ANTIMICROBIAL SENSITIVITY TESTING OF STORED BACTERIAL ISOLATES FROM 2000 TO 2021 IN THE AGRICULTURAL SECTOR

By

Mrs Masabata Annah Motaung

Submitted in partial fulfilment of the requirements

for the degree of

MASTER OF SCIENCE IN AGRICULTURE

In the Department of Agriculture and Animal Health

UNIVERSITY OF SOUTH AFRICA

SUPERVISOR: Dr P.N. Kayoka

CO-SUPERVISOR: Dr I. Matle

September 2023

DECLARATION

Name	MASABATA ANNAH MOTAUNG
Student number	66073669
Degree	Master Science in Agriculture (Full dissertation)
Title	Antimicrobial sensitivity testing of stored bacterial isolates from 2000 to 2021 in the agricultural sector.

"I, Masabata Annah Motaung, hereby declare that the dissertation submitted for the degree of Master of Science in Agriculture, at the University of South Africa is my own original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references."

SIGNATURE……  ……DATE…29/09/2023……

DEDICATION

This dissertation is dedicated to the Almighty who has been the light, courage, and strength through this journey. My late father Mr Simon Ramachona Macholo for believing in education and encouraging us to pursue education at any age. To my mother, Mrs Betty Macholo, my husband, Mr Aaron Motaung (the love of my life), my son, Mr Taunyana Thato Oretshoanetse Motaung and my daughter, Ms Kamohelo Retshidisitsoe Motaung, for their continuous support and love during my studies.

To my two beautiful sisters and friends for their endless words of encouragement when I needed it the most.

“Many discouraging hours will arise before the rainbow of accomplished goals will appear on the horizon” Haile Selassie.

ACKNOWLEDGEMENTS

I would like to acknowledge the following individuals/organizations:

My supervisors: Dr P.N. Kayoka and Dr I. Matle for their leadership, patience, professional advice, support, and encouragement throughout this work, without them this work would not have been finalized successfully.

Ms L Makhado, Ms C Motau and Ms P Nthaba for training on identification of bacteria.

Ms Cynthia Ngwane for assisting with statistical analysis.

ARC-OVR institute for funding and for providing me with the opportunity to study for this degree.

University of South Africa for awarding a bursary for my studies.

All colleagues who either wittingly or unwittingly contributed to this research project.

ABSTRACT

Antimicrobial resistance is a critical global concern that has a detrimental effects on both human and animal health as well as food security, development, and the economy. This study investigated the antimicrobial sensitivity of stored bacterial isolates from 2000 to 2021 in the agricultural sector across eight provinces in South Africa. A total of 216 bacterial isolates which represented four distinct bacterial pathogens: *Salmonella enterica* (*n* = 88), *Escherichia* coli (*n* = 30), *Staphylococcus aureus* (*n* = 64), and *Listeria monocytogenes* (*n* = 34) were revived and analyzed. Phenotypic antimicrobial profiling was determined against 12 antibiotics for *S. enterica* and *E. coli* isolates and 13 antibiotics for *S. aureus* and *L. monocytogenes,* using Kirby-Bauer disk diffusion and minimum inhibitory concentration methods. Conventional PCR was performed for confirmation of isolate species, serotypes, and screening of antimicrobial resistance genes (*bla*, *tet*, *Dfr*, *qnr*, *sul*, *erm*, *cat1*, *flo*, *cm1A* and *mecA*). The isolates that were lyophilized yielded a poor viability rate (31%), compared to 69% of the isolates preserved in tryptose broth-glycerol. *Salmonella enterica* and *E. coli* isolates were generally sensitive against all tested antibiotics except for tetracyclines. *S. aureus* showed high resistance (90-100%) to 11 antibiotics (penicillin, gentamycin, nitrofurantoin, chloramphenicol, linezolid, quinupristin-dalfopristin, tetracycline, vancomycin, erythromycin, kanamycin, and ciprofloxacin). All the tested *L. monocytogenes* showed a complete (100%) resistance against a wide range of antibiotics, including penicillin, gentamycin, kanamycin, vancomycin, ciprofloxacin, quinupristin-dalfopristin, tetracycline, and erythromycin. The *tetA* and bla_{PSE} genes were predominantly detected in *S. enterica* and *E. coli* while *S. aureus* isolates harbored a wide variety of antibiotic resistance genes namely *mecA* (44%), *blaZ* (39%), *aap-apl* (31%), and *tetM* (22%). Among the four serogroups of *L. monocytogenes* tested in this study, 71% of serogroup IIc harbored *ermB* and 42% of serogroup IVa carried the *tetA* gene. This retrospective study provides a glimpse into the antimicrobial sensitivity profile of major animal and foodborne pathogens over the years in the country. These data can be used in monitoring existing anti-microbial resistance (AMR) policies and strategies in South Africa.

Key words: Antibiotics, stored bacterial isolates, *Listeria monocytogenes, Salmonella enterica, Escherichia coli, Staphylococcus aureus*, antimicrobial resistance genes.

TABLE OF CONTENTS

DECLARATION ii

DEDICATION iii

ACKNOWLEDGEMENTS iv

ABSTRACT v

LIST OF TABLES ix

LIST OF ABBREVIATIONS xi

CHAPTER 1: GENERAL INTRODUCTION 1

1.1 BACKGROUND AND JUSTIFICATION 1

1.2 PROBLEM STATEMENT 3

1.3 AIM AND OBJECTIVES 4

1.4 RESEARCH QUESTIONS 4

1.5 BENEFIT OF THIS STUDY 4

1.6 DISSERTATION LAYOUT 5

CHAPTER 2: LITERATURE REVIEW 6

2.1 DEFINITION OF ANTIBIOTICS 6

2.2 A BRIEF HISTORY OF ANTIBIOTICS 6

2.3 ANTIBIOTICS CLASSIFICATION AND CATEGORIZATION 8

2.4 THE IMPORTANCE OF ANTIBIOTICS 10

2.4.1 The application and use of antibiotics in animal 10

2.5 DEVELOPMENT OF ANTIBACTERIAL RESISTANCE 11

2.5.1 Drug uptake limitation 11

2.5.2 Drug target modification 11

2.5.3 Drug inactivation 12

2.5.4 Drug efflux 12

2.6 POLICIES GOVERNING ANTIMICROBIAL RESISTANCE IN SOUTH AFRICA 13

2.7 SURVEILLANCE OF ANTIMICROBIAL RESISTANCE 14

2.8 SURVEILLANCE METHODS FOR ANTIMICROBIAL RESISTANCE 14

2.8.1 Laboratory based surveillance 15

2.8.2 Population based surveillance 16

2.8.2 Sentinel surveillance 16

2.8.3 Active surveillance 16

2.9 DIAGNOSTIC TESTING FOR ANTIMICROBIAL RESISTANCE 16

2.9.1 Phenotypic methods 16

2.9.2 Molecular based methods to detection antimicrobial resistance 20

2.9.3 Conventional PCR methods **21**
2.9.4 Isothermal amplification method **21**
2.9.5 DNA microarrays **21**
2.9.6 Other molecular methods **22**
2.10 CONCLUSION 23
CHAPTER 3: MATERIALS AND METHODOLOGIES **23**
3.1 STUDY DESIGN 23
3.2 SELECTION OF BACTERIAL STRAINS 24
3.3 SAMPLE ORIGIN AND SOURCES OF ISOLATES 24
3.4 SAMPLE SIZE 25
3.5 SAMPLING METHOD 27
3.6 MICROBIOLOGICAL ANALYSIS 27
3.6.1 Revival of isolates **27**
3.6.2 Bacterial confirmation of isolates **28**
3.6.3 Molecular confirmation of *Salmonella enterica* **28**
3.6.4 Biochemical tests confirmation **29**
3.6.5 Antimicrobial susceptibility test **30**
3.6.6 Identification of resistance genes by PCR **34**
3. 6.7 Molecular characterization (subtyping) **37**
3.6.8 Phenotypic serotyping **42**
3.7 ANTIBIOTIC RESISTANCE INDEX (ARI) 42
3.8 DATA ANALYSIS 42
CHAPTER 4: RESULTS **43**
4.1 Strain characterization 43
4.2 Frequency of recovery of the isolates 43
4.3 Phenotypic antibiotic results of isolates stored between 2000 and 2021 45
4.3.1 *Salmonella enterica* **45**
4.3.2 *Escherichia coli* **47**
4.3.3 *Listeria monocytogenes* **51**
4.3.4 *Staphylococcus aureus* **57**
4.3.4.1 The overall sensitivity of *Staphylococcus aureus* **57**
4.3.4.2 Analysis of multidrug resistance among *Staphylococcus aureus* strains **57**
4.4 Overall distribution of susceptibility by Minimum Inhibitory Concentration (MIC) method 59
4.4.1 *Salmonella enterica* **59**
4.4.2 *Escherichia coli* **61**
4.4.3 *Listeria monocytogenes* **61**

4.4.5 Examining antimicrobial resistance pattern of *Salmonella enterica* and *Escherichia coli* using MIC assay **63**
4.4.6 Change in Antimicrobial resistance pattern of *Listeria monocytogenes* using MIC **65**
4.5 Detection of antibiotic resistance genes 65
4.5.1. Prevalence of antibiotic resistance genes amongst gram negative isolates **65**
4.5.2 Antibiotic resistance genes among the Gram-positive pathogens **68**
CHAPTER 5: DISCUSSION **70**
5.1 Viability of the isolates 70
5.2.1 *Salmonella enterica* **71**
5.2.2 *Escherichia coli* **74**
5.2.3 *Staphylococcus aureus* **75**
5.2.4 *Listeria monocytogenes* **79**
5.2.5 The antibiotic resistance index (ARI) **80**
5.3 Detection of genotypic antimicrobial resistance in gram negative pathogens 81
5.3.1 *Salmonella enterica* **81**
5.3.2 *Escherichia coli* **84**
5.4 Detection of genotypic antimicrobial resistance in Gram positive pathogens 86
5.4.1 *Staphylococcus aureus* **86**
5.5 Summary remarks 90
CHAPTER 6: CONCLUSION & RECOMMENDATIONS **91**
6.1 CONCLUSION **91**
6.2 RECOMMENDATIONS **91**
6.3 FUTURE STUDIES **91**
CHAPTER 7: REFERENCES **92**
ANNEXURE A **116**

LIST OF TABLES

Table 3. 1: Summary of the sample stratum (subgroup of isolates)..... 26
Table 3. 2: Selective media used to revive bacterial isolates for selected pathogens 27
Table 3. 3: List of antibiotics disk and their concentration used in this study. 31
Table 3. 4: The antibiotics concentration used for Gram-negative pathogens..... 33
Table 3. 5: The antibiotics concentration used for Gram-positive pathogens..... 33
Table 3. 6: The primers used for PCR for resistance genes in *Staphylococcus aureus* isolates. 35
Table 3. 7: Primer sequences and PCR condition used for *Listeria monocytogenes* in the study 36
Table 4. 1: Summary of the recovery rate of revived stored isolates for specific pathogens over a 20-year period 44
Table 4. 2: Distribution of antibiotic resistance of *Escherichia coli* and *Salmonella enterica* isolates using disc diffusion method 46
Table 4. 3: Resistance patterns of *Salmonella enterica* strains from various time periods, sources, and animal species 49
Table 4. 4: Resistance patterns of *Escherichia coli* strains isolated from poultry meat..... 52
Table 4. 5: Overall distribution of antibiotic resistance of *Listeria monocytogenes* and *Staphylococcus aureus* isolates using disk diffusion method 54
Table 4. 6: Resistance patterns of *Listeria monocytogenes* strains from meat sources and various animal species between 2015 -2017 56
Table 4. 7: Resistance patterns of *Staphylococcus aureus* strains from meat sources and various species in 2015. 58
Table 4. 8: Antibiotic Resistance Index 59
Table 4. 9: Overall distribution of antibiotic resistance of *S. enterica* and *E. coli* isolates using MIC method..... 60
Table 4. 10: Overall distribution of antibiotic resistance of *Listeria monocytogenes* and *Staphylococcus aureus* isolates using MIC method..... 62
Table 4. 11: Antimicrobial resistance genes detected in *S. enterica and E. coli* 67
Table 4. 12: Selected antibiotic resistance genes of *Staphylococcus aureus* in this study. 69
Table 4. 13: Genes detected from *Listeria monocytogenes* 69

LIST OF FIGURES

Figure 2. 1: A timeline displaying the decade in which novel antibiotic classes were developed for use in human medicine. Antibiotics are represented in different colors: green for *actinomycete*s, blue for other bacteria, purple for fungi, and orange for synthetic. The first reports of drug-resistant strains were methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), vancomycin-resistant *S. aureus* (VRSA), and plasmid-borne colistin resistance Sengupta *et al*., (2013). 7

Figure 4. 1: Distribution of resistance for *Salmonella enterica* strains by the year of isolation. 47

Figure 4. 2: Distribution of resistance for *E. coli* strains by the year of isolation. 50

Figure 4. 3: Distribution of resistance for *Listeria monocytogenes* strains by the year of isolation. 55

Figure 4. 4*:* Antimicrobial sensitivity pattern shift towards resistance of *S. enterica* and *E. coli* pathogens over the time period :(CHL: chloramphenicol, TET: tetracycline, AXO: ceftriaxone, AUG: amoxicillin, CIP: ciprofloxacin, GEN: gentamycin, XNL: ceftiofur, FIS: sulfisoxazole, SXT: trimethoprim, AMP: ampicillin). 64

Figure 4. 5: Antimicrobial sensitivity pattern shift towards resistance of *L. monocytogenes* and *S. aureus* pathogens: (TGC: tigecycline, CHL: chloramphenicol, DAP: daptomycin, CIP: ciprofloxacin, SYN: quinupristin/dalfopristin, LZD: linezolid, NIT: nitrofurantoin, ERY: erythromycin, VAN: vancomycin). 66

Figure 4. 6: PCR for detection of mecA gene in *S. aureus* isolates. Lane M contains Fast Ruler LR DNA ladder, lane 1 a positive control *S. aureus* ATTC 25923, lanes 2-13 positive samples, and lane 14 a negative control containing nucleic free water (H_20). 116

Figure 4. 7: Detection of quinolones genes in *Salmonella enterica* isolates by PCR. Lane M contains Fast Ruler LR DNA ladder, lanes 1-2 unspecific bands, lane 3 negative sample, lanes 4,7 unspecific band samples, lane 8 negative control containing nucleic free water (H_20), lane 9 positive control *Salmonella* Typhimurium ATCC 14028, lanes 10-11 positive samples. 117

Figure 4. 8: Detection of tetracycline genes in *L. monocytogenes*. Lane M contains Fast Ruler LR DNA ladder, lanes 1 and 2 positive samples, lanes 3-4 negative samples, lane 5 non-specific band, lane 6 negative sample, lane 7 positive control *L. monocytogenes* ATTC 19111, lane 8 negative control containing nucleic free water (H_20), lanes 9-14 positive samples. 118

LIST OF ABBREVIATIONS

AMR	Antimicrobial resistance
AMU	Antimicrobial use
ARC	Agricultural Research Council
AST	Antimicrobial Susceptibility Testing
ASSuT	Ampicillin Streptomycin Sulphonamides Tetracycline
bla_{SEP}	Beta-lactam resistance gene (pseudomonas extended-spectrum)
bla_{TEM}	Beta-lactam resistance gene
bla_{SHV}	Beta-lactam resistance gene (pencillin)
bla_{CMY}	Beta-lactam resistance gene (cephalosporin)
BMD	Broth Micro-Dilution
BHI	Brain Heart infusion
catA1	Chloramphenicol resistance gene
cmlA	Chloramphenicol acetyltransferase
CLSI	Clinical and Laboratory Standards Institute
mecA	methicillin resistance gene
ermA,B, C	Erythromycin resistance genes
EML	Essential Medical List
ESBL	Extended Spectrum Beta-Lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization
GAP	Global Action Plan
aap-aph	Gentamycin resistance gene
GLASS	Global Antimicrobial Resistance Surveillance System

HGT	Horizontal Gene Transfer
MIC	Minimum Inhibitory Concentration
MDR	Multi-Drug Resistance
OIE	Office International des Epizooties (World Organisation for Animal Health)
PCR	Polymerase chain reaction
SA	South Africa
SCCmec	Staphylococcal cassette chromosome mec
sul1, sul2 and sul3	Sulphonamide resistance gene
tetA, tetB& tet M	Tetracycline resistance gene
WHO	World Health Organization
WGS	Whole Genome Sequence
Van-A, Van-B, Van-C, *Van-D, Van-E &Van-G*	Vancomycin resistance genes

CHAPTER 1: GENERAL INTRODUCTION

1.1 BACKGROUND AND JUSTIFICATION

Bacterial foodborne diseases have been reported more frequently worldwide than those caused by viruses and parasites (Bintsis, 2017). In the United States, New-Zealand and Canada, foodborne illnesses are major contributors of morbidity and loss of productivity (Newman *et al*., 2015). Bacteria have developed resistance to antibiotics used in the medical and veterinary sectors (Economou and Gousia, 2015). The impact of bacterial resistance to antibiotics includes, among others, threatening the effective prevention and treatment of bacterial infection, prolonging infections in humans, resulting in higher healthcare costs and, in some cases, deaths (Manyi-Loh *et al*., 2018; Prestinaci et al., 2015). Costs due to antimicrobial resistance (AMR) of bacteria are quite high and vary greatly between nations (Dadgostar, 2019). The Centre for Disease Control and Prevention (CDC) calculated the cost of diseases caused by antibiotic resistant bacteria to be $55 billion annually in the United States, with $20 billion going toward medical expenses and around $35 billion going toward lost productivity (Chokshi *et al*., 2019; Prestinaci *et al*., 2015). Inequity will significantly rise because of antimicrobial resistance (AMR), which will cause the gap between developing and developed nations to widen. The majority of those pushed into extreme poverty because of AMR will be from low-income countries (www.worldbank.org).

Antibiotic-resistant bacteria associated with animals can be transmitted to humans via food products, direct contact with animals and with contaminated environments (Manyi-Loh *et al*., 2018). The great similarities in the antibiotic resistance profiles of bacterial strains that were isolated from humans and animals provides valid evidence that these microorganisms may be transmitted from animals to humans (Argudín *et al*., 2017; Peterson and Kaur, 2018). In support of this notion, several studies have reported colistin (last defense group of antibiotics) plasmid-mediated resistance in *Escherichia coli*, *Salmonella* and *Klebsiella* species isolated from food of animal origin that was comparable to human isolates in Africa, Asia, Europe, and North America (Bastidas-Caldes *et al*., 2022; Binsker, Käsbohrer and Hammerl, 2022; Sharma *et al*., 2022).

According to a systematic review of the clinical and economic impact of antibiotic resistance, ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*,

Acinetobacter baumannii, *Pseudomonas aeruginosa*, and *Enterobacter species*) are associated with the highest risk of mortality, resulting in increased health-care costs. (Founou *et al*., 2017). Based on their antibiotic resistance profile, the World Health Organization has published 12 bacteria species divided into three categories namely critical, high and medium *(*Tacconelli *et al*., 2018). *Salmonella* species and *E. coli* have been catalogued under high category against fluoroquinolone and third generation cephalosporin-resistant respectively (Asokan *et al*., 2019). Antimicrobial resistance is a rapidly growing problem linked to *E. coli* and *Salmonella* infections in both animals and humans, and it is best documented in human bacterial isolates, particularly in developing countries (Fuhrmeister and Jones, 2019). Conversely, *S. aureus* is categorized as having a high level of resistance to vancomycin and methicillin, while some international and local studies have found that *Listeria monocytogenes* strains isolated from food products have a greatly revived level of resistance (Kallipolitis et al., 2020; Matle *et al*., 2020; Mpondo and Ebomah, 2021).

One of the approaches to reduce the threat of AMR bacteria is through monitoring and surveillance systems (Velazquez-meza and Galarde-lópez, 2022). While searching for alternatives to current antibiotics, establishing trends in pathogen antimicrobial resistance, identifying emerging pathogens at the national and global levels, continuing the identification of antibiotics capable of effectively and safely treating and preventing infectious diseases, and limiting the spread of antimicrobial resistant microorganisms are critical for antimicrobial surveillance studies (Iskandar *et al*., 2021).

Monitoring specific pathogens over time will determine the emergence of resistance in specific strains or species and detect changes in the organisms' AMR profile. When longitudinal surveillance covers a large geographic area, one can eventually develop a useful understanding of regional, national, or even global trends in the distribution of resistant bacterial species (Fuhrmeister and Jones, 2019). Surveillance studies in general aim at improving the recording of emerging AMR, increasing the active life of antimicrobial drugs, and providing guidance for the development and use of newer drugs (Sharma *et al*., 2018).

In South Africa (SA), there is a lack of data on AMR of bacterial pathogens which could aid risk management in assessing the effectiveness of efforts to ensure responsible and prudent use, as well as mitigation strategies such as identifying changes in the veterinary field, prescribing practices, and indicating where changes in antimicrobial usage practices might be

appropriate (Bharatha and Hilaire, 2020). Therefore, it is crucial to conduct surveys to assess trends of bacterial antibiotic resistance in livestock, environmental sources, feeds and in food products in South Africa. Using laboratory-stored isolates collected over a long period is an important epidemiological tool to monitor the evolution and spread of antibiotic resistant bacteria from different sources (Mafuna *et al*., 2022). Retrospective testing of laboratory-stored isolates is an uncomplicated method to assess the occurrence and resistance pattern among bacterial populations from different sources in the country (Matle *et al*., 2019). In addition, it is useful to identify trends and patterns in development and persistence of AMR among zoonotic bacteria important in the empiric management of diseases (Matle *et al*., 2019).

1.2 PROBLEM STATEMENT

The last national AMR surveillance program in livestock in South Africa was carried out in 2007 (Henton *et al*., 2011). This means that in the last 16 years, the country did not report the occurrence of AMR bacterial pathogens to the World Organization for Animal Health (WOAH) (formerly known as OIE) as a signatory member state (personal communication). There have been several studies undertaken in the country to assess AMR bacteria isolated from animals and food of animal origin (Al-nabulsi *et al*., 2014; Gahamanyi *et al*., 2020; Madoroba *et al*., 2016; Pekana and Green, 2018). Although these studies provide important information, they still lack a comprehensive overview of the national occurrence of bacterial AMR pathogens. They focus on limited animal species and small geographical locations. Most importantly, they do not comply with the WOAH guideline for national surveys for AMR programme (www.oie.int). Antimicrobial profile surveys have been limited in scope, primarily focusing on the Gauteng and Western Cape provinces of South Africa (Matle *et al.*, 2019; Dufailu *et al.*, 2021). These studies revealed the presence of multi-drug resistant strains of bacteria to commonly used antimicrobials such as penicillin, erythromycin, ampicillin, trimethoprim, and nitrofurantoin. Therefore, it is important to establish whether AMR genes are present in South African isolates. However, the data on the quantity and patterns of antimicrobial agents used in food-producing animals in SA is limited (Henton *et al*., 2011) as commercial farmers tend to keep their production information away from the public. There is a gap in knowledge on AMR profiles and genetic determinants of resistance for zoonotic bacteria isolated from environmental sources and food and animals in SA. A lack of surveillance on AMR makes it impossible to keep track of the pathogens of major public health threats and limits the ability to assess and monitor trends of resistance worldwide. Such a

situation may lead to increasing rates of AMR and inability to map the spread of resistance, detect early outbreaks and set the national health policy to tackle resistance (Iskandar *et al*., 2021).

1.3 AIM AND OBJECTIVES

The main aim of the study was to generate data that feeds into the national antimicrobial surveillance database.

The specific objectives of this study were:

a) To detect the AMR genes responsible for antibiotic resistance from the stored South African bacterial isolates.

1.4 RESEARCH QUESTIONS

a) Could lyophilized and glycerol stored bacterial isolates from 2000 to 2021 be successfully revived on agar plates?
b) ii) Was the record data on sources and identity of bacteria accurate and traceable?
c) Is there a link between resistance genes detected and phenotypic analysis?

1.5 BENEFIT OF THIS STUDY

According to Dhingra *et al.*, (2020), antibiotic resistance is a major threat to clinical medicine and public health, not just in the developing countries but globally. Fuhrmeister and Jones, (2019) further explain that the challenge of AMR has been a persistent issue in many parts of the world, with emergence of multidrug-resistant strains of bacteria, viruses and fungi hampering medical progress. Collecting AMR surveillance data is identified as a critical step in defining the scope of the resistance problem, developing interventions to improve the appropriate use of antimicrobial agents, reducing resistance selection pressure while searching for alternatives to current antimicrobials drugs. Antimicrobial resistance monitoring is critical in disease management because it provides data that influences clinical decision-making when selecting the appropriate antibiotics for the treatment of patients with suspected infections or prophylaxis in patients at high risk of infection (Johnson, 2015).

1.6 DISSERTATION LAYOUT

This study comprises six chapters, arranged as follows:

Chapter 1: Introduction

This first chapter provides an overview and historical context for the study related to antimicrobial resistance worldwide. This section also outlines the problem statement, which highlights the purpose of the study, aims, objectives, research questions and the dissertation layout.

Chapter 2: Literature review

Chapter 2 provides an overview of existing literature on antibiotic classifications, categories and importance, antibiotic resistance profiles for different pathogens including gram-negative and gram-positive bacteria.

Chapter 3: Research methods overview

The research area, sampling method, data collection method and instruments used were outlined in this chapter. The ethical principles followed were also discussed.

Chapter 4: Results

This chapter includes the rate of survival of stored isolates, information extracted from records and molecular assessment for the presence of different genes coding for resistance.

Chapter 5: Discussion

This chapter comprises a comparison of the results with those from published papers by other researchers.

Chapter 6: General conclusions and suggestions

Assessment of objectives, limitations and challenges during this research and future perspectives related to this study are discussed.

CHAPTER 2: LITERATURE REVIEW

2.1 DEFINITION OF ANTIBIOTICS

Antibiotics are generally produced in nature by soil bacteria and fungi; however, some laboratory-based antibiotics have been- developed (Kapoor *et al*., 2017). Serwecińska, (2020) defines antibiotics as natural or synthetic chemicals able to affect the survival of microorganisms by inhibiting their growth (bacteriostatic) or killing them (bactericidal). Antibiotics, according to Chunrong *et al*., (2021), are medications used to treat and cure bacterial infectious diseases in both humans and animals by killing bacteria or by inhibiting bacterial growth. Antibiotics are classified as bacteriostatic or bactericidal, and also as narrow-spectrum or broad-spectrum agents. Bacteriostatic antibiotics keep bacterial populations stable by preventing bacterial cell proliferation. This enables the host's immune system to fight the infection or to eradicate the bacteria (Mehdi *et al*., 2018).

2.2 HISTORY OF ANTIBIOTICS

The age of antibiotics began in the 1920s with Alexander Fleming's discovery of penicillin (Figure 2.1). However, it took several years for penicillin to be introduced as a therapeutic agent (Fleming, 1929). Antibiotics have since transformed modern medicine and saved millions of lives (www.cdc.gov). Sulphonamides was first introduced in 1930 as an effective antibiotic against septicaemia (Figure 2.1). In the 1940s, antibiotics were first used to treat serious infections (www.cdc.gov). Penicillin was effective in treating bacterial infections in soldiers during World War II (Fleming, 1929; Sengupta *et al*., 2013). However, penicillin resistance quickly became a significant clinical problem in the same years, threatening many of the previous decade's advances by the 1950s (Fleming, 1929; Spellberg and Gilbert, 2014). New beta-lactam antibiotics were discovered, developed, and deployed in response, restoring confidence (Sengupta *et al*., 2013; Spellberg and Gilbert, 2014).

The first cases of methicillin-resistant *S. aureus* (MRSA) were identified in the United Kingdom in 1962 and in the United States in 1968. (Figure 2.1). In 1972, vancomycin was approved for the treatment of methicillin resistant *S. aureus* and coagulase-negative staphylococci. Resistance to vancomycin was thought to be extremely difficult to achieve in human medicine (Ahmed and Baptiste, 2018). In 1979 and 1983, cases of vancomycin

resistance were reported in coagulase-negative staphylococci (www.cdc.gov). The pharmaceutical industry has introduced numerous antibiotics to combat resistance between the late 1960s and early 1980s. However, the antibiotic pipeline had since began to dry up (Sengupta *et al*., 2013). As a result, bacterial infections have re-emerged many decades after successful treatment with antibiotics (Ahmed and Baptiste, 2018). Sulphonamides are still used today in human and veterinary medicine, but their therapeutic applications have been hampered by the emergence of certain resistance mechanisms (Ovung and Bhattacharyya, 2021).

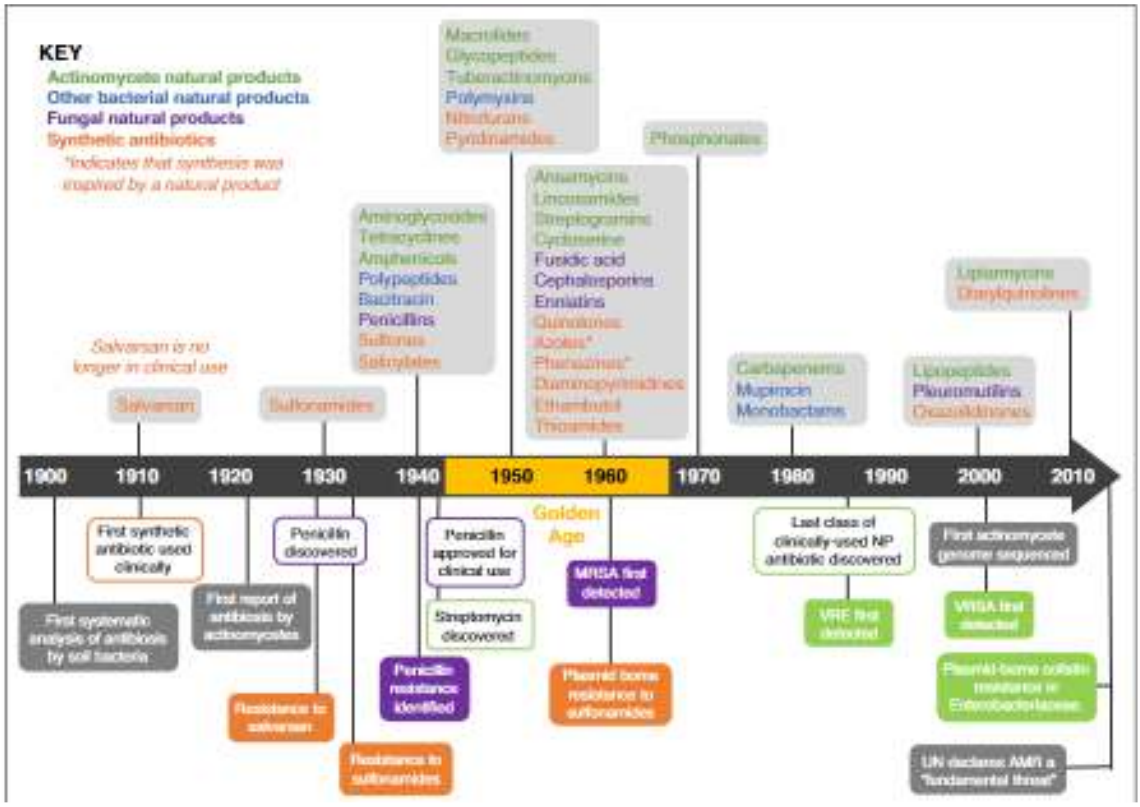

Figure 2. 1: A timeline displaying the decade in which novel antibiotic classes were developed for use in human medicine. (adopted from Sengupta *et al*., 2013).

2.3 ANTIBIOTICS CLASSIFICATION AND CATEGORIZATION

There are numerous ways of classifying antibiotics, most common classification schemes are based on their molecular structures, mode of action and spectrum of activity (Table 2.1). Other classifications are based on their effects as either bacteriostatic or bactericidal, and on their efficacy on the types of Gram stained bacteria (Gram negative and Gram positive) (Nwobodo *et al*., 2022). The antibiotics belonging to the same structural class often show similar pattern of effectiveness, toxicity, and allergic potential side effects (Ebimieowei Etebu, 2016). In veterinary medicine, common classes of antibiotics widely used include β-lactams, pleuromutilins, macrolides, tetracyclines, quinolones, aminoglycosides, sulphonamides, glycopeptides, and oxazolidinones. These antibiotics have raised concerned due to their potential adverse effects and risk management (Adzitey, 2015; Manyi-Loh *et al*., 2018; Van Hoek *et al*., 2011).

Table 2. 1: Classification of selected antibiotic groups based on mechanism of action.

Antibiotics Classes	Common antibiotic	Mode of action	Reference
B-Lactams	Penicillin Amoxicillin Ampicillin Menoprenem	Inhibit protein synthesis	
Glycopeptides	Vancomycin	Inhibit protein synthesis	
Aminoglycosides	Kanamycin Gentamycin	Inhibit protein synthesis	
Tetracyclines	Tetracycline Tigecycline	Inhibit protein synthesis	
Phenicols	Chloramphenicol	Inhibit protein synthesis	
Macrolides	Erythromycin Tylosin tartrate Azithromycin	Inhibit protein synthesis	(Reygaert, 2018)
Oxazolidinones	Linezolid	Inhibit protein synthesis	
Streptogramin	Quinupristin	Inhibit protein synthesis	
Nitrofurans	Nitrofurantoin	Inhibit protein synthesis	
Cephalosporins	Ceftiofur Cefoxitin Ceftriaxone	Inhibit protein synthesis	
Lipopeptides	Daptomycin	Depolarize cell membrane	
Quinolones	Ciprofloxacin	Inhibit nucleic acid synthesis	
Sulfonamides	Trimethoprim	Inhibit metabolic pathways	

2.4 THE IMPORTANCE OF ANTIBIOTICS

2.4.1 The application and use of antibiotics in animals

According to Nayiga *et al.*, (2020) and Caneschi *et al.*, (2023) antibiotics are used for various purposes in human and animal medicine, for the treatment and prevention of disease. In the veterinary field, antibiotics are used for treatment of diseases and for non-therapeutic purposes such as prophylaxis and metaphylaxis and as growth promoters (www.ahpsr.WHO.int/global-action-plan-on-antimicrobial-resistance). The application of antibiotics as feed proficiency enhancers and growth promoters in animals poses a serious challenge since many of them closely resemble drugs used in human medicine (Dufailu *et al*., 2021). To support this notion there have been reports indicating that the use of antibiotics in small doses in food animals is a significant driving factor to increasing antimicrobial resistance in humans (Choffnes *et al*., 2012; Lekshmi *et al*., 2017; Van Boeckel *et al*., 2015). For instance, Dutil *et al*., (2010) reported a strong correlation between ceftiofur-resistant *Salmonella* Heidelberg and *E. coli* in chicken and human infections in Canada. Their study showed that temporary withdrawal of ceftiofur usage in chicken led to reduction of resistance in human *Salmonella* isolates. However, the trend reversed upon the reuse of the same antibiotic. The high usage of antibiotics in agriculture due to intensive farming and globalization are a major risk for the transmission of AMR bacteria from livestock to humans (Zhao *et al*., 2021).

