* species positivity rate across different sources

Variable	Number of samples	*Listeria* isolates	Prevalence %	95%CI
Type of sample				
Poultry samples	120	51	42.5	33.6 – 51.4
RTE deli meats	52	12	23.1	11.5 – 34.7
Beef samples	200	29	14.5	9.6 – 19.4
Lettuce	52	7	13.5	4.1 – 22.8
Environmental samples	80	8	10.0	3.4 – 16.6
Fish samples	150	0	0	-
Total	654	107	16.4	13.5 – 19.2
Source				
Poultry abattoirs	150	59	39.3	31.5 – 47.2
Beef abattoirs	250	29	14.5	7.6– 15.6
Fish Plants	150	0	0	-
Retail markets	104	18	17.3	10.8 – 25.7
Location				
Lusaka	150	59	39.3	31.5 – 47.2
Namwala	250	29	14.5	7.6 – 15.6
Siavonga	150	0	-	-
Retail markets	104	18	17.3	10.8 – 25.7

4.7 Overall distribution of Antimicrobial Resistance specific *Listeria* species coming from different sources

Among the antimicrobial resistance of *Listeria* species from food sources and environmental samples, the most common resistance phenotype was clindamycin (*n*=17/107). Distributed as follows: *L. monocytogenes* (*n*=12/107) isolated from (6) poultry, (3) from RTE deli meats, (2) from beef samples, and one isolate came from environmental samples; *L. innocua* (*n*=5/107), (3) isolated from poultry samples and (2) from RTE deli meats. In Tetracycline (*n*=14 /107), the distribution was as follows: *L. monocytogenes* (*n*=10/107) (6) isolated from poultry, (3) from beef samples, and one was from RTE deli meats; *L .innocua* (*n*=3/107) all isolates came from poultry and *L. ivanovii* recorded only one isolate that came from poultry. Erythromycin resistance was also recorded (*n*=6/107); *L. innocua* (*n*=5/107) was all from poultry, and *L. ivanovii* (*n*=1/107) was only recorded in one isolate, which also came from poultry. Penicillin G (12/107) distributed as follows: *L. innocua* (6/107); (4) isolates came from poultry and (2) isolates came from RTE deli meats; *L. monocytogenes* (*n*=4/107); (3) isolated from RTE deli meats and one isolate came from lettuce samples; *L. ivanovii* and *L. seeligeri* each had one isolate sampled from poultry and lettuce respectively. Trimethoprim/sulfamethoxazole (*n*=8/107) distributed as follows: *L. monocytogenes* (*n*=3/107); (2) from RTE deli meats, and one came from beef samples, *L. innocua* (*n*=3/107); (2) samples were isolated from poultry samples and one came from RTE deli meats. *Listeria seeligeri* (*n*=2/107) one sample was isolated from poultry and the other from lettuce. Vancomycin was observed to be susceptible on both the disc and Etest methods. Gentamicin, levofloxacin, and chloramphenicol only showed susceptibility to the Etest method **(Table 4.9).**

Table 4.9. Antimicrobial Resistance specific *Listeria* species coming from food and environmental sources

	(Disc diffusion) *n*=107			(Etest) *n*=107		
Antibiotics	**R (%)**	**I (%)**	**S (%)**	**R (%)**	**I (%)**	**S (%)**
Penicillin G	12 (11.2)	28 (26.2)	67 (62.6)	1 (0.9)	16 (14.9)	90 (84.1)
Imipenem	-	-	-	0.0	14 (13.1)	93 (86.9)
Gentamicin	2 (1.9)	2 (1.9)	103 (96.3)	00	00	107 (100.0)
Levofloxacin	3 (2.8)	-	104 (97.2)	00	00	107 (100.0)
Trimethoprim/sulfamethoxazole	8 (7.5)	00	99 (92.5)	3 (2.8)	1 (0.9)	103 (96.3)
Clindamycin	17 (15.9)	7 (6.5)	83 (77.6)	14 (13.1)	00	93 (86.9)
Erythromycin	6 (5.6)	4 (3.7)	97 (90.7)	5 (4.7)	12 (11.2)	90 (84.1)
Vancomycin	-	-	-	00	00	107 (100.0)
Chloramphenicol	3 (2.8)	00	104 (97.2)	00	00	107 (100.0)
Tetracycline	14 (13.1)	7 (6.5)	86 (80.4)	7 (6.5)	1(0.9)	99 (92.5)

***(R) means resistance, (I) means intermediate, and (S) means susceptible**

4.8 Molecular characterization of isolated *Listeria* species

4.8.1 Pre-screening PCR amplification of *prs* gene for *Listeria* species identification

Deoxyribonucleic was extracted from 112 suspected *Listeria* species isolates. An expected 370bp PCR product amplified in some strains tested. The negative control used was nuclease-free water. After running the *prs* gene, only 107 isolates came out as *Listeria* species.

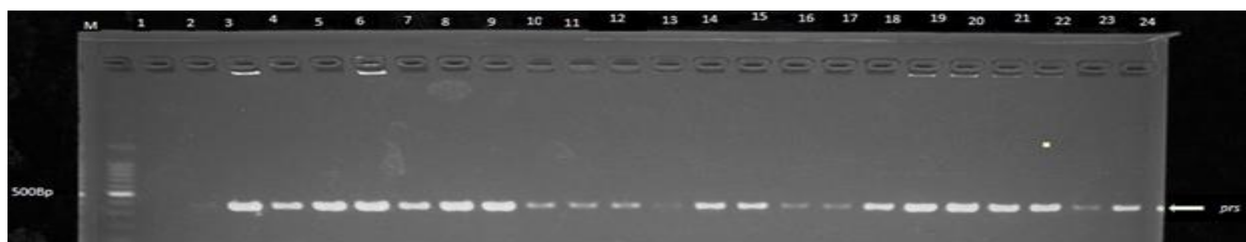


Fig. 4.2 Agarose gel electrophoresis of DNA fragments by PCR.

Fig.4.2. Shows an example of some strains that amplified the *prs* gene fragment (370bp). Lane 1 is a negative control, and Lane, 2 to 24 *Listeria* species genes corresponding to amplified fragments, is indicated on the right. Lane M, Promega, Benchtop 100 bp DNA Ladder. On the left, *Listeria* species molecular sizes are given (in base pairs).

4.9 Whole Genome Sequence results of *Listeria* isolates from Beef, poultry, RTE deli meats, and lettuce vegetables

4.9.1 Phylogenetic analysis

A total of 42 from 107 isolates identified previously by MALDI-TOF MS See **appendix 14** were subjected to WGS analysis. The selection included all isolates identified as *L. monocytogenes* and other species which showed antibiotic resistance phenotypically. Phylogenetic analysis of the WGS as well as the reference sequences showed the presence of five clusters, namely A1, A2, B, C, and D, based on *Listeria* species types (Fig.4.3). Cluster A1 comprised only *L. monocytogenes* reference sequences and study sequences (*n*=24) collected from chicken samples (*n*=10), beef (*n*= 6) and RTE deli meats (*n*=4), storage environment (*n*=2) and lettuce vegetables (*n*=1). The majority isolates came from poultry samples, followed by beef samples. On the other hand, cluster A2 consisted only of study sequences that did not cluster according to specific *Listeria* types because the majority of the *Listeria* species that were isolated in this study were represented, namely:, *L. ivanovii* (*n*=1), *L. monocytogenes* (*n*=4) and *L. innocua* (*n*=10) which had the most significant number. All sample sources were represented except for samples obtained from lettuce vegetable. Cluster B consisted of only reference sequences of *L. monocytogenes* isolated from various food and human sample sources. Cluster C only consisted of study sequences belonging to *L. seeligeri* obtained from lettuce vegetables only. While cluster

D excessively consisted of reference sequences, including an out-group category. Overall, phylogenetic analysis revealed that sequences collected from all samples in this study were represented in all clusters except in cluster C, where only sequences obtained from lettuce had representation. Furthermore, *L. ivanovii* and *L. seeligeri* were only represented in specific food samples of poultry and lettuce. However, clustering according to the source of samples or food types was not observed.