2.4.2 Important microorganisms in antimicrobial resistance

Ramsamy *et al.*, (2018) described the ESKAPE group, which consists of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp*., as requiring serious attention. This is due to their association with antibiotic resistance and infections which result in high mortality and morbidity rates; increased healthcare costs; diagnostic dilemma and difficulty in the initiation of treatment. These pathogens feature prominently in the global pathogen priority list of antibiotic-resistant bacteria as reported in 2021 by the World Health Organisation (www.hhs.gov/sites/default/files/who-update-amr.pdf), with the aim of tackling the threat of AMR. The list is ranked into three levels which is critical (top level), high, and medium priority. The bacteria from the *Enterobacteriaceae* family, such as *E. coli*, are regarded as critical priority pathogens, which pose threat to the life. Foodborne pathogens such as *Staphylococcus aureus*, are considered high priority pathogens. These pathogens are resistant to numerous

antibiotics such as vancomycin and quinolones. Pathogens in the medium category include *Shigella*, which may be resistant, but effective antibiotics are available to treat them.

2.5 DEVELOPMENT OF ANTIBACTERIAL RESISTANCE

There are various mechanisms involved in the development of bacterial resistance to antibiotics. These include drug uptake limitation, drug target modification, drug inactivation, and active efflux of a drug (Reygaert, 2018). These mechanisms could be native to the microorganisms or acquired from other microorganisms. Drug target modification, drug inactivation, and drug efflux are examples of intrinsic resistance mechanisms which can also be acquired. During these mechanisms, microorganisms change or destroy the antibiotics with enzymes and proteins that break down the drug. Gram negative bacteria, for example, use different mechanisms than Gram positive bacteria due to structural differences. Gram negative bacteria use all four major mechanisms, while Gram positive bacteria use limiting drug uptake due to the absence of a lipopolysaccharide (LPS) outer membrane and inability to use certain types of drug efflux mechanisms (Chancey *et al*., 2012).

2.5.1 Drug uptake limitation

Gram negative bacteria are intrinsically less permeable to certain antibiotics due to the presence of an LPS layer (Uddin *et al.*, (2021). The ineffectiveness of glycopeptide antibiotics, such as vancomycin, against Gram-negative bacteria due to lack of penetration through the outer membrane is a prime example of the effectiveness of this natural barrier (Uddin *et al*., 2021). Hydrophilic molecules, such as β-lactams, tetracyclines, and some fluoroquinolones diffuse through the outer membrane porin proteins(Blair *et al*., 2015).

2.5.2 Drug target modification

Acquired changes in antimicrobial target sites, which may prevent drug binding, are a common mechanism of resistance. The target site changes are the result of a chromosomal spontaneous mutation of a bacterial gene (Kapoor *et al*., 2017). Antibiotic interaction with target molecules is generally specific, minor changes to the bacterial target molecule can have a significant effect on antibiotic binding. For example the modification of the PBP (penicillin-binding protein) is a preferred mechanism of Gram-positive bacteria resistance (Kapoor *et al*., 2017). The presence

of a mutation in the PBP reduces its affinity for β-lactam antibiotics. A structural change, such as the development of the *mec*A gene in *S. aureus*, reduces or eliminates drug binding *(Guo et al*., 2020).

2.5.3 Drug inactivation

Bacteria inactivate antibiotics either by chemically altering or destroying the drug.

2.5.3.1 Chemical modification of the drug

Bacteria produce enzymes that can attach different chemical groups to drugs. This prevents the antibiotic from binding to its target in the bacterial cell. The transfer of phosphoryl, acetyl, and adenyl groups to the compound is the most effective method of drug inactivation of aminoglycosides, chloramphenicol, streptogramins, and fluoroquinolones. Aminoglycosides are thought to be formed via adenylation and phosphorylation *(Krause et al., 2016 ; Khan et al*., 2020).

2.5.3.2 Destroying the drug

The most used antimicrobial agents are β-lactam antibiotics such as penicillin and cephalosporins (Bush and Bradford, 2016). The central structure of this class of drugs is a four-sided -lactam loop that is shared by all members. The β-lactamase activity destroys the β-lactam loop, which is the key mechanism of β-lactam resistance. β-lactamases hydrolyze β-lactam ring formation, preventing it from binding to penicillin-binding proteins (PBP) (Bush and Bradford, 2016).

2.5.4 Drug efflux

Blanco et al., (2016) reported that efflux pumps were first identified as a tetracycline resistance mechanism in *Escherichia coli*. Although many antibiotics are actively transported out of the cell by bacterial efflux pumps, which play an important role in Gram-negative bacteria's intrinsic resistance. Efflux pumps are found in a wide variety of bacteria The five primary families of efflux pump are the ATP-binding cassette (ABC) family, small multidrug resistance (SMR) family, multidrug and toxic compound extrusion (MATE) family, resistance-nodulation-cell division (RND) family, and large facilitator superfamily (MFS) (Reygaert,

2018). Except for the RND family, which consists of multi-part pumps that efflux substrates across the cell envelope, all other efflux pump families are single-part pumps that transport substrates across the cytoplasmic membrane (Reygaert, 2018).

2.6 POLICIES GOVERNING ANTIMICROBIAL RESISTANCE IN SOUTH AFRICA

South Africa (SA) has two acts that govern the administration of antimicrobial agents in animals: the Fertilizers, Farm Feeds, Agricultural Remedies, and Stock Remedies Act (Act No. 36 of 1947) and the Medicines and Related Substances Control Act (Act No. 101 of 1965). Act No. 36 of 1947, which deals with over-the-counter medications, is controlled and administered by the Department of Agriculture, Land Reform, and Rural Development (DALRRD). Act No. 36 of 1947 governs the use of antimicrobials for growth promotion and the purchase of antimicrobials by the general public, specifically farmers, over the counter (OTC). The DALRRD is in charge of ensuring farmers' access to veterinary medicines for disease control and improved food production, as well as public safety by monitoring residues (including antibiotics) in food-producing animal products. It allows veterinarians to use off-label medications. Act No. 101 of 1965, which requires a veterinarian's prescription and is published in the government gazette, is overseen by the National Department of Health. The Medicines and Related Substances Act (Act 101 of 1965) establishes the legal framework for ensuring medication safety, efficacy, and quality. It also provides for the regulation of veterinary medicines to ensure that they are produced, distributed, and used in a way that does not endanger human or animal health. Only a veterinarian can administer or prescribe antimicrobials registered under Act 101 for use in animals. This act also permits the compounding of pharmaceuticals for use in animals (Sykes *et al*., 2019).

South Africa as other countries in the world has been sensitized about public health concern related to antimicrobial resistance. As part of South Africa strategy, few bodies were created namely National Veterinary Surveillance and Monitoring for Resistance to Antimicrobial Drugs (SANVAD) in 2003, Global Antibiotic Resistance Partnership- South Africa (GARP-SA) in 2011, Federation of Infectious Diseases Societies of South Africa (FIDSSA) and South African Stewardship Program (SAAP) which include practitioners from various fields who develop the South African Antimicrobial Resistance Strategy Framework (Moyane *et al*.,

2013). The main objectives of this framework were to enhance antimicrobial resistance surveillance, antimicrobial stewardship and improve prevention and control of infection.

2.7 SURVEILLANCE OF ANTIMICROBIAL RESISTANCE

AMR surveillance allows for continuous monitoring of trends in AMR prevalence and is an essential tool in combating the growing threat of AMR globally. To reduce AMR-related mortality and morbidity, surveillance is required to inform policymakers, regulators, and clinicians about international policy and local antimicrobial stewardship activities in health facilities (Sugianli *et al*., 2020). Surveillance and monitoring are widely recognized as crucial components of the antimicrobial resistance response from the Global Action Plan (GAP) on AMR's five strategic priorities (www.who.int). Surveillance is critical to any AMR containment strategy because it provides the data needed to locate an antimicrobial resistance problem, monitor its growth, transmission, and direction of travel, and assess the impact of interventions aimed at containing it (www.who.int) Because the barriers to establishing surveillance systems are high, particularly for poorer nations, collective action is required to produce effective surveillance systems.

The dominant strategies nationally, regionally and globally comprise the surveillance of AMR and the tracking of antimicrobial consumption, promoting research and development of new antimicrobials as well as alternative treatments, and effective implementation of measures ensuring appropriate and rational use of existing antimicrobials (www.who.int). According to OIE guidelines, South Africa's surveillance program should focus on three types of bacteria: indicator bacteria, zoonotic bacteria, and animal pathogenic bacteria. These categories offer the best opportunities to detect resistance when selective pressures are applied, carrier animals of zoonotic bacteria are treated, and clinically ill animals are treated, respectively (Mupfunya *et al*., 2021). The benefit of collecting data through various antibiotic resistance surveillance systems has been demonstrated to provide information on the actual burden of resistance at the local, national, and international levels (Diallo *et al*., 2020).

2.8 SURVEILLANCE METHODS FOR ANTIMICROBIAL RESISTANCE

Effective surveillance systems collect baseline data on the incidence trends and geographic distribution of known infectious agents. It is necessary to be able to provide such information

in order to detect new or re-emerging threats (American Centre for Disease Control, 2019). There are several approaches towards surveillance of AMR, which include laboratory-based, active, population-based and sentinel surveillance (Hazim *et al*., 2018). According to Eloit, (2018) antimicrobial resistance surveillance should be done on planned intervals to monitor, assess and determine the trends and sources of antimicrobial resistance in bacteria and detect the emergence of new bacterial antimicrobial resistance mechanisms. Bacteria to be evaluated include animal bacterial pathogens relevant to the countries' priorities. In SA, zoonotic bacteria such as *Salmonella* species, *Campylobacter jejune* and commensal bacteria such as *E. coli* and *Enterococcus* are considered important. Other animal bacterial pathogens, such as *S. aureus* and *Streptococcus* species, are primarily derived from severe or recurring clinical cases, including therapy failure, which might give biased data. Commensal bacteria are commonly utilized as indicators in surveillance and monitoring activities, providing information on a potential reservoir of antimicrobial resistance genes that could be passed on to pathogenic bacteria (Varga *et al*., 2008). According to OIE, (2017), the tests that should be performed to determine antimicrobial resistance or sensitivity should be reported quantitatively using minimum inhibitory concentration (MIC).

2.8.1 Laboratory based surveillance

The general perception is that laboratory-based surveillance is currently the most efficient method of surveillance of AMR, which is what the WHO and GHSA (global health security agenda) advocate. Laboratory-based AMR surveillance is recognized as a priority for developing antibiotic resistance strategies and assessing the impact of interventions (www.who.int). Laboratory-based surveillance with information traceable to that of the patient is regarded as the most efficient and feasible approach because the data are generated by laboratories that routinely identify and determine the susceptibility of bacteria isolated from clinical specimens. Laboratory-based surveillance plays an important role as it provides data on the location and frequency of isolation of specific pathogens, rapidly detecting unusual increases or clusters (Hutwagner *et al*., 1997). Despite the fact that laboratory-based surveillance is known to overestimate AMR prevalence due to bias, WHO recommends it to obtain actionable AMR data at both the local and national levels (Sugianli *et al*., 2020).

2.8.2 Population based surveillance

Population-based surveillance is based on the observation of individuals in a defined population who exhibit signs and symptoms consistent with a clinical case definition and provides more precise data on the prevalence of AMR in this population. However, population-based surveillance is frequently regarded as being too time-consuming and may necessitate resources and capacity that are not available in areas where patients present with symptoms (www.who.int).

2.8.3 Sentinel surveillance

Sentinel surveillance is a voluntary network of laboratories and public health departments that monitors the rate of occurrence of specific diseases in order to assess the stability or change in health levels of a population. It is also used to obtain data about a specific disease that cannot be obtained through a passive system, such as summarizing standard public health reports "Sentinel Surveillance-MeSH-NCBI" (www.ncbi.nlm.nih.gov). It is believed to be the best type of surveillance especially when an intensive investigation of each case is conducted to collect the necessary data. However, it requires more time and resources (www.who.int).

2.8.4 Active surveillance

When regulators initiate data collection from the lab, this is known as active surveillance. Because active surveillance achieves more complete and accurate reporting than passive surveillance, it is frequently used during outbreak investigations or research studies. The limitation is that it necessitates more resources from the public health agency responsible for active surveillance (www.who.int).

2.9 DIAGNOSTIC TESTING FOR ANTIMICROBIAL RESISTANCE

2.9.1 Phenotypic methods

Veterinary and public health AMR bacteria are routinely detected using standardized phenotypic methods (Anjum, 2017). The traditional method for screening for AMRs is to culture samples on non-selective or antibiotic-selective agar plates, purify bacterial colonies, and use various assays. AMR is measured or determined using agar dilution, disk diffusion, E-

test, broth dilution, gradient strip, and other similar methods (Randall *et al*., 2014). Bacterial isolate susceptibility testing allows not only for isolate differentiation, but also for the assessment of developing resistance. Susceptibility testing methods include disk diffusion (National Committee for Clinical Laboratory Standard; 2000), agar dilution (NCCLS, 1992), E-test (Brown & Brown, 1991) and broth micro-dilution assays (Burrows *et al*. 1993).

2.9.1.1 Agar dilution

The agar dilution method involves preparing a series of agar plates containing the antimicrobial agent to be tested in increasing concentrations, usually in doubling dilutions (i.e., 1, 2, 4, 8, 16, 32 µg/ml). A suspension of the organism tested is prepared to equal the turbidity of a 0.5 McFarland standard (1.5×10^8 colony forming units (CFU/ml) and 1–5 µl of this suspension is placed on each of the series of plates with increasing concentrations of the antimicrobial agent using a replicator device (final inoculum is 5×10^4 CFU/spot). The CFU are then counted after incubation depending on the type of pathogen tested. This method is advantageous because thirty different bacterial isolates including quality control organisms can be tested simultaneously on each agar plate. However, it is time-consuming because each set of agar plates for each antimicrobial agent to be tested must be prepared (Tenover *et al*., 2019).

2.9.1.2 Gradient test

To determine the MIC value, the antimicrobial gradient method combines the principles of dilution and diffusion methods. It is predicated on the ability to create a concentration gradient of the antimicrobial agent tested in agar medium. In this method, an agar surface previously inoculated with the microorganism tested is impregnated with an increasing concentration gradient of the antimicrobial agent from one end to the other (Balouiri *et al*., 2016). This assay is simple to perform and has an immobilized antimicrobial gradient indicated on the ruler, ensuring a simpler way of directly quantifying the susceptibility of microorganisms, particularly those that are difficult to culture, but it is very expensive (Galhano *et al*., 2021).

2.9.1.3 Disk diffusion

Since its inception in the 1940s, the disk diffusion method has remained the most widely used routine antimicrobial sensitivity test in clinical microbiological laboratories (Heatley and

Wiuiam, 1943). It was created to evaluate the susceptibility of the most common and clinically relevant bacteria that cause human diseases.
(CSLI,2018; www.eucast.org/clinical_breakpoints/). The disk diffusion method is regarded as the gold standard for confirming the susceptibility of bacteria which was standardized and introduced by Bauer and Kirby's experiments in 1956 (Bauer *et al*., 1966; Beargie *et al*., 1965). Disk diffusion refers to the diffusion of an antimicrobial agent in a specified concentration from disks, tablets, or strips into solid culture medium seeded with the selected inoculum isolated in a pure culture (Kahlmeter *et al*., 2006). The mechanism of disk diffusion is based on the formation of an inhibition zone proportional to the bacterial susceptibility to the antimicrobial present in the disk. The isolated bacterial colony is selected, suspended in growth media, and standardized using a turbidity test in this method (Kahlmeter *et al*., 2006).

The antibiotic-treated paper is then placed on the surface of the agar plate after the standardized suspension has been inoculated onto the solidified agar plate. After an overnight incubation at 35 °C, the antibiotic-containing disc is allowed to diffuse through the solidified agar, resulting in the formation of an inhibition zone. The size of the inhibition zone formed around the paper disc is then measured and compared to the published standard of measured zones of inhibition against reference strains of microorganisms. The main benefits are simplicity, reproducibility, ease of modifying antimicrobial discs, ability to use as a screening test against a large number of isolates, and low cost (Balouiri *et al*., 2016; Salam *et al*., 2023). However, the manual zone of inhibition measurement can be time consuming (Kahlmeter *et al*., 2006). The agar disk-diffusion method is ineffective for determining the minimum inhibitory concentration (MIC) because it is impossible to quantify the amount of antimicrobial agent diffused into the agar medium (Balouiri *et al*., 2016).

2.9.1.4 Broth micro-dilution

Aside from disk diffusion susceptibility tests, the most commonly used methods include broth macro- and microdilution, as well as agar dilution, which serve as reference methods (Moreira *et al.*, 2018; Gajic *et al.*, 2022). The minimum inhibitory concentrations (MICs) of antimicrobial agents (i.e., the lowest concentration at which the agent inhibits the growth of microorganisms) can be determined using broth and agar dilution methods (Gajic *et al*., 2022; Kowalska-krochmal and Dudek-wicher, 2021). Broth micro-dilution is the standard method used in most reference laboratories. The method typically tests two-fold dilutions of multiple

antimicrobial agents in 96-well disposable plastic trays. The test medium is typically cation-adjusted Mueller–Hinton broth, or for fastidious organisms, cation-adjusted Mueller–Hinton broth containing 5% lysed horse blood. A suspension of the organism to be tested is prepared in saline or Mueller–Hinton broth to the turbidity of a 0.5 McFarland standard (1×10^8CFU/ml). The suspension is diluted 1:20 in saline, and 1–5µl of this suspension is transferred to all but one well of the 96-well tray containing doubling dilutions of the antimicrobial agents to be tested (usually between 8 and 12 antimicrobial agents per tray) using a disposable plastic inoculator (the remaining well is a broth sterility control). The final inoculum size is 5×10^5CFU ml^{-1}or 5×10^4CFU/well (Tenover et al., 2019).

The benefits of the micro-dilution procedure include the generation of MICs, the reproducibility and convenience of having pre-prepared panels, and the savings in reagents and space as a result of the test's miniaturization (Benkova *et al*., 2020). Moreover, MIC value is used to assess the pathogen's susceptibility category to a given antibiotic for organisms that produce ambiguous results, and especially when no clinical breakpoints for disk diffusion are available. Unlike a qualitative method, the MIC value can be used to determine the degree of susceptibility or resistance to an antibiotic (Kowalska-krochmal and Dudek-wicher, 2021). The main disadvantage of the micro-dilution method is that it is less adaptable to changing monitoring needs than agar dilution or disk diffusion (Kahlmeter *et al*., 2006; Salam *et al*., 2023).

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) was used in the study to provide clinical breakpoints of MIC for different antimicrobials against veterinary and human pathogens. Clinical breakpoints are determined by a review of MIC distribution data, pharmacokinetic-pharmocodynamic parameters, and clinical response and used in routine clinical laboratory work to inform the choice of therapy in the patient (Silley, Simjee and Schwarz, 2012; EUCAST, 2013). Determination of MICs by means of the broth micro-dilution assay is particularly useful in evaluating incremental changes in the development of resistance (EUCAST 2022). According to Matuschek *et al*., (2014), the epidemiological cut-off values determine whether a bacterial isolate is sensitive or resistant to various antibiotics and is very informative. However, it is time consuming and may take several days to perform the full panel of MICs on isolates after purification.

2.9.1.5 MALDI- TOF spectrometry

Due to its high diagnostic accuracy, robustness, reliability, and rapid turn-around time, MALDI-TOF spectrometry has been introduced as a diagnostic technique in microbiology laboratories, replacing most other tools such as phenotypic tests, biochemical identification, and agglutination kits as the first-line pathogen identification method. MALDI-TOF spectrometry has several advantages over PCR assays, aside from the fact that it is commonly used for bacterial identification. Its primary advantage is that individual pathogen identification is inexpensive, and sample preparation does not necessitate highly skilled technicians or complex additional laboratory infrastructure. It is also less susceptible to contamination, and results are available in a matter of minutes. However, the technique requires a constant power supply, limiting its applicability in resource-constrained settings (Feucherolles *et al*., 2019).

2.9.2 Molecular based methods for the detection of antimicrobial resistance

Molecular methods are frequently used in conjunction with phenotypic methods, but they are set to replace them in many laboratories due to the increased speed and accuracy in detecting the underlying genetic mechanism(s) for AMR (Anjum *et al*., 2017) . Only the concentration of an antimicrobial that inhibits bacterial growth in vitro to breakpoints used to predict clinical success during treatment is referred to as phenotypic susceptibility. It provides no indication of the resistance mechanisms that may spread to other bacterial species via mobile genetic elements (MGEs). For this, molecular or genotypic characterization is required. There are currently a plethora of PCRs used to detect the presence of AMR genes, including standard, real-time, and multiplex PCRs (Anjum, 2015; Galhano *et al*., 2021). However, the limitation of using only molecular approaches is that new and emerging resistances to some of the last-line antibiotics may be overlooked (Yong *et al*., 2009). It is critical to conduct both genotypic and phenotypic screening, even if the latter is done on a small scale. It is also critical to track resistances in bacteria not only in clinical samples but also in healthy humans, animals, and the environment in order to gain a better understanding of the baseline levels of AMR in these niches and the potential measures that can be taken to control its rise in the future (Anjum, 2015).

2.9.3 Conventional PCR methods

According to Galhano *et al.*, (2021), in the testing laboratories, PCR is used to rapidly and efficiently identify bacteria and resistance genes from various environments. One of its significant advantages over traditional cultivation is the possibility of amplification of genes from existing microorganisms that are not cultivable or are dead, and thus cannot be identified using traditional methods. The RNA molecule is transcribed to a complementary DNA molecule (cDNA) and amplification is performed using standard PCR in an RT-PCR reaction. This method has high specificity, sensitivity, and reliability; however, it has the disadvantage of high RNA molecule instability, which makes sample processing the most difficult because it requires qualified and prepared personnel, making analysis time-consuming and expensive (Galhano *et al*., 2021).

2.9.4 Isothermal amplification method

Isothermal DNA amplification has recently been developed. It eliminates the need for thermo-cycling, which is required in traditional PCR methods. Several methods have been developed, including strand displacement amplification, transcription mediated amplification, nucleic acid sequence-based amplification, and others. The main advantages of isothermal over conventional PCR-based methods are the elimination of thermo-cycling and the use of a water-bath or hotplate to regulate the temperature, resulting in low power consumption and reduced analysis time. Furthermore, unlike PCR, isothermal amplification is faster and more sensitive. However, isothermal multiplexing approaches are less successful as the difficulty of the experimental design increases (Kaprou *et al*., 2021).

2.9.5 DNA microarrays

Another technique that can be used to investigate genetic AMR in bacteria is the microarray. This technology has long been considered the gold standard for studying transcriptomes. Furthermore, it enables the investigation of gene expression through the hybridization of oligonucleotide sequences that purify and amplify specific molecules of RNA from the sample of interest. The microarray technique is used for a variety of purposes, the most common of which is to determine the function of specific genes. However, its main limitation is the requirement for prior knowledge of the genomic regions to be studied, as well as hybridization

of similar sequences, which complicates target gene reading and analysis (Galhano *et al*., 2021).

2.9.6 Other molecular methods

2.9.6.1 Genomes sequencing

To identify AMR genes, whole-genome sequence (WGS) comparisons of isolates/genomes against known genes catalogued in several AMR reference gene databases, such as ResFinder and the Comprehensive Antibiotic Resistance Database, can be used. The database is well-known for housing thousands of publicly accessible gene sequences (Fair and Tor, 2014; Stoesser *et al*., 2013). WGS DNA extracted from tested samples is assembled using De Bruijn graph-based programs such as SPAdes, Velvet, ABySS, and SOAPdenovo. Contigs are assemblies formed from small sequencing reads that can be annotated to search for resistance genes. Resfinder, ARG-ANNOT, RGI, ARGs-OAP, RGI, ARGs-OAP (v2), ARIBA, PointFinder, NCBI-AMRFinder, SRST2, SEAR, ShortBRED, PATRIC, SSTAR, KmerResistance, GROOT, and DeepArgs are some of the methods used to find resistance genes. The purpose of each study (i.e., resistance genes, virulence genes, proteins) and the sequence confidence deposited in each database influence the database selection (Boolchandani and Dantas, 2020).

2.9.6.2 Metagenomics

Metagenomics is another molecular method that studies microbial genomes without cultivating them, allowing for culture-independent cloning and analysis of microbial DNA directly from the environment (Zhang *et al.,* 2021). It investigates microbial communities collectively, revealing the presence of new microbial species, genes, and functions. Metagenomics analysis employs two primary methods. i) Sequence-based metagenomics is the direct sequencing of large DNA libraries to gain access to community gene pools, whereas function-based metagenomics uses functional screens and selections to identify active clones in metagenomic libraries. The advantage of the sequence-based approach is that it does not rely on host cells to express the cloned genes, but it cannot screen for unknown genes with completely different sequences from existing genes (Chaudhari *et al.,* 2023). While the function-based approach is

simple, fast, and does not rely on known sequence information, it is dependent on the expression of functional genes in foreign hosts, which results in a low screening rate (Zhang *et al.,* 2021).

2.10 CONCLUSION

Antibiotics have made significant contributions to the advancement of healthcare over the last 50 years. As a result, the ongoing increases in AMR must be reversed or modern medicine will be relegated to the stone age. Antibiotic resistance is a naturally occurring mechanism that can be gradually slowed but not completely stopped because resistance is an unavoidable result of medication selective pressure. As a result, AMR necessitates a collaborative effort to combat, i.e., relationships are formed on a global scale between private and governmental organizations, researchers, manufacturers, pharmaceutical companies, hospitals, policymakers, regulatory bodies within the agriculture industry, and patients. The primary goal of this collaboration should be to slow the current trends in AMR to limit the negative consequences for society and the economy. Effective governance is critical to sustaining AMR performance and safety in health care systems. Agriculture and animal health should be added to this list.

CHAPTER 3: MATERIALS AND METHODS

3.1 STUDY DESIGN

This is a retrospective study, data collected were based on existing records. Bacteria were isolated for diagnostic purposes and stored as per standard operating procedure of the accredited General bacteriology laboratory, Agricultural Research Council, and thus not principally for use in this research. The identity of isolates were known and reconfirmed for the purpose of this study. This study examined stored bacterial isolates of sixteen *Salmonella* serotypes (*S*. Bareilly, *S*. Bovismorbificans, *S*. Dusseldorf, *S*. Enteritidis, *S*. Infantis, *S*. Isangi, *S*. Muenchen, *S*. Orion, *S*. Schwarzengrun, *S*. Senftenburg, *S*. Typhimirium, *S*. Ablogame, *S*. Choleraesuis, *S*. Coela, *S*. Derby and *S*. Hadar), *E. coli*, *L. monocytogenes*, and *S. aureus* from animal, food of animal origin, animal feed, and the environmental sources for antibiotic

resistance and molecular profiling. The stored bacterial isolates were recovered from samples collected across nine provinces of SA between 2000 and 2021.

3.2 SELECTION OF BACTERIAL STRAINS

Bacteria belonging to the ESKAPE group such as *E.coli* and *S. aureus* and those commonly reported in food and animal samples in the country namely *L. monocytogenes* and *S. enterica* were selected for this study (Thwala *et al*., 2021). The ESKAPE pathogens require special attention due to their relationship with illnesses that have significant mortality and morbidity rates and drug resistance. The ESKAPE pathogens are known for their ability to escape the effect of bactericidal activity of antibiotics (Mulani *et al*., 2019). Furthermore, *S. enterica*, *E. coli*, and *L. monocytogenes* were chosen because they are the most common foodborne bacterial pathogens that pose a significant burden in the food industry and health sector in South Africa (Abatcha *et al*., 2020; Matle *et al*., 2019; Thwala *et al*., 2021). The World Health Organization published the global pathogen priority list of antibiotic-resistant bacteria in 2017 to combat the threat of AMR, and the majority of selected bacterial pathogens are prominently represented (Asokan *et al*., 2019).

3.3 SAMPLE ORIGIN AND SOURCES OF ISOLATES

The bacterial isolates used in this study were stored at the national reference laboratory hosted at Agricultural Research Council for the identification of many bacterial agents and is mandated by Veterinary Procedural Notification (VPN) 56 to store bacterial strains for research purposes (www.dalrrd.gov.za/vetweb/VPN).

The stored bacterial isolates were recovered from samples submitted by animal health technicians, veterinarians, farmers, and the public, for routine diagnostic services at the General bacteriology laboratory. A variety of samples from different sources were processed and comprised of samples from abattoirs, farms, butcheries, informal markets and retail outlets across the nine provinces of SA between the years 2000 and 2021. The types of bacteria used in this study were isolated from various animal species including, swine, bovine, poultry, and wildlife. The sample type ranged from animal food products (i.e., ready-to-eat meat, raw meat, processed meat and milk), and environmental samples.

3.4 SAMPLE SIZE

The overall population (total number of bacterial isolates stored in the laboratory) of this study consisted of 2752 isolates which were divided into 2 groups namely Gram negative and Gram positive. The Gram-negative group consisted of 1268 isolates while Gram positive was 1484. The Gram negative were further subdivided into two subgroups (*S. enterica* and *E. coli* while Gram positive comprised of two subgroups (*L. monocytogenes* and *S. aureus*).

This study adopted sample size formula cited in Israel, (2013); Puszczak et al., (2013); Sarmah and Hazarika, (2012), to determine minimum representative sample size of isolates out of 2752 (N) population of isolates. Given the known population proportion $\hat{p}$ of 25% i.e., $\hat{p}$= 0.25 and using 7% as margin of error (e = 0.07), where confidence level is 95% with corresponding standard normal deviation (z) of 1.96, then the initial sample size is given by:

$$n_0 = \frac{\hat{p}(1-\hat{p})Z^2_{\alpha/2}}{e^2}$$

$$n_0 = \frac{(0.25)(0.75)(1.96)^2}{(0.07)^2}$$

$$n_0 = \frac{(0.1875)(3.8416)}{0.0049}$$

$$n_0 = \frac{0.7203}{0.0049}$$

$$n_0 = 147$$

Given the population (N) of 2752 then the final sample size n which is calculated as:

$$n = \frac{n_0}{1 + \frac{(n_0 - 1)}{N}}$$

$$n = \frac{147}{1 + \frac{(147 - 1)}{2752}}$$

$$n = \frac{147}{1.0531}$$

$$n = 140$$

Applying the formula, the minimum sample size of this study was 140 isolates. However, 216 isolates were selected for this study. Furthermore, the study had determined the sample size proportional to each stratum (subgroup of isolates) using stratified proportional sampling. Stratified proportional random sampling breaks subgroups into stratums. The division of population by strata helps to easily choose the appropriate number of individuals from each stratum based on proportions of the population. Therefore, the Noor *et al*., (2022)study was adopted in order to determine the sample size of each stratum (subgroup of isolates) and is given by the following formula:

$$n_i = n.\frac{N_i}{N}$$

Where, n_i = required sample size for each stratum, n = sample size (163, N_i = population size for each stratum and N = size of the population (2752). The sub-population (N_i) consists of *S. enterica* (n = 898), *E. coli (*n = 370), *L. monocytogenes* (n=398) and *S. aureus* (n=1086) isolates. Applying equation 2, the proportional sample size for within each stratum (subgroup of isolates) to be used in this study as illustrated in Table 3.1.

Table 3. 1: Summary of the sample stratum (subgroup of isolates).

Isolates	**Pathogens**			
	Gram negative (1268)		**Gram positive (1484)**	
	S. enterica	*E. coli*	*L. monocytogenes*	*S. aureus*
N_i	898	370	398	1086
n_i	88	30	34	64

n_i = required sample size for each stratum, Ni = population size for each stratum

3.5 SAMPLING METHOD

A simple random sampling without replacement (SRSWOR) was used to select 216 samples of isolates (Pathak, 1988). This process ensures that each pathogen in the population of pathogens has a known and equal probability to be selected and be part of the trial. This was achieved by assigning each pathogen a code to a unique computerized number using randomized number generation in MS excel programme. The first 216 pathogens after randomization were used as the selected pathogens to be used in the trial.

3.6 MICROBIOLOGICAL ANALYSIS

3.6.1 Revival of isolates

The isolates were preserved as either freezer-dried (lyophilized) or in 40% glycerol (1/1 volume, stored at -80 ºC). For isolates preserved in glycerol, they were placed at room temperature to allow thawing and centrifuged at 13,000 rpm for 1 min (Biorad, Cape Town, South Africa). The supernatant was discarded, and 3 mL Brain Heart Infusion (BHI) broth (Thermo-Fisher Scientific, Hampshire, United Kingdom) was added to the pellet and incubated at 36±1 ºC for 48 hrs. To the isolates preserved as freeze-dried, 3 mL of BHI was added to the isolate vial, thoroughly mixed using glass Pasteur pipette and then incubated at 36±1 ºC for 48 hrs. Following the incubation, the inoculated BHI broth was streaked on the selective media for each pathogen Table 3.2.

Table 3. 2: Selective media used to revive bacterial isolates for selected pathogens.

Pathogens	**Selective medium**	**Typical morphology**
E. coli	Eosin Methylene Blue (EMB)	Colonies with greenish metallic sheen in light
S. enterica	Xylose Lysine Deoxycholate (XLD) Agar	Red colonies with black centres
S. aureus	Baird Parker Agar	Formation of black, shiny, convex colonies surrounded by a lightening halo
L. monocytogenes	Brilliance Listeria Agar Base	Formation of green colonies surrounded by a lightening halo

3.6.2 Bacterial confirmation of isolates

To ascertain that the revived isolates were the targeted bacteria, preliminary identification tests were conducted by classical assay using Gram staining and phenotypical tests (catalase, oxidase, and indole). Preliminary identification was performed using the ARC-OVR general bacteriology laboratory standard operating procedures. confirmation of *S. enterica*, was performed by PCR while for *E. coli, L. monocytogenes* and *S. aureus* biochemical tests was used following preliminary identification.

3.6.3 Molecular confirmation of *Salmonella enterica*

3.6.3.1 DNA extraction

DNA for PCR was extracted using cell lysis boiling method as described by Madoroba *et al*., (2016), where, a loop full of a colony from a pure culture plate not older than 24 hrs was suspended into 100 µL of DNA free water (Thermo-Fisher Scientific, Hampshire, United Kingdom) in an Eppendorf tube (Lasec, Johannesburg, South Africa). The suspension was vortexed (Lasec, Johannesburg, South Africa) at a speed of 3200 rpm for 30 seconds and heated at 96 ºC for 15 minutes using PCR thermal cycler-C1000™ (Bio-Rad, Cape Town, South Africa). The suspension was allowed to cool down for 5 minutes at room temperature and then centrifuged using Mini Spin Plus, Eppendorf (Lasec, Johannesburg, South Africa) at 13000 rpm for 5 min. The supernatant containing the DNA, was transferred into a new Eppendorf tube, and stored at -20 ºC for use over a period not exceeding 8 weeks.

3.6.3.2 *Salmonella enterica*

Confirmation of S. *enterica* strains was done using the PCR assay by targeting the *invA* gene as reported by (Barmak et al., 2021). PCR reaction was performed in a total of 25 µl, comprising 12.5 µL of Red Taq Master mix (Thermofischer, Hampshire, United Kingdom), 2.0 µl of 10mM primer (inVA-F: 5'- GTGAAATTATCGCCACGTTCGG-3'; inVA-R: 5'-ATCGCCATTTACGCGGGTCA-3'), 5.5 µl Nuclease-free water and 5 µl of DNA. *Salmonella* Typhimurium ATCC 14028 and *E. coli* ATCC 25922 served as a positive and negative control respectively. In every PCR experiment, water was utilized as the negative DNA control.

3.6.3.3Agarose gel electrophoresis of PCR amplicons

The primers used in this study were synthesized by Inqaba biotech, Pretoria, South Africa. The PCR amplification products were electrophoresed in 1.5% agarose gel stained with ethidium bromide at 3 V/cm for approximately 1 hr as described by (Madoroba *et al*., 2022).A molecular weight marker with (100 bp) was included in each gel prepared to determine the amplicon sizes. The stained agarose gels were visualized under ultraviolet light and the results were captured using a gel documentation system (BIO-RAD; California; United States of America) (Madoroba *et al*., 2022).

3.6.4 Biochemical tests confirmation

3.6.4.1 Sta*phylococcus aureus*

Colonies that were black or greyish black on Baird-Parker agar (BPA) plates were tentatively identified as staphylococci. Initial identification tests on suspect staphylococcal colonies on BPA plates included the following: Tests for coagulase, catalase, oxidase, indole, and gram-stain (Abolghait *et al*., 2020).To find coagulase production, staphylase test kit (Thermo-Fisher Scientific, Hampshire, United Kingdom) was employed, where a drop of test latex was dispensed onto one circle on the test card and 1 drop of control reagent onto a second circle and each labelled with ‘Test' and 'Control'. Five suspect colonies were picked with a loop and mixed into the drop of test reagent and spread within the circle so that a diameter of about 2 cm is achieved. A sterile loop was used, and the procedure repeated with the control latex. The card was picked up and rocked so that the reagents moved from side to side for up to 20 seconds and observed for agglutination. The presence of bound coagulase was demonstrated by the appearance of microscopic clumping.

3.6.4.2 *Escherichia coli*

Confirmation of *E. coli* was done using to RapID™ ONE biochemical System (Thermo-Fisher Scientific, Hampshire, United Kingdom) according to the manufacturer’s instructions. In summary, the three to five presumptive colonies obtained from a pure culture not older than 24 hrs were inoculated in 2 ml of RapID inoculation fluid to achieve a turbidity equivalent to a 2.0 McFarland standard. The inoculated solution was gently transferred to RapID™ ONE cavities using a micro-pipette (Lasec, Cape Town, South Africa) and followed by aerobic

incubation (Club refrigeration, Pretoria, South Africa) at 35°C for 4 hrs. Analysis of results was done based on interpretations of ERIC Software for RapID™ Systems (Thermo-Fisher Scientific, Hampshire, United Kingdom).

3.6.4.3 *Listeria monocytogenes*

The Oxoid Biochemical Identification System (O.B.I.S.) biochemical test was used to confirm the isolates as *L. monocytogenes* in accordance with manufacturer's instructions. The O.B.I.S test card was inoculated with the pure colonies of presumed *L. monocytogenes* from Brilliance-*Listeria* plates, and then a drop of O.B.I.S buffer was added to the inoculated test card. After that, the inoculated O.B.I.S test card was incubated at 36 º C for 10 min. Following incubation, a drop of developing solution was added onto each well of the test card. Within 20 seconds of applying the developing solution, the outcomes were assessed. If a purple hue appeared, the bacterium was not *L. monocytogenes*. The organism was confirmed to be *L. monocytogenes* if no colour appeared after 20 seconds.

3.6.5 Antimicrobial susceptibility test

3.6.5.1 Kirby-Bauer Disk Diffusion

All viable isolates were subjected to antimicrobial susceptibility testing against a total of 25 antibiotics: twelve for Gram negative pathogens and thirteen for Gram positive pathogens (Thermo-Fisher Scientific, Hampshire, United Kingdom) as listed in Table 3.4. The Kirby-Bauer disk diffusion was performed as described in European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2021). Mueller-Hinton agar (MHA) plates supplemented with 5% sheep blood were used for Gram positive bacteria while plain MHA plates were used from Gram negative bacteria. The plates were swabbed with a bacteria tested by dipping a sterile swab into the 0.5 McFarland standard prepared by using 0.85% saline (Labchem, Johannesburg, South Africa). The excess of saline from the swab was removed by pressing the swab against the sides of the tube. The surface of the Mueller-Hinton agar was swabbed completely without leaving any un-swabbed agar areas at all. After completely swabbing the plate, it was turned 90 º and the swabbing process repeated so that there were no spaces missed. The surface was allowed to dry for 5 minutes before the antibiotic discs (Thermo-Fisher Scientific, Hampshire, United Kingdom) were placed on the agar with a disc dispenser and

incubated within 15 minutes at 37 ºC for 18 - 24 hours. After incubation, the clear inhibition zone around individual antibiotic disc was measured and interpreted as resistant or susceptible using the EUCAST guidelines.

3.6.5.2 Control strains for Kirby-Bauer Disk Diffusion

Salmonella Typhimurium ATCC 14028, *E. coli* ATCC 25922, *S. aureus* ATTC 25923 and *L. monocytogenes* ATTC 19111 reference strains were used as positive and negative controls.

Table 3. 3: List of antibiotic disks and their concentration used in this study.

Antibiotics Classes	**Antibiotics name**	**Concentrations**	**Antibiotics used for Gram (+) or (-) pathogens**
β-lactamans	Penicillin	10ug	Gram (+)
	Ampicillin	10ug	Gram (-)
	Amoxicillin	10ug	Gram (-)
	Meropenem	10ug	Gram (-)
Phenicols	Chloramphenicol	30ug	Gram (-) and (+)
Oxazolidinones	Linezolid	10ug	Gram (+)
Nitrofurans	Nitrofurantoin	100ug	Gram (+)
Macrolides	Erythromycin	15ug	Gram (+)
	Tylosin tartrate	30ug	Gram (+)
	Azithromycin	15ug	Gram (-)
Lipopeptides	Daptomycin	10ug	Gram (+)
Glycopeptides	Vancomycin	30ug	Gram (+)
Fluoroquinolones	Ciprofloxacin	30ug & 5ug	Gram (-) and (+)
Aminoglycosides	Kanamycin	30ug	Gram (+)
	Gentamycin	30ug &10ug	Gram (-) and (+)
Tetracyclines	Tetracycline	30ug	Gram (-) and (+)
	Tigecycline	15ug	Gram (+)
Streptogramin	Quinupristin	15ug	Gram (+)
Cephalosporin	Ceftriaxone Ceftiofur Cefoxitin	30ug	Gram (-)
Sulphonamide	Trimethoprim	5ug	Gram (-)

3.6.5.3 Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration micro-dilution method was performed using 96 well plates (Thermofischer, Hampsphire, United Kingdom). The antibiotic concentrations used for pathogens from Gram negative group are listed in Table 3.4 and those used for Gram positive group are listed in Table 3.5. A loop full of a colony from a pure culture was transferred into a 5ml distilled/demineralized water (Thermofischer, Hampshire, United Kingdom) to prepare a suspension. A 10µL turbidity equal to a 0.5 McFarland standard was transferred into a 9ml of Mueller Hinton broth (Thermofischer, Hampshire, United Kingdom) using 1-20 µL Eppendorf micropipette (Lasec, Johannesburg, South Africa). The inoculated 50µl Mueller Hinton broth was transferred to all wells of the 96 antibiotics coated plates using calibrated multichannel micropipettes (Lasec, Johannesburg, South Africa). The plates used for Gram negative pathogens were then incubated with a cultured nutrient agar plate (Thermofischer, Hampshire, United Kingdom) and Gram-positive plates incubated with blood agar plates (Thermofischer, Hampshire, United Kingdom), for sterility check purposes at 37 ºC for 18-24 hrs or 42 - 48 hrs in suitable environmental conditions depending on the bacteria (acid fast/non fastidious). The ATCC strains (*Salmonella* Typhimurium ATCC 14028, *E. coli* ATCC 25922, S. *aureus* ATTC 25923 and *L. monocytogenes* ATTC 19111 were included as controls for specific pathogens to ensure validity of results for each plate. The results were then read using a magnified mirror and interpreted against the EUCAST endpoints.

Table 3. 4: The antibiotics concentration used for Gram-negative pathogens

Antibiotic classes	Antibiotic Name	Concentration range (mg/L)
Phenicols	Chloramphenicol (CHL)	32-8
Tetracyclines	Tetracycline (TET)	16-4
Cephalosporins	Ceftriaxone (AXO)	2-1
Penicillins	Amoxicillin (AUG2)	32/16-8/4
Fluoroquinolones	Ciprofloxacin (CIP)	0.5-0.25
Aminoglycosides	Gentamycin (CN)	64-16
Cephalosporins	Ceftiofur (XNL)	8-2
Sulfonamides	Sulfisoxazole (FIS)	512-256
Sulfonamides	Trimethoprim (SXT)	4/76-2/38
Penicillins	Ampicillin (AMP)	32-8

Table 3. 5: The antibiotics concentration used for Gram-positive pathogens

Antibiotic classes	Antibiotic Name	Concentration range (mg/L)
Glycylcyclines	Tigecycline (TGC)	0.5 - 0.015
Tetracyclines	Tetracycline (TET)	32 – 1
Phenicols	Chloramphenicol (CHL)	32 – 2
Cyclic lipopeptides	Daptomycin (DAP)	4 - 0.25
Fluoroquinolones	Ciprofloxacin (CIP)	4 - 0.12
Streptogramin	Quinupristin (SYN)	32 - 0.5
Oxazolidinones	Linezolid (LZD)	8 - 0.5
Nitrofurans	Nitrofurantoin (NIT)	64 - 2
Beta-lactams	Penicillin (PEN)	16 - 0.25
Macrolides	Erythromycin (ERY)	8 - 0.25
Glycopeptides	Vancomycin (VA)	4 - 0.25
Aminoglycosides	Gentamycin (CN)	1024 – 128

3.6.6 Identification of resistance genes by PCR

3.6.6.1 DNA Extraction

DNA extraction was performed by boiling method as indicated in section 3.5.3.1 above.

3.5.6.2 Detection of antibiotic resistant genes for *Staphylococcus aureus*

The PCR amplification as outlined by Adigun *et al*., (2020) was performed to amplify the β-lactams (*blaZ,* and *mecA),* macrolides (*ermA, ermB,* and *ermC*) and vacomycin (*vanA* and *vanB*) antibiotic resistance genes. The primers (Thermofischer, Hampshire, United Kingdom), and the PCR conditions are outlined in Table 3.6. A PCR reaction with a total volume of 25µL consisting of 2µL of each primer (forward and reverse) which were hybridized to the 5 and 3' borders added to 12.5µL of 2×Taq PCR Master mix (Thermofischer, Hampshire, United Kingdom) 7.5 µL nuclease-free water then 1µL DNA template which was added last in the amplification room. PCR protocol was carried out as follows: 5 min initial denaturation at 95 ℃, 30 cycles of amplification at 95 ℃ for 30 sec, annealing for 45 sec (see annealing temperature for each gene in Table 3.6), extension at 72 ℃ for 45 s and final extension at 72 ℃ for 15 min.

3.6.6.3 Detection of antibiotic resistant genes for *Listeria monocytogenes*

In total, 10 commercially available primers (Table 3.7) from various antibiotic classes were used in duplex-PCR for detection of resistance genes in *L. monocytogenes*. A total of 50 µL volume of PCR product was used which contained 25-µL of Red Taq Master mix (Thermofischer, Hampshire, United Kingdom), 4 µL of each primer, and 16 µL of nuclease-free water and 5 µL DNA template was used to conduct the PCR. The PCR amplification was done in a thermal cycler (Bio-Rad, Germany) using the following parameters: initial denaturation at 94 °C for 1 minute, 35 cycles of PCR at 94 °C for 1 min for denaturation, various relevant annealing temperatures for the appropriate genes as optimized (Table 3.8), extension at 72 °C for 1 min, and final extension at 72 °C for 10 min.

Table 3. 6: The primers used for PCR for resistance genes in *Staphylococcus aureus* isolates.

Primer name	Sequence (5'-3')	Annealing temperature (°C)	Size (bp)	References
blaZ	F: AACACCTGCTGCTTTCGGTA	55.5	314	(Gan *et al*., 2021)
blaZ	R: CACTCTTGGCGGTTTCACTT			
mecA	F: CTTTGCTAGAGTAGCACTCG	55.5	533	(Rocchetti et al.,
mecA	R: GCTAGCCATTCCTTTATCTTG			2018)
ermA	F: CTACACTTGGCTTAGGATGA	56.5	311	(Gan *et al*., 2021)
ermA	R: AGTGACTAAAGAAGCGGTAA			
ermB	F: TAACGACGAAACTGGCTAA	56.0	414	
ermB	R: CTGTGGTATGGCGGGTAA			
ermC	F: GAGGCTCATAGACGAAGAAA	54.5	375	
ermC	R: AAGTTCCCAAATTCGAGTAA			
vanA	F: CATGACGTATCGGTAAAATC R: ACCGGGCAGRGTATTGAC	56	732	(Patel *et al*., 1998)
vanB	F: CATGACGTATCGGTAAAATC R: ACCGGGCAGRGTATTGAC		885	

F- forward primer, R- reverse primer

Table 3. 7: Primer sequences and PCR condition used for *Listeria monocytogenes* in the study

Antibiotic class	Target gene	Sequence (5'-3')	Product sizes (bp)	Annealing temperature (°C)	Final primer concentration (µM)	Reference
Tetracycline	*tetA*	F: GGCCTCAATTTCCTGACG R: AAGCAGGATGTAGCCTGTGC	372	63	0.1	(Guillaume *et al*., 2000)
	tetM	F: GTGGACAAAGGTACAACGAG R: CGGTAAAGTTCGTCACACAC	405		0.2	
Erythromycin	*ermA*	F: AACACCCTGAACCCAAGGGACG R: CTTCACATCCGGATTCGCTCGA	420	55	0.2	(El-Banna *et a*l., 2016)
	ermB	F: GAAAAGGTACTCAACCAAATA R: AGTAACGGTACTTAAATTGTTTAC	639			
	ermC	F: GAGGCTCATAGACGAAGAAA R: AAGTTCCCAAATTCGAGTAA	375			
Penicillin	*PenA*	F: ATCGAACAGGCGACGATGTC R: GATTAAGACGGTGTTTACGG	500	54	0.2	(El-Banna *et al*., 2016)
Vancomycin	*vanA*	F: CATGACGTATCGGTAAAATC R: ACCGGGCAGRGTATTGAC	732	56	0.2	(Patel *et al*., 1998)
	vanB	F: CATGACGTATCGGTAAAATC R: ACCGGGCAGRGTATTGAC	885			
Aminoglycoside	*aphA1*	F: ATGCATTCGCGATAATGTC R: CTCACCGAGGCAGTTCCAT	600	55	0.4	(Travis *et al*., 2006)
	aphA2	F: GATTGAACAAGATGGATTGC R: CCATGATGGATACTTTCTCG	347		0.1	

3.6.6.4 Detection of AMR genes for Gram negative:

Screening for the presence of 18 AMR genes was carried out using conventional PCR assays as listed in Table 3.8. PCR reactions were carried out in 25 µL (total volume) which consisted of 12.5 µL 2× Taq master mix, 4.5 µL nuclease free water (with exception β-lactams; 3.5 µL and tetracycline 5.5 µL), 5 µL template DNA and 3 µL primers. Thirty (30) cycle PCR reaction was conducted with the following conditions: Two step denaturation at 94 ºC for 3 min and 30 sec respectively followed by annealing of different genes at different temperatures Table 3.8, extension and final extension at 72 ºC for 1 min and 10 min respectively.

3. 6.7 Molecular characterization (subtyping)

3.6.7.1 *Listeria monocytogenes* serogrouping

Serogrouping was performed using a multiplex-PCR assay that targets the five genes (*ORF2819*, *ORF2110*, *lmo0737*, *lmo1118* and *prs*) of *Listeria* spp., and *L. monocytogenes* as previously described by Doumith *et al.*, (2004). The primers used in this study were synthesized by Inqaba biotec, South Africa (Table 3.9). This PCR allowed the differentiation of *L. monocytogenes* strains into five major molecular serogroups (IIa, IIb, IIc, Ivb and Iva) based on the PCR amplification patterns to targeted genes. PCR was performed in a total volume of 25 µL which consisted of 12.5 µL 2× DreamTaq master mix, 4.5 µL nuclease free water, 5 µL template DNA, 3 µL primer mix (five primer sets). Initial denaturation step at 94 °C for 3 min; 35 cycles of 94 °C for 0.40 min, 53 °C for 1.15 min, and 72 °C for 1.15 min; and one final cycle of 72 °C for 7 min in a thermocycler.

Table 3. 8: Primer sequences and PCR conditions for screening of AMR genes

Antibiotic class	Target gene	Sequence (5'-3')	Product sizes (bp)	Annealing Temperature (°C)	Reference
Tetracycline	*tetA*	F: GGCGGTCTTCTT CATCATCATGC R: CGGCAGGCAGAGCAGTAGA	502	59	(Lanz *et al*., 2003)
	tetB	F: CGCCCAGTGCTGTTGTTGTC R: CGCGTTGAGAAGAAGCTGAGGTG	173		
Trimethoprim	*Dfrl*	F: CGGTCGTAACACGTTCAAGT R: CTGGGGATTTCAGGAAAGTA	220	55.3	
	DfrXII	F: AAATTCCGGGTGAGCAGAAG R: CCCGTTGACGGAATGGTTAG	429		
	DfrXIII	F: GCAGTCGCCCTAAAACAAAG R: GATACGTGTGACAGCGTTGA	294		
Sulphonamides	*sul1*	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCCG	433	63	
	sul2	F: GCGCTCAAGGCAGATGGCATT R: GCGTTTGATACCGGCACCCGT	293		
	sul3	F: CAACGGAAGTGGGCGTTGTGGA R: GCTGCACCAATTCGCTGAACG	244		
Phenicol	*cat1*	F: CTTGTCGCCTTGCGTATAAT	508		

		R: ATCCCAATGGCATCGTAAAG			
	Flo	F: CTGAGGGTGTCGTCATCTAC R: GCTCCGACAATGCTGACTAT	673	53.3	
	cmlA	F: CGCCACGGTGTTGTTGTTAT R: GCGACCTGCGTAAATGTCAC	394		
β- lactams	bla_{TEM}	F: TTAACTGGCGAACTACTTAC R: GTCTATTTCGTTCATCCATA	247	55.3	(Lanz *et al*., 2003)
	bla_{CMY-2}	F: GACAGCCTCTTTCTCCACA R: TGGACACGAAGGCTACGTA	1000		
	bla_{SHV}	F: AGGATTGACTGCCTTTTTG R: ATTTGCTGATTTCGCTCG	393		
	bla_{PSE}	F: TGCTTCGCAACTATGCTAC R: AGCCTGTGTTTGAGCTAGAT	438		
Quinolones	*qnrA*	F: TCAGCAAGAGGATTTCTCA R: GGCAGCACTATTACTCCCA	516	53	
	qnrB	F: GATCGTGAAAGCCAGAAAGG R: ACGATGCCTGGTAGTTGTCC	469		
	qnrS	F: ACGACATTCGTCAACTGCAA R: TAAATTGGCACCCTGTAGGC	417		

Table 3. 9: Primer sequences used for serotyping of *Listeria monocytogenes* in the study

Target Genes	Primer sequences (5'-3')	Product sizes (bp)
ORF2110	F: AGTGGACAATTGATTGGTGAA, R: CATCCATCCCTTACTTTGGAC;	597
ORF2819	F: AGCAAAATGCCAAAACTCGT, R: CATCACTAAAGCCTCCCATTG;	471
lmo1118	F: AGGGGTCTTAAATCCTGGAA, R: CGGCTTGTTCGGCATACTTA;	906
lmo0737	F: AGGGCTTCAAGGACTTACCC, R: ACGATTTCTGCTTGCCATTC;	691
Prs	F: GCTGAAGAGATTGCGAAAGAAG, R: CAAAGAAACCTTGGATTTGCGG	370

3.6.7.2 *Staphylococcus aureus* toxin typing

To identify *S. aureus* toxin and resistance genes, PCR multiplex assays for two sets (A and B) were carried out as described by Mehrotra, Wang and Johnson, (2000). To summarize, set A had the genes *sea*, *seb*, *sec*, *sed*, and *femA*, whereas set B contained the genes *femA*, *eta*, and *ts*t-1, as shown in Table 3.10. which lists the primer sequences and predicted amplicon sizes. 50 µL ml of total volume was used for the Set A reaction, which included 25 µL of red mix (Lasec, Johannesburg, South Africa), 4 µL of each primer (Inqaba, Pretoria, South Africa), 5 µL of DNA, and 16 µL of DNA-free water (BioConcept, Allschwil, Switzerland). Using 34 cycles of denaturation for 30 seconds at 95 °C, annealing for 1 min at 55 °C, extension for 1 min at 72 °C, and final extension for 7 min at 72 °C, amplification was performed in a thermocycler. The only difference between Set A and Set B amplification was the annealing temperature, which was changed from 55°C to 58°C.

3.6.7.3 Agarose gel electrophoresis of PCR amplicons

The primers used for testing of resistance genes were synthesized by Inqaba biotech, Pretoria, South Africa. The PCR amplification products 4.5g of agarose in 300ml TAE buffer were electrophoresed in 1.5% (singleplex-PCR) or 3% (multiplex-PCR) agarose gel stained with 5µL ethidium bromide at 3 V/cm for approximately 1 hr or 3hrs respectively as described by Madoroba *et al*., (2022). A 10 µL molecular weight marker with (100 bp) was included in each

gel prepared to determine the amplicon sizes. The stained agarose gels were visualized under ultraviolet light and the results were captured using a gel documentation system (BIO-RAD; California; United States of America) (Madoroba *et al*., 2022).

3.6.7.4 Control for resistance genes

PCR amplicons of various genes were submitted to Inqaba Biotech (Pretoria, South Africa) for sequencing and were used in this study as either positive or negative controls.

Table 3. 10: Oligonucleotide sequences and expected amplicon sizes for *S. aureus* toxin gene

Target gene	Primer sequence (5'-3')	PCR product size (bp)
sea	F: GGT TAT CAA TGT GCG GGT GG R: CGG CAC TTT TTT CTC TTC GG	102
seb	F: GTA TGG TGG TGT TAA CTG AGC R: CCA AAT AGT GAC GAG TTA GG	164
sec	F: AGA TGA AGT AGT TGA TGT GTA TGG R: CAC ACT TTT AGA ATC CAA CCG	451
sed	F: CCA ATA ATA GGA GAA AAT AAA AG R: ATT GGT ATT TTT TTT CGT TC	278
sema	F: AAA AAA GCA CAT AAC AAG CG R: CTG GTG AAG TTG TAA TCT GG	132
eta	F: GCA GGT GTT GAT TTA GCA TT R: AGA TGT CCC TAT TTT TGC TG	93
tst-1	F: ACC CCT GTT CCC TTA TCA TC R: TTT TCA GTA TTT GTA ACG CC	326

3.6.8 Phenotypic serotyping

3.6.8.1 Serotyping of *Salmonella enterica*

The Kauffman-White method for serotyping *Salmonella* was used in this study. Briefly, the presence of agglutination was examined by testing the *Salmonella* isolates against polyvalent and monovalent antisera. The isolates were tested for the presence of flagellar (H), somatic (O), and vi antigens (Uelze *et al*., 2020).

3.7 ANTIBIOTIC RESISTANCE INDEX (ARI)

The antibiotic resistance index (ARI) measures the frequency of antibiotic resistance among isolates obtained from a specific sample. ARI was calculated using the formula below:

ARI = A/NY

where A is a total number of resistance determinants recorded in the population, N is the number of isolates in the population, and Y is the total number of antibiotics tested.

3.8 DATA ANALYSIS

Recording, storage interpretation of data and Statistical analysis

The frequencies were summarized in one- and two-way tables and subjected to the appropriate chi-square ($\chi 2$) to test whether the proportions vary significantly over the different classifications (Caruso *et al*., 2018; Siegel, 1930).

The data set for random forest analysis were classified based on four sources (food of animal origin, animals, dairy environment) and four hosts (poultry, wildlife, bovine and swine). To detect the AMR genes responsible for antibiotic resistance among South African bacterial isolates.

CHAPTER 4: RESULTS

4.1 Strain characterization

A total of 216 bacterial isolates were included in the study. The isolates in the current study represented four distinct bacterial pathogens: *S. enterica* (n = 88), *S. aureus* (n = 64), *E. coli* (n = 30), and *L. monocytogenes* (n = 34) (Table 4.1). All strains were isolated across the eight provinces of South Africa i.e., Gauteng (n = 97), Limpopo (n = 36) KwaZulu Natal (n = 37), Western Cape (n = 15) and Mpumalanga (n = 4), Free State (n = 15), Eastern Cape (n = 6) and Northwest (n = 6). The distribution of strains in this study includes isolates from food of animal origin, animals, dairy and the environment (n = 179, n = 24, n = 12 and n = 1, respectively), with an uneven distribution in terms of isolation time (Table 4.1). Only 60% (n = 53) of *Salmonella* isolates were viable, and 73% (n = 22) for *E. coli* strains. *Staphylococcus aureus* strains were all viable (n = 64), but *L. monocytogenes* were only 71% viable (n = 24).

4.2 Frequency of recovery of the isolates

Table 4.1 also shows the recovery rate for specific pathogens based on the total number of isolates tested versus revived isolates. For this study, 216 bacterial isolates that had been either freeze-dried (lyophilized) (n = 78) or stored in glycerol (n = 138) were selected. Only 31% (n = 53) of the lyophilized and 69% (n = 119) glycerol isolates were viable on Blood Tryptone Agar. All (n = 53) viable isolates represented various strains of *S. enterica*, that were preserved between the years 2000 and 2016, were from poultry 66% (n= 35), followed by bovine 15 % (n = 8), swine 15 % (n = 8) and environmental sources 2 % (n = 1) wildlife sources (Table 4.1). The lyophilized isolates (n = 35; 40%) which did not yield growth upon revival represented *S.* Enteritidis (23%; n =8) and *S.* Typhimurium (77%; n =27) which were preserved in the year 2000 (n = 3), 2001 (n = 11), 2002 (n = 2), 2004 (n = 3), 2007 (n = 2), 2010 (n = 3), 2015 (n = 9) and 2019 (n = 2) respectively. Of the 30 viable *E. coli* isolates, 73%; (n = 22) were preserved between the years 2000 and 2021, 77% (n = 17) came from poultry species, 9% (n = 2) from bovine and unknown species, and 5% (n = 1) from swine species. From the 34 *L. monocytogenes* isolated between the years 2015 and 2017, 71% (n = 24) were revived and 88% (n=21) came from bovine, 8% (n = 2) from poultry, and 4% (n = 1) from swine species. All 64 of *S. aureus* were viable; 88% (n = 56) were from bovine, 9% (n = 6) from swine, and 3% (n = 2) from poultry species.

Table 4. 1: Summary of the recovery rate of revived stored isolates for specific pathogens over a 20-year period

Bacterial pathogen	**Number of isolates tested (%)**	**Number of viable isolates (%)**	**Number of non-viable isolates (%)**	**Year of isolation for viable isolates**	**Year of isolation for non-viable isolates**
E. coli	30 (13)	22 (73)	8 (27)	1996 (n = 1); 2005 (n = 1); 2009 (n = 1); 2011 (n = 1); 2017 (n = 1); 2018 (n = 7); 2021 (n = 10)	2015 (n = 8); 2021 (n =11)
S. aureus	64 (28)	64 (100)	0 (0)	1992 (n = 1); 2015 (n = 63)	-
L. monocytogenes	34 (15)	24 (71)	10 (29)	2015 (n = 11); 2016 (n = 1); 2017 (n = 12)	2015 (n = 8); 2016 (n =2)
S. enterica	88 (39)	53 (60)	35 (40)	2000 (n = 1); 2001 (n = 3) 2002 (n = 2); 2003 (n = 7) 2004 (n = 23); 2005 (n = 1) 2007 (n = 5); 2008 (n = 2) 2010 (n =3); 2016 (n =6)	2000 (n = 3); 2001 (n = 11) 2002 (n = 2); 2004 (n = 3) 2007 (n = 2); 2010 (n = 3) 2015 (n = 9); 2019 (n = 2)
Total	**216**	**163 (75%)**	**53 (25%)**	**-**	**-**

4.3 Phenotypic antibiotic results of isolates stored between 2000 and 2021

4.3.1 *Salmonella enterica*

4.3.1.1 Overall sensitivity of *Salmonella enterica*

Table 4.2 shows the antibiotic results of *S. enterica* (n = 53) isolates, tested using the disk diffusion method. Among the 12 tested antibiotics the highest susceptibility (100%) was observed against azithromycin, meropenem and ciprofloxacin, followed by amoxicillin (96%; n = 51), chloramphenicol and gentamycin (both 94%; n = 50), cefoxitin and ceftriaxone (both at 93%; n = 49), trimethoprim (91%; n = 48) and ampicillin (87 %; n = 46). The differences were statistically significant (P<0.00001). Eighty three percent (n = 44) of the *S. enterica* isolates were sensitive to ceftiofur while 17% (n = 9) of the isolates showed intermediate resistance to the same antibiotic and 2% (n=1) to tetracycline and chloramphenicol. Sixty-eight percent (n = 36) demonstrated sensitive to tetracycline.

4.3.1.2 Analysis of resistance among *Salmonella enterica* strains isolated in different time periods

A total of 53 strains of *S. enterica* used in this study were grouped into four time periods, including 2000–2004 (n = 36), 2005–2010 (n = 11), 2011–2015 (n = 0), and 2016–2021 (n = 6). The analysis of resistance among strains isolated in different time periods showed that the percentage of resistant strains of *S. enterica* was low throughout the period under review (Figure 4.1). The percentage of *S. enterica* strains with resistance to more than one antibiotic was highest among isolates obtained in 2000–2004 and 2016–2021. Among the isolates obtained in 2000–2004, resistance was 11.1% (n = 4), 2.8% (n = 1), 5.6% (n = 2), and 2.8% (n = 1) against two, three, four, and five antibiotics, respectively. Isolates obtained between 2016 and 2021 showed a resistance of 33.3% (n = 2) and 16.7% (n = 1) against three and four antibiotics, respectively. No multiple (more than one antibiotic) resistance was observed against *S. enterica* isolates obtained between 2006–2010 and 2011–2015.

Table 4. 2: Distribution of antibiotic resistance of *Escherichia coli* and *Salmonella enterica* isolates using disc diffusion method

Antibiotic class	**Antibiotic**	**No. of isolates resistant (%)**		**No. of isolates sensitive (%)**		**Intermediate (%)**	
		E. coli **(n=22)**	***Salmonella*** **(n=53)**	***E. coli*** **(n=22)**	***Salmonella*** **(n=53)**	***E. coli*** **(n=22)**	***Salmonella*** **(n=53)**
β-Lactam	Ampicillin (AMP)	4 (18)	7 (13)	17 (77)	46 (87)	1 (4)	0 (0)
	Amoxycillin (AMO)	4 (18)	2 (4)	16 (73)	51 (96)	0 (0)	0 (0)
Cephalosporin	Ceftriaxone (CFTR)	1 (4)	4 (8)	21 (96)	49 (92)	0 (0)	0 (0)
	Ceftiofur (CEF)	0 (0)	0 (0)	21 (96)	44 (83)	1 (4)	9 (17)
	Cefoxitin (CEFO)	3 (13)	4 (8)	19 (86)	49 (93)	0 (0)	0 (0)
Aminoglycosides	Gentamycin (CN)	0 (0)	3 (6)	22 (100)	50 (94)	0 (0)	0 (0)
Tetracycline	Tetracycline (TET)	8 (36)	16 (30)	14 (64)	36 (68)	0 (0)	1 (2)
Macrolides	Azithromycin (AZI)	0 (0)	0 (0)	22 (100)	53 (100)	0 (0)	0 (0)
Carbepenes	Meropenem (MEM)	0 (0)	0 (0)	22 (100)	53 (100)	0 (0)	0 (0)
Fluoroquinolones	Ciprofloxacin (CIP)	1 (5)	0 (0)	21 (96)	53 (100)	0 (0)	0 (0)
Phenicols	Chloramphenicols (CHL)	0 (0)	2 (4)	22 (100)	50 (94)	0 (0)	1 (2)
Sulphonamides	Trimethoprim (TRIM)	0 (0)	5 (9)	21 (96)	48 (91)	1 (4)	0 (0)

Figure 4. 1: Distribution of resistance for *Salmonella enterica* strains by the year of isolation.