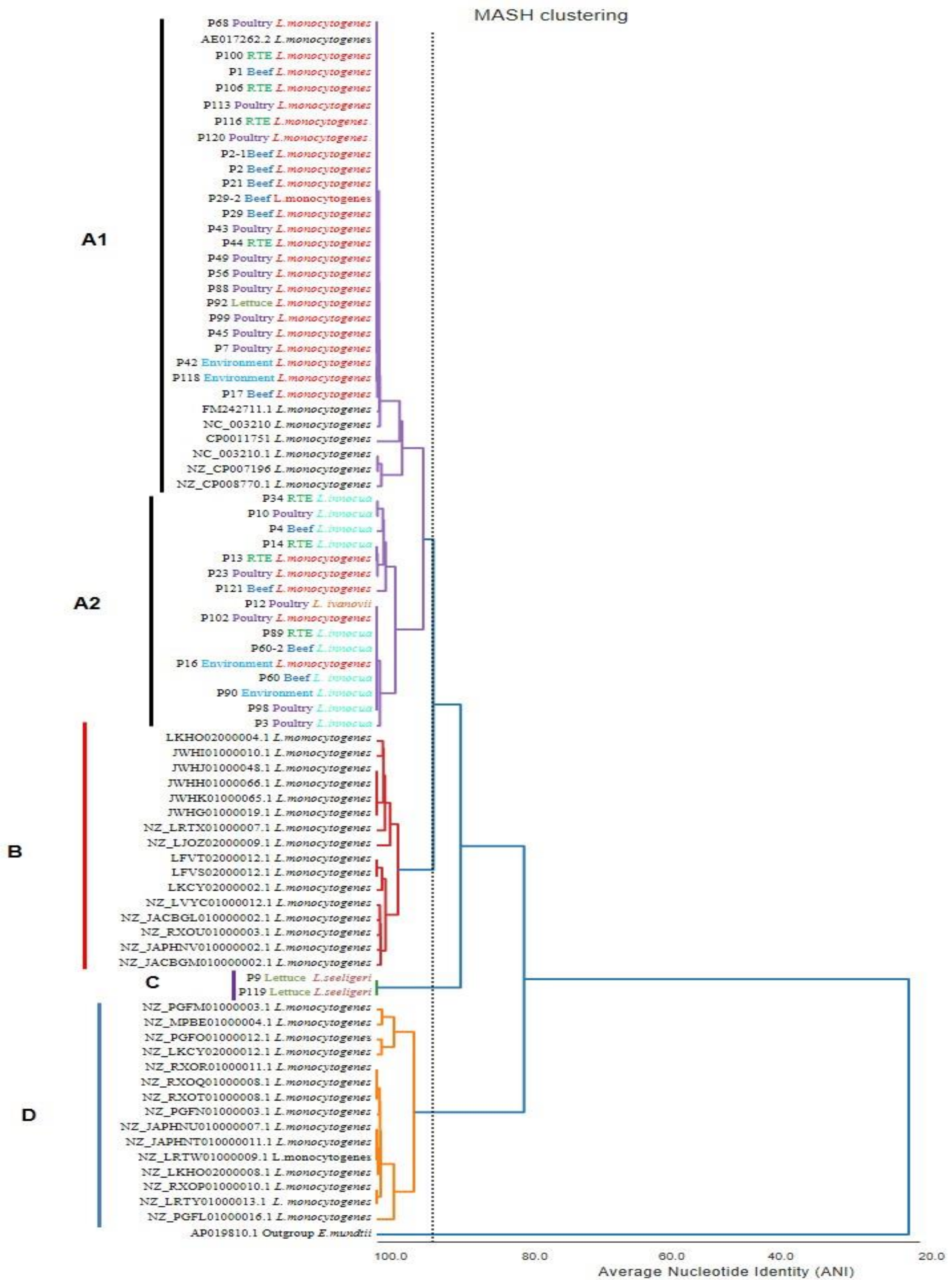


Fig. 4.3 The phylogenetic tree of WGS

Fig. 4.3 the phylogenetic tree consisting of 81 sequences (42 obtained in the study and 39 downloaded sequences from (GenBank). Genome comparison of sequences understudy and those downloaded from genbank in a pairwise manner was achieved using dRep. This allowed the identification of sequences with similar DNA content with regards to the Average Nucleotide Identity. The colour codes represent the sample source (e.g, poultry), and species of *Listeria* of the study sequences, and the number at the beginning indicates the sequence identification code.

4.9.2 Resistance gene identification

Out of the 42 isolates analyzed using WGS, 8 expressed resistant genes.Of which 7 expressed *Tet(M)* (resistance to tetracycline) resistant gene distributed as follows: 2 isolates belonged *to L. innocua,* and 5 were *L. monocytogenes*. In comparison, only one isolate expressed *Tet(L)* (resistance to tetracycline), *dfrE* (resistance to trimethoprim), *erm(B)* (macrolide), and *lsa(A)* (resistance to lincosamide, streptogramin). All isolates harbored *FosX* (resistance to fosfomycin) and *lmo0919*_fam (resistance to lincosamides) to which all *Listeria* species are intrinsically resistant.

CHAPTER FIVE

DISCUSSION

This study aimed to evaluate the public health importance and antibiotic resistance patterns of *Listeria* species in Zambia as they relate to food and food processing environments. Overall, this study has elucidated the significance and public health importance of *Listeria* species in Zambia across different food sources and environments. Additionally, it has profiled the antibiotic resistance patterns of *Listeria* species of clinical, environmental, and general significance for the first time. To our awareness, this study is also the first in Zambia to evaluate this pathogen's public health significance precisely. One of the significant findings is the isolation of *L. monocytogenes* across all food sources (encompassing food of animal as well as plant origin). Genetic relatedness was observed among *Listeria* strains of the same species, which clustered together, including closely related ones, as was the case with *L. monocytogenes* and *L. innocua.* Other studies have equally exemplified the close relatedness between *L. monocytogenes* and *L. innocua* (Doumith et al., 2004, Milillo et al., 2012). This study reveals overlapping or shared environment for individual species, which is consistent with a previous study in other pathogenic species (Brown et al., 2003). Concurrently, the clusters in the present study were observed to be discriminated according to specie type regardless of the source or food type. However, results different from the finding of this study reported *Listeria* species to have clustered according to different sources (Sauders et al., 2012). They attributed their findings to having been influenced by the ecological preferences of some types of *Listeria* species (Sauders et al., 2012). A total of five genes conferring resistance were recorded as follows: *tet(M), tet(L), dfrE*, *erm(B),* and *lsa(A).* Most of the resistance genes from this study came from poultry samples, and results similar to these findings were equally recorded (Hanes et al., 2022). This current study equally detected intrinsic resistant genes, namely, *fosX* and *lmo0919.* The isolation of *fosX* was also

observed in a study by (Hanes et al., 2022), of which it was reported to be among the commonly resistant genes together with *tet(M).*

The genus *Listeria* currently consists of 17 recognized species, and only two of these, *L. monocytogenes* and *L. ivanovii,* are considered pathogenic (Scallan et al., 2011). In this particular study, out of the 17 species, we isolated five *Listeria* species across the sampled sites, namely: *L. innocua* (66.4%*), L. monocytogenes* (21.5%), *L. ivanovii* (5.6%), *L. marthii* (1.9%), and *L. seeligeri* (4.7%). The results obtained from this study are comparable to other similar studies conducted by others (Jalali and Abedi, 2008, Vallim et al., 2015). In the above two studies, *L. innocua* was equally reported to be the significant contaminant in foods and food processing environments; the results are congruent with our present findings. The likely reason for the high isolation rate of *L. innocua* in most foods and food processing environments may partly be attributed to either its ubiquitous nature compared to other subspecies as well as the probability of higher propensity of establishment, more than others (Korsak et al., 2012, Jadhav et al., 2013). On a similar subject area, other workers reported that *L. innocua* occupies the same ecological space as *L. monocytogenes;* however, it has been reported to have a higher incidence than *L. monocytogenes* (Scotter et al., 2001, Zitz et al., 2011). Unfortunately, these workers were not conclusive regarding elaborating the reasons for their observations. Furthermore, *L. monocytogenes* during the stages of selective enrichment is overshadowed by the growth of *L. innocua* (Scotter et al., 2001). Other findings indicate that isolation of *L. innocua* is more linked to specific food types (Milillo et al., 2012, Vallim et al., 2015). Similarly, in this particular study, there was no *L. innocua* isolated from lettuce. Vallim and co-workers established that *L. innocua* was less likely to be isolated in unprocessed meat products than in processed ones (Vallim et al., 2015). The assertion by Vallim and co-workers was at variance with the findings from this present study

because *L. innocua* was isolated more in the unprocessed food samples than in the processed ones. The sampling process and distribution could partly explain the differences in this particular study; more samples were derived from unprocessed beef and poultry. Other authors have equally elaborated on the persistence of *L. innocua* in various foods and processing environments (Korsak et al., 2012, Jadhav et al., 2013). The findings of lower levels and distribution of other *Listeria* species, such as *L. marthii* and *L. seeligeri*, agree with the earlier author's findings (Gebretsadik et al., 2011, Simmons et al., 2014). In our present findings, *L. seeligeri* was only isolated in lettuce vegetables. This finding of *L. seeligeri* in lettuce is exemplified by MacGowan and co-workers, who reported that this type of *Listeria* species is found in soil (MacGowan et al., 1994). On the other hand, *L. ivanovii* though not very common, has been associated with human cases (Guillet et al., 2010), with its finding in foods being more associated or linked to human contamination.

Antimicrobial resistance reports in *Listeria* species, including *L. monocytogenes*, differ from when the first report of antibiotic-resistant isolates was discovered (Bockserman, 2000). In this current study, clindamycin, antimicrobial resistance, emerged with the highest resistance 15.9 %, and similar findings were also reported by (Poyart-Salmeron et al., 1990, Kanarat et al., 2011). Most importantly, *L. monocytogenes* showed the highest resistance to clindamycin (11.2%) and tetracycline (9.3%). Further, from the results of this present study, there is an indication that resistance was not clustered according to food type or surface swabbed but randomly distributed. The results above have revealed a considerable level of resistance to clindamycin, tetracycline, and erythromycin, which are antimicrobial agents classified among the most important in veterinary medicine, especially tetracycline (Skovgaard and Morgen, 1988). From the results, *L. monocytogenes* remains susceptible to the first line treatment of listeriosis, according to the

prescription of CLSI guidelines (Sader et al., 2020). However, limited studies have recorded high levels of *L. monocytogenes* resistance to clindamycin, especially from food isolates (Bankole et al., 2013). According to the study by Bockserman (2000), increased clindamycin resistance was seen to influence induction with erythromycin (Bockserman, 2000). Clindamycin mechanism activity is like erythromycin, especially since they share their binding site (Escolar et al., 2017). The clindamycin resistance reported in this current study could partly relate to enzyme in-activation due to possible cross-resistance of the isolation of resistant erythromycin isolates. Tetracycline had the second-highest resistance, with most isolates belonging to *L. monocytogenes*. Other authors have reported similar results to the ones recorded in this study (Srinivasan et al., 2005, Fallah et al., 2012). A systematic review conducted in RTE foods reported tetracycline as the common antibiotic intrinsically resistant to *L. monocytogenes* classified in the third-generation antibiotics (Mpundu et al., 2021a). Additionally, tetracycline is among the most frequently reported resistance phenotype, especially in *L. monocytogenes* sampled from different sources (Poyart-Salmeron et al., 1990, Charpentier and Courvalin, 1999). Although, the major factors that might be contributing to enhanced antibiotic resistance could be complex and may point to many existing lapses. The augmented resistance of tetracycline recorded in this study can be attributed to extensive animal feed additives and veterinary treatment (Gómez et al., 2014). The need to produce more directly affects the increased use of antibiotics such as tetracycline, especially in foods like poultry (Fakhri et al., 2019), which is the cheapest source of protein (Amoako et al., 2020). This was comparable to the results recorded from beef samples from this study which had a relatively lower antimicrobial resistance. Inadequate regulation of antibiotic use, particularly in poultry, can partly influence the abuse of certain drugs (Fakhri et al., 2019, Mudenda et al., 2022). The other factor links the farm to folk concept that should ensure the prudent use of antibiotics in animal foods (Munita and Arias,

2016). Erythromycin resistance was also recorded in this present study, results concurrent to our finding by Escolar and others reported erythromycin resistance in *Listeria* species (Escolar et al., 2017). The isolation of erythromycin-resistant isolates in this study could have partly been influenced by the higher clindamycin resistance recorded due to the possibility of inducible resistance. Equally *L. innocua* recorded considerable resistance in most antibiotics including clindamycin, which indicates the possible specie differences concerning antibiotic resistance acquisition rates. Other studies also recorded variations in antibiotic resistance in different *Listeria* species, especially in *L. monocytogenes* and *L. innocua* (Franco Abuin et al., 1994, Rota et al., 1996, Gómez et al., 2014). In this study, there were considerable variations in antibiotic resistance according to species. Correspondingly, most studies that have reported specie-dependent differences to antibiotic resistance have been on a case by case issue (Espaze and Reynaud, 1988, MacGowan et al., 1994). On the other hand, Troxler and others reported a different perspective of other factors that can influence differences in observed antibiotic resistance patterns on *Listeria* species, not just specie related (Troxler et al., 2000). Therefore, from this study, at phenotypic level, it can be concluded that the antimicrobials with the highest resistance were clindamycin 15.9%, tetracycline 13.1%, penicillin G 11.2%, trimethoprim / sulfamethoxazole 7.5% and erythromycin 5.6 %.

Beef carcasses were recorded to have multiple *Listeria* species contamination. The sampling of beef carcasses in this study was in various abattoirs having different environmental and human factors. Typically, *L. monocytogenes* is a frequent contaminant of raw foods; studies have shown that food premises that lack adequate environmental management are more likely to have contaminated products than abattoirs that have (Bakhtiary et al., 2016). More robust information on *L. monocytogenes* in various food processing environments and approaches for control have

been provided (Kornacki and Gurtler, 2007). Furthermore, control measures of *Listeria* in food processing environments are highlighted in a study by (Tompkin, 2002). Food handlers are also key players in influencing the contamination of a specific food premise because of the high correlation between food safety training and contamination reports (Al-Kandari et al., 2019). Research studies have revealed the role that food safety training plays in most food premises (Medeiros et al., 2011; Sani and Siow, 2014). The other factor that could have contributed to beef carcass contamination was increased process throughput per day, and this may negatively impact the maintenance of hygienic practices (Hichaambwa, 2012). Beef abattoirs contributing to significant contamination levels were associated with high through-puts daily. Lapses introduced due to high throughput might lead to the introduction of *Listeria* in the food chain if domestic animals are exposed to the organism, resulting in long-term persistent contamination (Haileselassie et al., 2013). Beef, like poultry, is a good source of *Listeria* species because it supports the growth and survival of several microbes (Mpundu et al., 2021a).

Raw beef and poultry contamination is of public health concern when raw or undercooked. Equally, part-swabbed in beef samples was significantly associated to contamination of *Listeria* species, with the interior part recording (17.0%). Contrary to the current finding of this study, no substantial *Listeria* contamination with the parts swabbed was recorded by (Eruteya et al., 2014). Inconsistencies recorded in the two studies could also be linked to the beef samples from Eruteya and contemporaries who tested cut pieces at the market. Unvarying dissemination may signify the presence of bacteria across the beef meat, unlike the carcasses sampled from the abattoirs. Some portions, like external parts, were less exposed to cutting than internal parts. The association is attributable to the handling and use of unsterilized knives or equipment from one carcass to another. As earlier communicated, the possibility of intestinal contaminants may support the

spreading through the internal part. The beef carcasses investigated in this study were sourced from various abattoirs farms with unfamiliar risk limitations that we may not explain due to a lack of auxiliary facts. In abattoirs where observance to good hygienic practices lacks the likelihood of cross-contamination may occur through various utensils used in the abattoirs. Other studies have expounded the role of knives used for evisceration and cutting carcasses to be among the significant drivers of cross-contamination (Fasanmi et al., 2018, Wambui et al., 2018, Wardhana, 2019). Meanwhile, the lack of *Listeria* species isolation from beef abattoir storage environments could have partly been influenced by the temporary storage of carcasses from these abattoirs after slaughter compared to poultry abattoirs. The carcasses from these abattoirs were only kept in cold rooms for a maximum of two days and were later dispatched to their final destination. To a greater extent, this could have facilitated adequate cleaning and disinfection, which would not be possible if carcasses had been kept longer in the abattoir (Matthews et al., 2017). Results contrary to the finding of this study were reported by Kells and co-workers (2004), who isolated *Listeria* species on environmental samples collected from beef (Kells and Gilmour, 2004). These discrepancies in isolation can probably be attributable to the differences in the sampled environment concerning the storage system of carcasses and the period of storage in the two studies. The other difference can be linked to the sanitary conditions of specific abattoir facilities because *Listeria* is known to be persistent in such environments by forming biofilms (McDermott et al., 2002). This current study has also revealed that carcass contamination does not always mean environmental contamination, such as cold- rooms.

Poultry emerged as a significant food contaminated with *Listeria* species. Results are comparable to several other studies (Alonso-Hernando et al., 2012, González et al., 2013, Archer, 2018). Also more studies reported contamination of poultry at 16% to 58% (Siriken et al., 2014, Saludes et

al., 2015). Differences in isolation variations may be due to slaughter processes since the sampled chickens in this study did not come from the same abattoir as beef samples. Poultry samples reported in this study came from different abattoirs with different environmental factors. Some abattoirs slaughtered large numbers of poultry, which may introduce compromised standards due to fecal spillage on the surface of the meat. Leading to an increased probability of introducing *Listeria* into the food chain if any slaughtered poultry had prior exposure to the organism resulting in long-term persistent infections (Ferreira et al., 2014a). Therefore, partly influencing the positivity. The introduction of *L. monocytogenes* into meat products is possible during processing, and depending on the hygienic measures by food handlers, cross-contamination may occur (Zhu et al., 2012, Ristori et al., 2014). Additionally, most chickens sampled in this study were kept under refrigeration, allowing *L. monocytogenes* to multiply (Zhu et al., 2012). Equally, abattoirs that contributed to the higher isolation of *Listeria* species in this study were characterized by inadequate cleaning of the environment and utensils such as knives. *Listeria* species can form biofilms at refrigeration temperatures, thus contributing to the persistence and dissemination of the pathogen (Ristori et al., 2014). In another study of fresh chicken carcasses purchased in retail stores, out of 100 chicken samples collected, 95% of these samples had *Listeria* species (Capita et al., 2001). Prevalence contamination was higher than what has been reported in this study. The differences in isolation between the two studies can partly be attributed to the isolation method used. Capita and others used cultural methods to isolate *Listeria* species, while in this current study, molecular methods were used. Molecular methods were utilized to target the DNA because of their high accuracy and help to reflect genetic relationships between isolates (Gasanov et al., 2005). This is in comparison to cultural methods, if used singularly, which may be surrounded by false positive results even if they are considered the gold standard (Allerberger, 2003, Gasanov et al., 2005). The other thing that could be attributed to the

differences in the analyzed samples was that Capita and others collected the skin carcasses where 25g was harmonized together in the *Listeria*-specific broth (Capita et al., 2001). This method increases the surface area of the part being considered for bacteria isolation which may increase the chances of bacteria isolation if it is present (Gasanov et al., 2005). While in this current study, poultry swabs were collected by swabbing a 5cm^2 x 5cm^2 surface area using a swab stick. Although this method may be reliable may sometimes yield negative results if the swabbing is not done properly (Huang et al., 2016). The other difference is linked to point of sampling; the current study sampled chickens in the abattoirs while Capita and others collected samples from retail shops. Retail samples are more likely to be exposed to several post-processing contaminants through cross-contamination. Mainly, the quality of the final product is influenced by the source of the sample from primary to secondary processes (Mpundu et al., 2019). Osaili and others reported 33% of *Listeria* species in RTE poultry products, which to some extent can clearly indicate the link that exist between the eminence of the raw food and its finished product such as the RTE foods (Osaili et al., 2011). Poultry carcass yielded different results with regards to part swabbed, whether it was Cloacal or exterior surface; when specific part swabbed was considered, a higher prevalence of *L. monocytogenes* was obtained from exterior swabs (60.0 %), indicating higher carcass contamination, possibly because of prior exposure of the domestic animal to *Listeria* (Haileselassie et al., 2013). Further, contamination could have arisen from unwholesome contacts of carcasses with excretions cumulating from the food handler's skin, mouth, and nose on the processing line (Okonko et al., 2008, Omoruyi et al., 2011). *Listeria* contamination is suspected to result from cross-contamination of raw processed chicken by inadequately cleaned and disinfected processing environments and, to a lesser probability, from the live chickens. This is because the cloacal revealed lower *Listeria* contamination by 25 %; this, to some extent, reveals the actual status of the source of contamination. The findings of this study

concur with similar findings by (Cox et al., 1997) and Kanarate and others in (2011), who reported processing as a significant cross-contamination hazard in other studies (Kanarat et al., 2011, Mpundu et al., 2019). Results different from our findings were reported by Ishola and others, who recorded a higher incidence of 65% on Cloacal swabs than other parts (Ishola et al., 2016). They attributed poultry contaminants to be the reason why the increased prevalence of contamination with *Listeria* was observed. These findings were similar to those (Gravani and safety., 1999) and (Schlech III et al., 1983), stating that *Listeria* species in soil, water, and hay, including bird guts, as the usual habitat for *L. monocytogenes* (Bockserman, 2000), including animal feces (Skovgaard and Morgen, 1988, Bankole et al., 2013). On the other hand, most poultry storage was in cold rooms already packaged without direct contact with the storage environment. This probably minimized cross-contamination from poultry to the cold-room environment or vice versa, affecting the isolation rate. The swabbed environment and food can also influence the isolation of the *Listeria* species. Results similar to the finding of this study revealed a significant difference in isolation of *Listeria* species on environmental swabs regarding the type of food processed (Pritchard et al., 1995). Storage poultry environments yielded (10.0%) *Listeria* species contamination. The reasons for lower isolation in this study can partly be due to the sample size allocated to storage environments. The positive samples were only recorded in poultry abattoirs and none from beef abattoirs. The finding of positive samples in a poultry cold-room environment can be attributable to the poultry-dressed chickens storing system. Most of the poultry was being stored for a long period depending on the supply-demand; this could have contributed to the recording of the current results of *Listeria* contamination.

More importantly, no *Listeria* species were recorded from tilapia fish sampled from (aquaculture cage farms and capture fisheries) in the Siavonga District of Zambia. Samples collected from this

study were exterior surface skin and gills. A similar study in India showed that *Listeria* species surface swabs had (72.4%) of tropical fish and fishing environments (Jeyasekaran et al., 1996). The significant difference is deduced from the differences in the sampled fish; Jeyasekaran and others collected sample swabs from fish markets and processing units. Onjong and others conducted a study on the microbiological safety of fresh tilapia in value chains, and a 2% prevalence of *L. monocytogenes* sampled at landing was recorded (Onjong et al., 2018). The differences in isolation with this present study deduction are from the sampled tilapia fish characteristics and techniques used in the isolation method. This present study utilized molecular methods to confirm the *Listeria* genus. At the same time, Onjong and co-workers used VITEK 2 system version 0.8.01 biomerieux, Inc. Hazelwood, MO. These two methods may yield different results due to differences in sensitivity (Martins et al., 2018). Further, this study only collected fish samples at the landing stage without multiple processes. Human activities occurring near sampled water bodies may thus influence the level of *Listeria* contamination in the fish. In the Siavonga district, cattle and crop farming are rare, and most people solely depend on fishing for a living. The none-detection of *Listeria* contamination on PCR observed in the present study could also be explained because sampling was at the primary production stage. Furthermore, sampling was from a single lake, where environmental factors are assumed to be similar, previous studies reporting a higher prevalence of *Listeria* species mainly sampled fish from different water bodies (Jami et al., 2014). Climatic conditions, mainly rainfall, contribute to bacterial contamination of surface water. The collection of samples was during the rainy season, when the probability of isolating *Listeria* species is usually higher than during the dry season, as reported in other studies. However, Hansen and others (2006) failed to find significant seasonal variations of *L. monocytogenes* levels in water from freshwater fish farms (Hansen et al., 2006) .