4.3.1.3 Analysis of multidrug resistance among *Salmonella enterica* strains isolated from different animal species and sources

A strain of *S*. Düsseldorf, *S*. Orion, and *S.* Typhimurium that was recovered in 2003 and 2004 from samples of poultry and cattle, respectively, was shown to be resistant to two antibiotics with the resistance patterns TET-AMO, AMP-CN, and CETR-AMP (Table 4.3). A strain of *S.* Hadar isolated from poultry in 2004 was resistant to three antibiotics (ME-TET-AMO). Similarly, two strains of *S*. Typhimurium isolated in 2004 (TET- AMP-TRI) and 2016 (TET-CETR-TRI) from swine and poultry were resistant to all three antibiotics. *Salmonella* Senfenburg isolated from an abattoir environment in 2004 was resistant to four antibiotics (TET- AMO- AMP- CN), while *S*. Typhimurium from 2016 isolated in beef (bovine meat) was also resistant to four antibiotics (CETR-AMO-AMP-TRI), and *S*. Typhimurium isolated in 2002 from a bovine animal sample was resistant to five antibiotics (TET-AMP- TRIM- CHL-CEF) (Table 4.3). The occurrence of multidrug resistance among the tested (n = 53) strains of *S. enterica* was very low at 1.9% (n = 1) across 10 observed resistance patterns with a high MDR pattern against five antibiotics (TET-AMP-TRIM-CHL-CEF) (Table 4.3).

4.3.2 *Escherichia coli*

4.3.2.1 Overall sensitivity of *Escherichia coli* strains

The disk diffusion method results of *E. coli* isolates (n = 22) tested in this study are shown in Table 4.2. Chloramphenicol, azithromycin, meropenem, and gentamycin showed the highest (100%; n = 22) susceptibility level among the 12 tested antibiotics followed by trimethoprim, ciprofloxacin, ceftriaxone, and ceftiofur (96%; n = 21), cefoxitin (86%; n = 19), ampicillin (77%; n = 17), and amoxicillin (73%; n =16). Tetracycline resistance was observed in 36% (n = 8) of the isolates. Nine percent (n = 2) of the isolates exhibited intermediate amoxicillin resistance, while 4% (n = 1) exhibited intermediate trimethoprim and ampicillin resistance. The differences were statistically significant ($P<0.0001$).

Table 4. 3: Resistance patterns of *Salmonella enterica* strains from various time periods, sources, and animal species

Resistance pattern exhibited by *S. enterica*						
Resistance pattern	**No. (%) of isolates exhibiting resistance patterns**	**No. of resistance phenotype patterns observed**	**Serotype**	**Year of isolation**	**Source of isolation**	**Animal species**
AMP, GEN	1 (1.9)	2	*S*. Orion	2004	Meat	Poultry
TET, AMP	1 (1.9)	2	*S*. Typhimurium	2004	Animal	Bovine
CETR, AMP	1 (1.9)	2	*S*. Dusseldorf	2003	Meat	Poultry
ME, TET, AMO	1 (1.9)	3	*S*. Hadar	2004	Animal	Poultry
TET, AMP, TRI	1 (1.9)	3	*S*. Typhimurium	2004	Animal	Swine
TET, CETR, TRI	1 (1.9)	3	*S*. Typhimurium	2016	Meat	Bovine
TET, AMP, TRIM, CHL	1 (1.9)	4	*S*. Typhimurium	2001	Animal	Bovine
TET, AMO, AMP, CN	1 (1.9)	4	*S*. Senfenburg	2004	Environment	Swab
CETR, AMO, AMP, TRI	1 (1.9)	4	*S*. Typhimurium	2016	Meat	Bovine
TET, AMP, TRIM, CHL, CEF	1 (1.9)	5	*S*. Typhimurium	2002	Animal	Bovine

TET=tetracycline, AMP=ampicillin, TRI=trimethoprim, CHL= chloramphenicol, CEF=cefoxitin, AMO, amoxicillin, CN=gentamycin, ME=meropenem, CETR= ceftriaxone.

4.3.2.2 The analysis of resistance among *Escherichia coli* strains isolated in different time periods

Twenty-two strains of *E. coli* used in this study were divided into three time periods: 2005 - 2009 (n = 3), 2010 - 2015 (n = 1), and 2016 - 2021 (n = 18). The investigation of resistance among strains isolated at various times revealed that the proportion of resistant *E. coli* was also lower throughout the duration of the study (Figure 4.2). The percentage of *E. coli* strains with resistance to more than one antibiotic was highest among isolates obtained in 2016–2021 with resistance of 18.2% (n = 4), and 13.6% (n =3) against three and two antibiotics, respectively. Isolates obtained between 2005 - 2009 and 2010-2015 showed resistance of 4.5% (n = 1) against one antibiotic, in both periods.

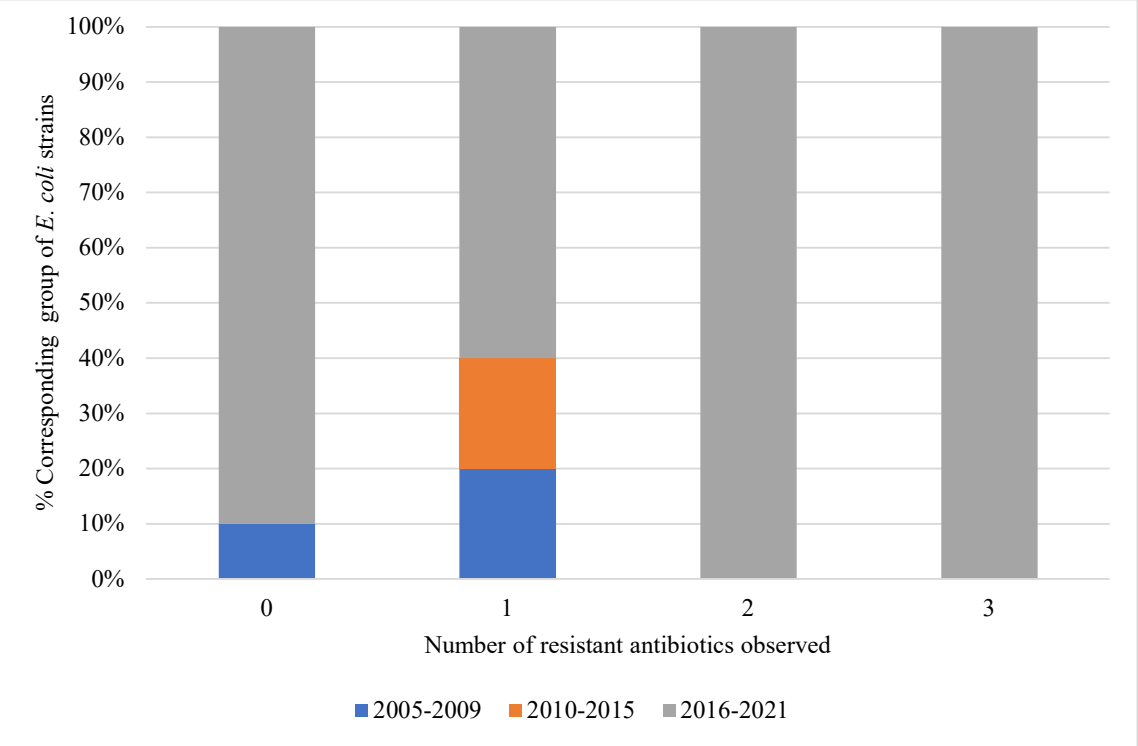

Figure 4. 2: Distribution of resistance for *E. coli* strains by the year of isolation.

4.3.2.3 Analysis of resistance multidrug resistance among *Escherichia coli*

Among the 22 tested *E. coli* isolates, a total of five resistance patterns were observed, of which 5 isolates (23%) showed multidrug resistance (MDR). Isolates that exhibited MDR were all recovered from poultry meat samples that were isolated in 2021 (Table 4.4). The resistance pattern represented by ME- CETR- AMP and TET- AMP- CIP were most detected among *E. coli* isolates.

4.3.3 *Listeria monocytogenes*

4.3.3.1 The overall sensitivity of *Listeria monocytogenes*

Table 4.5 shows the antibiotic results of *L. monocytogenes* (n = 24) isolates tested using the disk diffusion method. Among the 13 tested antibiotics, a statistically significant higher susceptibility was observed against linezolid and tylosin tartrate (100%; n = 24) compared to 33% (n = 8) chloramphenicol. All tested *L. monocytogenes* isolates showed complete (100%) resistance against a wide range of antibiotics, including penicillin, gentamycin, kanamycin, vancomycin, ciprofloxacin, quinupristin-dalfopristin, tetracycline, and erythromycin. A high percentage (92%, n=22) of the tested isolates of *L. monocytogenes* were resistant to Tigecycline, while 67% (n = 16) of the isolates were resistant to chloramphenicol. The differences were statistically significant ($P< 0.00001$).

Table 4. 4: Resistance patterns of *Escherichia coli* strains isolated from poultry meat

Resistance pattern exhibited by *E. coli*					
Resistance pattern	**No. (%) of isolates exhibiting resistance patterns**	**No. of agents involved in the pattern**	**Year of isolation**	**Source of isolation**	**Animal species**
ME, CETR	1(4.6)	2	2021	Meat	Poultry
AMO, AMP	1 (4.6)	2			
ME, CETR, AMP	2 (9.1)	3			
AMO, AMP, CEF	2 (9.1)	3			
TET, AMP, CIP	1 (4.6)	3			

ME= meropenem, CETR= ceftriaxone, AMP= ampicillin, AMO= amoxicillin, CEF= cefoxitin, TET=tetracycline, CIP=ciprofloxacin.

4.3.3.2 The analysis of resistance among *Listeria monocytogenes* strains isolated from 2014 -2017

The tested strains of *L. monocytogenes* (n =24) in this study were divided into two time periods: 2014-2015 (n = 11) and 2016 - 2017 (n = 13). The investigation of resistance among strains isolated at various times revealed that the proportion of resistant *L. monocytogenes* was below 30% throughout the duration of the study (Figure 4.3). Over the course of the study, 29.2% (n = 7) of *L. monocytogenes* strains showed resistance to more than one antibiotic while isolates from 2014 and 2015 were resistant to nine and ten antibiotics. Isolates from 2016 and 2017 demonstrated resistance to eight to ten antibiotics.

4.3.3.3 Analysis of multidrug resistance among *Listeria monocytogenes* strains

Among the 24 isolates of *L. monocytogenes*, a total of 3 resistant patterns were observed. The number of antibiotic agents responsible for MDR varied from 8 to 10 antibiotics (Table 4.6). Over 58% (n = 14) of the isolates were resistant to 10 antibiotics (TGC, TET, CHL, CIP, SYN, NIT, PEN, ERY, VA, CN), while 37.5% (n = 9) were resistant to 9 antibiotics (TGC, TET, CIP, SYN, NIT, PEN, ERY, VA, CN). The lowest number of antibiotic agents detected in the MDR strain was one isolate against 8 antibiotics (TET, CIP, SYN, NIT, PEN, ERY, VA, CN). Resistance patterns varied among different serogroups across years and animal species.

Table 4. 5: Overall distribution of antibiotic resistance of *Listeria monocytogenes* and *Staphylococcus aureus* isolates using disk diffusion method

Antibiotic class	**Antibiotic**	**No. of isolates resistant (%)**		**No. of isolates sensitive (%)**	
		L. monocytogenes **(n = 24)**	*S. aureus* **(n = 64)**	*L. monocytogenes* **(n = 24)**	*S. aureus* **(n = 64)**
β-Lactam	Penicillin (PEN)	24 (100)	64 (100)	0 (0)	0(0)
Glycopeptides	Vancomycin (VA)	24 (100)	61 (95)	0 (0)	9 (5)
Aminoglycosides	Gentamycin (CN)	24 (100)	64 (100)	0 (0)	0 (0)
	Kanamycin (KAN)	24 (100)	56 (88)	0 (0)	0 (0)
Tetracycline	Tetracycline (TET)	24 (100)	61 (95)	0 (0)	3 (5)
	Tigecycline (TGC)	22 (92)	17 (27)	2 (8.3)	47(73)
Macrolides	Erythromycin (ERY)	24 (100)	60 (94)	0 (0)	4 (6)
	Tylosin Tartrate (TYLT)	0 (0)	0 (0)	24 (100)	64 (100)
Oxazolidinones	Linezolid (LZD)	0 (0)	62 (97)	22 (100)	2(3)
Fluoroquinolones	Ciprofloxacin (CIP)	24 (100)	45 (70)	0 (0)	19(30)
Phenicols	Chloramphenicols (CHL)	16 (67)	62 (97)	8 (33.3)	2 (3)
Nitrofuran	Nitrofurantoin (NIT)	24 (100)	63 (98)	0 (0)	1 (2)
Macrolide-lincosamide-streptogramin	Quinupristin-dalfopristin (SYN)	24 (100)	62 (97)	0 (0)	2(3)

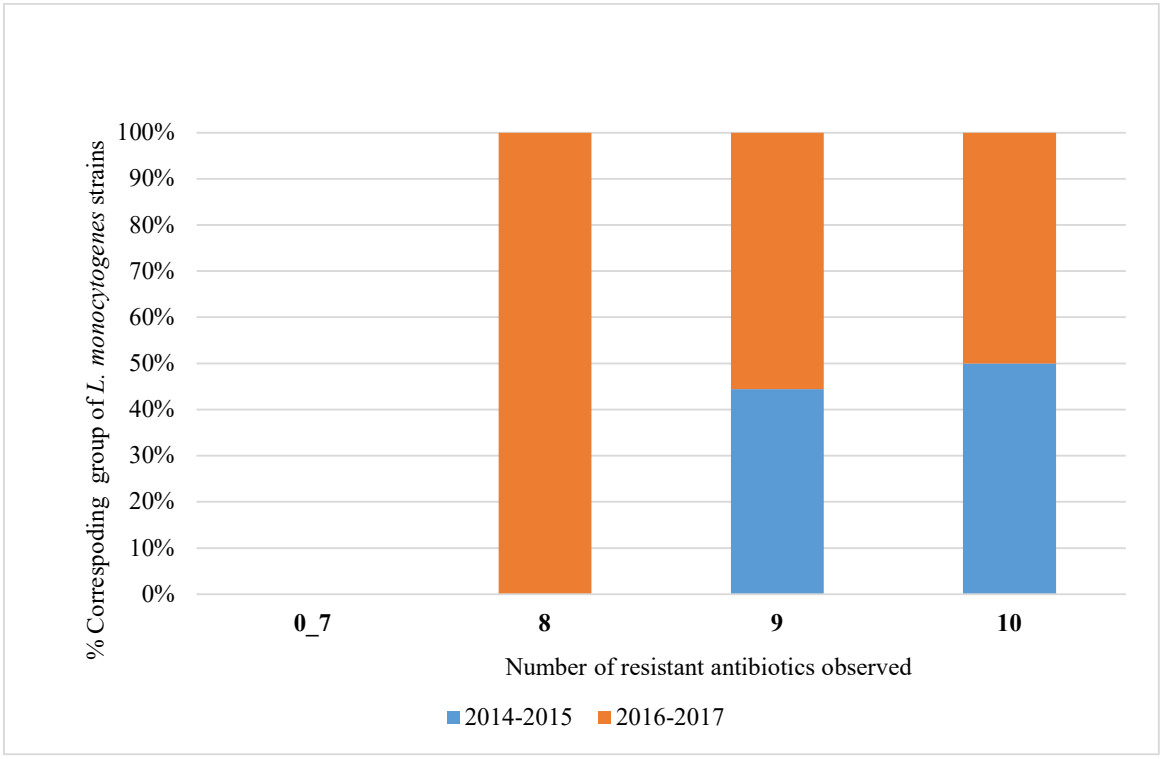

Figure 4. 3: Distribution of resistance for *Listeria monocytogenes* strains by the year of isolation.

Table 4. 6: Resistance patterns of *Listeria monocytogenes* strains from meat sources and various animal species between 2015 -2017

Resistance pattern	No. (%) of isolates exhibiting resistance patterns	No. of agents involved in the pattern	Year of isolation	Serogroup	Source of isolation	Animal species
TET, CIP, SYN, NIT, PEN, ERY, VA, CN	1 (4)	8	2017	IIb	Meat	Bovine
TGC, TET, CIP, SYN, NIT, PEN, ERY, VA, CN	9 (38)	9	2015 (n = 4) 2017 (n = 5)	IIa (n = 4) Ivb (n = 5)	Meat	Bovine (n = 8) Poultry (n = 1)
TGC, TET, CHL, CIP, SYN, NIT, PEN, ERY, VA, CN	14 (58)	10	2015 (n = 7) 2016 (n = 1) 2017 (n = 6)	IIa (n = 4) IIb (n = 2) Ivb (n =5) Ungroupable (n = 3)	Meat	Bovine (n = 12) Poultry (n =1) Swine (n = 1)

TGC=tigecycline, TET=tetracycline, CHL=chloramphenicol, CIP=ciprofloxacin, SYN= Quinupristin-dalfopristin, NIT= Nitrofurantoin, PEN= penicillin, ERY= erythromycin, VA= Vancomycin, CN=gentamycin

4.3.4 *Staphylococcus aureus*

4.3.4.1 The overall sensitivity of *Staphylococcus aureus*

The antibiotic results of *S. aureus* isolates (n = 64) tested using the disk diffusion method are shown in Table 4.5. Tylosin tartrate showed the highest susceptibility (100%; n = 64) of the 13 antibiotics tested, followed by tigecycine (73%; n = 47) and ciprofloxacin (30%; n = 19). Penicillin and gentamycin showed the highest resistance (100%; n = 64) against 13 antibiotics tested followed by nitrofurantoin (98%; n = 63), chloramphenicol, linezolid, and quinupristin-dalfopristin (all three at 97%; n = 62), tetracycline and vancomycin (both at 95%; n = 61), erythromycin (94%; n = 60), kanamycin (88%; n = 56) and ciprofloxacin (90%; n = 64). The differences were statistically significant ($P<0.0001$). The analysis of the variation in isolation source and isolation year was not possible since all tested strains of *S. aureus* were isolated from meat samples in 2015. On kanamycin antibiotics, eight isolates (12%) produced intermediate results.

4.3.4.2 Analysis of multidrug resistance among *Staphylococcus aureus* strains

Among the 64 isolates of *S. aureus*, a total of nine resistant patterns were observed. The highest number of *S aureus* isolates exhibiting MDR was 46.9% (n = 30). They were resistant against 11 antibiotics (TGC, TET, CHL, CIP, SYN, LZD, NIT, PEN, ERY, VA, CN). At least 21.9% (n =14) of the isolates were resistant to 10 antibiotics (TGC, TET, CHL, CIP, SYN, NIT, PEN, VA, CN) while 12.5% (n = 8) and 4.7% (n = 3) were resistant to eight antibiotics (Table 4.7).

Table 4. 7: Resistance patterns of *Staphylococcus aureus* strains from meat sources and various species in 2015.

Resistance pattern	No. (%) of isolates exhibiting resistance patterns	No. of agents involved in the pattern	Year of isolation	Source of isolation	Animal species
LZD, NIT, PEN, CN	1 (2)	4	2015	Meat	Bovine
TGC, NIT, PEN, ERY, CN	1 (2)	5			Bovine
TET, CHL, SYN, LZD, PEN, CN	1 (2)	6			Bovine
TET, CHL, SYN, LZD, NIT, PEN, VA, CN	3 (5)	8			Swine (n = 1) Bovine (n = 2)
CHL, SYN, LZD, NIT, PEN, ERY, VA, CN	1 (2)	8			Bovine
TGC, TET, CHL, SYN, LZD, PEN, ERY, VAN, CN	1 (2)	9			Bovine
TET, CHL, CIP, SYN, LZD, NIT, PEN, ERY, VA, CN	8 (13)	10			Poultry (n = 1) Bovine (n = 7)
TGC, TET, CHL, CIP, SYN, NIT, PEN, VA, CN	14 (22)	10			Swine (n = 1) Bovine (n = 13)
TGC, TET, CHL, CIP, SYN, LZD, NIT, PEN, ERY, VA, CN	30 (47)	11			Swine (n = 3) Bovine (n = 27)

TGC=tigecycline, TET=tetracycline, CHL=chloramphenicol, CIP=ciprofloxacin, SYN= Quinupristin-dalfopristin, LZD= linezolid, NIT= Nitrofurantoin, PEN= penicillin, ERY= erythromycin, VA= Vancomycin, CN=gentamycin

4.3.4.3 The antibiotic resistance index (ARI)

The antibiotic resistance index (ARI) of our isolates from different periods was calculated (Table 4.8). The ARI scores for all groups studied were less than 0.1, indicating relatively low environmental contamination. The findings showed that the percentage of antibiotic-resistant strains increased between 2016 and 2021.

Table 4. 8: Antibiotic Resistance Index

Period of Isolation	Gram Negative Pathogens			Gram Positive Pathogens		
	S. enterica (53)	*E. coli* (22)	All collection	*S. aureus* (64)	*L. monocytogenes* (24)	All Collection
2000- 2004	0.012	n.a	0.012	n.a	n.a	n.a
2011- 2015	n.a	n.a	n.a	n.a	0.035	0.035
2016- 2021	0.003	0.011	0.014	0.072	0.041	0.113

n.a.–not applicable

4.4 Overall distribution of susceptibility by Minimum Inhibitory Concentration (MIC) method

4.4.1 *Salmonella enterica*

On *S. enterica* isolates (n = 53), antibiotic susceptibility was also determined using MIC methods. Sulfisoxazole, gentamycin, and ceftriaxone were found to be the most effective antibiotics against all isolates (100%; n = 53). Trimethoprim (87%, n = 47), Ciprofloxacin (77%, n = 41), tetracycline (62%, n = 33), ciprofloxacin (45%, n = 24), chloramphenicol (26%, n = 14), amoxicillin (25%, n = 13), and ceftiofur (2%, n = 1) were the next most effective. Trimethoprim (87%; n = 46), ceftiofur (32%; n = 17), ampicillin (26%; n = 14), chloramphenicol (21%; n = 11), amoxicillin (13%; n = 7), ceftiofur (11%; n = 6), and tetracycline (6%; n=3) produced intermediates (Table 4.9).

Table 4. 9: Overall distribution of antibiotic resistance of *S. enterica* and *E. coli* isolates using MIC method

Antibiotic class	**Antibiotic**	**No. of isolates resistant (%)**		**No. of isolates sensitive (%)**		**Intermediate (%)**	
		E. coli **(n = 22)**	***S. enterica*** **(n = 53)**	***E. coli*** **(n = 22)**	***S. enterica*** **(n = 53)**	***E. coli*** **(n = 22)**	***S. enterica*** **(n = 53)**
β-Lactam	Ampicillin (AMP)	9 (41)	26 (49)	7 (32)	13 (25)	6 (27)	14 (26)
	Amoxycillin (AMO)	16 (73)	22 (42)	0 (0)	24 (45)	6 (27)	7 (13)
Cephalosporin	Ceftriaxone (AXO)	6 (27)	0 (0)	16 (73)	53 (100)	0 (0)	0 (0)
	Ceftiofur (XNL)	18 (82)	1 (2)	1 (5)	52 (81)	3 (13)	0 (0)
Aminoglycosides	Gentamycin (CN)	0 (0)	0 (0)	22 (100)	53 (100)	0 (0)	0 (0)
Tetracycline	Tetracycline (TET)	4 (18)	17 (32)	17 (77)	33 (62)	1 (5)	3 (6)
Sulfonamides	Sulfisoxazole (FIS)	0 (0)	0 (0)	22 (100)	53 (100)	0 (0)	0 (0)
	Trimethoprim (TRIM)	0 (0)	7 (13)	22 (100)	46 (87)	0 (0)	0 (0)
Fluoroquinolones	Ciprofloxacin (CIP)	11 (50)	12 (23)	11 (50)	41 (77)	0 (0)	0 (0)
Phenicols	Chloramphenicol (CHL)	9 (41)	28 (52)	2 (9)	14 (26)	11 (50)	11 (21)

4.4.2 *Escherichia coli*

Table 4.9 shows the overall sensitivity of *E. coli* isolates to antibiotics using the MIC method, with all *E. coli* isolates (100%; n = 22) showing susceptibility to sulfisoxazole, trimethoprim and gentamycin. Only (77%; n = 17) isolates were susceptible to tetracycline, (73%; n = 16) to ceftriaxone, (50%; n = 11) to ciprofloxacin, (32%; n = 7) to amoxicillin, (9%; n = 2) to chloramphenicol, and (5%; n = 1) to ceftiofur. Intermediate sensitivity was observed with chloramphenicol (50%; n = 11), ampicillin and amoxicillin (27%; n = 6), ceftiofur (13%; n = 3), and tetracycline (5%; n = 1).

4.4.3 *Listeria monocytogenes*

Table 4.10. displays the minimum inhibitory concentration method results for *L. monocytogenes* isolates (n = 24) tested in this study. Of the 13 antibiotics tested, sensitivity of *L. monocytogenes* was highest with linezolid (58%; n=14) followed by chloramphenicol (38%; n = 9). All *L. monocytogenes* isolates were resistant to penicillin, vancomycin, gentamycin, tetracycline, tigecycline, ciprofloxacin and quinupristin-dalfopristin (100%; n=24). In addition, intermediates were observed with nitrofurantoin (100%; n = 24), erythromycin (96%; n = 23), and chloramphenicol (63%; n = 15). The differences were statistically significant (P<0.0001).

4.4.4 *Staphylococcus aureus*

Resistance of *S. aureus* was highest against gentamycin and penicillin (100%; n = 64), followed by tetracycline and linezolid (97%; n = 62), vancomycin (95%; n = 61), chloramphenicol (93%; n = 60), erythromycin (88%; n = 56), tigecycline and ciprofloxacin (73%; n = 47) (Table 4.10). Nitrofurantoin (97%; n = 62), erythromycin (3%; n= 2) and chloramphenicol (2%; n = 1) were intermediate. The differences were statistically significant (P<0.0001).

Table 4. 10: Overall distribution of antibiotic resistance of *Listeria monocytogenes* and *Staphylococcus aureus* isolates using MIC method

Antibiotic class	**Antibiotic**	**No. of isolates resistant (%)**		**No. of isolates sensitive (%)**		**Intermediate (%)**	
		L. monocytogenes **(n=24)**	***S. aureus*** **(n=64)**	***L. monocytogenes*** **(n=24)**	***S. aureus*** **(n=64)**	***L. monocytogenes*** **(n=24**	***S. aureus*** **(n=64)**
β-Lactam	Penicillin (PEN))	24 (100)	64 (100)	0 (0)	0 (0	0 (0)	0 (0)
Glycopeptides	Vancomycin (VA)	24 (100)	61 (95)	0 (0)	3 (5)	0 (0)	0 (0)
Aminoglycosides	Gentamycin (CN)	24 (100)	64 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Tetracycline	Tetracycline (TET)	24 (100)	62 (97)	0 (0)	2 (3)	0 (0)	0 (0)
	Tigecycline (TGC)	24 (100)	47 (73)	0 (0)	17 (27)	0 (0)	0 (0)
Macrolides	Erythromycin (ERY)	1 (4)	56 (88)	0 (0)	6 (9)	23 (96)	2 (3)
Oxazolidinones	Linezolid (LZD)	10 (42)	62 (97)	14 (58)	2(3)	0 (0)	0 (0)
Fluoroquinolones	Ciprofloxacin (CIP)	24 (100)	47 (73)	0 (0)	17(27)	0 (0)	0 (0)
Phenicols	Chloramphenicols (CHL)	0 (0)	60 (93)	9 (38)	3 (5)	15 (63)	1 (2)
Nitrofuran	Nitrofurantoin (NIT)	0 (0)	0 (0)	0 (0)	2 (3)	24 (100)	62 (97)
Macrolide-lincosamide-streptogramin	Quinupristin-dalfopristin (SYN)	24 (100)	62 (97)	0 (0)	2(3)	0 (0)	0 (0)

4.4.5 Examining antimicrobial resistance pattern of *Salmonella enterica* and *Escherichia coli* using MIC assay

During different time periods under review in this study, a pattern of susceptibility shifting toward resistance was observed. This was most visible in the *S. enterica* and *E. coli* strains which were isolated between 2000 and 2005, 2006 and 2010, and 2016 and 2020, and 2021, respectively (Figure 4.4). To clearly support this notion, all isolates tested between 2000 and 2005 showed a shift in the form of increasing concentrations toward resistance against all ten antibiotics. For example, these isolates were sensitive to chloramphenicol at a range of 2–4 mg/L between 2000 and 2004; however, towards the year 2005, the isolates were sensitive to the same antibiotic at a concentration of 8 mg/L, which shifted toward resistance. During the years 2000-2005, tetracycline concentrations increased from 4mg/L to 8mg/L, amoxicillin concentrations increased from 2/1mg/L to 4/2mg/L, ceftriaxone concentrations increased from 0.25mg/L to 1mg/L during 2021, ciprofloxacin concentrations increased from 0.12mg/L to 0.25mg/L, gentamycin concentrations increased 8mg/L to 16mg/L, ceftiofur at 2mg/L to 4mg/L, trimethoprim at 0.12/2.38 to 0.25/4.75mg/L and ampicillin at 2mg/L increasing to 4mg/L concentration in 2021. Although this shift was not statistically significant, it represents a serious observation.

A similar pattern was observed against seven of ten antibiotics showing the shift towards resistance during 2006-2010, tetracycline at 4mg/L, ceftriaxone at 0.25mg/L, amoxicillin at 4/2mg/L, ciprofloxacin at 0.25mg/L, gentamycin at 0.5mg/L, ceftiofur at 1mg/L and trimethoprim at 0.12/2.28 and 2/38 concentrations. Significant changes or a shift toward resistance were observed between 2011 and 2015, with the same pattern with minimal picks obtained across the six antibiotics except for ceftriaxone and ciprofloxacin, which showed a shift at 0.25mg/L, ceftiofur at 2mg/L, and trimethoprim at 0.5/95. Tetracycline (8mg/L), ciprofloxacin (0.12mg/L), gentamycin (16mg/L), ceftiofur (4mg/L), sulfisoxazole (64mg/L), and trimethoprim (0.12/2.38) were found to have evidence of susceptibility shift between 2016 and 2020. In 2021, a similar pattern of six antibiotics was observed, including chloramphenicol at 16mg/L, tetracycline at 4mg/L, ceftriaxone at 0.25mg/L, ciprofloxacin at 0.25 mg/L, ceftiofur at 4mg/L, and trimethoprim at 0.25/4.4.75. Tetracycline, ceftriaxone, ciprofloxacin, chloramphenicol, and trimethoprim were common antibiotics that demonstrated susceptibility shifts across all four time periods.

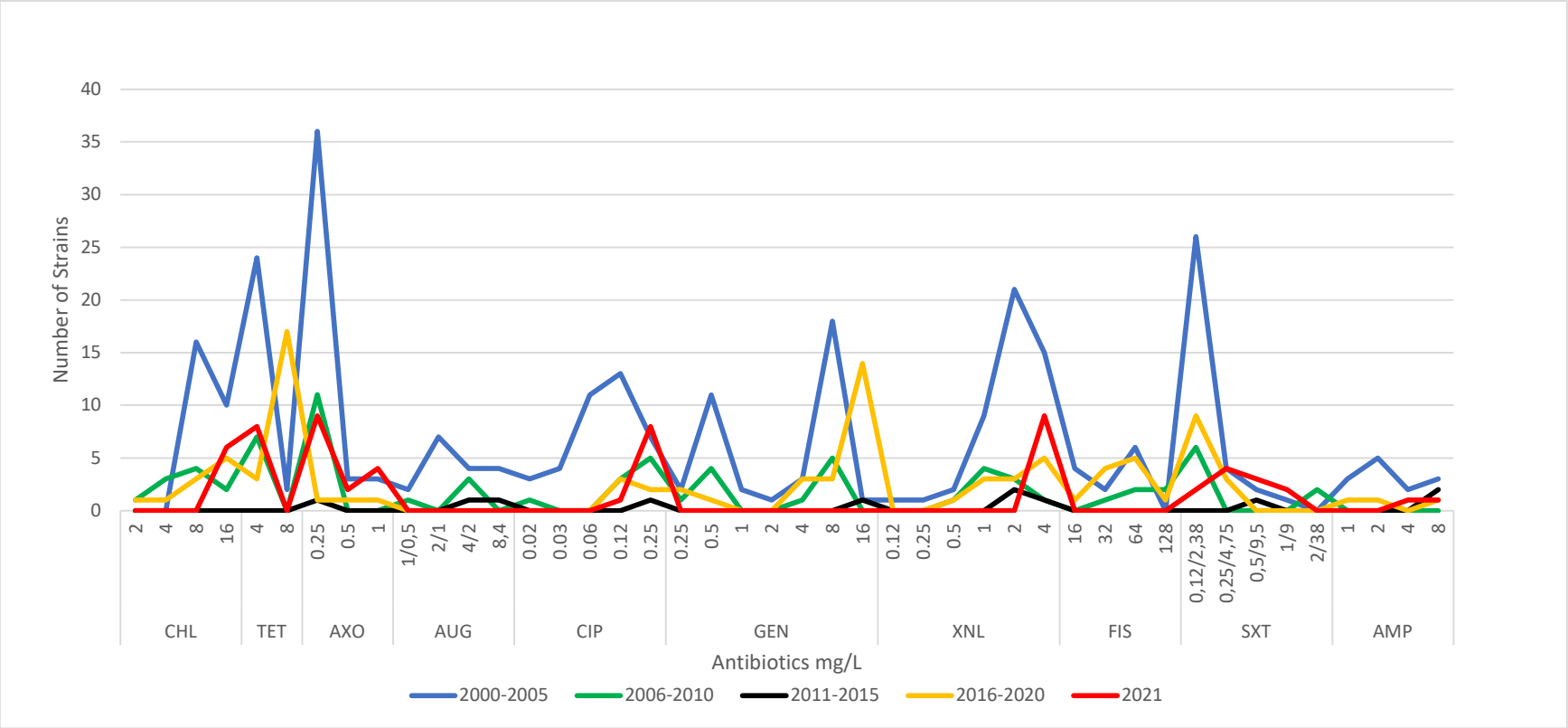

Figure 4. 4*:* Antimicrobial sensitivity pattern shift towards resistance of *S. enterica* and *E. coli* pathogens over the time period :(CHL: chloramphenicol, TET: tetracycline, AXO: ceftriaxone, AUG: amoxicillin, CIP: ciprofloxacin, GEN: gentamycin, XNL: ceftiofur, FIS: sulfisoxazole, SXT: trimethoprim, AMP: ampicillin).

4.4.6 Change in Antimicrobial resistance pattern of *Listeria monocytogenes* using MIC

Figure 4.5 depicts the sensitivity shift toward resistance observed in *L. monocytogenes* from 2015 to 2017. Picks were observed from tigecycline at 0.12 and 0.25 mg/L, ciprofloxacin at 0.120.25 mg/L, chloramphenicol at 8mg/L, and linezolid at 2mg/L between 2015 and 2017.

4.5 Detection of antibiotic resistance genes

4.5.1. Prevalence of antibiotic resistance genes amongst Gram negative isolates

Seventy-five isolates consisting of *E. coli* (n = 22) and *S. enterica* (n = 53), were screened for the presence of 18 antibiotic resistance genes using PCR. Tetracycline resistance encoding genes, *tetA* and *tetB* were indiscriminately (p: 0.2634 and 0.8270 respectively) present in strains of *Salmonella enterica* in this study. The *tetA* and *tetB* genes were detected in 38% (n = 20) and 32% (n = 7) of the *Salmonella enterica* isolates. *E. coli* isolates harbored 32% of the *tetA* genes while *tetB* genes were absent (Table 4.11). Furthermore, three quinolone resistance encoding genes, 9% (n=5) *qnrA*, 17% (n=9) *qnrB* and 25% (n=13) *qnrS* were detected in *S. enterica* isolates, while 32%, 41% and 4% were detected respectively for *E. coli* isolates. However, there was no statistical significance (with the highest p = 0.3450) on the presence of quinolone resistance genes between the *E. coli* and *Salmonella* isolates. β-lactamase resistance coding genes, n=8 (15%) bla_{PSE} and n=2 (4%) bla_{TEM}, *were* detected from *S. enterica*, while for *E. coli* isolates only 36% (n=8) of bla_{PSE} was detected. Other genes encoding for resistance against phenicols were detected at lower percentages of 2% (n=4) *cat1* and both *flo* and *cm1*A at (9%; n=5) in *Salmonella* isolates. While 5% (n=1) *cat1*, 9% (n=2) *flo* and 14% (n=3) *cm1A* genes were detected from *E. coli* isolates as shown in Table 4.11. Notably, all isolates lacked bla_{CMY-2}, bla_{SHV}, *sul1, sul2, sul3*, and *DfrII* and *DfrIII* genes, which encode resistance to β-lactamase, sulfonamides, and trimethoprim, respectively. There was no statistical difference on the presence of resistance genes among the tested Gram-negative pathogens in this study.

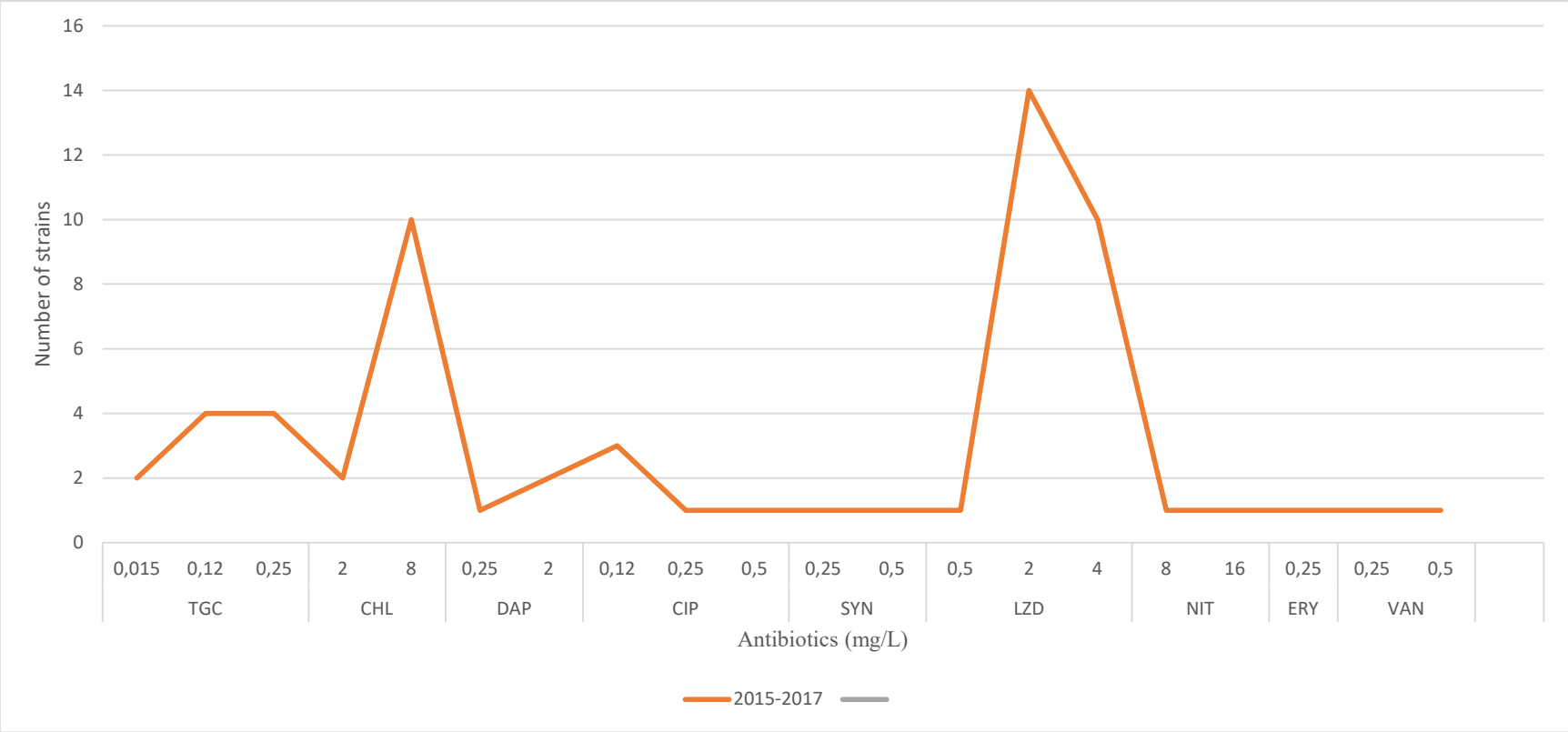

Figure 4. 5: Antimicrobial sensitivity pattern shift towards resistance of *L. monocytogenes* and *S. aureus* pathogens: (TGC: tigecycline, CHL: chloramphenicol, DAP: daptomycin, CIP: ciprofloxacin, SYN: quinupristin/dalfopristin, LZD: linezolid, NIT: nitrofurantoin, ERY: erythromycin, VAN: vancomycin).

Table 4. 11: Antimicrobial resistance genes detected in *S. enterica and E. coli*

Pathogens	**Isolates**	**Antibiotic resistance genes (%)**																	
		tetA	***tetB***	***DfrI***	***DfrII***	***DfrIII***	***sul1***	***sul2***	***sul3***	***cat1***	***flo***	***cm1A***	***bla$_{TEM}$***	***bla$_{CMY-2}$***	***bla$_{SHV}$***	***bla$_{PSE}$***	***qnrA***	***qnrB***	***qnrS***
S. enterica	53 (71)	20 (38)	21 (40)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (8)	5 (9)	5 (9)	2 (4)	0 (0)	0 (0)	8 (15)	5 (9)	9 (17)	13 (25)
E. coli	22 (29)	7 (32)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)	2 (9)	3 (14)	0 (0)	0 (0)	0 (0)	8 (36)	7 (32)	9 (41)	8 (4)
Total	75	29 (34)	24 (28)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (6)	7 (8)	8 (9)	2 (3)	0 (0)	0 (0)	16 (21)	12 (14)	18 (21)	21 (25)
p-value	-	0.2634	0.8270	0.9140	-	-	-	-	-	0.5238	0.8330	0.5932	0.1631	-	-	0.1304	0.3450		0.081

4.5.2 Antibiotic resistance genes among the Gram-positive pathogens

4.5.2.1 *Staphylococcus aureus*

A total of 28 (44%) isolates carried the *mecA* gene, which encodes methicillin resistance, while 25 (39%) carried the *blaZ* gene, which encodes β-lactamase resistance. Erythromycin resistance encoding genes, *ermA*, *ermB*, and *ermC*, were present in 20% (n = 13), 17% (n = 11), and 20% (n = 13) of isolates. Twenty (31%) isolates carried *aap-apl*, which encodes for aminoglycoside resistance. A total of 14 (22%) and 5 (8%) isolates had *tetA* and *tetM* genes, which are responsible for tetracycline resistance (Table 4.12). The *vanA* and *vanB* genes were not present in all the tested isolates in this study. Gel picture of PCR result are shown in annexure A.

4.5.2.2 *Listeria monocytogenes*

Listeria monocytogenes isolates tested in this study were grouped into five different serogroups (Table 4.13) which were screened against eight resistance genes. Penicillin resistance encoding gene (penA) was discovered commonly in isolates belonging to serogroup Iva (13%; n = 3), followed by IIb (8%; n = 2), IIa and Ivb (4%; n = 1). The Iva serogroup was found to harbor *tetA* 10 (42%) and *tetM* (21%; n=5) genes which encodes for tetracycline resistance, followed by IIc 8(33%; n=8) on both, IIb (33%; n=8), (21%; n=5), and IIa (21%; n=5), respectively. Furthermore, eight percent (n= 4) of erythromycin resistance encoding gene *ermA* was discovered from the isolates belonging to the three serogroups IIa, Iva, Ivb and IIb (4%; n=1), followed by *ermB* from serogroups IIc (71% ; n=17), IIb (33% ; n=8), IIa and Ivb (4%; n=1) and *ermC* from Iva (50%; n=12), IIb (13%; n=3), IIc (8%; n=2) and IIa (4%; n=1).

Table 4. 12**:** Selected antibiotic resistance genes of *Staphylococcus aureus* in this study.

	Antibiotic resistance genes (%)								
	β-lactam	**Erythromycin**			**Aminoglycoside**	**Tetracycline**		**Methicillin**	**Vancomycin**
	blaZ	*ermA*	*ermB*	*ermC*	*aap-apl*	*tetA*	*tetM*	*mecA*	*vanA & vanB*
Number of positive isolates	25 (39)	13 (20)	11 (17)	13 (20)	20 (31)	5 (8)	14 (22)	28 (44)	0 (0)

Table 4. 13: Genes detected from *Listeria monocytogenes*

Serogroups	**Antibiotic resistance genes (%)**							
	Penicillin	**Tetracycline**		**Erythromycin**			**Vancomycin**	
	penA	*tetA*	*tetM*	*ermA*	*ermB*	*ermC*	*van A*	*van B*
IIa	1 (4)	5 (21)	3 (13)	2 (8)	1 (4)	1 (4)	0	0
IIb	2 (8)	8 (33)	5 (21)	1 (4)	8 (33)	3 (13)	0	0
IIc	0 (0)	8 (33)	8 (33)	0 (0)	17 (71)	2 (8)	0	0
Ivb	1 (4)	7 (29)	6 (25)	2 (8)	1 (4)	0 (0)	0	0
Iva	3 (13)	10 (42)	5 (21)	2 (8)	0 (0)	12 (50)	0	0
p-value	0.980	0.001	0.023	0.071	0.085	0.002	-	-

CHAPTER 5: DISCUSSION

5.1 Viability of the isolates

This study investigated the antibiotic sensitivity of stored bacterial isolates over a 20-year period. The bacterial isolates were stored as lyophilized (n = 88) or in glycerol (n =138). These methods are generally used to stabilizes or preserve the bacterial cultures for long-term storage (Bircher *et al*., 2018; Guo *et al*., 2020). Bacterial lyophilization, also known as freeze-drying, is a process used to preserve bacteria by removing water from the cells and subsequently storing them in a dry state. It is a commonly employed method in microbiology, biotechnology, and pharmaceutical industries to extend the shelf life of bacterial cultures and maintain their viability over long periods (Wessman *et al*., 2013). Surprisingly, only 31% of the lyophilized bacterial isolates were viable in the current study. This finding was inconsistent with a study by Bircher *et al*., (2018) which reported a high (< 90%) recovery rate for various bacterial isolates that were lyophilizates for 30 years. Bellali *et al*., (2020), argue that lyophilization of bacterial culture has been widely used for decades as a suitable technique for preserving microorganisms and also reported a recovery rate of 98% in isolates that were stored for over 20 years. Kupletskaya and Netrusov, (2011), recovered 100% of bacterial isolates which were stored for more than 50 years. It is worth noting that the success of bacterial lyophilization depends on various factors, including the specific bacterial strain, the composition of the protective medium used during the process, and the optimization of the freeze-drying conditions (Nasran *et al*., 2020). Therefore, a low recovery rate observed in the current study might be influence by these factors. Moreover, this method can also at times damage cell membranes and proteins without a suitable protectant medium, threatening the bacteria's viability (Bellali *et al*., 2020) which might have also been the case in this study.

The bacterial isolates that did not show viability with lyophilization include *S.* Enteritidis (9%), *S.* Typhimurium (31%), *E. coli* (33%), and *L. monocytogenes* (29%). *Salmonella* Enteritidis and *S*. Typhimurium, which did not yield growth upon revival, were persevered between 2000 and 2019, with the majority coming from 2001 (n = 11) and 2015 (n = 9). Notably, most of the *E. coli* and *L. monocytogenes* isolates that were not viable were from 2015. This poor recovery rate associated with the year 2015 was due to a change in laboratory personnel as new staff started operating the freeze-drying machine.

All *S. aureus* isolates used in the current study were preserved in glycerol and yielded 100% viability rate. This result was consistent with those reported by Mahmmoud, (2020) who discovered that preservation of *S. aureus* using glycerol for long periods of time (40 years) allows 100% recovery rate. According to Bircher *et al*., (2018), preservation of bacterial isolates in glycerol is the most effective way of storing samples indefinitely, hence all isolates in our study were still viable because they were preserved for less than 5 years. This notion is also supported by American Tissue Culture Centre (Prakash *et al*., 2020). Freitas-Ribeiro, Reis and Pirraco, (2022), reported this method as harmless to microorganism at any concentration of glycerol and can maintain cells during gradual freezing by reducing or delaying the cells' osmotic-derived shrinking to a lower temperature, hence there was 100% recovery rate in this study.

5.2 Detection of phenotypic antimicrobial resistance

In the current study phenotypic antimicrobial resistance was determined using both Disk diffusion and minimum Inhibitory Concentration (MIC) methods. These methods are commonly used in microbiology to determine the susceptibility of bacteria to antimicrobial agents. They help in evaluating the effectiveness of antibiotics or other antimicrobial drugs against specific bacterial strains. They guide clinicians in selecting appropriate antibiotics for treating bacterial infections (Leekha *et al*., 2011). The MIC method determines the lowest concentration of an antimicrobial agent that inhibits the visible growth of bacteria. It provides a quantitative measure of susceptibility whereas disk diffusion method measures the ability of an antimicrobial agent to create a concentration gradient in the agar medium (Gajic *et al*., 2022).

5.2.1 *Salmonella enterica*

Generally, the results from both disk diffusion and MIC assays revealed that the *S. enterica* isolates were sensitive to a wide range of important clinical and veterinary antibiotics tested in this study. This comparatively high sensitivity rate suggests that the majority of antimicrobial agents used in the current study might be effective in the treatment of *Salmonella* infections associated with animal and animal-derived food products in South Africa. The high antibiotic sensitivity of *Salmonella* isolates reported in this study was inconsistent with numerous previous studies that were conducted in South Africa over the years which reported a high prevalence of resistant *Salmonella* in animal and food of animal origin (Jaja *et al*., 2019;

Madoroba *et al*., 2016; Mathole *et al*., 2017). For example, Zishiri *et al*., (2016) reported that *Salmonella* strains isolated from animals exhibited resistance to ten antibiotics at frequencies ranging between 93% and 12%. The variation of the results between studies can be attributed to the low sample size of this study.

However, 32% of *S. enterica* isolates in our study were resistant to tetracycline using disk diffusion method. This finding was supported by the MIC results which reported a resistance of 38%. This resistance level was not surprising because tetracycline is one of the most commonly used antibiotics in South African livestock production systems. Tetracycline has been approved for use in animal feed under the Fertilizers, Farm Feeds, and Agricultural Remedies Act (Act No. 36 of 1947), and it has been reported that tetracyclines are the second most commonly used antibiotic class in the South African animal production industry (Eagar *et al*., 2012). Moreover, tetracycline is relatively cheap and available from diverse sources which makes access very easy. Similarly, frequency of detection of resistance to tetracycline which has been reported in our study was somewhat consistent with what other studies had documented in South Africa (Mathole *et al*., 2017; Zishiri *et al*., 2016). The resistance level towards tetracycline observed in the current study can be concerning as this antibiotic is commonly used in the first-line treatment of human and animal salmonellosis and other bacterial infections (Peruzy *et al.*, 2020; European Medicine Agency, 2019) Furthermore, the Stock Remedies Act of 1947 in South Africa allows tetracycline to be purchased over the counter (OTC) without a veterinary prescription (Henton *et al*., 2011).

Intermediate resistance to ceftiofur, a third generation cephalosporin, was also discovered to be moderate (17%) on both the disk diffusion and MIC assays, albeit slightly higher than previously reported by European (European Food Safety Agency, 2023) and American (Dutil *et al*., 2010) counterparts in food and animal samples. The only application of ceftiofur is in veterinary medicine in many countries and is a common therapeutic drug used to treat postpartum metritis in cows and respiratory disease in feedlot cattle (Fan *et al*., 2021). It is also one of the most effective and preferred antimicrobials for lactating dairy cows due to zero withdrawal time for milk (Chambers *et al*., 2015). However, ceftiofur shares structural similarities with the widely used antibiotic ceftriaxone in human medicine, and the two antibiotics operate in a similar way (Dutil *et al.*, 2010; Schmidt *et al.*, 2013). Therefore, there are concerns that using ceftiofur for cattle ailments like respiratory disease, metritis, and enteric disease may result in ceftriaxone resistance in bacteria that cause foodborne illnesses like *S.*

enterica (Fan *et al*., 2021). Intermediate resistance to ceftiofur highlights a need for targeted resistance surveillance among animal isolates populations and in animal-derived food products in the country since it is authorized for use on food producing animals (Eagar *et al*., 2012).

Over prescription and misuse of antibiotics in clinical and veterinary setting play a significant role in the development of resistance of *S. enterica* and other pathogens to antibiotics leading to multidrug resistance (Manishimwe *et al*., 2017). Ten multidrug resistance patterns were detected across the 53 tested *S. enterica* strains in the current study and 1.9% (n = 1) of all the multi-drug resistant strains of *S. enterica* exhibited resistance to 3 – 5 antimicrobial agents. The predominant resistance patterns with a high MDR pattern against five antibiotics (TET-AMP-TRIM-CHL-CEF) represent five different classes. Similar profiles have been confirmed where *S. enterica* isolated from animal samples and food of animal origin in South Africa (Jaja *et al*., 2019) and other countries such as Portugal (Silva et al., 2022) and Mexico (Aguilar-Montes de Oca *et al*., 2018) where tetracycline, trimethoprim and ampicillin were resistant.

Salmonella Typhimurium has been among the most frequently isolated serotypes from animals in South Africa for years (Carroll *et al*., 2021; Gelaw *et al*., 2018; Magwedere *et al*., 2015). Intriguingly, among the tested *Salmonella* serotypes in our study, *S*. Typhimurium isolates from poultry, swine, and cattle samples recovered between 2002 and 2016 exhibited multidrug resistance primarily to ampicillin, chloramphenicol, trimethoprim, tetracyclines and ceftiofur. This MDR pattern is one of the most important globally as it is commonly associated with *S*. Typhimurium DT193 strains which are known to cause significant human infection globally (Prats *et al*., 2000; Threlfall *et al*., 2000). Although this MDR pattern was detected in 2% of the isolates in the current study, it should be noted that some of these antibiotics are important for treatment of human salmonellosis. For instance, antibiotics from ceftiofur are important to treat *Salmonella* infections in children and pregnant women (Collignon and McEwen, 2019). Moreover, *S*. Typhimurium with MDR with a similar pattern have become more common in many countries including Italy since 2000 (Dionisi *et al*., 2009; Graziani *et al*., 2008) and South Africa (Carroll *et al*., 2021). Similar *S.* Typhimurium isolates with this MDR pattern were associated with swine populations in the United Kingdom (Wang *et al*., 2019) and poultry in Ethiopia (Carroll *et al*., 2021). Notably, the most common MDR pattern identified in this study was in 2002, which is consistent with those reported ASSuT pattern multidrug resistance in Italy (Dionisi *et al*., 2009; Graziani *et al*., 2008).

5.2.2 *Escherichia coli*

Similar to the results of *S. enterica*, the majority of antibiotics tested on the *E. coli* isolates in the current study, were sensitive especially towards those that are used as first-line therapy for colibacillosis in humans and animals. This is an indication that most of the tested antibiotics in our study might be effective in the treatment of *E. coli* infections in animals and possibly in humans in South Africa.

Eighteen percent of *the E. coli* isolates in this study were resistant to ampicillin. This is a public concern as ampicillin is important and is used in treating bacterial infections in both humans and animals, therefore its effectiveness may be compromised. Furthermore, it raises concerns about the potential transfer of antibiotic-resistant bacteria from animals to humans through the consumption of contaminated meat(Manyi-Loh *et al.*, 2018). Ampicillin is a broad-spectrum antibiotic that is commonly used to treat bacterial infections caused by susceptible strains of *E. coli*. Ampicillin belongs to the class of antibiotics called penicillin. It works by interfering with the synthesis of the bacterial cell wall, leading to cell lysis and the death of the bacteria (Poirel *et al*., 2018). Our findings don't match those reported by Dsani *et al.*, (2020), who discovered that 57% of *E. coli* strains isolated from foods and animals were resistant to ampicillin. Ampicillin was one of the antibiotics investigated by Parvin *et al.*, (2022), who found multidrug resistance in all *E. coli* strains recovered from animal sources. Additionally, research from Bangladesh and China found that all *E. coli* bacteria found in poultry meat were ampicillin-resistant (Sarker et al., 2023). Over 85% of *E. coli* strains were shown to co-resist ampicillin and other antibiotics like tetracycline in other studies (Abdelwahab *et al*., 2022; Haulisah *et al*., 2021; Kozak *et al*., 2009). The prevalence of ampicillin-resistant *E. coli* in animals and food can vary depending on many factors, including local practices, antibiotic usage patterns, and the presence of resistant strains in the environment (Nkansa-Gyamfi *et al*., 2019). In 2017, in South Africa, ampicillin was among the most commonly administrated antibiotics in hospitals for treatment of human infections with daily doses of 132 100 (National Department of Health, 2017).

It's worth noting that 23% of *E. coli* isolates recovered from poultry meat in this study exhibited MDR pattern with ME, CETR, AMP and AMO, AMP, CEF predominating. Although the percentage of these MDR patterns is low individually, it is important to note that the development of multidrug resistance in *E. coli* is a significant public health concern, as it limits

treatment options and can lead to more severe infections (Poirel et al., 2018). Therefore, MDR *E. coli* strains that exhibit resistance to meropenem, ceftriaxone, ampicillin, amoxicillin, and cefoxitin can pose significant challenges in the treatment of infections. This is because some of the antibiotics are considered last-resort or third generation in the treatment of *E. coli* and other bacterial infections. For example, meropenem belongs to the carbapenem class of antibiotics and is used to treat serious infections. It is often considered the last-resort antibiotic for treating gram-negative bacteria. Whereas ceftriaxone is a third-generation cephalosporin antibiotic commonly used to treat a range of bacterial infections (Berhe *et al*., 2019). Additionally, it has been shown that the treatment of *E. coli* infections, particularly in the elderly, children, and immune-compromised people, is negatively impacted by *E. coli* isolates with various antibiotic resistance patterns similar to the one seen here (Wu *et al*., 2021). The results of our study concur with those reported in food and animals from Tanzania *(*Sonola *et al*., 2021).

5.2.3 *Staphylococcus aureus*

In this study, the majority of *S. aureus* isolates showed resistance to up to 11 antimicrobial agents on both MIC and disk diffusion assays. Resistance to gentamicin, penicillin, erythromycin, tetracycline, ciprofloxacin, nitrofurantoin, linezolid, quinupristin-dalfopristin, vancomycin and kanamycin were common in the current study. It has been reported that this bacterium has shown resistance to several available antimicrobial agents in the last few decades, and most recently, it developed resistance to some of the chemotherapeutic agents considered last-resort treatment, daptomycin and linezolid (Jubeh and Breijyeh, 2020). Antibiotic resistance in *S. aureus* is a significant concern in both human and animal health. In humans and animals, *Staphylococcus aureus* can cause a variety of infections including skin and soft tissue infections, pneumonia, bloodstream infections, and foodborne infections. The emergence and spread of antibiotic-resistant strains of *Staphylococcus aureus* has become a worldwide health concern (Chinemerem Nwobodo *et al*., 2022; Rasha *et al*., 2018).

High resistance to penicillin and gentamycin was observed in the current study which was not surprising given that these antibiotics are commonly used to treat infections in both humans and animals (Cave et al., 2021), moreover penicillin has developed resistance to *S. aureus* since the 1960s (Lowy, 2003; Thwala *et al*., 2021). Gentamycin is an antibiotic that belongs to the aminoglycoside class of drugs. It is often used to treat infections caused by bacterial infections,

including those caused by *S. aureus* (Serio *et al*., 2018). Gentamycin is generally effective against methicillin-susceptible *Staphylococcus aureus* (MSSA), which is a strain of *S. aureus* that is sensitive to methicillin and other beta-lactam antibiotics. Resistance of *S. aureus* from animals, foods and humans to gentamycin has been well documented in South Africa (Amoako *et al*., 2016; Marais *et al*., 2009; Sineke, 2021) and other countries (Yılmaz and Aslantaş, 2017). Other studies in South Africa found that 99.7% to 100% of *S. aureus* isolates were resistant to penicillin and gentamycin *(Akindolire et al., 2015; Buzón-Durán et al., 2017; Li et al*., 2018). These findings are most likely the result of easy access to these antimicrobial agents in South Africa (Henton *et al*., 2011).

Nitrofurantoin is typically used to treat urinary tract infections and is not considered a first-line treatment for *Staphylococcus aureus* infections. As a result, nitrofurantoin resistance in *S. aureus* is relatively uncommon. Resistance mechanisms can develop in rare cases where nitrofurantoin is used to treat *S. aureus* infections or co-infections with other bacteria (Squadrito and Portal, 2020). Although nitrofurantoin resistance mechanisms in *S. aureus* may differ, mutation in chromosomal genes involved in activation or bacterial targets of nitrofurantoin are one common mechanism (Squadrito and Portal, 2020). High resistance (98%) of *S. aureus* isolates to nitrofurantoin was obtained in this study. These findings were higher than the 7.02% and 5.72% reported by Morobe *et al*., (2009) and *Safarpoor Dehkordi et al.*, (2013) respectively from South Africa and India. However, this is not the case with this study as high resistance was detected by both disk diffusion and MIC assays. In pregnant women, nitrofurantoin is recommended as the first-line treatment for uncomplicated cystitis and urinary tract infections. It is therefore regarded as a bactericidal antibiotic against uropathogens in humans (Shakti and Veeraraghavan, 2015; Squadrito and Portal, 2020), but it is not widely used in veterinary medicine (Jaja *et al*., 2020). The high resistance to nitrofurantoin in our isolates is worrying, as animal pathogens such as *S. aureus* can spread to humans by multiple routes and introduce resistant strains to the human population.

In the current study, 97% of *S. aureus* isolates were resistant to both linezolid and quinupristin-dalfopristin. Linezolid is an antibiotic that belongs to the class of oxazolidinones with activity against Gram-positive organisms such as staphylococci, enterococci and streptococci (Butin et al., 2019). Linezolid inhibits bacterial protein synthesis by targeting the ribosomes and is often used as an alternative treatment option for MRSA infections in human or when patients cannot

tolerate other options (Butin *et al*., 2019). According to Bortolaia *et al*., (2016), linezolid can be used in poultry production but is rarely used in food-producing animals in South Africa (Moyane *et al*., 2013). High resistance observed in our study is alarming since linezolid was first introduced and approved in 2000 and it's rarely used in veterinary medicine (Hashemian *et al*., 2018) . This high resistance level may aid in the rapid zoonotic transmission to humans. Contradictory to the findings of this study, the prevalence of linezolid resistance *S. aureus* associated with meat and meat products in African countries was reported at 8.8% (Hashemian, Farhadi and Ganjparvar, 2018).

Quinupristin-dalfopristin, also known as Synercid, is a combination antibiotic that consists of two streptogramin antibiotics. Quinupristin-dalfopristin works by inhibiting bacterial protein synthesis. When other treatment options have failed or are not suitable for humans, this antibiotic is frequently reserved for severe infections caused by multidrug-resistant gram-positive organisms, such as MRSA (Rivera and Boucher, 2011). The high resistance observed in this study might be due to overuse in pig production, as the pig veterinary society of the South African Veterinary Association approved the use of virginiamycin (streptogramin associated with resistance to quinupristin-dalfopristin) for growth enhancement in pig production (Eagar *et al*., 2012). This is concerning because virginiamycin, was banned in the EU based on WHO recommendations due to chemical and structural similarities to antibiotics used in humans (Marshall and Levy, 2011). Therefore, the findings of this study suggest a need to establish the extent of the use of Quinupristin-dalfopristin in pig production in South Africa.

Chloramphenicol is an antibiotic that has long been used to treat various bacterial infections including *S. aureus*. It is a broad-spectrum antibiotic, capable of working against a wide variety of bacteria. Chloramphenicol works by inhibiting the synthesis of bacterial proteins (Reygaert, 2018). It binds to bacterial ribosomes, which are in charge of producing proteins that are required for bacterial growth and survival. Chloramphenicol prevents bacteria from multiplying and eventually kills them by interfering with protein synthesis (Foster, 2017). *S. aureus* isolates demonstrated 93% resistance against Chloramphenicol, which is similar to what Akanbi *et al*., (2017) reported. The resistance may be due to the production of an enzyme called chloramphenicol acetyltransferase (CAT), which inactivates it or due to antibiotic misuse or misapplication. It is worth noting that chloramphenicol was banned in South Africa and Europe (Eagar *et al*., 2012). In addition, phenicols are not among the main antimicrobial agents authorized under Acts 36 of 1947 and 101 of 1965. This confirms antibiotic misuse and easy

access in South Africa when compared to other countries such as Denmark and Europe (Eagar *et al*., 2012).

The observed vancomycin-resistant enterococci (VRE) and vancomycin-resistant *Staphylococcus aureus* (VRSA) confirms vancomycin resistance (Elsayed *et al*., 2018). In the present study, ninety-five percent of *S. aureus* isolates tested positive for vancomycin resistance (VRSA), implying a major challenge in the treatment of infections caused by this bacterium. This is a cause for concern, as these antibiotics are considered drugs of last resort for treating serious infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) or other multidrug-resistant strains (Ventola, 2015). Furthermore, for infection control and epidemiological purposes. Vancomycin resistance in *S. aureus* is typically associated with the acquisition of the *vanA* or *vanB* gene cluster, which alters the structure of the bacterial cell wall, making it less susceptible to the action of vancomycin (Aqib and Alsayeqh, 2022). Vancomycin efficacy has since been called into question as recent research has revealed that *S. aureus* is a vancomycin-resistant pathogen, increasing the burden of antimicrobial drug resistance (Aqib and Alsayeqh, 2022; Riaz *et al*., 2021). The main reasons for the high risk of therapeutic failure with glycopeptides in life-threatening MRSA infections are thought to be ineffective pharmacokinetic/pharmacodynamic properties (Holmes *et al*., 2015). Daptomycin is the only antibiotic that has been shown to be noninferior to vancomycin in the treatment of MRSA bacteremia.

A 73% ciprofloxacin resistance has been reported from both disk diffusion and MIC assays in the current study. Although the prevalence of ciprofloxacin resistance is not as high in other studies, similar results have been noted in other studies (Afzal *et al*., 2021; Van Boeckel *et al*., 2015). Ciprofloxacin is a fluoroquinolone antibiotic that is commonly used to treat bacterial infections. High levels of resistance, however, can limit its effectiveness as a treatment option (Elsayed et al., 2018). This is a concern because it has been recommended as first-line treatment for CAUTIs (community-acquired urinary tract infections) (South African antibiotic stewardship programme, 2015; the Essential Medicines List for SA, 2018) (Fourie *et al*., 2021). High resistance rate (90.9%) against ciprofloxacin has been reported by Elsayed, Ashour and Amine, (2018), on isolates which were previously confirmed as VRSA.