The other result reported in this study were the isolation of *Listeria* species in RTE deli meats, accounting for 23.1 %. Literature has shown that foodborne disease control programs have identified RTE deli meats as significant sources of global outbreaks that put humans at risk (Gottlieb et al., 2006). Even in the most significant outbreak in SA, the primary foods involved were deli meats (Sibisi, 2019). The isolation of *Listeria* species in RTE deli meats is of public health concern, especially if the isolated strains are pathogenic (Rodriguez and McLandsborough, 2007, Paudyal et al., 2017). This is because these foods do not undergo any heating step before consumption, and if the food is contaminated, the consumer will most likely eat the viable pathogens. In the last decades, consumption of RTE deli meats has increased due to their selling convenience (Tooby et al., 2021). More importantly, in this study, most of the *Listeria* species, including *L. monocytogenes,* came from the deli meats sliced at the retail markets compared to those packaged by the producers. Cross-contamination can happen if pathogens are present on the meat's surface or on processing tools item such as deli slicers introducing pathogens to the surface of the meat when it is being sliced (Kurpas et al., 2018). In addition, further processing and handling of meat, such as cutting and coating with spices, increase the risk of contamination with *Listeria* species (Karakolev, 2009). The introduction of *Listeria* on food is done at primary processing stages such as slaughter and later passed to RTE foods. Comparatively, a study conducted on RTE meat products reported an overall 37.0% *Listeria* species results higher than what this study reported. *L. innocua* was the major contaminant, 32.0 %, and *L. monocytogenes* accounted for 3.0 % (Calvo-Arrieta et al., 2021). They attributed the higher isolation of *Listeria* species to be associated with handling practices within the retail shops. Equally, types of equipment, such as cutting boards and the type of material may influence bacterial contamination (Calvo-Arrieta et al., 2021). According to their results, wood-made cutting boards were less likely to be contaminated than plastic ones (Calvo-Arrieta et al., 2021). The differences in

contamination with this current study can be deduced from the differences in sample size Calvo-Arrieta and others had a bigger sample size than this current study. Partly this increased the probability of high isolation rates. The sample size is one of the tools that help the findings to be reliable and more generalizable (Singh and Masuku, 2013). The other factor is related to the point of sampling; this current study only sampled retail markets with perquisite standards for maintaining good hygienic practices. Most retail markets get their supplies of deli meats from reputable producers with prerequisite control systems. While Calvo-Arrieta and colleagues collected samples from small butcheries and public markets. The characteristics of deli meat samples took an upper prospect of *Listeria* contamination because of practices attributed to sampled environments. As reported by Calvo-Arrieta and others , the deli meats were being cut at the point of sale using knives without prior disinfection before the next cut was done (Calvo-Arrieta et al., 2021). Further, authors indicated that the same knife was being used to cut across different types of meat products. In this current study we sampled sliced deli meats which were repackaged and displayed for sale. The differences in the characteristics of the samples can help explain the isolation rates observed. Majority of food contaminations occur at the retail plants and less likely from the source of food production. Therefore, there is need to ensure the safeness of the food to reduce exposure to the consumers.

Contamination of *Listeria* was also recorded in lettuce vegetables accounting for 13.5% of the total allocated sample size. Similar results of the isolation of *Listeria* in lettuce vegetables are also reported in other studies (Kyere et al., 2019, Kyere et al., 2020, Balali et al., 2020). *Listeria* contamination in lettuce in this study can partly be attributed to retailer's inadequate enforcement of standard quality control systems. Isolation of *Listeria* species like *L. seeligeri* and *L. marthii* is rare in food and food processing environments. Additionally, most of the lettuce vegetables we

sampled did not undergo further processing and was sold as supplied by the suppliers. Therefore, the contamination may be traced back to the environmental factors where the lettuce was processed and produced. Other studies concur with the possible link between the contaminations on the final product and environmental sources (Toussaint et al., 2019, Mpundu et al., 2021b). Most retail markets have prerequisite standards for food suppliers to meet before supplying to maintain food quality from primary to secondary production. However, when retail owners do not maintain quality control checks, lapses come in. MacGowan and others (1994) reported *L.seeligeri* as a major *Listeria* species found in the soil (MacGowan et al., 1994). *Listeria monocytogenes* contamination in fresh vegetables has contributed to foodborne illness (CDC, 2017, NSW, 2018, Self et al., 2019). Another report involving the biggest salad producer in New Zealand where all the produced lettuce in March 2017 had *Listeria* contamination (Zhu et al., 2017). In a study done in Zambia by Nguz and co-workers (2005), a recorded prevalence of 20% of *L. monocytogenes* in freshly cut mixed vegetables and green beans for export records (Nguz et al., 2005). To some extent, inconsistency in prevalence with this present study may relate to differences in the identification methods. Nguz et al. (2005) and co-workers only utilized distinct selective agars (PALCAM and OXFORD agars) without any definitive molecular methods (Nguz et al., 2005) . In this current study, analysis was with molecular techniques. Vegetables are usually less contaminated than meat products (Zhu et al., 2017, Mpundu et al., 2021a). However, *Listeria* species are ubiquitous and can contaminate a broader range of food sources, including vegetables (Ikeh et al., 2010). Lettuce is primarily available throughout the year in Zambia due to staggered irrigation production. Most farmers get their water from wetlands/streams, which can also serve as sources of contamination for the lettuce (Ngoma et al., 2019). Furthermore, farming practices like human compost and animal dung can also influence the presence of *Listeria* species in vegetables like lettuce (Oliveira et al., 2011). Nwachukwu and colleagues reported evidence

of contamination in water used for irrigation, and the occurrence of *L. monocytogenes* in the two lakes in Abia State was 92% and 79% each (Nwachukwu et al., 2010). Equally, odjadjare and others reported the abundance of human pathogens and *Listeria* species in wastewater used for irrigation in SA. Lakes and other water bodies are primary water sources for irrigation, and contaminants can quickly transfer to the vegetables (Odjadjare et al., 2011). *Listeria* species, including *L. monocytogenes*, have been isolated from animal dung and poultry droppings (David and Odeyemi, 2007, Umeh and Okpokwasili, 2009). Conversely, these serve as a significant component of manure applied to the soil to improve the yield of agricultural produce. The public health concern is that lettuce is consumed raw, a leafy vegetable that may allow bacteria within the leaves, especially if inadequately washed. Also, contaminated vegetables such as lettuce may serve as reservoirs for *Listeria* in salad vegetables as reported from the results conducted by (Ieren et al., 2013).

Bacterial identification by WGS is based on sequence identity and alignment of the rRNA gene, specific to the species level (Ranjan et al., 2016). On the other hand, traditional culture and biochemical rely on the phenotypic identification of tested bacteria via bacterial growth on selective media and bacteria metabolism of various nutrients (Lagier et al., 2015). From time immemorial this approach has been the gold standard in diagnostic microbiology and undoubtedly resolved the identification of bacterial pathogens (Chao et al., 2006, El-Shenawy et al., 2011). One major disadvantage is that biochemical results could be arbitrary and operator-dependent, especially if not cultured in correct conditions; results of culture will be unspecified, failing to identify the infecting pathogens (Salipante et al., 2013, Cummings et al., 2016). However, this is not the case with WGS. MALDI-TOF MS is another culture method used for quick identification of bacteria, including *Listeria* species which uses analysis of the whole cell

proteomes of microbes in the mass range of 2-20KDa. In the case of *Listeria,* timely information especially in the food industry is cardinal to avoid product recalls or delays in product release. MALDI-TOF MS has a significant advantage of rapid analysis; it represents an attractive universal detection and subtyping tool for multiple pathogens. Other studies have equally explored the identification of *L. monocytogenes* using MALDI-TOF MS (Barbuddhe et al., 2008, Hsueh et al., 2014). Although MALDI-TOF MS is associated with less waiting time, it may not be highly discriminatory compared to WGS. Molecular methods using the *prs,* a general marker gene in all *Listeria* species, are also used to determine the genus (Nightingale et al., 2005). Other writers have also used *prs* to screen the occurrence of *Listeria* species because it is known as the housekeeping gene (Nightingale et al., 2005, Graves and Swaminathan, 2001). This method has high discriminatory power but cannot identify the stains of *Listeria*, making it less useful in outbreak tracking. However acts as an indicator of *Listeria* species in the given samples (Nightingale et al., 2005, Doumith et al., 2004). For most samples included in this study, *Listeria* species could be identified by culture 17.1% (112/653). Overall, there were 107 samples where culture and molecular methods were in agreement. The agreement is attributable to the greater diversity found by the molecular methods vs. culture (Gasanov et al., 2005). This is also consistent with similar comparative studies of other microorganisms evaluating microbial detection methods (Frece et al., 2010, Maktabi et al., 2015). In the remaining five samples, the disagreement between culture and molecular methods could have been caused by the misidentification of samples at the culture level. Also, the presence of live-injured bacterial cells could not grow properly on culture. Three of these samples came from fish, and two came from lettuce vegetables samples. MALDI-TOF MS was used to identify specific *Listeria* species. Whole Genome Sequencing was also performed to confirm specific *Listeria* isolates and genes conferring resistance from the phenotypic results. The classification of *Listeria* species from

MALDI-TOF MS differed with WGS as most of the previously identified isolates were reclassified. As mentioned earlier, MALDI-TOF MS may lack specificity provided by molecular techniques like WGS, which is exceptionally high and accurate (Anjum et al., 2018).

Blast analysis of the obtained sequences identified 29 isolates as *L. monocytogenes* with similarity scores ranging from 87.18% to 100.00% (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Ten isolates belonged to the *L. innocua* species with a similarity score of 100.00%, except for one, which had 98.41%. The other species were *L. ivanovii* and *L. seeligeri,* with a 100.00% similarity score each. Phylogenetic analysis exposed clustering of sequences under study with reference sequences belonging to *L. monocytogenes* 4b only. The study sequences in this cluster were more highly similar to the reference sequences they clustered with, as seen from the similarity score by blast analysis. This, in part, may explain why *L. monocytogenes* strains from this study clustered differently due to differences in lineages as exemplified in population genetic studies and typing studies (Bibb et al., 1989, Brosch et al., 1994, Graves et al., 1994).

Cluster A2 sequences did not group according to *Listeria* specie types because all *Listeria* species isolated in this study were represented except for *L. seeligeri*. Results in agreement with the findings of this study were reported indicating the evidence of interspecies recombination between (i) *L. innocua* and *L. monocytogenes,* (ii) *L. innocua* and *L. marthii* (iii) *L. monocytogenes* and *L. marthii* (iv) *L. ivanovii* and *L. seeligeri* (den Bakker et al., 2010). This observation can be attributed to the close relatedness and shared niches that exist between species, especially *L. monocytogenes* and *L. innocua* (Rahimi et al., 2012). Other techniques have been recommended apart from WGS comparisons (Alm et al., 1999, Perna et al., 2001),

such as micro and macroarray are extensively used to study inter and interspecies diversity (Fitzgerald and Musser, 2001, Joyce et al., 2002).

Cluster B excessively consisted of reference sequences belonging to *L. monocytogenes* 1/2a. As discussed earlier within the species, some differences exist, causing the observed clusters despite belonging to the same *Listeria* species group (Joyce et al., 2002).

Cluster C only had study references belonging to *L. seeligeri* isolated from lettuce vegetables. *Listeria seeligeri* was probably only isolated in lettuce because it is attributed to be the most common *Listeria* species isolated from the soil (MacGowan et al., 1994) . This was elaborated in earlier studies conducted by MacGowan and others (1994) about *L. seeligeri* bringing, a major *Listeria* species found in the soil (MacGowan et al., 1994). Another study found an association between the isolation of *L. seeligeri* and the natural environments, such as the soil (Sauders et al., 2012). The other factor that may be linked to lettuce vegetable is leafy and it's able to hide the soil within their leaves. If cleaning is inadequately done on vegetables previous exposure to *Listeria* species, they may remain contaminated. Isolation of *L. seeligeri* was also documented in a study (Orsi et al., 2016). Lastly, cluster D only had reference sequences together with the out-group strain, which is a reference outside the group of interest. Sequences in cluster D grouped differently regardless of them being *L. monocytogenes* because none belonged to either 4b or 1/2a, the main serotypes identified in this study. However, other techniques are needed to conclusively confirm the serotypes isolated in this study (Alm et al., 1999, Perna et al., 2001).

Furthermore, sequences from different locations and food types clustered together irrespective of the sample origin, implying that the same species are being circulated in Namwala, Lusaka, and retail shops. Furthermore, this indicates that RTE deli meats are contaminated by raw foods such as poultry and beef products, which is a public health concern. The above finding can partly be attributed to poor hygienic practices associated with food processing facilities. Facilitating cross-contamination of bacteria from one food to the other. The close relatedness recorded in this study also implies lapses in the food continuum. Other studies have emphasized poor hygienic practices role in food contamination (Guthmann et al., 2006, Fasanmi et al., 2018). Equally, evidence of the role of knives used for evisceration and cutting foods are among the drivers of cross-contamination (Fasanmi et al., 2018, Wambui et al., 2018, Wardhana, 2019). *Listeria monocytogenes* is a post-processing bacterial contaminant that usually results from lapses in standard operating procedures (Milillo et al., 2012, Wu et al., 2020). *Listeria monocytogenes* species in consumed raw food products, such as beef and chicken carcasses, are a public health threat mainly when raw or undercooked. The other risk is cross-contamination during production at retail shops, especially in processing areas with poor hygienic practices. Incidences of listeriosis are due to the consumption of contaminated food items like RTE foods of both animal and vegetable origin (Rahimi et al., 2012, Shamloo et al., 2019). Emphasis should be channeled to maintaining natural product safety to ensure the quality of the final product. In the past decade's consumption trends have evolved, such as eating raw beef, and undercooked foods are among the primary reasons for causing foodborne infections (Singh et al.)

In this study, we recorded a total of 8 isolates that expressed antibiotic resistant genes, of which 7 had *tet(M),* 2 were *L. innocua* and five belonged to *L. monocytogenes*. These isolates came from the following sources: 3 were from chicken samples, two came from chicken abattoir storage

environments, and the remaining two were isolated from RTE deli meats and beef samples. While only one isolate from the eight belonging to *L. innocua* from chicken samples expressed *tet(L), dfrE*, *erm(B)* and *lsa* (*A*). Tetracycline resistance can be transferred by conjugative method between the different bacterial strains, such as *Enterococcus* and *Listeria* species (Korsak et al., 2012, Tresse et al., 2017). Literature indicates *tet(M)* as the commonest in *Listeria* and among the gram-positive bacteria of the genera *Enterococcus*, *Streptococcus,* and *Staphylococcus* (Charpentier et al., 1999). The *tet(M)* is responsible for directing the synthesizing of a cytoplasmic protein that protects the ribosome from inhibition by the antibiotic (Speer et al., 1992). While *tet(L)* gene codes are responsible for a protein that promotes the active efflux of tetracycline from the bacteria (Speer et al., 1992). These tetracycline resistance determinants are common in *Enterococcus* and *Streptococcus* (Charpentier et al., 1994, Speer et al., 1992). Therefore the findings indicate that two types of moveable genetic elements, namely conjugative plasmids and transposons originating from *Enterococcus*, *Streptococcus* is responsible for the emergence of resistance to tetracycline in *L. monocytogenes* isolated from food environment (Poyart-Salmeron et al., 1992). In recent years the incidence of tetracycline has been increasing in strains of *Listeria* species isolated from food and environmental sources (Walsh et al., 2001, Gómez et al., 2014). The initial strains of *L. innocua* resistant to tetracycline were reported in a study (Schuchat et al., 1991) on susceptibilities to nine antibiotics of 26 strains isolated from milk in Canada. Furthermore, 11 tetracycline-resistant strains were reported among 85 strains of *L. innocua* isolated from meat and cheese in Italy (Facinelli et al., 1991, Facinelli et al., 1993). Additionally, a genetic study of tetracycline resistance was performed in 11 strains from cheese (Facinelli et al., 1993), *tet(M)* was recorded in 9 strains, *tet(K)* was recorded in one strain. The *tet(M)* has also been shown to have a link to large integrative and conjugative elements with a board range (Yan et al., 2010). Other studies have suggested that *L. innocua* strains constitute a

reservoir of antibiotic resistance transferable to *L. monocytogenes* (Bertrand et al., 2005). Similarly Li and others detected the *tet(M)* gene that confers resistance to tetracycline's in AMR *L. innocua* from bison with potential to transfer resistance to *L. monocytogenes* (Li et al., 2007). Additionally, (Chen et al., 2010) equally found the *tet(M)* gene in *L. monocytogenes* that was genetically similar to *L. innocua,* and this was well reported by (Milillo et al., 2012). Tetracycline resistance incidence is increasingly common among strains of *Listeria* species coming from foodstuffs and the environment (Gómez et al., 2014). The findings confirm the easy exchange of tetracycline resistance genes under natural conditions between the genera *Enterococcus*, *Streptococcus*, and different species of *Listeria* (White et al., 2002, Roberts and Schwarz, 2016). A study of investigation of AMR genes from 2010 to 2021 reported *tet(M)* and *fosX* to be the most common resistance in *L. monocytogenes* in Europe, Australia, and New Zealand, South America, UK/Ireland and South Africa (Hanes et al., 2022). The efficiency of tetracycline is diminishing in the preceding decades owing to the widespread existence of resistance genes (Gómez et al., 2014). Probably this could be as a result of the prolonged overuse of these antimicrobials in human beings and in food animals (Gómez et al., 2014). From the results the majority of resistant genes of *tet(M)* were from chicken isolates including *tet(L)*. This could have partly been influenced by extensive over use of this antibiotics in poultry. Also easy access of purchase and inadequate regulation also may influence the high numbers of recorded resistance patterns in poultry. Equally the rising demand of poultry meat due to being among the cheaper sources of proteins and its universal religious acceptance adds to extensive use of antimicrobials (Loh et al., 2004). The other factor can be attributed to tetracycline which belongs to third-generation antibiotics of which most *L. monocytogenes* are intrinsically resistant (Byrne et al., 2016). Resistance against macrolides in *Listeria* species such as erythromycin is connected with the presence of two genes *ermB* and *ermC*. In this study only *ermB* was detected in one isolate of

chickens. Antibacterial activity of macrolides is with binding to the 50S ribosomal submit which inhibit the biosynthesis of 23S ribosomal RNA (rRNA). The *erm* genes encode methyltransferases that modify 23S rRNA (Kwiatkowska and Maślińska, 2012). Furthermore, as expected intrinsic resistant genes were also detected in this present study namely; *fosX* and *lmo0919*. These molecular markers were also detected in *L. monocytogenes* by other authors (Kuch et al., 2018, Hurley et al., 2019, Camargo et al., 2019, Matle et al., 2019). *L. monocytogenes* and other *Listeria* species previously, has been described to have low resistance to antimicrobials (Sosnowski et al., 2019). However, lack of detection of beta-lactam genes despite recording penicillin G at phenotypic level gives an indication of possibility of point mutation which affects the gene expression or alteration in encoded proteins (Schwan et al., 2021). Specifically, in some isolates, AMR genes were detected in both the WGS and phenotypic level. Genotypes predicated phenotypes with 62% sensitivity and 100% specificity for resistance to tetracycline's. Sensitivity of genotypic predication was less than 100% for resistance to gentamicin, levofloxacin, trimethoprim, sulfamethoxazole, clindamycin, erythromycin and chloramphenicol. Although some isolates that recorded resistance phenotypically at genotypic level no resistant genes were detected. Nevertheless, genotypic and phenotypic AMR mismatches have also been observed in other studies in *L. monocytogenes* and other species such as *Salmonella*, *Escherichia coli* and *Vibrio parahaemolyticus* (Deekshit et al., 2012, Ortiz and López, Lou et al., 2016, Do Nascimento et al., 2017, Neuert et al., 2018).