Moreover, 94% of *S. aureus* isolates showed resistance towards erythromycin. Erythromycin is an antibiotic that belongs to the macrolide class of antibiotics. It is commonly used in both human and veterinary medicine to treat various bacterial infections. In veterinary medicine,

erythromycin is approved for treatment of respiratory infections in several animal species, including dogs, cats and livestock (Palma et al., 2020). It has also been used in the treatment of mastitis, an infection of the mammary glands in dairy cows. Resistance to erythromycin is relatively common in *S. aureus* isolates recovered from commercial broiler abattoirs (57.1%) and chicken retail outlets (83.3%) in South Africa (Mkize, Zishiri and Mukaratirwa, 2017), and Egypt (Elsayed *et al*., 2018) which is lower than what the current study has found.

The most common *S. aureus* multidrug resistance patterns ranging from 9 to 11, (TGC, TET, CHL, SYN, LZD, PEN, ERY, VAN, CN); (TGC, TET, CHL, CIP, SYN, NIT, PEN, VA, CN) and (TGC, TET, CHL, CIP, SYN, LZD, NIT, PEN, ERY, VA, CN) with overall percentage of 2%, 22% and 47% respectively were observed. Similar profiles have been confirmed for *S. aureus* strains from South African poultry meat (100%; (Mkize, Zishiri and Mukaratirwa1, 2017), from food-animals in Canada (Rubin *et al*., 2011) in China (87.2%;Li *et al.*, 2018), and Korea (100%;Kim *et al.*, 2018). According to Adigun *et al.*, (2020) inappropriate antimicrobial agent use will keep promoting *S. aureus* and other pathogens' resistance to antimicrobial agents, eventually leading to multi-drug resistance. The MDR patterns observed in this study are vital because some antibiotic classes used in food-producing animals are also used to treat human infections. As a result, excessive utilization in animal production may result in resistance, jeopardizing their efficacy in human infections (Lekagul *et al*., 2019). Although the European Union has banned some of these antibiotics as growth promoters, South Africa continues to use them *(*Eagar *et al*., 2012; Sineke, 2021). Some antimicrobial agents such as glycopeptides are important in treating multidrug-resistant infections, hence it is critical to prevent the development of vancomycin resistance.

5.2.4 *Listeria monocytogenes*

This study found all *L. monocytogenes* isolates resistant against penicillin, gentamycin, tetracycline, erythromycin, and ciprofloxacin. This is concerning since *L. monocytogenes* is generally considered to be an antibiotic-susceptible pathogen. Moreover, penicillin in combination with gentamycin are regarded as the standard treatment for human listeriosis globally including South Africa (Keet and Rip, 2021; Tchatchouang *et al*., 2020). However, there have been reports of antibiotic resistance in certain strains of *L. monocytogenes*, particularly in recent years for example, Andriyanov *et al.*, (2021) discovered 100% penicillin

and gentamycin resistance in *L. monocytogenes* strains from various sources, which is consistent with the findings of this study.

Fifty-eight percent of the *L. monocytogenes* strains were resistant to ten antibiotics, including TGC, TET, CHL, CIP, SYN, NIT, PEN, ERY, VA, and CN. This is a concern because trimethoprim/sulfamethoxazole, erythromycin, vancomycin, and fluoroquinolones are second-line agents for listeriosis treatment, according to Andriyanov *et al.*, (2021). TET, CIP, SYN, NIT, PEN, ERY, VA, and CN were the antibiotics that contributed to MDR in our study with 28% being from IIa serotype. Antibiotic resistance, particularly multi-resistance, is a public health issue because it can lead to therapeutic treatment failure. As a result, tracking changes in *L. monocytogenes* antibiotic resistance due to the emergence of resistant strains is necessary, particularly through the incorporation of phenotypic and genotypic techniques (Tchatchouang *et al*., 2020).

5.2.5 The antibiotic resistance index (ARI)

The antibiotic resistance index (ARI) is a measure used to quantify the overall level of antibiotic resistance in a population of a specific bacterial strain. It provides a numerical value that reflects the resistance profile of the bacteria to a set of antibiotics. The ARI is a useful tool in surveillance studies and epidemiological investigations to monitor the level of antibiotic resistance in bacterial populations over time (Davis and Brown, 2016). In our study the overall collection (2000-2021) ARI ranged between 0.035 and 0.113 which indicates that the bacterial population has a low level of antibiotic resistance. This suggests that a majority of the antibiotics tested in this study will be effective in treating infections caused by these bacteria. It is typically considered favorable from a public and animal health perspective, as it indicates that antibiotic treatment options are still viable.

A value of 0.035 in the ARI suggests a very low level of antibiotic resistance in the bacterial population or strain being analyzed. It indicates that all bacteria strains in our study were resistant to only a small fraction of the antibiotics tested, with the majority of antibiotics remaining effective. This is considered favorable from a clinical and public health standpoint, as it indicates a high susceptibility to most antibiotics and a greater likelihood of successful treatment options. On the other hand, a value of 0.113 in the ARI indicates a higher level of antibiotic resistance compared to 0.003. In this case, the bacterial strain particularly *S. aureus*

was resistant to a larger proportion of the antibiotics tested. While it is still lower than 1, indicating that there are still effective treatment options available, the higher ARI value suggests a need for closer monitoring and potentially targeted interventions to prevent further spread of resistance in *S. aureus*.

5.3 Detection of genotypic antimicrobial resistance in gram negative pathogens

5.3.1 *Salmonella enterica*

Salmonella enterica strains were screened for the presence of four β-lactamase resistance coding genes (bla_{TEM}, bla_{CMY-2}, bla_{SHV} and bla_{PSE}). Only two of these genes were detected among the isolates at relatively low proportion of 15% and 4% for bla_{PSE} and bla_{TEM} respectively. The bla_{PSE} and bla_{TEM} genes are well-known resistance genes that are commonly found in various bacteria, including *Salmonella* (Eguale *et al*., 2017). These genes encode for an enzyme which confers resistance to certain beta-lactam antibiotics, such as penicillins and cephalosporins, rendering them ineffective against the bacteria (Bush and Bradford, 2020). The low presence of these genes in the isolates tested in this study corresponds to the phenotypic resistance.

A similar observation of bla_{PSE} and bla_{TEM} genes in *Salmonella* isolates has been documented in Japan (EFSA, 2011).The reason for this strong relationship is unknown, but it suggests that *Salmonella* may have beta-lactamase genes of various types, conferring resistance to β-lactamase antibiotics which is not unusual (Igbinosa *et al*., 2015). In the studies conducted by Tajbakhsh and Hendriksen, (2012) and (Li *et al*., 2014), the prevalence of bla_{PSE} and bla_{TEM} in *Salmonella* isolates was reported to be 63% and 18%, 3.36%, and 2.42%, respectively. *Salmonella* species are diverse, and resistance patterns and genes can vary between different strains and regions (Eguale *et al*., 2017).

The bla_{PSE} gene has also been reported in *Salmonella* isolates from food-producing animals in many countries (Caleja *et al*., 2011)including South Africa (Mkize, Zishiri and Mukaratirwa, 2017). It has been hypothesized that this gene is widely distributed in the South African aquatic ecosystem (Igbinosa *et al*., 2015). As a result, water might be the source for these antibiotic-resistant determinants in the food-producing animals in the country. Therefore, good farming practices, good hygiene, and safe drinking water are being promoted on animal farms.

Another study reported that over 19% of *Salmonella* strains from food-producing animals harbor the *bla$_{TEM}$* gene (Igbinosa, 2015). Aslam *et al.*, (2012) reported that the percentage of *bla$_{TEM}$* gene in *Salmonella* isolated from retail meats in Canada was 17% and this gene was the most common resistance genes detected. Beta-lactam resistant bla$_{TEM}$ gene was also found 73.3%, 63.6%, and 50% in *Salmonella* isolates of broiler, sonali and native chicken, respectively in Dhaka, Bangladesh. The emergence of *bla$_{TEM}$* genes in *Salmonella enterica* serovars is often associated overuse of beta-lactam antibiotics in poultry farming practices (Siddiky *et al*., 2021). Therefore, the low percentage of isolates harboring *bla$_{TEM}$* gene indicates the possibility of the prudent use of beta-lactam antibiotics in animal production systems.

Tetracycline resistance encoding *tetA* and *tetB* genes were detected in 38% and 32% of *Salmonella* isolates in the current study which was consistent with the phenotypic resistance results. These genes encode for efflux pumps that actively pump tetracycline antibiotics out of bacterial cells, reducing the intracellular concentration of the antibiotic and rendering it less effective (Zishiri *et al*., 2019). The presence of *tetA* and *tetB* genes in Salmonella is a result of complex interactions between various factors, including antibiotic usage, bacterial genetics, and environmental conditions (Mthembu *et al*., 2021). For example, Zhang *et al.*, (2019) reported the frequent detection of 80.9%, and only 4.8% for *tetA* and *tetB* genes respectively in tetracycline resistant *Salmonella* isolates. Mattiello and Drescher, (2015) *Salmonella enterica* strains isolated from Brazilian poultry production were tested for antimicrobial resistance, and the genes *tetA, tetB*, and *tetC* were found in 60%, 5%, and 5% of these isolates, respectively. Sanchez-maldonado *et al*., (2017) investigated the antimicrobial resistance of *Salmonella* isolated from two Canadian pork processing plants, and the most prevalent genes were *tetB*, which was found in 21.3% of isolates, and *tetA*, which was found in 12.6% of isolates. It is well documented that tetracycline resistance *tetA* and *tetB* genes were the most frequently isolated from *Salmonella* in South Africa (Mthembu *et al*., 2019).

The *qnr* genes are a group of plasmid-mediated quinolone resistance genes. They encode proteins that protect DNA gyrase and topoisomerase IV, the targets of quinolone antibiotics, from the inhibitory effects of these drugs (Hossain et al., 2023) . In this study, *qnrS* (25%) gene was more prevalent than the *qnrA* (9%) and *qnrB* (17%) genes. This finding was inconsistence with those reported by Mood *et al*., (2015) and Abbasi and Rad, (2021)who found that *qnrA* gene was more common among *Salmonella* isolates in Iran. Jacoby *et al*., (2006) also obtain

similar results to those reported by Mood *et al*., (2015) and Abbasi and Rad, (2021) in the United State of America. The genes *qnrA*, *qnrB*, and *qnrS* were observed in 12.9%, 39.3%, and 61.2% respectively in *S. enterica* isolated from slaughtered pigs in Metro Manila, Philippines (Calayag *et al*., 2021). In South Africa, Keddy *et al.*, (2010), discovered *S. enterica* isolates that were resistant to ciprofloxacin and carried the *qnrA* gene. It's worth noting that the presence of *qnrA, qnrB*, or *qnrS* genes in *Salmonella* strains varies depending on geographical location and *Salmonella* serotype. Despite having the genes for quinolone and fluoroquinolone resistance in our *Salmonella* isolates, the phenotypic results showed that none of them were resistant to ciprofloxacin. Perhaps the lack of an efficient promoter region explains this contradiction, or perhaps these genes grant greater resistance or susceptibility to nalidixic acid than ciprofloxacin. However, the presence of *qnrA*, *qnrB*, and *qnrS* genes in our isolates is worrying because quinolones are approved for therapeutic and preventative use in animal health in South Africa. It is suspected that their misuse may contribute to the emergence of resistant bacteria in humans (Eagar *et al*., 2012; Yin *et al*., 2022).

The *flo* gene encodes a membrane protein that is a member of the MFS (major facilitator superfamily) of efflux pumps and confers antibiotic resistance, particularly to florfenicol while cat genes are associated with chloramphenicol resistance, a broad-spectrum antibiotic used in veterinary medicine (Andersen *et al*., 2015). The *cat* genes encode CATs (chloramphenicol acetyltransferases), enzymes that acetylate chloramphenicol. This modification reduces chloramphenicol's binding to bacterial ribosomes, reducing its effectiveness as an antibiotic (Alcala *et al*., 2020). In this study, a low percentage (9%) of *Salmonella* isolates contained both the *flo* and *cat* genes, which was expected given the low phenotypic resistance detected. Resistance to chloramphenicol can also be mediated by chloramphenicol efflux pumps encoded by the genes *cmlA* and *flo* (Mąka and Popowska, 2016). Several studies conducted in South Africa were consistent with the reported low prevalence of *flo* and *cat* genes (2.4%) in this study (Adefisoye and Okoh, 2016; Mbelle *et al*., 2020). Even though this is the case, it is important to note that the presence of *cat* genes in *Salmonella* isolates can limit treatment options and make infection control more difficult, hence the importance of antibiotic stewardship and surveillance to track the emergence and spread of antibiotic resistance in bacteria. It is critical to monitor *Salmonella* isolate resistance because globalization of trade, which results in long-distance movement of goods, animals, and food products, promotes the spread of resistant pathogens around the world (Mąka and Popowska, 2016).

The genes *bla*$_{CMY-2}$ and *bla*$_{SHV}$ are linked to β-lactamase enzymes, which confer resistance to β-lactam antibiotics such as cephalosporins (Liakopoulos *et al*., 2016). While trimethoprim resistance genes *DrfI* and *DrfII* encode enzymes that modify or bypass the target site of trimethoprim, an antibiotic commonly used to treat urinary tract infections and other bacterial infections. Sulfonamide resistance is linked to the genes *sul1, sul2,* and *sul3*. These genes generate enzymes that modify or deactivate sulfonamide antibiotics, which are used to treat a variety of bacterial infections (Poirel *et al*., 2018). In this study, *bla*$_{CMY-2}$, *bla*$_{SHV}$, *DrfI, DrfII, sul1, sul2* and *sul3* genes were not detected in *Salmonella* isolates. This is not surprising following the reports by Deekshit and Srikumar, (2022), that the presence of a resistance gene does not always result in resistance to the antibiotic in question. The presence of resistance phenotypes without the corresponding ARGs may suggests that other mechanisms are at work, and more research is needed to fully understand and characterize these mechanisms (Reygaert, 2018).

5.3.2 *Escherichia coli*

Similar to *S. enterica*, *E. coli* strains were screened for the presence of four β-lactamase resistance coding genes (*bla*$_{TEM}$, *bla*$_{CMY-2}$, *bla*$_{SHV}$ and *bla*$_{PSE}$). Only one of these genes was detected among the isolates at 38% *bla*$_{PSE}$ which is a member of the PSE (Pseudomonas extended-spectrum) class of β-lactamases. These enzymes were discovered in *Pseudomonas aeruginosa* and can be acquired by other bacteria, including *E. coli*, via horizontal gene transfer (Bush and Bradford, 2020). Furthermore *bla*$_{PSE}$ genes are capable of hydrolyzing and inactivating β-lactam antibiotics, including penicillin and cephalosporins (Bush and Bradford, 2020). In another study (Eguale *et al*., 2017), *bla*$_{PSE}$ genes were detected from 50% of meat isolates in Ethiopia. The presence of *bla* genes could be attributed to the fact that antibiotics like ampicillin and amoxicillin are frequently used, resulting in selection pressure with *bla*$_{TEM}$ variants dominating (Eguale *et al*., 2017). The presence of these genes suggests the resistance mechanism as relatively common among these isolates (Bush and Bradford, 2016). The high prevalence of *bla*$_{PSE}$ in *E. coli* isolates is cause for concern as it suggests the reduction of the effectiveness of β-lactam antibiotics, which are commonly used to treat bacterial infections (Bajaj *et al*., 2016). The *bla*$_{PSE}$ mediated resistance can significantly limit treatment options for infections caused by these resistant *E. coli* strains leading to treatment failures, prolonged illness, and increased healthcare costs (Mahmud *et al*., 2020).

In this study, *E. coli* isolates contained 5% *cat1*, 9% *flo*, and 14% *cm1A* genes encoding for chloramphenicol resistance. In other studies, Jaja *et al.*, (2020) discovered low prevalence (1.7%) of *cat* in South Africa. Despite the fact that it is present in a minority of the tested *E. coli* isolates, the prevalence of antibiotic resistance genes varies over time and across regions. A variety of factors contributing to this include antibiotic usage patterns, environmental factors, and horizontal gene transfer between bacteria. While phenotypic findings were higher at 40% and 60% for MIC and disk diffusion respectively, this raises concerns because it may indicate that tetracycline was misapplied to treat unrelated diseases, exerting selective pressure and sustaining the emergence of resistant bacterial strains (Jaja *et al*., 2020). Despite the fact that some isolates with resistance phenotypes lacked the corresponding ARGs tested in this study, this could indicate the presence of multi-gene mediated AMR. While ARGs play a significant role in resistance, they are not the only determinant. Other factors, such as mutations in target genes, efflux pumps, or alternative resistance mechanisms, can contribute to resistance phenotypes even when the ARGs targeted in the study are not present. Previously, similar AMR phenomena were reported (Hossain *et al*., 2023; Rosengren *et al*., 2009). The presence of these antibiotic resistance genes encoding chloramphenicol resistance emphasizes the importance of antibiotic stewardship, surveillance, and infection control measures (Manyi-Loh et al., 2018). It also emphasizes the importance of ongoing research and development of alternative treatment options for infections caused by antibiotic-resistant *E. coli* strains (Velazquez-meza and Galarde-lópez, 2022).

Tetracycline resistance gene *tetA*, like *tetB*, is a common tetracycline resistance gene which encodes an efflux pump capable of removing tetracycline antibiotics from bacterial cells. In this study, *tetA* genes were however not detected. The absence of the *tetA* genes indicates that the tested *E. coli* isolates lacked this specific tetracycline resistance mechanism. However, its absence does not rule out the possibility of tetracycline resistance in general, as other resistance mechanisms or genes may be involved (Grossman, 2016). Thirty-six percent of *E. coli* isolates with tetracycline resistance possessed *tetB* genes, according to this study. These findings are similar to 30% reported by Lanz, Kuhnert and Boerlin, (2003) in Switzerland even though there are other studies that have reported higher percentages namely 42% by (Poirel et al., 2018) in Korea and 49% by Hölzel *et al.*, (2012) in Germany. Furthermore, these findings supported the hypothesis that these efflux genes are the most common *tet* genes found in *Enterobacteriaceae* (Hossain *et al*., 2023).

Quinolones and fluoroquinolones have been used to treat salmonellosis for more than 40 years (Balasundaram, Veerappapillai and Karuppasamy, 2017). In this study 32% *qnrA,* 41% *qnrB*, and 4% *qnrS* encoding for quinolones were detected, similar results were reported from meat samples in Europe by (Caruso *et al*., 2018) and by Calayag, Widmer and Rivera, (2021) in Korea. In South Africa, Ramatla, (2019), reported 22% of *Salmonella* isolates harboring *qnrA* genes. Several studies reported the association between *qnr* and *bla* genes (Antonio *et al*., 2019; Moawad *et al*., 2017), which has been observed in this study.

5.4 Detection of genotypic antimicrobial resistance in Gram positive pathogens

5.4.1 *Staphylococcus aureus*

In the current study, the isolates were screened for the ten-antibiotic resistance genes (*blaZ*, *mecA*, *ermB*, *ermA*, *ermC*, *aap-aph*, *tetA*, *tetM*, *vanA* and *vanB).* All *S. aureus* harboured at least one or more resistant genes except for vancomycin resistance encoding genes *vanA* and *vanB*. The presence of penicillin-binding proteins, PBP2' (PBP2a), encoded by the *mecA* gene, has been linked to methicillin resistance in staphylococci. Because the *mecA* gene can be expressed in a variety of ways, not all methicillin-resistant staphylococcal strains may be detectable using phenotypic methods (Bakheet *et al*., 2018). In the current study, the *mecA* gene, which encodes methicillin resistance, was found in 44% of the *S. aureus* isolates. The PCR positive percent for the *mecA* gene was slightly lower than the antibiotic susceptibility test, which revealed 100% resistance to penicillin and gentamycin. These findings were nearly identical to those reported by Mamza *et al*., 2010; Nemati et al., 2008), who all reported 100% penicillin resistance. *S. aureus* methicillin resistance is mediated by the mecA gene, which is carried by the staphylococcal cassette chromosome mec (SCCmec) (Asante *et al*., 2021). According to Thwala *et al.*, (2021), the *mecA* gene is the most common in meat and meat products in African countries including South Africa, which is in agreement with our findings. This is a concern as it poses a public health risk. Govender *et al.*, (2019), reported 21% methicillin resistance in *S. aureus* isolated from South African poultry meat products. Furthermore, the *mecA* gene was detected in *S. aureus* strains circulating in poultry and farm workers at a South African intensive poultry production system (Amoako *et al*., 2019),

Furthermore, 39% of the *blaZ* genes, which encodes β-lactamase (penicillin) resistance were detected in this study which is low compared to 100% resistance obtained phenotypically. This

could be due to mutations or antibiotic tolerance causing the phenotypic resistance (Croes *et al*., 2009). The *blaZ* genes are found in *S. aureus* on transposon Tn522 in plasmid pI524. This configuration implies that these genes, along with the transposon and plasmid sequences, could be transferred to other bacteria via horizontal gene transfer mechanism, producing β-lactamase, which hydrolyzes and activates penicillin's β-lactam ring (Foster, 2017). Compared to our study, Sineke (2021) found a higher prevalence of the *blaZ* gene in 88.75% of penicillin-resistant *S. aureus* isolates in South Africa, while Amoako et al., (2016) found a low rate of *blaZ* genes. In another study, Zehra *et al*., (2017) previously discovered the *blaZ* resistance gene in *S. aureus* in bovine and swine from Punjab, India.

The *erm* genes, which code for a macrolide called erythromycin resistant methylase, are primarily responsible for erythromycin resistance in staphylococci (Akanbi *et al*., 2017). Erythromycin is known to reduce protein synthesis by interfering with ribosome function. The detection of *ermB* genes from *S. aureus* is predominant in east Asia, Europe, and South Africa (Harimaya et al., 2007). However, the current study reported moderate findings of *ermA* (20%), *ermB* (17%) and *ermC* (20%) genes detected from *S. aureus* isolates which implies that a significant but not overwhelming proportion of the isolates carry these genes. Furthermore, these results may suggest that macrolide resistance exists among the *Staphylococcus* isolates. This may have implications for treatment options, since macrolides are commonly used antibiotics for treating *Staphylococcus* infections (Miklasinka-Majdanik, 2021). Notably, erythromycin is a first-generation macrolide antibiotic with some drawbacks and side effects. While it is effective against many Gram-positive bacteria, its spectrum of activity and susceptibility to resistance mechanisms are limitations (Dinos, 2017). Alternative antibiotics such as azithromycin that are effective against strains carrying *erm* genes may be required in such cases.

Thirty-one percent of *aap-aph* genes encoding aminoglycoside (gentamycin) resistance in *S. aureus* were reported in this study. Mesbah, *et al*., (2021), reported similar findings from ready to eat meat in Iran. The presence of these genes indicates a potential reduced susceptibility to gentamycin among the tested *S. aureus* isolates. This resistance can pose challenges in the treatment of infections caused by these bacteria, as gentamicin may be less effective in inhibiting bacterial growth. Resistance to gentamicin can arise through several mechanisms, including the acquisition of genes that encode enzymes called aminoglycoside-modifying enzymes (AMEs) or through alterations in the bacterial ribosomal target site (Munita *et al*.,

2016). It has been reported that *S. aureus* resistance to a wide variety of antibiotics has resulted in limited beneficial options to treat its infections (Foster, 2017; Sineke, 2021).

One of the mechanisms involved in tetracycline resistance is the protection of the bacterial ribosomes, which are the cellular structures responsible for protein synthesis and the primary target of tetracycline. Certain resistance genes, such as *tetM* and *tetO*, produce proteins that bind to the ribosomes and prevent tetracycline from binding to its target site (Rao *et al*., 2022). Eight percent of the *tetA* and 22% of *tetM* encoding for tetracycline antibiotics were detected from *S. aureus* isolates in the current study. The tetracycline resistance of *S. aureus* from meat strains may result from the host-switching from human to animals (Richardson *et al*., 2018). Furthermore, it could be linked to the widespread use of feed additives in production of animals. The tetracycline resistance gene *tetM* has been found in all MRSA isolates associated with livestock but not in human isolates (Rao *et al*., 2022). Tetracyclines are also used in greater quantities in farm animals than in humans, which could explain why this resistance is still being detected.

Vancomycin resistance conciliated by the *vanA* or *vanB* gene in staphylococci is uncommon and is usually associated with vancomycin resistance in enterococci (Lowy, 2003) . In this study, all the *S. aureus* isolates which were resistant to vancomycin phenotypically (95%), lacked the genes (*vanA* and *vanB*). According to Khalili *et al*., (2012), the disk diffusion test does not reliably detect vancomycin resistance in *S. aureus*. Although other studies found lower resistance rates of vancomycin, our study is consistent with another South African study on *Staphylococcus* in farm animals which discovered a low 12% of vancomycin-resistant MRSA that lacked the *vanA* and *vanB* resistance genes (Mehndiratta and Bhalla, 2014). This could be because other plasmid-mediated vancomycin genes, such as *vanC*, *vanD*, *vanE*, *vanF*, and *vanG*, were not studied in this study (Adegoke and Okoh, 2014). The presence of vancomycin could be due to the use of gentamycin, and other aminoglycoside antibiotics commonly used in South Africa as growth promoters (Sineke, 2021).

5.4.2 *Listeria monocytogenes*

Similar to *S. aureus*, nine of 10 ARGs were detected in the *L. monocytogenes* isolates. In the current study, erythromycin resistance genes *ermB* and *ermC* were detected from serotypes IIc (71%) and Iva (50%) respectively. *Listeria monocytogenes* isolates from food are typically from serogroup IIa or IIb, thus the information about *L. monocytogenes* Ivb could be useful in understanding the epidemiological chain of food-borne listeriosis (Alvarez-Molina *et al*., 2021; Henriques and Cristino, 2017). The isolation of erythromycin resistance genes from *L. monocytogenes* serotypes suggests resistance to macrolide-lincosamide-streptogramin-B (MLSB) antibiotics through ribosomal modification within these strains (Khodabandeh *et al*., 2019). This is concerning as erythromycin has been reported to play a vital role in animals and humans, particularly its most common use for treating listeriosis in pregnant women (Keet and Rip, 2021). Heidarzadeh and Pourmand, (2021) reported prevalence of 83.3% *ermB* genes which is close to what our study has revealed. Erythromycin has been used to treat Listeria infections as an alternative to penicillin and gentamicin (Thomas *et al*., 2019).

Among the well-known genes associated with tetracycline resistance are the *tetA* and *tetM* genes (Grossman, 2016). Tetracycline is actively pumped out of the bacterial cell by the *tetA* gene, while the *tetM* gene encodes a ribosomal protection protein that prevents tetracycline from binding to the ribosomes and inhibiting protein synthesis. In this study 33% of *tetA* and 42% of *tetM* were respectively detected from IIc and Iva *L. monocytogenes* serotype, Wu *et al., (2021)*, reported similar findings in China. The presence of these resistance genes in *L. monocytogenes* isolates, particularly those belonging to the IIc and Iva serotypes, suggests that tetracycline resistance is widespread in these strains. This data is useful for understanding the antibiotic resistance profiles of *L. monocytogenes* populations, evaluating the potential impact on treatment options, and informing surveillance and control measures. It's worth noting that the presence of tetracycline resistance genes does not necessarily imply active resistance in all instances, as the expression and functionality of these genes can vary. Furthermore, Ivb 29% serotype contained *tetA* genes, these serotypes are of clinical importance as they were reported to be responsible for 55% of invasive Listeriosis cases in Poland (Kuch *et al*., 2018).

PenA genes were found in 13% of Iva and 8% of IIa *L. monocytogenes* serotypes. The *penA* gene encodes a penicillin-binding protein enzyme that is required for bacterial cell wall synthesis. Penicillin antibiotics' affinity to the target site can be reduced by mutations or

changes in *penA*, making them less effective in inhibiting cell wall synthesis and leading to penicillin resistance. However, penicillin resistance in *L. monocytogenes* is considered uncommon, which is consistent with the low prevalence of these genes in all four serotypes.

5.5 Summary remarks

The study examined the antimicrobial profile of four bacterial pathogens from 2000 to 2021. Despite a low antimicrobial resistance level in Gram-negative bacteria (*S. enterica* and *E. coli*), susceptibility values were shifting towards resistance. The isolates were sensitive to chloramphenicol between 2000 and 2004 but showed sensitivity to the same antibiotic at 8 mg/L over the following years. This shift requires intervention to change farmers' knowledge and practices regarding antibiotic use. Furthermore, S. *aureus* and *L. monocytogenes* were found to resistant to various antibiotics, with an ARI score of 0.113. These isolates carried diverse genes encoding resistance, potentially contributing to their high phenotypic resistance. The presence of these genes emphasizes the importance of infection control measures and prudent antibiotic use in the veterinary fields.

Key words: Antibiotics, antibiotic use, antibiotic resistance, disc diffusion, minimum inhibitory concentration, phenotypic resistance, multidrug resistance, antibiotic resistance genes, antibiotic resistance index.

CHAPTER 6: CONCLUSION & RECOMMENDATIONS

6.1 CONCLUSION

A retrospective laboratory-based study such as this one is an easy method to survey the antimicrobial sensitivity patterns over the years. The findings of this study provided a glimpse into the changing patterns of antimicrobial sensitivity of four bacterial pathogens of veterinary and public health importance in South Africa over a period of 21 years. It can therefore be concluded that phenotypic and genotypic antimicrobial resistance in South Africa was very low in the period under review. It can also be concluded there is need for the government, farmers, and veterinary professional to pay special attention to the development of antibiotics against *S. aureus* and *L. monocytogenes* as their resistance presents a potential public health risk in the country.

6.2 RECOMMENDATIONS

It is recommended that the MIC results of this study be submitted to the veterinary antimicrobial resistance working group. It is also recommended that more studies similar to this one be carried out with the isolates stored in veterinary provincial laboratories which will provide more comprehensive details on the extent of AMR in the country. Easy access and misuse of antibiotics, especially tetracyclines for animal husbandry has proven to be the source of emerging antibiotic resistance Therefore it is recommended that stricter policies especially on the dispensation and usage of antibiotics for growth be put in place.

6.3 FUTURE PERSPECTIVES

Molecular characterization of these isolates will be interesting for epidemiological purposes and traceability of antimicrobial resistance. Molecular analyses should include DNA fingerprinting, studying of integrons and plasmids as well as advanced genomic work such as whole genome sequencing.

REFERENCES

Abatcha, M.G., Goni, M.D., Abbas, M.A., Jalo, I.M., Mohammed, G., 2020. A review of *Listeria* and *Salmonella*: An update on description, characteristics, incidence, and antibiotic susceptibility. Adv. Anim. Vet. Sci. 8, 1232–1249.

Abbasi, E., Rad, E.G., 2021. Quinolone resistant *Salmonella* species isolated from pediatric patients with diarrhea in central Iran. BMC Gastroenterol. 3, 1–6.

Abdelwahab, G.E., Ishag, H.Z.A., Al Hammadi, Z.M., Al Yammahi, S.M.S., Mohd Yusof, M.F. Bin, Al Yassi, M.S.Y., Al Neyadi, S.S.A., Al Mansoori, A.M.A., Al Hamadi, F.H.A., Al Hamadi, I.A.S., Hosani, M.A.A. Al, Mohammed Al Muhairi, S.S., 2022. Antibiotics Resistance in *Escherichia coli* isolated from livestock in the Emirate of Abu Dhabi, UAE, 2014-2019. Int. J. Microbiol. 2022.

Abolghait, S.K., Fathi, A.G., Youssef, F.M., Algammal, A.M., 2020. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chicken meat and giblets often produces staphylococcal enterotoxin B (SEB) in non-refrigerated raw chicken livers. Int. J. Food Microbiol. 328, 108669.

Adefisoye, M.A., Okoh, A.I., 2016. Identification and antimicrobial resistance prevalence of pathogenic *Escherichia coli* strains from treated wastewater effluents in Eastern Cape, South Africa. Microbiology open 5, 143–151.

Adegoke, A.A., Okoh, A.I., 2014. Species diversity and antibiotic resistance properties of *Staphylococcus* of farm animal origin in Nkonkobe Municipality , South Africa. Folia Microbiol. 59, 133–140.

Adigun, O., Gcebe, N., Jambwa, K., Fasina, F., Adesiyun, A.A., 2020. Molecular and phenotypic characterization of *Staphylococcus aureus* strains isolated from carcass swabs and carcass drips of chickens slaughtered in the informal market in Gauteng Province, South Africa. J. Food Saf. 40, 1–29.

Adzitey, F., 2015. Antibiotic Resistance of escherichia coli isolated from beef and its related samples in techiman municipality of Ghana. Asian J. Anim. Sci. 9, 233–240.

Afzal, M., Vijay, A.K., Stapleton, F., Willcox, M.D.P., 2021. Susceptibility of ocular *Staphylococcus aureus* to antibiotics and multipurpose disinfecting solutions. Antibiotics 10, 1–14.

Aguilar-Montes de Oca, S., Talavera-Rojas, M., Soriano-Vargas, E., Barba-León, J., Vázquez-Navarrete, J., Acosta-Dibarrat, J., Salgado-Miranda, C., 2018. Phenotypic and genotypic

profile of clinical and animal multidrug-resistant *Salmonella enterica* isolates from Mexico. J. Appl. Microbiol. 124, 67–74.

Akanbi, O.E., Njom, H.A., Fri, J., Otigbu, A.C., Clarke, A.M., 2017. Antimicrobial Susceptibility of *Staphylococcus aureus* isolated from recreational waters and beach Sand in Eastern Cape province of South Africa. Int. J. Environ. Res. Public Health 14, 1–15.

Akindolire, M.A., Babalola, O.O., Ateba, C.N., 2015. Detection of antibiotic resistant *Staphylococcus aureus* from milk: A public health implication. Int. J. Environ. Res. Public Health 12, 10254–10275.