However, despite the above unique and, to a greater extent, novel findings, our study had some limitations and challenges. Beef carcasses sampled from this study came from different abattoirs, and cattle was sourced from mixed farms. Moreover, environmental factors were not investigated at the farm level to ascertain the *Listeria* contamination. Therefore our conclusion was limited in

terms of the cradle of *Listeria* contamination, whether at the abattoir or farm level. Furthermore, the inability to isolate *Listeria* from the beef storage environment and tilapia fish. This limits the ability of this study to link the beef storage environment and the tilapia fish as a possible source of *Listeria* contamination. Additionally, the other limiting factor may link to the approach used. Including entire fish or the enteric contents would have increased the likelihood of isolating *Listeria*. On the other hand, large freshwater bodies have a practical dilution effect. Another insidious factor that we might have inadvertently ignored may have significantly affected the final results. Despite these challenges and limitations, the current study design was based on the standard protocol for investigation of *Listeria* species contamination in food as recommended by (Alsheikh et al., 2013). Therefore, the results observed are likely to reflect the true prevalence of *Listeria* species in beef, poultry and there processing environment, fish, lettuce and deli meats. It further determined the antibiotic resistance patterns of *Listeria* species in selected districts of Zambia as they relate to food and food processing environments.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATION

6.1 Conclusion

This study aimed to evaluate the public health importance and antibiotic resistance patterns of *Listeria* species in Zambia as they relate to food and food processing environments. The results indicate that *L. innocua* was the major *Listeria* species contaminant, followed by *L. monocytogenes*. Interestingly *L. seeligeri* was only isolated in lettuce vegetables. Furthermore, this study indicates the absence of any *Listeria* species in both wild-caught and farmed tilapia from a freshwater lake sampled in the Siavonga district in Zambia. Further, findings revealed that clindamycin, tetracycline, and erythromycin had the highest prevalence of antibiotic resistance, although the first drugs of choice remained susceptible. No multiple drug resistance was recorded in this present study. However, this study recorded genes that are responsible for antibiotic resistant and the majority of these genes were isolated from poultry samples. This work's findings are important for determining food safety from primary to secondary processes and treating listeriosis with antibiotics. These results also suggest the need for improved food safety through implementing hygienic measures at all levels, from production to secondary processes, with particular emphasis on RTE food items that do not go through a heating step before consumption. Regarding RTE deli meats, post-processing manipulations appear to be how the products are re-contaminated. The risk of cross-contamination from raw to cooked foods during storage and preparation necessitates a control program for *L. monocytogenes* with strict hygienic processing of foods. The results also indicates that carcass contamination does not always mean cold-room storage contamination.

6.2 Recommendations

The following are the recommendations:

1. There is need to develop *Listeria* surveillance programmmes in beef and poultry carcasses, abattoirs and fish and processing plants, vegetables and stored deli products.
2. That *L. innocua* may be used as an indicator organism for *L. monocytogenes* (given by the results and that it is closely similar in many aspects) in the *Listeria* surveillance programmes.
3. There is need to monitor the emergence of AMR in *Listeria* species, given their public health importance.
4. There is need to upscale awareness education campaigns
5. Further research is required on the strains of *L. monocytogenes* circulating in food animal origin and the emergence of multidrug-resistant strains which may have a public health impact.

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LIST OF APPENDICS

Appendix 1: Publications during the thesis period

1. Mpundu, P., Mbewe, A., Muma, J., Mwasinga, W., Mukumbuta, N. & Munyeme, M. A global perspective of antibiotic-resistant Listeria monocytogenes prevalence in assorted ready to eat foods: A systematic review, Veterinary World, 14 (8): 2219-2229. 2021.
2. Mpundu P, Muma JB, Mukubesa AN, Kainga H, Mudenda S, Bumbangi FN, Muleya W, Katemangwe P, Munyeme M: Antibiotic Resistance Patterns of Listeria Species Isolated from Broiler Abattoirs in Lusaka, Zambia. 2022, 11(5):591
3. Mpundu, P., Muma, J.M., Mukumbuta N., Mukubesa, A. N., Muleya, W, Kapila, P, Hang'ombe B.M. & Munyeme, M. Isolation, discrimination, and molecular identification of Listeria species from slaughtered cattle in Namwala District, Zambia. (BMC Journal of Microbiology).
4. Mpundu,P, Aspholm E.M, Muleya W, Mukumbuta N, Muma J.B, Munyeme M. Polymerase chain reaction fails to detect Listeria species from wild and cultured Nile tilapia (Oreochromis niloticus) caught from a large freshwater lake in Zambia. 2022, 9 (19). (10.11604/pamj-oh.2022.9.19.37355)

Appendix 2: Participant Information Sheet

Study Title: Isolation and characterization of Antimicrobial Resistant *Listeria* speciesin food and food processing environments in selected districts, Zambia

Name of Investigator: Prudence Mpundu

Institution: University of Zambia

Phone: +260976905391

Introduction:

I am a student doing my Doctor of Philosophy Degree in Infectious diseases at the University of Zambia under the Disease Control [Public Health Unit]. As partial requirement for the completion of the degree programme am required to do the research. I ask that you read through this form and ask any questions before you proceed to taking part in the study.

Purpose of Study:

The purpose of this study is to find out the quality of various foods in relation to *Listeria* contamination and Antimicrobial resistance. You have been selected at random and I would like to ask if you can allow me collect some food samples processed in your plant/ abattoir.

Confidentiality:

Be assured that all the samples which will be collected in your plant /abattoir will remain confidential. I will not write down the name of the business so that no one will know where the samples were collected.

Participation in this research is completely voluntary. You may withdraw at any time when you feel you cannot continue. However, hope you will participate in the research since knowing the quality of the food being processed in your plant/abattoir is very important. The sampling process will be taking less than one hour a day.

Right to Ask Questions and Report Concerns

You have the right to ask questions about this research study and to have those questions answered by me before, during or after the research. If you have any further questions about the study, at any time feel free to contact me, Prudence Mpundu at *email: prudencezimba@gmail.com* or by cell phone number 0976905391 or Levy Mwanawasa Medical University P.O Box 33991 Lusaka, Zambia . You can also contact the research Ethics Committee that reviewed this study at ERES Converge IRB Plot No. 1 Cnr. Great East Road & Joseph Mwilwa Road | Rhodes Park Lusaka Zambia Office: +260 955 155633 | +260 955 155634 Mobile: +260 966 765503 E-mail: eresconverge@yahoo.co.uk 0955 155633/4 and the responsible city councils that permitted the study to go ahead

Appendix 3: Consent Form

Study Title: Public health importance and antibiotic resistance patterns of *Listeria* species in Zambia in food and food processing environments.

At this time, do you want to take part in this research?

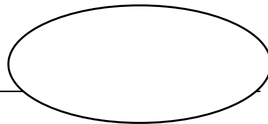
Respondent Agrees 1----- Go ahead

Respondent Disagrees 2------ End

Your signature below indicates that you have decided to volunteer your plant/abattoir to participant in this study, and that you have read and understood the information provided in the information sheet. You may withdraw at any time when you feel you cannot continue however, know that your participation in this research is important. You will be given a signed and dated copy of this form to keep.

Participants Name (print): ______________________________

Participants Signature/Thump print: ______________________________ Date: ____________________



Investigator's Signature: ______________________________ Date: ____________________

Appendix 4: Cichewa Translation

Zowonjezera 1: Chidziwitso cha Gulu Lophunzira

Mutu Wophunzirira: Kupatula ndi mawonekedwe a Antimicrobial Resistant Listeria speciesm'malo opezeka chakudya ndi zakudya m'malo osankhidwa, Zambia

Dzina la Wofufuza: Prudence Mpundu

Sukulu: University of Zambia

Foni: +260976905391

Mawu oyambira:

Ndine wophunzira wochita Doctor wanga wa Philosophy Degree mu matenda opatsirana ku Yunivesite ya Zambia pansi pa matenda a Disease Control [Public Health Unit]. Pakufunika pang'ono kuti pulogalamuyo imalize ndikufunika kuchita kafukufukuyu. Ndikufunsani kuti muwerenge nkhaniyi ndikufunsa mafunso musanatenge nawo phunziroli.

Cholinga cha Phunziro:

Cholinga cha phunziroli ndikuwonetsetsa mtundu wa zakudya zosiyanasiyana mokhudzana ndi kuipitsidwa kwa Listeria ndi antimicrobial. Mwasankhidwa mwachisawawa ndipo ndikufuna kufunsa ngati mungandilole kuti nditengere zitsanzo za zakudya zomwe zimapangidwa mu chomera chanu / malo ogulitsira.

Chinsinsi:

Dziwani kuti zitsanzo zonse zomwe zikusungidwa mu chomera chanu / malo ogulitsira zidzakhalabe zachinsinsi. Sindingalembe dzina la bizinesiyo kuti pasadzapezeke munthu amene angadziwe komwe ma sampuli anasonkhanitsira

Kutenga nawo mbali pa kafukufukuyu ndikudzifunira ndekha. Mutha kusiya nthawi iliyonse mukaona kuti simungathe kupitiliza. Komabe, mukukhulupirira kuti mutenga nawo gawo pofufuzira popeza kudziwa kuchuluka kwa zakudya zomwe zimapangidwira mmera / malo ogulitsira ndikofunikira kwambiri. Njira zowerengera izi zimatenga ola limodzi patsiku.

Ufulu Wofunsa Mafunso ndi Kupereka Zovuta

Mulinso ndi ufulu wofunsa mafunso okhudza kafukufukuyu komanso kuti mafunso amenewa ayankhidwe ndi ine kale, mkati mwake kapena pambuyo pa kafukufukuyu. Ngati muli ndi mafunso ena okhudza kafukufukuyu, nthawi iliyonse musayankhule ndi ine, Prudence Mpundu pa imelo: prudencezimba@gmail.com kapena patelefoni nambala ya 0976905391 kapena Levy Mwanawasa Medical University P.O Box 33991 Lusaka, Zambia. Mutha kulankhulanso ndi Ethics Ethics Committee yomwe yawunikira kafukufukuyu ku ERES Converge IRB Plot No. 1 Cnr. Main East Road & Joseph Mwilwa Road | Rhode Park Lusaka Zambia. Ofesi: +260 955 155633 | +260 955 155634 Telefoni: +260 966 765503 Imelo: eresconverge@yahoo.co.uk 0955 155633/4 ndi makhonsolo a mzindawu omwe alola kuti phunziroli lipitirire

Kumapeto 2: Fomu Yovomerezeka

Mutu Wophunzirira: Kupatula ndi mawonekedwe a Antimicrobial Resistant Listeria speciesm'malo opangira zakudya ndi zakudya m'malo osankhidwa, Zambia

Pakadali pano, mukufuna kutenga nawo mbali pa kafukufukuyu?