Al-nabulsi, A.A., Olaimat, A.N., Osaili, T.M., Shaker, R.R., Zein, N., Jaradat, Z.W., Abushelaibi, A., Holley, R.A., 2014. Use of acetic and citric acids to control *Salmonella* Typhimurium in tahini (sesame paste). Food Microbiol. 42, 102–108.

Alcala, A., Ramirez, G., Solis, A., Kim, Y., Tan, K., Luna, O., Nguyen, K., Vazquez, D., Ward, M., Zhou, M., Mulligan, R., Maltseva, N., Kuhn, M.L., 2020. Structural and functional characterization of three type B and C chloramphenicol acetyltransferases from Vibrio species. Protein Sci. 29, 695–710.

Alvarez-Molina, A., Cobo-díaz, F., Mercedes, L., Prieto, M., de Toro, M., Alvarez-Ordonez, A., 2021. Unraveling the emergence and population diversity of *Listeria monocytogenes* in a newly built meat facility through whole genome sequencing. J. food Microbiol. 340, 1–13.

Amoako, D.G., Bester, L.A., Somboro, A.M., Baijnath, S., Govind, C.N., Essack, S.Y., 2016. Plasmid-mediated resistance and virulence mechanisms in the private health sector in KwaZulu-Natal, South Africa: An investigation of methicillin resistant *Staphylococcus aureus* (MRSA) clinical isolates collected during a three month period. Int. J. Infect. Dis. 46, 38–41.

Amoako, G.D., Somboro, M.A., Arabia, L.K.A., Allam, M., Ismail, A., Bester, L., Essack, Y.S., 2019. Science of the total environment genomic analysis of methicillin-resistant *Staphylococcus aureus* isolated from poultry and occupational farm workers in Umgungundlovu District , South Africa. Sci. Total Environ. J. 670, 704–716.

Andersen, J.L., He, G.X., Kakarla, P., Ranjana, K.C., Kumar, S., Lakra, W.S., Mukherjee, M.M., Ranaweera, I., Shrestha, U., Tran, T., Varela, M.F., 2015. Multidrug efflux pumps from enterobacteriaceae, Vibrio cholerae and *Staphylococcus aureus* bacterial food pathogens. Int. J. Environ. Res. Public Health 12, 1487–1547.

Andriyanov, P.A., Zhurilov, P.A., Liskova, E.A., Karpova, T.I., Sokolova, E. V., Yushina, Y.K., Zaiko, E. V., Bataeva, D.S., Voronina, O.L., Psareva, E.K., Tartakovsky, I.S.,

Kolbasov, D. V., Ermolaeva, S.A., 2021. Antimicrobial resistance of *Listeria monocytogenes* strains isolated from humans, animals, and food products in russia in 1950–1980, 2000–2005, and 2018–2021. Antibiotics 10, 2000–2005.

Anjum, M.F., 2015. Screening methods for the detection of antimicrobial resistance genes present in bacterial isolates and the microbiota. Future Microbiol. 10, 317–320.

Anjum, M.F., Zankari, E.A., Hasman, H., 2017. Molecular methods for detection of antimicrobial resistance. Microbiol. Spectr. 5, 1–17.

Antonio, R., Penha, C., Cristina, J., Maria, A., Kanashiro, I., Berchieri, A., Lúcia, A., 2019. Emergent multidrug-resistant nontyphoidal *Salmonella* serovars isolated from poultry in Brazil coharboring bla CTX-M-2 and qnr B or bla-CMY-2 in large plasmids. Diagnostic Microbiol. Infect. Dis. 95, 93–98.

https://ahpsr.who.int/publications/i/item/global-action-plan-on-antimicrobial-resistance[Accessed on 21 October 2022].

Aqib, A.I., Alsayeqh, A.F., 2022. Vancomycin drug resistance , an emerging threat to animal and public health. Front. Vet. Sci. 9, 1–9.

Argudín, M.A., Deplano, A., Meghraoui, A., Dodémont, M., Heinrichs, A., Olivier Denis, C.N. 1 and S.R., 2017. Bacteria from animals as a pool of antimicrobial resistance genes. Antibiotics 6, 1–38.

Asante, J., Hetsa, B.A., Amoako, D.G., Abia, A.L.K., Bester, L.A., Essack, S.Y., 2021. Multidrug-resistant coagulase-negative staphylococci isolated from bloodstream in the umgungundlovu district of Kwazulu-Natal province in South Africa: emerging pathogens. Antibiotics 10, 1–11.

Aslam, M., Checkley, S., Avery, B., Chalmers, G., Bohaychuk, V., Gensler, G., Reid-Smith, R., Boerlin, P., 2012. Phenotypic and genetic characterization of antimicrobial resistance in *Salmonella* serovars isolated from retail meats in Alberta, Canada. Food Microbiol. 32, 110–117.

Asokan, G. V., Ramadhan, T., Ahmed, E., Sanad, H., 2019. WHO global priority pathogens list: A bibliometric analysis of medline- pubmed for knowledge mobilization to infection prevention and control practices in Bahrain. Oman Med. J. 34, 184–193.

Bajaj, P., Singh, N.S., Virdi, J.S., 2016. *Escherichia coli* β-lactamases: What really matters. Front. Microbiol. 7, 1–14.

Bakheet, A.A., Amen, O., Habaty, S.H.A.L., Darwish, S.F., 2018. Prevalence of *Staphylococcus aureus* in broiler chickens with special reference to beta-lactam resistance genes in the isolated strains. Alexandria J. Vet. Sci. 57, 25–33.

Balasundaram, P. Veerappapillai, S., Karuppasamy, P., 2017. Quinolones and fluoroquinolones to treat *Salmonella* Typhimurium: A review of metabolism and pharmacokinetics. Curr. Drug Metab. 18, 1085–1094.

Balouiri, M., Sadiki, M., Ibnsouda, S.K., 2016. Methods for in vitro evaluating antimicrobial activity : A review. J. Pharm. Anal. 6, 71–79.

Barmak, S.M., Sinyavskiy, Y.A., Berdygaliev, A.B., Sharmanov, T.S., Savitskaya, I.S., Sultankulova, G.T., Zholdybayeva, E. V., 2021. Development and evaluation of alternative methods to identify the three most common serotypes of *Salmonella enterica* causing clinical infections in Kazakhstan. Microorganisms 9, 2319.

Bastidas-Caldes, C., de Waard, J.H., Salgado, M.S., Villacís, M.J., Coral-Almeida, M., Yamamoto, Y., Calvopiña, M., 2022. Worldwide prevalence of mcr-mediated colistin-resistance *Escherichia coli* in isolates of clinical samples, healthy humans, and livestock—A systematic review and meta-analysis. Pathogens 11, 659.

Bauer, A., Kirby, W.M.M., Sheriss, J.C., Truck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45, 493–496.

Beargie, R.A., Bracken, E.C., Riley, H.D., 1965. Micromethod (Spot-Plate) Determination of in vitro antibiotic susceptibility. Appl. Microbiol. 13, 279–280.

Bellali, S., Bou Khalil, J., Fontanini, A., Raoult, D., Lagier, J.C., 2020. A new protectant medium preserving bacterial viability after freeze drying. Microbiol. Res. 236, 126454.

Benkova, M., Soukup, O., Marek, J., 2020. Antimicrobial susceptibility testing : currently used methods and devices and the near future in clinical practice. J. Appl. Microbiol. 129, 806–822.

Berhe, Y.H., Amaha, N.D., Ghebrenegus, A.S., 2019. Evaluation of ceftriaxone use in the medical ward of Halibet National Referral and teaching hospital in 2017 in Asmara, Eritrea: A cross sectional retrospective study. BMC Infect. Dis. 19, 1–7.

Bharatha, A., Hilaire, M.G., 2020. Antimicrobial Stewardship : Fighting antimicrobial resistance and protecting global public health. Infect. Drug Resist. 13, 4713–4738.

Bintsis, T., 2017. Foodborne pathogens. Microbiology 3, 529–563.

Binsker, U., Käsbohrer, A., and Hammerl, J. E. 2022. Global colistin use: a review of the emergence of resistant Enterobacterales and the impact on their genetic basis, FEMS Microbiology Reviews, Volume 46, Issue 1, fuab049.

Bircher, L., Geirnaert, A., Hammes, F., Lacroix, C., Schwab, C., 2018. Effect of cryopreservation and lyophilization on viability and growth of strict anaerobic human gut microbes. Microb. Biotechnol. 11, 721–733.

Blair, J.M.A., Webber, M.A., Baylay, A.J., Ogbolu, D.O., Piddock, L.J.V., 2015. Molecular mechanisms of antibiotic resistance. Nat. Rev. Microbiol. 13, 42–51.

Boolchandani, M., Dantas, G., 2020. Sequencing-based methods and resources to study antimicrobial resistance. Nat. Rev. Genet. 20, 356–370.

Bortolaia, V., Espinosa-Gongora, C., Guardabassi, L., 2016. Human health risks associated with antimicrobial-resistant enterococci and *Staphylococcus aureus* on poultry meat. Clin. Microbiol. Infect. 22, 130–140.

Bush, K., Bradford, P.A., 2016. β -Lactams and β -Lactamase Inhibitors: An Overview. Cold Spring Harb. Perspectine Med. 6, 1–22.

Bush, K., Bradford, P.A., 2020. Epidemiology of β-lactamase-producing pathogens. Clin. Microbiol. Rev. 33, 1–37.

Butin, M., Dumont, Y., Monteix, A., Raphard, A., Roques, C., Martins Simoes, P., Picaud, J.C., Laurent, F., 2019. Sources and reservoirs of *Staphylococcus capitis* NRCS-A inside a NICU. Antimicrob. Resist. Infect. Control 8, 0–6.

Buzón-Durán, L., Capita, R., Alonso-Calleja, C., 2017. Microbial loads and antibiotic resistance patterns of *Staphylococcus aureus* in different types of raw poultry-based meat preparations. Poult. Sci. 96, 4046–4052.

Calayag, A.M.B., Widmer, K.W., Rivera, W.L., 2021. Antimicrobial susceptibility and frequency of *bla* and *qnr* genes in *Salmonella enterica*Isolated from slaughtered pigs. Antibiotics 10, 1–11.

Caleja, C., de Toro, M., Gonçalves, A., Themudo, P., Vieira-Pinto, M., Monteiro, D., Rodrigues, J., Sáenz, Y., Carvalho, C., Igrejas, G., Torres, C., Poeta, P., 2011. Antimicrobial resistance and class I integrons in *Salmonella enterica* isolates from wild boars and Bísaro pigs. Int. Microbiol. 14, 19–24.

Caneschi, A., Bardhi, A., Barbarossa, A., Zaghini, A., 2023. The Use of Antibiotics and Antimicrobial Resistance in veterinary medicine, a complex phenomenon: A narrative review. Antibiotics 12, 487.

Carroll, L.M., Pierneef, R., Mathole, M., Matle, I., 2021. Genomic characterization of endemic and ecdemic non-typhoidal *Salmonella enterica* lineages circulating among animals and animal products in South Africa. Front. Microbiol. 12, 1–19.

Caruso, G., Giammanco, A., Cardamone, C., Oliveri, G., Mascarella, C., Capra, G., Fasciana, T., 2018. Extra-intestinal fluoroquinolone-resistant *Escherichia coli* strains isolated from meat 2018. Biomed Res Int. 2018:8714975.

Cave, R., Cole, J., Mkrtchyan, H. V., 2021. Surveillance and prevalence of antimicrobial

resistant bacteria from public settings within urban built environments: Challenges and opportunities for hygiene and infection control. Environ. Int. 157, 106836.

Http://www.cdc.gov [Accessed on 20-August-2022]

Chaudhari, H . G., Prajapati S., Wardah, Z . H., Raol G., Prajapati V., Patel R., Shati A. A., Alfaifi M. Y., Elbehairi S. E. I., Sayyed R. Z., 2023. Decoding the microbial universe with metagenomics: a brief insight. Front. Genet. 14, 1119740

Chambers, L., Yang, Y., Littier, H., Ray, P., Zhang, T., Pruden, A., Strickland, M., Knowlton, K., 2015. Metagenomic analysis of antibiotic resistance genes in dairy cow feces following therapeutic administration of third generation cephalosporin. PLoS One 10, 1–18.

Chancey, S.T., Zähner, D., Stephens, D.S., 2012. Acquired inducible antimicrobial resistance in gram-positive bacteria. Future Microbiol. 7, 959–978.

Chinemerem Nwobodo, D., Ugwu, M.C., Oliseloke Anie, C., Al-Ouqaili, M.T.S., Chinedu Ikem, J., Victor Chigozie, U., Saki, M., 2022. Antibiotic resistance: the challenges and some emerging strategies for tackling a global menace. J. Clin. Lab. Anal. 36 (9): 24655.

Choffnes, E.R., Relman, D.A., Olsen, L., Hutton, R., Mack, A., 2012. Improving food safety through a one health approach: workshop summary. Washington (DC): National Academies Press (US); 2012.

Chokshi, A., Sifri, Z., Cennimo, D., Horng, H., 2019. Global contributors to antibiotic resistance. Orig. Pap. 11 (1): 36–42. doi: 10.4103/jgid.jgid_110_18

Chunrong, L., Hongxia, F., Puxuan, L., Lecai, J., 2021. WHO 2021年全球结核病报告 ： 全球与中国关键数据分析.

Collignon, P.J., McEwen, S.A., 2019. One health-its importance in helping to better control antimicrobial resistance. Trop. Med. Infect. Dis. 4 (1): 22. doi: 10.3390/tropicalmed4010022

Croes, S., Deurenberg, R.H., Boumans, M.L., Beisser, P.S., Neef, C., Stobberingh, E.E., 2009. concentration depends on the *S. aureus* lineage. BMC Microbiol. 9, 229. doi: 10.1186/1471-2180-9-229

Dadgostar, P., 2019. Antimicrobial Resistance : Implications and costs. Infect. Drug Resist. 12, 3903-3910. doi: 10.2147/IDR.S234610.

https://www.dalrrd.gov.za/vetweb/VPN. [Accessed on 12 September 2022].

Davis, R., Brown, P.D., 2016. Multiple antibiotic resistance index, fitness and virulence potential in respiratory *Pseudomonas aeruginosa* from Jamaica. J. Med. Microbiol. 65(4):

261–271. doi: 10.1099/jmm.0.000229.

Deekshit, V.K., ShabarinathSrikumar, 2022. To be, or not to be'—The dilemma of 'silent' antimicrobial resistance genes in bacteria. Appl. Microbiol. 133 (5): 2902–2914. doi: 10.1111/jam.15738.

Dhingra, S., Rahman, N.A.A., Peile, E., Rahman, M., 2020. Microbial resistance movements : An overview of global public health threats posed by antimicrobial resistance, and how best to counter. Front. Public Heal. 8, 1–22. doi: 10.3389/fpubh.2020.535668.

Diallo, O.O., Baron, S.A., Abat, C., Colson, P., Chaudet, H., Rolain, J.M., 2020. Antibiotic resistance surveillance systems: A review. J. Glob. Antimicrob. Resist. 23, 430–438. doi: 10.1016/j.jgar.2020.10.009.

Dinos, G.P., 2017. The macrolide antibiotic renaissance. 174 (18):2967–2983. doi: 10.1111/bph.13936.

Dionisi, A.M., Graziani, C., Lucarelli, C., Filetici, E., Villa, L., Owczarek, S., Caprioli, A., Luzzi, I., 2009. Molecular characterization of multidrug-resistant strains of *Salmonella enterica* serotype typhimurium and monophasic variant (s. 4,[5],12:i:-) isolated from human infections in Italy. Foodborne Pathog. Dis. 6(6): 711–717. doi: 10.1089/fpd.2008.0240.

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., Martin, P., 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J. Clin. Microbiol. 42 (8):3819–3822. doi: 10.1128/JCM.42.8.3819-3822.2004.

Dsani, E., Afari, E.A., Danso-appiah, A., Kenu, E., Kaburi, B.B., Egyir, B., 2020. Antimicrobial resistance and molecular detection of extended spectrum β - lactamase producing *Escherichia coli* isolates from raw meat in Greater Accra region , Ghana. BMC Microbiol 20, 253. doi: 10.1186/s12866-020-01935-z.

Dufailu, O.A., Yaqub, M.O., Owusu-kwarteng, J., Addy, F., 2021. Prevalence and characteristics of *Listeria species* from selected African countries. Trop. Dis. Travel Med. Vaccines 7 (26):1–9. doi.org/10.1186/s40794-021-00151-5.

Dutil, L., Irwin, R., Finley, R., Ng, L.K., Avery, B., Boerlin, P., Bourgault, A.M., Cole, L., Daignault, D., Desruisseau, A., Demczuk, W., Hoang, L., Horsman, G.B., Ismail, J., Jamieson, F., Maki, A., Pacagnella, A., Pillai, D.R., 2010. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerg. Infect. Dis. 16, 48–54.

Eagar, H., Swan, G., Van Vuuren, M., 2012. A survey of antimicrobial usage in animals in South Africa with specific reference to food animals. J. S. Afr. Vet. Assoc. 83 (1):16. doi:

10.4102/jsava.v83i1.16.

Ebimieowei Etebu, I.A., 2016. Antibiotics: Classification and mechanisms of action with. Int. J. Appl. Micrrobiology Biotechnol. Res. 96, 90–101.

Economou, V., Gousia, P., 2015. Agriculture and food animals as a source of antimicrobial-resistant bacteria. Infect. Drug Resist. 8, 49–61. doi: 10.2147/IDR.S55778.

EFSA, 2011. Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals. EFSA J.9(8):2322. doi:10.2903/j.efsa.2011.2322.

Eguale, T., Birungi, J., Asrat, D., Njahira, M.N., Njuguna, J., Gebreyes, W.A., Gunn, J.S., Djikeng, A., Engidawork, E., 2017. Genetic markers associated with resistance to beta-lactam and quinolone antimicrobials in non-typhoidal *Salmonella* isolates from humans and animals in central Ethiopia. Antimicrob. Resist. Infect. Control 6, 1–10. doi: 10.1186/s13756-017-0171-6.

El-Banna, T., Ibrahim, F., Sonbol, F.I., Zaki, M.., El-Sayyad, H.H.I., 2016. Clinical Microbiology : Open access clinical and environmental prevalence and antibiotic susceptibility of *Listeria*. Clin. Microbiol. 5(3):249. doi: 10.4172/2327-5073.1000249.

Eloit, M., 2018. OIE Standards , Guidelines and resolutions on antimicrobial resistance and the use of antimicrobial agents, International Office of Epizootics.

Elsayed, N., Ashour, M., Amine, A.E.K., 2018. Vancomycin resistance among *Staphylococcus aureus* isolates in a rural setting, Egypt. Germs 8 (3):134–139. doi: 10.18683/germs.2018.1140.

EUCAST: Breaking point tables for interpretation of MICs and zone diameters, 2022. v_12.0_Breakpoint_Tables 1–110.

https://www.eucast.org/presentations_and_statistics/eucast_at_eccmid/eucast_at_eccmid_2013. [Accessed 22/10/2022]

Fair, R.J., Tor, Y., 2014. The rise of antibiotic resistance. Perspect. Med. Chem. 6.

Fan, S., Foster, D., Miller, W.G., Osborne, J., Kathariou, S., 2021. Impact of ceftiofur administration in steers on the prevalence and antimicrobial resistance of campylobacter spp. Microorganisms 9 (2):318. doi: 10.3390/microorganisms9020318.

Feucherolles, M., Poppert, S., Utzinger, J., Becker, S.L., 2019. MALDI - TOF mass spectrometry as a diagnostic tool in human and veterinary helminthology : a systematic review. Parasit. Vectors 12, 245. doi.org/10.1186/s13071-019-3493-9.

Fleming, A., 1929. The antibacterial action of cultures of a penicillium, with special reference to their use in isolation of B. influenza. Br. J. Exp. Pathol.10(3): 226-236.

Foster, T.J., 2017. Antibiotic resistance in *Staphylococcus aureus* . Current status and future prospects. FEMS Microbiol. Rev. 41(3): 430–449. doi: 10.1093/femsre/fux007.

Founou, R.C., Founou, L.L., Essack, S.Y., 2017. Clinical and economic impact of antibiotic resistance in developing countries : A systematic review and meta-analysis. PLoS One 12(12):1–18. doi: 10.1371/journal.pone.0189621.

Fourie, J.L., Claassen, F.M., Myburgh, J.J., 2021. Causative pathogens and antibiotic resistance in community-acquired urinary tract infections in central South Africa. South African Med. J. 111(2):124–128. doi:10.7196/SAMJ.2021.v111i2.

Freitas-Ribeiro, S., Reis, R.L., Pirraco, R.P., 2022. Long-term and short-term preservation strategies for tissue engineering and regenerative medicine products: state of the art and emerging trends. PNAS Nexus 1(4):1–15. doi:10.1093/pnasnexus/pgac212.

Fuhrmeister, A.S., Jones, R.N., 2019. The importance of antimicrobial resistance monitoring worldwide and the origins of SENTRY antimicrobial surveillance program. Open Forum Infect. Dis. 6 (1), 1–4. doi:10.1093/ofid/ofy346.

Gahamanyi, N., Mboera, L.E.G., Matee, M.I., Komba, E.V.G., 2020. Prevalence, risk factors , and antimicrobial resistance profiles of thermophilic *Campylobacter species* in humans and animals in Sub-Saharan Africa : A systematic review. Int. J. Microbiol. 2020, 1–13. doi: 10.1155/2020/2092478.

Gajic, I., Kabic, J., Kekic, D., Jovicevic, M., Milenkovic, M., Mitic Culafic, D., Trudic, A., Ranin, L., Opavski, N., 2022. Antimicrobial susceptibility testing: A comprehensivereview of currently used methods. Antibiotics 11 (4):427. doi:10.3390/antibiotics11040427.

Galhano, B.S.P., Ferrari, R.G., Panzenhagen, P., Jesus, A.C.S. De, Conte-junior, C.A., 2021. Antimicrobial resistance gene detection methods for bacteria in animal-based foods : A brief review of highlights and advantages. Microorganisms 9 (5):923. doi: 10.3390/microorganisms9050923. .

Gan, T., Shu, G., Fu, H., Yan, Q., Zhang, W., Tang, H., Yin, L., Zhao, L., Lin, J., 2021. Antimicrobial resistance and genotyping of *Staphylococcus aureus* obtained from food animals in Sichuan Province, China. BMC Vet. Res. 17(1):177. doi: 10.1186/s12917-021-02884-z.

Gelaw, A.K., Nthaba, P., Matle, I., 2018. Detection of *Salmonella* from animal sources in South Africa between 2007 and 2014. J. S. Afr. Vet. Assoc. 89(0):1–10. doi: 10.4102/jsava.v89i0.1643.

Govender, G., Madoroba, E., Magwedere, K., Fosgate, G., Kuonza, L., 2019. Prevalence and

risk factors contributing to antibiotic-resistant *Staphylococcus aureus* isolates from poultry meat products in South Africa , 2015 – 2016. J. S. Afr. Vet. Assoc. 90(0):1–8. doi: 10.4102/jsava.v90i0.1738.

Graziani, C., Busani, L., Dionisi, A.M., Lucarelli, C., Owczarek, S., Ricci, A., Mancin, M., Caprioli, A., Luzzi, I., 2008. Antimicrobial resistance in *Salmonella enterica* serovar Typhimurium from human and animal sources in Italy. Vet. Microbiol. 128 (3-4):414–418. doi: 10.1016/j.vetmic.2007.10.017.

Grossman, T.H., 2016. Tetracycline antibiotics and resistance. Cold Spring Harb. Perspect. Med. 6 (4):1–24. doi: 10.1101/cshperspect.a025387.

Guillaume, G., Verbrugge, D., Chasseur-libotte, M., Moens, W., Y, J.C., 2000. PCR typing of tetracycline resistance determinants (Tet A ^ E) in *Salmonella enterica* serotype Hadar and in the microbial community of activated sludges from hospital and urban wastewater treatment facilities in Belgium. FEMS Microbiol. Ecol. 32(1):77–85. doi: 10.1111/j.1574-6941.2000.tb00701.x.

Guo, N., Wei, Q., Xu, Y., 2020. Optimization of cryopreservation of pathogenic microbial strains. J. Biosaf. Biosecurity 2 (2):66–70. doi:10.1016/j.jobb.2020.11.003.

Harimaya, A., Shin-ichi, Y., Sato, K., Yamazaki, N., Himi, T., Fuji, N., 2007. High prevalence of erythromycin resistance and macrolide-resistance genes , mefA and ermB , in *Streptococcus pneumoniae* isolates from the upper respiratory tracts of children in the Sapporo district , Japan. J. Infect. Chemother. 13(4):219–223. doi: 10.1007/s10156-007-0528-5.

Hashemian, S.M.R., Farhadi, T., Ganjparvar, M., 2018. Linezolid: A review of its properties, function, and use in critical care. Drug Des. Devel. Ther. 12, 1759–1767. doi: 10.2147/DDDT.S164515.

Haulisah, N.A., Hassan, L., Bejo, S.K., Jajere, S.M., Ahmad, N.I., 2021. High levels of antibiotic resistance in isolates from diseased livestock. Front. Vet. Sci. 8. doi: 10.3389/fvets.2021.652351.

Hazim, C., Abubeker Ibrahim, R., Westercamp, M., Belete, G.A., Amare Kibret, B., Kanter, T., Yimer, G., Adem, T.S., Stevenson, K.B., Urrego, M., Kale, K.N., Omondi, M.W., Vanderende, D., Park, B.J., Parsons, M.M.B., Gallagher, K.M., 2018. Establishment of a sentinel laboratory-based antimicrobial resistance surveillance network in Ethiopia. Heal. Secur. 16 (1):S30–S36. doi: 10.1089/hs.2018.0052.

Heatley, B.Y.N.G., Wiuiam, S., 1944. A Method for the Assay of Penicillin 38 (1):61-65. doi:10.1042/bj0380061.

Heidarzadeh, S., Pourmand, M.R., 2021. Antimicrobial susceptibility , serotyping , and molecular characterization of antibiotic resistance genes in *Listeria monocytogenes* isolated from pregnant women with a history of abortion. Iran J. Public Heal. 50 (1), 170–179. doi:10.18502/ijph.v50i1.5084.

Henriques, A.R., Cristino, J.M., 2017. Genetic characterization of *Listeria monocytogenes* isolates from industrial and retail ready-to-eat meat-based foods and their relationship with clinical strains from human listeriosis in Portugal. J. Food Prot. 80(4):551–560. doi:10.4315/0362-028X.JFP-16-310.

Henton, M.M., Eagar, H.A., Swan, G.E., van Vuuren, M., 2011. Part VI. Antibiotic management and resistance in livestock production. South African Med. J. 101(8):583–586.

https://www.hhs.gov/sites/default/files/who-update-amr.pdf. [Accessed on 12 September 2022].

Holmes, N.E., Tong, S.Y.C., Davis, J.S., Hal, S.J. Van, 2015. Treatment of methicillin-resistant *Staphylococcus aureus* : vancomycin and beyond. 36(1):17-30. doi:10.1055/s-0034-1397040.

Hölzel, C.S., Harms, K.S., Bauer, J., Bauer-Unkauf, I., Hörmansdorfer, S., Kämpf, P., Mölle, G., Oehme, C., Preikschat, P., Schwaiger, K., 2012. Diversity of antimicrobial resistance genes and class-1-integrons in phylogenetically related porcine and human *Escherichia coli.* Vet. Microbiol. 160 (3-4):403–412. doi:10.1016/j.vetmic.2012.06.010.

Hossain, M.K., Islam, S., Uddin, S., Rahman, A.T.M.M., Jahan, N., Li, J., Alam, J., 2023. isolation , identification and genetic characterization of antibiotic resistant *Escherichia coli* from frozen chicken meat obtained from supermarkets at Dhaka City in Bangladesh. Antibiot. Artic.

Hutwagner, L.C., Maloney, E.K., Bean, N.H., Slutsker, L., Martin, S.M., 1997. Using laboratory-based surveillance data for prevention: An Algorithm for Detecting Salmonella Outbreaks. Emerg. Infect. Dis. 3(3), 395–400. doi:10.3201/eid0303.970322.

Igbinosa, I.H., Igbinosa, E.O., Okoh, A.I., 2015. Detection of antibiotic resistance , virulence gene determinants and biofilm formation in Aeromonas species isolated from cattle. Environ. Sci. Pollut. Res. 22, 17596–17605. doi.org/10.1007/s11356-015-4934-4.

Iskandar, K., Molinier, L., Hallit, S., Sartelli, M., Hardcastle, T.C., 2021. Surveillance of antimicrobial resistance in low - and middle - income countries : a scattered picture. Antimicrob Resist Infect Control 10, 1–19.

Israel, G.., 2013. Determining sample size. Institute of food and agricultural sciences (IFAS).

Sci. Res. 1–5.

Jacoby, G.A., Walsh, K.E., Mills, D.M., Walker, V.J., Oh, H., Robicsek, A., Hooper, D.C., 2006. qnrB , another plasmid-mediated gene for quinolone resistance. Antimicrob. Agents Chemother. 50, 1178–1182.

Jaja, I.F., Bhembe, N.L., Green, E., Oguttu, J., Muchenje, V., 2019. Molecular characterisation of antibiotic-resistant *Salmonella enterica* isolates recovered from meat in South Africa. Acta Trop. 190, 129–136.

Jaja, I.F., Jaja, C.J.I., Chigor, N.V., Anyanwu, M.U., Maduabuchi, E.K., Oguttu, J.W., Green, E., 2020. Antimicrobial resistance phenotype of *Staphylococcus aureus* and *Escherichia coli* isolates obtained from meat in the formal and informal sectors in South Africa. Biomed Res. Int. 2020.

Johnson, A.P., 2015. Surveillance of antibiotic resistance. Philos. Trans. R. Soc. 1–12.

Jubeh, B., Breijyeh, Z., 2020. Resistance of gram-positive bacteria to current antibacterial agents and overcoming approaches. Molecules 25, 1–22.

Kahlmeter, G., Brown, D.F.J., Goldstein, F.W., Macgowan, A.P., Mouton, J.W., Odenholt, I., 2006. European committee on antimicrobial susceptibility testing (EUCAST) Technical Notes on antimicrobial susceptibility testing. Eur. Soc. Clin. Infect. Dis. 12(6), 501–503. doi: 10.1111/j.1469-0691.2006.01454.x.

Kallipolitis, B., Gahan, C.G.M., Piveteau, P., 2020. ScienceDirect Factors contributing to *Listeria monocytogenes* transmission and impact on food safety. Curr. Opin. Food Sci. 36, 9–17.

Kapoor, G., Saigal, S., Elongavan, A., 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians. J. Anaesthesiol. Clin. Pharmacol. 33, 300–305.

Kaprou, G.D., Bergšpica, I., Alexa, E.A., Alvarez-ord, A., Prieto, M., 2021. Rapid methods for antimicrobial resistance diagnostics. Antibiotics 10, 1–30.

Keddy, K.H., Smith, A.M., Arvinda, S., Ismail, H., Oliver, S., 2010. Fluoroquinolone resistant typhoid, South Africa. Emerg. Infect. Dis. 16, 879–880.

Keet, R., Rip, D., 2021. *Listeria monocytogenes* isolates from western cape, south africa exhibit resistance to multiple antibiotics and contradicts certain global resistance patterns. AIMS Microbiol. 7, 40–58.

Khalili, H., Soltani, R., Negahban, S., 2012. Reliability of disk diffusion test results for the antimicrobial susceptibility testing of nosocomial gram-positive microorganisms : is e-test method better ? Iran. J. Pharm. Res. 11, 559–563.

Khan, F., Pham D.T. N and Kim Y., 2020. Alternative strategies for the application of

aminoglycoside. Applied Microbiology and Biotechnology. 104,1955–1976.

antibiotics against the biofilm-forming human pathogenic bacteria

Khodabandeh, M., Mohammadi, M., Abdolsalehi, M.R., Alvandimanesh, A., Gholami, M., Bibalan, M.H., Pournajaf, A., Kafshgari, R., Rajabnia, R., 2019. Analysis of resistance to macrolide-lincosamide-streptogramin B among mecA-positive *Staphylococcus aureus* Isolates. Osong Public Heal. Res. Perspect. 10, 25–31.

Kim, Y.B., Seo, K.W., Jeon, H.Y., Lim, S.K., Lee, Y.J., 2018. Characteristics of the antimicrobial resistance of *Staphylococcus aureu*s isolated from chicken meat produced by different integrated broiler operations in Korea. Poult. Sci. 97, 962–969.

Kowalska-krochmal, B., Dudek-wicher, R., 2021. The minimum inhibitory concentration of antibiotics : methods , interpretation , clinical relevance. Pathogens 10.

Kozak, G.K., Boerlin, P., Janecko, N., Reid-Smith, R.J., Jardine, C., 2009. Antimicrobial resistance in *Escherichia coli* isolates from Swine and wild small mammals in the proximity of swine farms and in natural environments in Ontario, Canada. Appl. Environ. Microbiol. 75, 559–566.

Krause, K.M., Serio, A.W., Kane, T.R., Connolly, L.E., 2016. Aminoglycosides : An overview. Cold spring harb perspect med. 6(6), a027029. doi:10.1101/cshperspect.a027029.

Kuch, A., Goc, A., Belkiewicz, K., Filipello, V., Ronkiewicz, P., Gołębiewska, A., Wróbel, I., Kiedrowska, M., Waśko, I., 2018. Molecular diversity and antimicrobial susceptibility of *Listeria monocytogenes* isolates from invasive infections in Poland. Sci Rep 8, 14562.

Kupletskaya, M.B., Netrusov, A.I., 2011. Viability of lyophilized microorganisms after 50-year storage. Microbiology 80, 850–853.

Lanz, R., Kuhnert, P., Boerlin, P., 2003. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. Vet. Microbiol. 91, 73–84.

Leekha, S., Terrell, C.L., Edson, R.S., 2011. General principles of antimicrobial therapy. Mayo Clin. Proc. 86, 156–167.

Lekshmi, M., Ammini, P., Kumar, S., Varela, M.F., 2017. The food production environment and the development of antimicrobial resistance in human pathogens of animal origin. Microorganisms 5, 1–15.