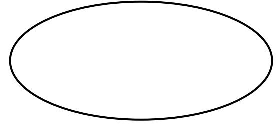
Milandu Yoyankha 1 ----- Pitirirani

Zosemphana ndi Zotsutsana 2 ------ Mapeto

Siginecha yanu m'munsimu ikuwonetsa kuti mwaganiza zodzipereka kudyera / malo ogulitsira kuti mukhale nawo phunziroli, komanso kuti mwawerenga ndikumvetsetsa zomwe zapezeka patsamba lazidziwitso. Mutha kusiya nthawi ina iliyonse mukaona ngati simungathe kupitiliza, dziwani kuti kutengapo gawo pakufufuza uku ndikofunikira. Mudzapatsidwa fayilo yosainidwa ndi detiyi kuti musunge.

Code Number

Ophunzira ______________________________

Chizindikiro /chosindikizidwa:  Tsiku: ____________

Saina Ya Wofufuza: ______________________________ Tsiku: ____________

Appendix 5: Tonga Translation

Pepa Lyakukumbalizya 1: Cipepa Catwaambo Cabasikutola Lubazu

Mutwe Walwiiyo: Ku pima akutonde zya musyobo watu zunda tutafwi kumusamu lisilika tutegwe *Lisitiliya* na nkotuli muli cakulya amuma sene mubambilwa zyakulya muzilikiti zyandeene mucisi ca Zambia

Izyina Lyamuvuntauzyi:	Prudence Mpundu
Kwazyilwa:	University of Zambia
Nambala Ya Luwaile:	+260976905391

Kulipandulula:

Ndili sichikolo acikolo cipati ca University of Zambia nditola lwiiyo lwabu dokotela wa philosophy degree mu malwazi ayambukila, oolu lwiiyo lwendelezegwa amutabi ukwabilila malwazi (Disease Control) ku (Mutabi ulanganya nseba zyaba mubuleya Public Health Unit). Mbuli cibeela cimwi ciyandikana kutegwa ndimanizye lwiiyo lwangu oolu ndileelede kucita buvuntauzyi oobu. Ndamulomba ikuti mubale cipepa eeci akundibuzya mibuzyo kamutanaya kumpela mukutola lubazu mulwiiyo oolu.

Muzeezo Wa Lwiiyo:

Kuvuntau zya nazyakulya zyandeene zelaatu zunda tutegwe Lisiteliya,alimwi nadtu zunda ootu tulasile kika naape.

Maseseke:

Amushomezegwe ikuti tubeela toonse twazyakulya tutibwezegwe omuno mumambilo/mumajailo abanyama alayobolwa mumaseseke. Tandiko kulemba izina lyamakwebo eenu kutegwa kukabule uzyiba tubeela twa zyakulya nkutwakazyila. Kutola lubazu mubuvuntauzyi oobu nkwakulya aba biya. Inga mwacileka kutola lubazu kufumbwa ciindi nchimwayanda. Nokuba boobo, kulombozya nkwakuti mutole lubazu mubuvuntauzyi oobu nkaambo kakuti ikuzyi bubotu bwachakulya cibambiwa mumabambilo/mumajailo abanyama nchipati kampatila. Kubweza tubeela twa zakulya kunakutola biyo ciindi cisyoonto citaindilili awoola lyomwe abuzuba.

Nguzu zya kubuzya mibuzyo alimwi akwaambilizya kutasalalilwa

Mulijisi nguzu zyakubuzya mibuzyo ijatikizya buvuntauzyi bwa lwiiyo oolu akubaabwinguzi kumibuzyo yenu kakutaninga, akatikati nokuba kumamanino abuvuntauzyi. Nakuti kamujisi mibuzyo imbi iya kumpela ijatikizya lwiiyo oolu, kufumbwa ciindi amulinvwe kulikwya/kwanguluka kunditumina, Prudence Mpundu at *email: prudencezimba@gmail.com* or by cell phone number 0976905391 or Levy Mwanawasa Medical University P.O Box 33991 Lusaka, Zambia. alimwi inga mwatumina kukabunga kalangwanya milawo yabuvuntauzyi research Ethics Committee kakalanganya lwiiyo oolu ku ERES Converge IRB Plot No. 1 Cnr. Great East Road & Joseph Mwilwa Road | Rhodes Park Lusaka Zambia. Office: +260 955 155633 | +260 955 155634 Mobile : +260 966 765503 E-mail : eresconverge@yahoo.co.uk 0955 155633/4 alimwi kwanganya ama kkanulu amumadolopo akazumizya kuti lwiiyo oolu luzwide mbele.

Pepa Lyakukumbatizya 2: Pepa Lyacizuminano Lyaba Sikutola Lubazu

Mutwe Wa Lwilyo: Ku pima akutonde zya musyobo watu zunda tutafwi kumusamu lisilika tutegwe *Lisitiliya* na nkotuli muli cakulya amuma sene mubambilwa zyakulya muzilikiti zyandeene mucisi ca Zambia

Mugindi egno sena mulayanda kutola lubazu mabuvuntauzyi Oobu?

Basikuvwiila Bazumina 1----- Amuye Ambele Akuvuntauzya

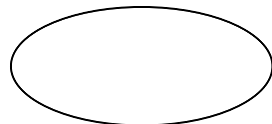
Basikuvwiila Bakaka 2------ Amusimpe Mpona Aawa

Kusaina kwaunu munselelo oomu caamba kuti mwazumizya caku lyaaba kuti mabambilo/majailo abanyama benu kutole lubazu mulwiiyo oolu akuti mwabala akuteelela twaambo toonse twapegwa mucipepacatwaambo cabasi- kutola lubazu. Inga mwacileka kufumbwa oindi chimwalivwa kuti inga timwazumanana nekuba boobo amuzyibe kuti kutola lubazu kwanu mubuyuntauzyi oobu nkupati kampatila. Muyakupengwa cipepa eeci cisainidwe akulembwa buzuba ketegwa anywebo mwayobola.

Code Number:

Utola lubazu ________________

Kusaina/ Kudyamba munwe Ng'anda:



Buzuba: ________________

Kusaina Kwasikuvuntauzya: ________________ Buzuba: ________________

Appendix 6: Sampling form for chicken *Listeria* community enumeration

Time Sampling Start…………………… Time Sampling End………………………….

DATE OF SAMPLING……………………………………

S/N	ABATTOIR ID	Batch/Source ID	Swabs		
			Cloacal	External surface	Environmental

Appendix 7: Sampling form for fish *Listeria* community enumeration

Time Sampling Start……………………………………….

Time Sampling End………………………….

DATE OF SAMPLING……………………………………

S/N	**Fish Plant ID**	**Batch/Source ID**	**Swabs**	
			Surface swabs	**Gills**

Appendix 8: Sampling form for beef *Listeria* community enumeration

Time Sampling Start……………………………………….

Time Sampling End………………………….

DATE OF SAMPLING……………………………………

S/N	**ABATTOIR ID**	**Batch/Source ID**	**Swabs**	
			Internal surface	**External surface**

Appendix 9: Sampling form for ready to eat food *Listeria* community enumeration

Time Sampling Start…………………… Time Sampling End………………………….

DATE OF SAMPLING……………………………………

S/N	Retail Markets ID	Batch/Source ID	25g of RTE food	
			Point of Sale	Point of Sale

Appendix 10: Pictures of *listeria* types isolated in this study on blood agar

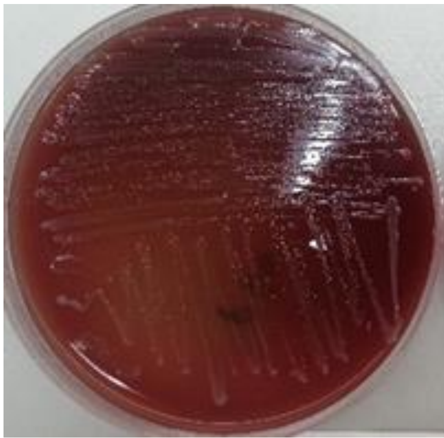


Fig 1. *L. innocua*

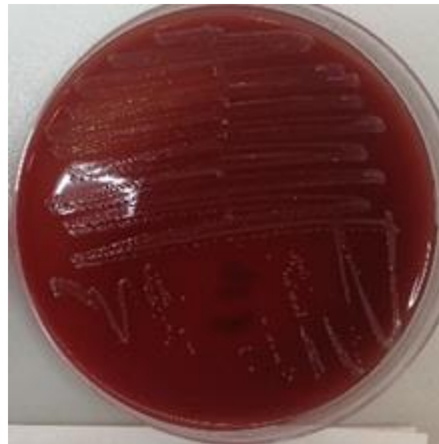


Fig 2. *L. welshimeri*

Fig 3. *L. ivanovii*

Fig 4. *L. monocytogenes*

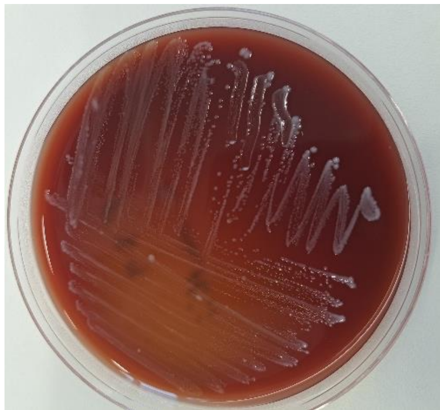


Fig 5. *L. marthii*

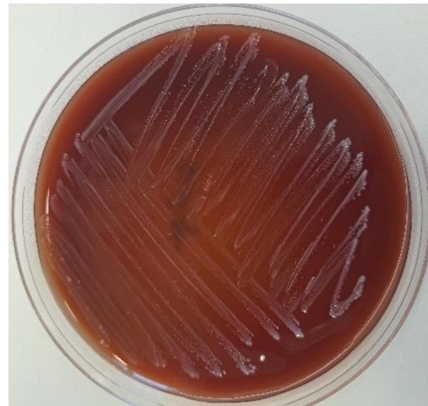


Fig 6. *L. seeligeri*

Appendix 11: Identification of specific *Listeria* isolates using MALDI-TOF MS

Site of swabbing	Source of sample	*Listeria* species	Similarity
External surface	Poultry abattoirs	*L .innocua*	99.9
Cloacal	Poultry abattoirs	*L. innocua*	99.9
Cloacal	Poultry abattoirs	*L. ivanovii*	99.9
External surface	Poultry abattoirs	*L. monocytogenes*	99.9
External surface	Poultry abattoirs	*L. innocua*	99.9
External surface	Poultry abattoirs	*L. monocytogenes*	99.9
Cloacal	Poultry abattoirs	*L.seeligeri*	99.9
External surface	Poultry abattoirs	*L. monocytogenes*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
Cloacal	Poultry abattoirs	*L.innocua*	99.9
Cloacal	Poultry abattoirs	*L.innocua*	99.9
Cloacal	Poultry abattoirs	*L. innocua*	99.9
Cloacal	Poultry abattoirs	*L.innocua*	99.9
Environmental swab	Poultry abattoirs	*L. monocytogenes*	99.9
External surface	Poultry abattoirs	*L. monocytogenes*	99.9
Environmental swab	Poultry abattoirs	*L. innocua*	99.9
Cloacal	Poultry abattoirs	*L .monocytogenes*	99.9
External surface	Poultry abattoirs	*L. innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
Cloacal	Poultry abattoirs	*L.ivanovii*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
Environmental swab	Poultry abattoirs	*L.innocua*	99.9
Environmental swab	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
Cloacal	Poultry abattoirs	*L.innocua*	99.9
Exterior surface	Poultry abattoirs	*L.ivanovii*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
Cloacal	Poultry abattoirs	*L.innocua*	99.9
Environmental swab	Poultry abattoirs	*L.innocua*	99.9
Cloacal	Poultry abattoirs	*L.innocua*	99.9
Cloacal	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L. monocytogenes*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
Cloacal	Poultry abattoirs	*L.innocua*	99.9

External surface	Poultry abattoirs	*L.innocua*	99.9
Environmental swab	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L. monocytogenes*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L. monocytogenes*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
Environmental swab	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
Environmental swab	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L. monocytogenes*	99.9
Cloacal	Poultry abattoirs	*L. innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L.innocua*	99.9
External surface	Poultry abattoirs	*L. monocytogenes*	99.9
External surface	Poultry abattoirs	*L. monocytogenes*	99.9
External surface	Beef abattoirs	*L.innocua*	99.9
Internal surface	Beef abattoirs	*L.innocua*	99.9
Internal surface	Beef abattoirs	*L. monocytogenes*	99.9
External surface	Beef abattoirs	*L.innocua*	99.9
Internal surface	Beef abattoirs	*L.innocua*	99.9
Internal surface	Beef abattoirs	*L. monocytogenes*	99.9
External surface	Beef abattoirs	*L. innocua*	99.9
Internal surface	Beef abattoirs	*L. innocua*	99.9
External surface	Beef abattoirs	*L.innocua*	99.9
External surface	Beef abattoirs	*L.innocua*	99.9
Internal surface	Beef abattoirs	*L.innocua*	99.9
External surface	Beef abattoirs	*L.innocua*	99.9
Internal surface	Beef abattoirs	*L. monocytogenes*	99.9

Internal surface	Beef abattoirs	*L.ivanovii*	99.9
Internal surface	Beef abattoirs	*L.innocua*	99.9
External surface	Beef abattoirs	*L.innocua*	99.9
Internal Surface	Beef abattoirs	*L. monocytogenes*	99.9
Internal surface	Beef abattoirs	*L.innocua*	99.9
Internal surface	Beef abattoirs	*L. monocytogenes*	99.9
External surface	Beef abattoirs	*L.innocua*	99.9
Internal surface	Beef abattoirs	*L. innocua*	99.9
External surface	Beef abattoirs	*L.ivanovii*	99.9
External surface	Beef abattoirs	*L .innocua*	99.9
External surface	Beef abattoirs	*L.innocua*	99.9
Internal surface	Beef abattoirs	*L.innocua*	99.9
External surface	Beef abattoirs	*L.innocua*	99.9
Internal surface	Beef abattoirs	*L.innocua*	99.9
Internal surface	Beef abattoirs	*L.innocua*	99.9
Internal surface	Beef abattoirs	*L.innocua*	99.9
RTE deli meats	RTE deli meats	*L. monocytogenes*	99.9
RTE deli meats	RTE deli meats	*L. monocytogenes*	99.9
RTE deli meats	RTE deli meats	*L. monocytogenes*	99.9
RTE deli meats	RTE deli meats	*L.innocua*	99.9
RTE deli meats	RTE deli meats	*L.innocua*	99.9
RTE deli meats	RTE deli meats	*L. monocytogenes*	99.9
RTE deli meats	RTE deli meats	*L.innocua*	99.9
RTE deli meats	RTE deli meats	*L.innocua*	99.9
RTE deli meats	RTE deli meats	*L.innocua*	99.9
RTE deli meats	RTE deli meats	*L. monocytogenes*	99.9
RTE deli meats	RTE deli meats	*L.ivanovii*	99.9
RTE deli meats	RTE deli meats	*L.innocua*	99.9
Lettuce	Lettuce	*L. Seeligeri*	99.9
Lettuce	Lettuce	*L. monocytogenes*	99.9
Lettuce	Lettuce	*L. Seeligeri*	99.9
Lettuce	Lettuce	*L. Seeligeri*	99.9
Lettuce	Lettuce	*L. marthii*	99.9
Lettuce	Lettuce	*L. Seeligeri*	99.9
Lettuce	Lettuce	*L. marthii*	99.9

Apendix 12: Blast analysis

Source of samples	*Listeria* spp.	Resistant genes	Blast analysis similarity score (%)	Reference accession number
Beef abattoir	*L. monocytogenes*	-	100.00	CP054846.1
Beef abattoir	*L. monocytogenes*	-	100.00	CP001604.1
Beef abattoirs	*L. innocua*	-	98.41	CP045744.1
Poultry abattoirs	*L. monocytogenes*	-	96.97	CP054846.1
Poultry abattoirs	*L. monocytogenes*	*Tet(M)*	97.87	CP114254.1
Poultry abattoir	*L.innocua*	-	100.0	CP045743.1
Poultry abattoir	*L. innocua*	*tet(L), dfrE, erm(B), lsa(A).*	100.00	CP102627.1
Storage environment (chicken abattoir) p14	*L. monocytogenes*	*Tet(M)*	100.00	CP032668.1
Storage environment (chicken abattoirs)	*L. monocytogenes*	-	100.00	CP001604.1
Beef abattoirs	*L. monocytogenes*		86.67	CP054040.1
Poultry abattoirs	*L. monocytogenes*	*Tet(M)*	100.00	CP020833.1
Beef abattoirs	*L. monocytogenes*	*Tet(M)*	87.18	CP054039.1
Retail markets (deli meats)	*L. innocua*	-	100.00	CP102627.1
Storage environment (chicken abattoirs)	*L. innocua*	-	100.00	CP102626.1
Chicken abattoirs	*L. monocytogenes*	-	100.00	CP020774.1

Retail markets (deli meats)	*L. monocytogenes*	-	100.00	CP054846.1
Chicken abattoirs	*L. monocytogenes*	-	97.46	CP054846.1
Chicken abattoirs	*L. monocytogenes*	-	100.00	CP054846.1
Chicken abattoirs	*L. monocytogenes*	-	100.00	CP054846.1
Chicken abattoirs	*L. innocua*	*Tet(M)*	100.00	CP071155.1
Beef abattoirs	*L.innocua*	-	100.00	CP071155.1
Chicken abattoirs	*L. monocytogenes*	-	97.14	CP043177.2
Retail markets (deli meats)	*L. innocua*	-	100.00	CP045743.1
Storage environment (chicken abattoirs)	*L.innocua*	*Tet(M)*	100.00	CP054846.1
Chicken abattoirs	*L. monocytogenes*	-	100.00	CP053628.1
Chicken abattoirs	*L.monocytogenes*	-	100.00	CP001604.1
Beef abattoirs	*L.monocytogenes*	-	100.00	CP054846.1
Retail markets (deli meats)	*L.monocytogenes*	-	100.00	CP0544848
Retail markets (deli meats)	*L.monocytogene*	-	100.00	CP054846.1
Retail markets (deli meats)	*L.monocytogenes*	-	100.00	CP054846.1
Storage environment (chicken abattoirs)	*L.monocytogenes*	*Tet(-M)*	100.00	CP034771.1
Chicken abattoirs	*L.ivanovii*	-	100.00	CP043177.2
Retail markets (Lettuce)	*L.monocytogenes*	-	100.00	CP054846.1

Appendix 13: Ethical approval

PlotNo.1.Cnr JosephMwilwa & Great East Road

Plot No. 1, Cnr Joseph Mwilwa & Great East Road
Rhodes Park, Lusaka - Zambia
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 977 493220
Email: eresconvergeltd@gmail.com

I.R.B. No. 00005948
F.W.A. No. 00011697

20th January, 2020

Ref. No. 2020-Jan-006

The Principal Investigator

Ms. Prudence Mpundu

Levy Mwanawasa Medical University P. O.
Box 33991 LUSAKA.

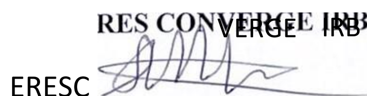
Dear Ms. Mpundu,

RE: ISOLATION AND CHARACTERIZATION OF ANTIMICROBIAL RESISTANT LISTERIA spp. IN FOOD AND FOOD PROCESSING ENVIRONMENTS IN SELECTED DISTRICTS, ZAMBIA.

Reference is made to your protocol dated 1 0th December, 2019. Verdict;
Approved Subject to Corrections.

1. You may need to translate the information sheet and consent form to common local language spoken in the study areas.
2. Avoid use of personal pronouns.
3. Namwala is a district council not a city council.
4. Avoid writing authors names in capital letters.

Yours faithfully,

ERESC 

VERGE IRB

Dr. Jason Mwanza

Dip. Clin. Med. sc., BA., M.Soc., PhD

CHAIRPERSON

Appendix 14 School Approval letter

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VETERINARY MEDICINE

OFFICE OF THE ASSISTANT DEAN (POSTGRADUATE)

Telephone : 293727
Telegrams: UNZA LUSAKA
Telex: UNZALUZA 44370
Fax: 293727/253952
School Fax 293727
Vet, Clinic Telephone 291515

P.O 32379
Lusaka, Zambia

Your Ref:

Our Ref:

26th September, 2019

Prudence Mpundu

School of Veterinary Medicine
University of Zambia P.O.
Box 32379 LUSAKA

Dear Prudence Mpundu,

RE: APPROVAL OF RESEARCH PROPOSAL

At the meeting of the School Board of Graduate Studies held on 17th September, 2019, your research proposal entitled 'Isolation and characterization of antimicrobial resistant Listeria spp. In food and food processing environments in selected districts of Zambia' was tabled and discussed. I am therefore pleased to inform you that the research proposal was subsequently approved by the Board.

On behalf of the Board, I wish you success as you apply for ethical approval and carry on with your research activities.

Yours sincerely

Dr Edgar Simulundu
ASSISTANT DEAN (PG), SCHOOL OF VETERINARY MEDICINE

cc Director, DRGS
Dean, School of Veterinary Medicine
Head, Department ofDisease Control
Dr. Musso Munyeme
Prof J. B. Muma
Prof. B. M. Hang'ombe