Li, R., Lai, J., Wang, Y., Liu, S., Li, Y., Liu, K., Shen, J.Z., Wu, C., 2014. Prevalence and characterization of *Salmonella* species isolated from pigs, ducks and chickens in Sichuan Province, China. Int. J. Food Microbiol. 163, 14–18.

Li, S.M., Zhou, Y.F., Li, L., Fang, L.X., Duan, J.H., Liu, F.R., Liang, H.Q., Wu, Y.T., Gu,

W.Q., Liao, X.P., Sun, J., Xiong, Y.Q., Liu, Y.H., 2018. Characterization of the multi-drug resistance gene cfr in methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from animals and humans in China. Front. Microbiol. 9, 1–10.

Liakopoulos, A., Mevius, D., Ceccarelli, D., 2016. A review of SHV extended-spectrum β -lactamases : neglected yet ubiquitous. Front. Microbiol. 7, 1–27.

Lowy, F.D., 2003. Antimicrobial resistance: The example of *Staphylococcus aureus*. J. Clin. Invest. 111, 1265–1273.

Madoroba, E., Kapeta, D., Gelaw, A.K., 2016. *Salmonella* contamination, serovars and antimicrobial resistance profiles of cattle slaughtered in South Africa. Onderstepoort J. Vet. Res. 83, 1–8.

Madoroba, E., Malokotsa, K.P., Ngwane, C., Lebelo, S., Magwedere, K., 2022. Presence and Virulence characteristics of shiga toxin *Escherichia coli* and non-shiga toxin-producing *Escherichia coli* O157 in products from animal protein supply chain enterprises in South Africa. Food borne Pathog. Dis. 19, 386–393.

Mafuna, T., Matle, I., Magwedere, K., Pierneef, R.E., Reva, O.N., 2022. Comparative genomics of listeria species recovered from meat and food processing facilities. Microbiol. Spectr. 10, 1–11.

Magwedere, K., Rauff, D., De Klerk, G., Keddy, K.H., Dziva, F., 2015. Incidence of nontyphoidal salmonella in food-producing animals, animal feed, and the associated environment in South Africa, 2012-2014. Clin. Infect. Dis. 61, S283–S289.

Mahmmoud, E.N., 2020. Comparison of preservation methods of *Staphylococcus aureus* and *Escherichia coli* bacteria. J. Pure Appl. Microbiol. 14, 2173–2180.

Mahmud, Z.H., Kabir, M.H., Ali, S., Moniruzzaman, M., Adnan, S., Hakim, I., Worth, M., Ahmed, D., Johnston, D., Ahmed, N., 2020. Extended-spectrum *Escherichia coli* in drinking water samples from a forcibly displaced , densely populated community setting in Bangladesh. Orig. Res. 8, 1–14.

Mąka, Ł., Popowska, M., 2016. Antimicrobial resistance of *Salmonella spp* . Natl. Inst. Public Heal. 67, 343–357.

Mamza, S.A., Egwu, G.O., Mshelia, G.D., 2010. Antibiotic susceptibility patterns of beta-lactamase-producing *Escherichia coli* and *Staphylococcus aureus* is. Vet. Arh. 80, 283–297.

Manishimwe, R., Nishimwe, K., Ojok, L., 2017. Assessment of antibiotic use in farm animals in Rwanda. Trop. Anim. Health Prod. 49, 1101–1106.

Manyi-Loh, C., Mamphweli, S., Meyer, E., Okoh, A., 2018. Antibiotic use in agriculture and

its consequential resistance in environmental sources: Potential public health implications, Molecules. 23(4), 795.

Marais, E., Aithma, N., Perovic, O., Oosthuysen, W.F., Musenge, E., Dusé, A.G., 2009. Antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolates from South Africa. South African Med. J. 99, 170–173.

Marshall, B.M., Levy, S.B., 2011. Food animals and antimicrobials: Impacts on human health. Clin. Microbiol. Rev. 24, 718–733.

Mathole, M.A., Muchadeyi, F.C., Mdladla, K., Malatji, D.P., Dzomba, E.F., Madoroba, E., 2017. Presence, distribution, serotypes and antimicrobial resistance profiles of *Salmonella* among pigs, chickens and goats in South Africa. Food Control 72, 219–224.

Matle, I., Mbatha, K.R., Lentsoane, O., Magwedere, K., Morey, L., Madoroba, E., 2019. Occurrence, serotypes, and characteristics of *Listeria monocytogenes* in meat and meat products in South Africa between 2014 and 2016. J. Food Saf. 39, 1–14.

Matle, I., Mbatha, K.R., Madoroba, E., 2020. A review of *Listeria monocytogenes* from meat and meat products : Epidemiology , virulence factors , antimicrobial resistance and diagnosis Listeria species. Onderstepoort J. Vet. Res. 87, 1–20.

Mattiello, S.P., Drescher, G., 2015. Characterization of antimicrobial resistance in *Salmonella enterica* strains isolated from Brazilian poultry production characterization of antimicrobial resistance in *Salmonella enterica* strains isolated from Brazilian poultry production. CrossMark Orig. Pap. 108, 1227–1228.

Matuschek, E., Brown, D.F.J., Kahlmeter, G., 2014. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clin. Microbiol. Infect. 20(4), O255-66

Mbelle, N.M., Feldman, C., Sekyere, J.O., Maningi, N.E., Modipane, L., Essack, S.Y., 2020. Pathogenomics and evolutionary epidemiology of multi-drug resistant clinical *Klebsiella pneumoniae* isolated from Pretoria, South Africa. Sci. Rep. Nat. Res. 10, 1–17.

Mehdi, Y., Létourneau-Montminy, M.P., Gaucher, M. Lou, Chorfi, Y., Suresh, G., Rouissi, T., Brar, S.K., Côté, C., Ramirez, A.A., Godbout, S., 2018. Use of antibiotics in broiler production: Global impacts and alternatives. Anim. Nutr. 4, 170–178.

Mehndiratta, P.L., Bhalla, P., 2014. Use of antibiotics in animal agriculture & emergence of methicillin- resistant *Staphylococcus aureus* (MRSA) clones : need to assess the impact on public health. Indian J. Med. Res. 140(3), 339–344.

Mehrotra, M., Wang, G., Johnson, W.M., 2000. Multiplex PCR for detection of genes for *Staphylococcus aureu*s enterotoxins , exfoliative toxins , toxic shock syndrome toxin 1 ,

and methicillin resistance. J. Clin. Microbiol. 38(3), 1032–1035.

Mesbah, A., Mashak, Z., Abdolmaleki, Z., 2021. A survey of prevalence and phenotypic and genotypic assessment of antibiotic resistance in *Staphylococcus aureus* bacteria isolated from ready - to - eat food samples collected from Tehran Province , Iran. Trop. Med. Health 49(1), 1–12.

Miklasinka-Majdanik, M., 2021. Mechanisms of resistance to macrolide antibiotics among *Staphylococcus aureus*. Antobiotics 10(11), 1406.

Mkize, N., Zishiri, O.T., Mukaratirwa, S., 2017. Genetic characterisation of antimicrobial resistance and virulence genes in *Staphylococcus aureus* isolated from commercial broiler chickens in the Durban metropolitan area , South Africa. J. S. Afr. Vet. Assoc.88(0), 1–7.

Moawad, A.A., Hotzel, H., Awad, O., Tomaso, H., Neubauer, H., Hafez, H.M., Adawy, H. El, 2017. Occurrence of *Salmonella enterica* and *Escherichia coli* in raw chicken and beef meat in northern Egypt and dissemination of their antibiotic resistance markers. Gut Pathog. 9, 57.

Mood, E.H., Meshkat, Z., Izadi, N., Rezaei, M., Jamehdar, S.A., 2015. Prevalence of quinolone resistance genes among extended-spectrum b-lactamase-producing *Escherichia coli* in Mashhad , Iran. Jundishapur J. Microbiol. 8(12), e16217.

Moreira, M., Nicolete, G., Lopes, B.D., Morita, C.H., Souza, J.M. De, Winkelstroter, L.K., 2018. Evaluation of antimicrobial susceptibility by *Staphylococcus aureus* isolated from the nutrition service of a teaching hospital. Adv. Microbiol. 8, 270–285.

Morobe, I.C., Obi, C.L., Nyila, M.A., Gashe, B.A., Matsheka, M.I., 2009. Prevalence, antimicrobial resistance profiles of *Listeria monocytognes* from various foods in Gaborone, Botswana. African J. Biotechnol. 8, 6383–6387.

Moyane, J.N., Jideani, A.I.O., Aiyegoro, A.O., 2013. Antibiotics usage in food-producing animals in South Africa and impact on human: antibiotic resistance. African J. Microbiol. Res. 7, 2990–2997.

Mpondo, L., Ebomah, K.E., 2021. Multidrug-resistant *Listeria species* shows abundance in environmental waters of a key district municipality in South Africa. Int. J. Environ. Res. Public Health 18(2), 481.

Mthembu, T.P., Zishiri, O.T., El Zowalaty, M.E., 2019. Detection and molecular identification of *Salmonella* virulence genes in livestock production systems in South Africa. Pathogens 8.

Mthembu, T.P., Zishiri, O.T., El Zowalaty, M.E., 2021. Genomic characterization of antimicrobial resistance in food chain and livestock-associated *Salmonella species*.

Animals 11, 1–16.

Mulani, M.S., Kamble, E.E., Kumkar, S.N., Tawre, M.S., Pardesi, K.R., 2019. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review. Front. Microbiol. 10.

Munita, J.M., Arias, C.A., Unit, A.R., Santiago, A. De, 2016. Mechanisms of antibiotic resistance. Microbiol. Spectr. 4, 1–37.

Mupfunya, C.R., Qekwana, D.N., Naidoo, V., 2021. Antimicrobial use practices and resistance in indicator bacteria in communal cattle in the Mnisi community, Mpumalanga, South Africa. Vet. Med. Sci. 7, 112–121.

Nasran, H.S., Yusof, H.M., Halim, M., Rahman, N.A., 2020. Optimization of protective agents for the freeze-drying of *Paenibacillus polymyxa* Kp10 as a potential biofungicide. Molecules 25(11), 2618. doi: 10.3390/molecules25112618.

Nayiga, S., Kayendeke, M., Nabirye, C., Willis, L.D., Chandler, C.I.R., Staedke, S.G., 2020. Use of antibiotics to treat humans and animals in Uganda: A cross-sectional survey of households and farmers in rural, urban and peri-urban settings. JAC-Antimicrobial Resist. 2, 1–11.

https:// www.ncbi.nlm.nih.gov. [Accessed on 12 September 2022].

Nemati, M., Hermans, K., Lipinska, U., Denis, O., Deplano, A., Struelens, M., Devriese, L.A., Pasmans, F., Haesebrouck, F., 2008. Antimicrobial resistance of old and recent *Staphylococcus aureus* isolates from poultry : First detection of livestock-associated methicillin-resistant antimicrobial resistance of old and recent *Staphylococcus aureus* isolates from poultry : First detection. Antimicrob. Agents Chemother. 52, 3817–3819.

Nkansa-Gyamfi, N.A., Kazibwe, J., Traore, D.A.K., Nji, E., 2019. Prevalence of multidrug-, extensive drug-, and pandrug-resistant commensal *Escherichia coli* isolated from healthy humans in community settings in low- and middle-income countries: a systematic review and meta-analysis. Glob. Health Action 12 (sup 1), 1815272. doi: 10.1080/16549716.2020.1815272.

Noor, S., Taji, O., Golzar, J., 2022. Simple random sampling. Int. J. Educ. Lang. Stud. 1, 79–82.

https://www.oie.int. [Accessed on 04 October 2022].

Palma, E., Tilocca, B., Roncada, P., 2020. Antimicrobial resistance in veterinary medicine: An overview. Int. J. Mol. Sci. 21(6), 1914. doi: 10.3390/ijms21061914.

Parvin, M.S., Ali, M.Y., Mandal, A.K., Talukder, S., Islam, M.T., 2022. Sink survey to investigate multidrug resistance pattern of common foodborne bacteria from wholesale

chicken markets in Dhaka city of Bangladesh, Sci Rep. Nature Publishing Group UK.12, Article number: 108.

Patel, R., Rouse, M.S., Piper, K.E., Cockerill, F.R., Steckelberg, J.M., 1998. In vitro activity of LY333328 against vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, and penicillin-resistant *Streptococcus pneumoniae*. Diagn. Microbiol. Infect. Dis. 30, 89–92.

Pathak, P.K., 1988. 4 Simple random sampling. pp. 97–109.

Pekana, A., Green, E., 2018. Antimicrobial resistance profiles of *Staphylococcus aureus* isolated from meat carcasses and bovine milk in abattoirs and dairy farms of the Eastern Cape , South Africa. Int. J. Environ. Res. Public Health 15, 2–13.

Peruzy, M.F., Capuano, F., Proroga, Y.T.R., Cristiano, D., Carullo, M.R., Murru, N., 2020. Antimicrobial susceptibility testing for *Salmonella* serovars isolated from food samples: Five-year monitoring (2015–2019). Antibiotics 9, 1–17.

Peterson, E., Kaur, P., 2018. Antibiotic resistance mechanisms in bacteria: Relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. Front. Microbiol. 9, 1–21.

Poirel, L., Madec, J., Lupo, A., Schink, A., Kieffer, N., Nordmann, P., Schwarz, S., 2018. Antimicrobial Resistance in *Escherichia coli.* Microbiol. Spectr. 6, 1–32.

Prakash, O., Nimonkar, Y., Desai, D., 2020. A recent overview of microbes and microbiome preservation. Indian J. Microbiol. 60, 297–309.

Prats, G., Mirelis, B., Llovet, T., Muñoz, C., Miró, E., Navarro, F., 2000. Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985-1987 and 1995-1998 in Barcelona. Antimicrob. Agents Chemother. 44, 1140–1145.

Prestinaci, F., Pezzotti, P., Pantosti, A., 2015. Antimicrobial resistance: A global multifaceted phenomenon. Pathog. Glob. Health.109(7), 309-18. doi: 10.1179/2047773215Y.0000000030.

Puszczak, K., Fronczyk, A., Urbański, M., 2013. Analysis of sample size in consumer surveys, Uma ética para quantos?

Ramatla, T.A., 2019. The virulence and antimicrobial resistance of *Salmonella spp* . isolates from rodents inhabiting chicken farms in Mafikeng , South Africa. PhD dessertation.

Ramsamy, Y., Essack, S.Y., Sartorius, B., Patel, M., Mlisana, K.P., 2018. Antibiotic resistance trends of ESKAPE pathogens in Kwazulu-Natal, South Africa: A five-year retrospective analysis. Afr. J. Lab. Med. 7, 1–8.

Rao, S., Linke, L., Magnuson, R., Jauch, L., Hyatt, D.R., 2022. Antimicrobial resistance and

genetic diversity of *Staphylococcus aureus* collected from livestock , poultry and humans. One Heal. 15, 100407.

Rasha, I.M., Mohamed, A.A., Heba, M.A., 2018. Virulence and antimicrobial susceptibility profile of *Listeria monocytogenes* isolated from frozen vegetables available in the Egyptian market. African J. Microbiol. Res. 12(9), 218–224.

Reygaert, W.C., 2018. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol. 4(3), 482-501. doi: 10.3934/microbiol.2018.3.482

Riaz, S., Hussain, A., Muhammad, S. Shafiq UR, M.S., Javed, N., Abbas, Z., 2021. Isolation and characterization of vancomycin resistant *Staphylococcus aureus* (VRSA) from Intensive Care Units (ICU) of different hospitals in Lahore, Pakistan. Adv. Life Sci. 8, 339–344.

Richardson, J.E., Bacigalupe, R., Ewan, M Harrison. Weinert, L.A., Lycett, Samantha. Vrieling, M., Robb, K., Hoskisson, Paul A. Holden, Matthew T G. Feil, E.J., Paterson, G.K., Tong, S.Y.C., Shittu, A., van Wamel, Willem. Aanensen, David M Parkhill, J., Sharon J. Peacock. Corande, J., Mark, H., Holmes, Ross, J.F., 2018. Gene exchange drives the ecological success of a multi-host bacterial pathogen. Natl. Ecol. Evol. 2, 1468–1478.

Rivera, A.M., Boucher, H.W., 2011. Current concepts in antimicrobial therapy against select gram-positive organisms: Methicillin-resistant *Staphylococcus aureus*, penicillin-resistant pneumococci, and vancomycin-resistant enterococci. Mayo Clin. Proc. 86, 1230–1243.

Rocchetti, T.T., Martins, K.B., Martins, P.Y.F., Oliveira, R.A. de, Mondelli, A.L., Fortaleza, C.M.C.B., Cunha, M. de L.R. de S. da, 2018. Detection of the mecA gene and identification of *Staphylococcus* directly from blood culture bottles by multiplex polymerase chain reaction. Brazilian J. Infect. Dis. 22, 99–105.

Rosengren, L.B., Waldner, C.L., Reid-smith, R.J., 2009. Associations between Antimicrobial Resistance phenotypes , antimicrobial resistance genes , and virulence genes of fecal *Escherichia coli* isolates from healthy grow-finish pigs. Appl. Environ. Microbiol. 75(5), 1373–1380.

Rubin, J.E., Ball, K.R., Chirino-trejo, M., 2011. Antimicrobial susceptibility of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolated from various animals. Can. Vet. J. 52(2), 153–157.

Safarpoor Dehkordi, F., Barati, S., Momtaz, H., Hosseini Ahari, S.N., Nejat Dehkordi, S., 2013. Comparison of shedding, and antibiotic resistance properties of listeria monocytogenes isolated from milk, feces, urine, and vaginal secretion of bovine, ovine, caprine, buffalo,

and camel species in Iran. Jundishapur J. Microbiol. 6(1), 147-153. doi: 10.1002/vms3.199.

Salam, A., Al-amin, Y., Singh, J., Akhter, N., Banu, I., 2023. Saudi journal of biological sciences conventional methods and future trends in antimicrobial susceptibility testing. Saudi J. Biol. Sci. 30, 103582.

Sanchez-maldonado, A.F., Aslam, M., Service, C., Narváez-bravo, C., Avery, B.P., Johnson, R., Jones, T.H., 2017. International journal of food microbiology prevalence and antimicrobial resistance of *Salmonella* isolated from two pork processing plants in Alberta, Canada. Int. J. Food Microbiol. 241, 49–59.

Sarker, M.S., Kabir, M.E., Mahua, F.A., Ahmed, A., Ripon, R.K., Silva, V., Poeta, P., Ahad, A., 2023. Antibiotic-resistant *Escherichia coli* isolated from duck cloacal and tap water samples at live bird markets in Bangladesh. Poult. Sci. J. 11, 19–27.

Sarmah, H., Hazarika, B., 2012. Importance of the size of Sample and its determination in the context of data related to the schools of greater Guwahati. Bull. Gauhati Univ. Math. Assoc. ISSN 0975-4148 12, 55–76.

Schmidt, J.W., Griffin, D., Kuehn, L.A., Brichta-Harhay, D.M., 2013. Influence of therapeutic ceftiofur treatments of feedlot cattle on fecal and hide prevalences of commensal *Escherichia coli* resistant to expanded-spectrum cephalosporins, and molecular characterization of resistant isolates. Appl. Environ. Microbiol. 79, 2273–2283.

Sengupta, S., Chattopadhyay, M.K., Grossart, H.P., 2013. The multifaceted roles of antibiotics and antibiotic resistance in nature. Front. Microbiol. 4, 47.

Serio, A.W., Keepers, T., Andrews, L., Krause, K.M., 2018. Aminoglycoside revival: review of a historically important class of antimicrobials undergoing rejuvenation. EcoSal Plus 8, 1–20.

Serwecińska, 2020. Antimicrobials and Antibiotic-Resistant Bacteria : Water 12, 1–17.

Shakti, L., Veeraraghavan, B., 2015. Advantage and limitations of nitrofurantoin in multi-drug resistant Indian scenario. Indian J. Med. Microbiol. 33, 477–481.

Sharma, C., Rokana, N., Chandra, M., Singh, B.P., Gulhane, R.D., Gill, J.P.S., Ray, P., Puniya, A.K., Panwar, and H., 2018. Antimicrobial resistance : its surveillance , impact and alternative management strategies in dairy animals. Front. Vet. Sci. 4, 237. doi: 10.3389/fvets.2017.00237.

Sharma, J., Sharma D., Singh, A., Sunita, K., 2022. Colistin resistance and management of drug resistant infections. Can J Infect Dis Med Microbiol. 4315030.

Siddiky, N.A., Sarker, M.S., Khan, M.S.R., Begum, R., Kabir, M.E., Karim, M.R., Rahman,

M.T., Mahmud, A., Samad, M.A., 2021. Virulence and antimicrobial resistance profiles of *Salmonella enterica* serovars isolated from chicken at wet markets in dhaka, bangladesh. Microorganisms 9(5), 952. doi: 10.3390/microorganisms9050952.

Siegel, S., 1930. Nonparametric statistics for the behavioral sciences. Science. 80, 266-312.

Silley, P., Simjee, S., Schwarz, S., 2012. Surveillance and monitoring of antimicrobial resistance and antibiotic surveillance and monitoring methodologies of antimicrobial. Rev. sci. tech. Off. int. Epiz 31, 105–120.

Silva, V., Correia, S., Rocha, J., Manaia, C.M., Silva, A., García-Díez, J., Pereira, J.E., Semedo-Lemsaddek, T., Igrejas, G., Poeta, P., 2022. antimicrobial resistance and clonal lineages of *Staphylococcus aureus* from cattle, their handlers, and their surroundings: A cross-sectional study from the one health perspective. Microorganisms 10(5), 941.

Sineke, N., 2021. Molecular characterization of antibiotic-resistant *Staphylococcus aureus* in an intensive pig production system in KwaZulu-Natal, South Africa. Univ. Kwazulu-Natal.

Sonola et al, 2021. Occurrence of multi-drug-resistant escherichia coli in chickens, humans, rodents and household soil in Karatu, Northern Tanzania. Antibiotics 10(9), 1137. doi: 10.3390/antibiotics10091137.

Spellberg, B., Gilbert, D.N., 2014. The future of antibiotics and resistance: A tribute to a career of leadership by John Bartlett. Clin. Infect. Dis. 59 (Sup 2), S71–S75.

Squadrito, F.J., Portal, D. del, 2020. Nitrofurantoin - StatPearls - NCBI Bookshelf.

Stoesser, N., Batty, E.M., Eyre, D.W., Morgan, M., Wyllie, D.H., Elias, C.D.O., Johnson, J.R., Walker, A.S., Peto, T.E.A., Crook, D.W., 2013. Predicting antimicrobial susceptibilities for *Escherichia coli* and *Klebsiella pneumoniae* isolates using whole genomic sequence data. J. Antimicrob Chemother. 68(10), 2234–2244.

Sugianli, A.K., Ginting, F., Lia Kusumawati, R., Parwati, I., de Jong, M.D., van Leth, F., Schultsz, C., 2020. Laboratory-based versus population-based surveillance of antimicrobial resistance to inform empirical treatment for suspected urinary tract infection in Indonesia. PLoS One 15(3), e0230489. doi: 10.1371/journal.pone.0230489.

Sykes, R.D., Swan, G.E., Banoo, S., Naidoo, V., 2019. Veterinary drug control and management for the practising veterinarian in South Africa 1–40.

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E.M., Houchens, C.R., Grayson, L.M., Hansen, P., Singh, N., Theuretzbacher, U., Magrini, N., WHO Pathogens Priority List Working Group, 2018.

Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Infect. Dis. (Auckl). 18, 318–327.

Tajbakhsh, M., Hendriksen, R.S., 2012. Antimicrobial resistance in *Salmonella spp* . recovered from patients admitted to six different hospitals in Tehran , Iran from 2007 to 2008. Folia Microbiol. 57, 91–97.

Tchatchouang, C.D.K., Fri, J., De Santi, M., Brandi, G., Schiavano, G.F., Amagliani, G., Ateba, C.N., 2020. Listeriosis outbreak in south africa: A comparative analysis with previously reported cases worldwide. Microorganisms 8, 1–18.

Tenover, F.C., Tickler, I.A., Le, V.M., Dewell, S., Mendes, R.E., Goering, R. V, 2019. Updating molecular diagnostics for detecting methicillin- isolates in blood culture bottles. Clin. Microbiol. 57, 1–11.

Thomas, R.K., Melton, R., Asbell, P.A., 2019. Antibiotic resistance among ocular pathogens: Current trends from the ARMOR surveillance study (2009–2016). Clin. Optom. 11, 15–26.

Threlfall, E.J., Ward, L.R., Frost, J.A., Willshaw, G.A., 2000. The emergence and spread of antibiotic resistance in food-borne bacteria. Int. J. Food Microbiol. 62, 1–5.

Thwala, T., Madoroba, E., Basson, A., Butaye, P., 2021. Prevalence and characteristics of *Staphylococcus aureus* associated with meat and meat products in african countries: A review. Antibiotics 10, 1–16.

Travis, L.B., Rabkin, C.S., Brown, L.M., Allan, J.M., Alter, B.P., Ambrosone, C.B., Begg, C.B., Caporaso, N., Chanock, S., DeMichele, A., Figg, W.D., Gospodarowicz, M.K., Hall, E.J., Hisada, M., Inskip, P., Kleinerman, R., Little, J.B., Malkin, D., Ng, A.K., Offit, K., Pui, C.H., Robison, L.L., Rothman, N., Shields, P.G., Strong, L., Taniguchi, T., Tucker, M.A., Greene, M.H., 2006. Cancer survivorship - Genetic susceptibility and second primary cancers: Research strategies and recommendations. J. Natl. Cancer Inst. 98, 15–25.

Uddin, T.M., Chakraborty, A.J., Khusro, A., Zidan, B.R.M., Mitra, S., Emran, T. Bin, Dhama, K., Ripon, M.K.H., Gajdács, M., Sahibzada, M.U.K., Hossain, M.J., Koirala, N., 2021. Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. J. Infect. Public Health 14, 1750–1766.

Uelze, L., Borowiak, M., Deneke, C., Szabó, I., Fischer, J., Tausch, S.H., 2020. crossm Performance and accuracy of four open-source tools for in Silico serotyping of *Salmonella spp* based on whole genome short read sequencing data. Appl. Environ. Microbiol. 86(5), e02265-19.

Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A., Laxminarayan, R., 2015. Global trends in antimicrobial use in food animals. Proc. Natl. Acad. Sci. U. S. A. 112, 5649–5654.

Van Hoek, A.H.A.M., Mevius, D., Guerra, B., Mullany, P., Roberts, A.P., Aarts, H.J.M., 2011. Acquired antibiotic resistance genes: An overview. Front. Microbiol. 2, 1–27.

Varga, C., Rajić, A., McFall, M.E., Reid-Smith, R.J., Deckert, A.E., Pearl, D.L., Avery, B.P., Checkley, S.L., McEwen, S.A., 2008. Comparison of antimicrobial resistance in generic *Escherichia coli* and *Salmonella spp*. cultured from identical fecal samples in finishing swine. Can. J. Vet. Res. 72, 181–187.

Velazquez-meza, M.E., Galarde-lópez, M., 2022. Antimicrobial resistance : One Health approach. Vet. World 15, 742–749.

Ventola, M.., 2015. The Antibiotic Resistance Crisis Part 1: Causes and Threats 40, 277–283.

Wessman, P., Håkansson, S., Leifer, K., Rubino, S., 2013. Formulations for freeze-drying of bacteria and their influence on cell survival. J. Vis. Exp. 1–5.

https://www.who.int. [Accessed on 04 October 2022].

Wang, X., Biswas, S., Paudyal, N., Pan, H., Li, X., Fang, W., Yue, M., 2019. Antibiotic Resistance in *S*almonella Typhimurium isolates recovered from the food chain through national antimicrobial resistance monitoring system between 1996 and 2016. Front Microbiol. 10, 985.

Wu, L., Bao, H., Yang, Z., He, T., Tian, Y., Zhou, Y., Pang, M., Wang, R., 2021. Antimicrobial susceptibility , multilocus sequence typing , and virulence of listeria isolated from a slaughterhouse in Jiangsu , China. BMC Microbiol. 21, 1–13.

Yin, X., Dudley, E.G., Pinto, C.N., M'ikanatha, N.M., 2022. Fluoroquinolone sales in food animals and quinolone resistance in non-typhoidal *Salmonella* from retail meats: United States, 2009–2018. J. Glob. Antimicrob. Resist. 29, 163–167.

Yılmaz, E.Ş., Aslantaş, Ö., 2017. Antimicrobial resistance and underlying mechanisms in *Staphylococcus aureus* isolates. Asian Pac. J. Trop. Med. 10, 1059–1064.

Yong, D., Toleman, M.A., Giske, C.G., Cho, H.S., Sundman, K., Lee, K., Walsh, T.R., 2009. Characterization of a new metallo- β -lactamase gene , bla NDM-1 , and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India β. Antimicrob. Agents Chemother. 53, 5046–5054.

Zehra, A., Singh, R., Kaur, S., Gill, J.P.S., 2017. Molecular characterization of antibiotic-resistant *Staphylococcus aureus* from livestock (bovine and swine). Vet. World 10, 598–604.

Zhang, C., Xu, L., Mou, X., Xu, H., Liu, J., Miao, Y., Wang, X.C., Li, X., 2019. Characterization and evolution of antibiotic resistance of *Salmonella* in municipal wastewater treatment plants. J. Environ. Manage. 251, 1–8.

Zhang, L., Chen, F., Zeng, Z., Xu, M., Sun, F., Yang, L., Bi, X., Lin, Y., Gao, Y., Hao, H., Yi, W., Li, M., Xie, Y., 2021. Advances in metagenomics and its application in environmental microorganisms. Front. Microbiol. 12, 766364.

Zhao, Y., Yang, Q.E., Zhou, X., Wang, F.H., Muurinen, J., Virta, M.P., Brandt, K.K., Zhu, Y.G., 2021. Antibiotic resistome in the livestock and aquaculture industries: Status and solutions. Crit. Rev. Environ. Sci. Technol. 51, 2159–2196.

Zishiri, O.T., Mkhize, N., Mukaratirwa, S., 2016. Prevalence of virulence and antimicrobial resistance genes in *Salmonella spp*. isolated from commercial chickens and human clinical isolates from south Africa and Brazil. Onderstepoort J. Vet. Res. 83, 1–11.

Zishiri, O.T., Mthembu, T.P., El Zowalaty Mohamed E, 2019. Molecular detection of multidrug-resistant *Salmonella* isolated from livestock production systems in South Africa. Infect. Drug Resist. 12, 3537–3548.

ANNEXURE A

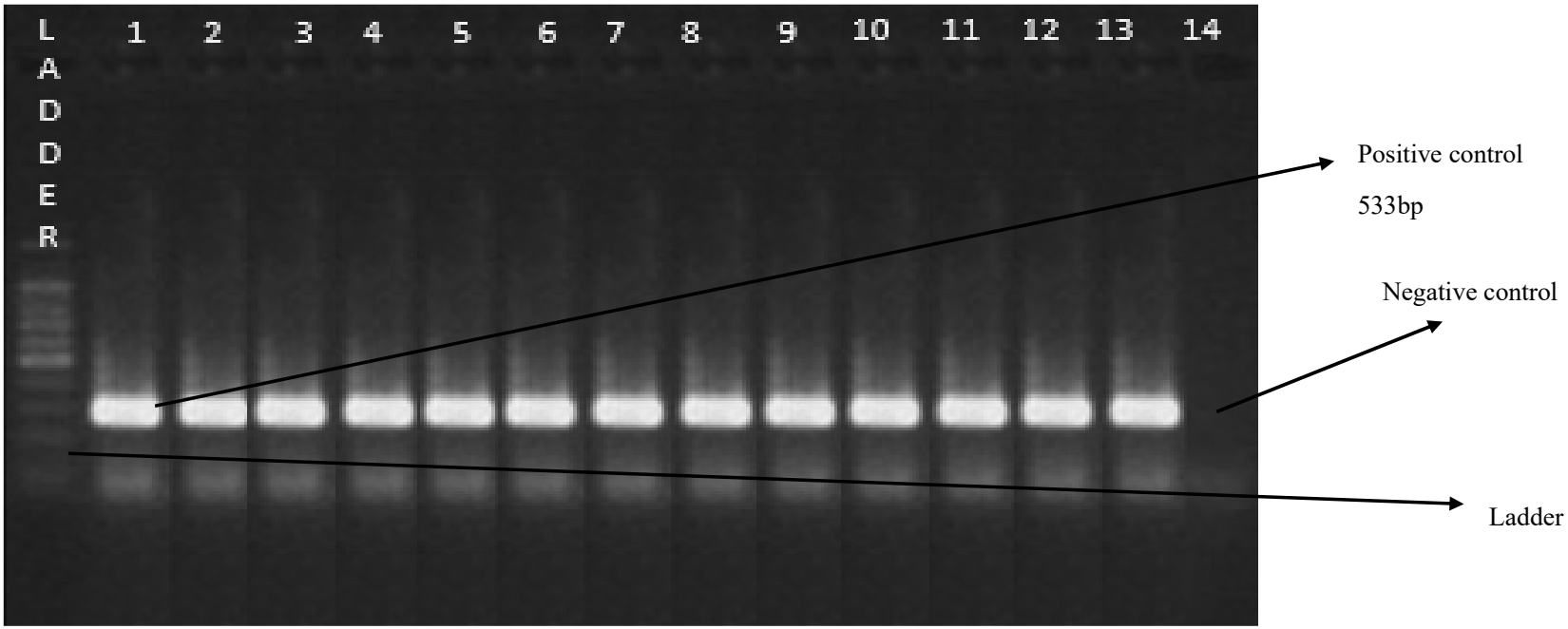

Figure 4. 6: PCR for detection of *mecA* gene in *S. aureus* isolates. Ladder lane contains fast ruler (100bp) LR DNA ladder, lane 1 a positive control *S. aureus* ATTC 25923, lanes 2-13 positive samples, and lane 14 a negative control containing nuclease free water (H_20).

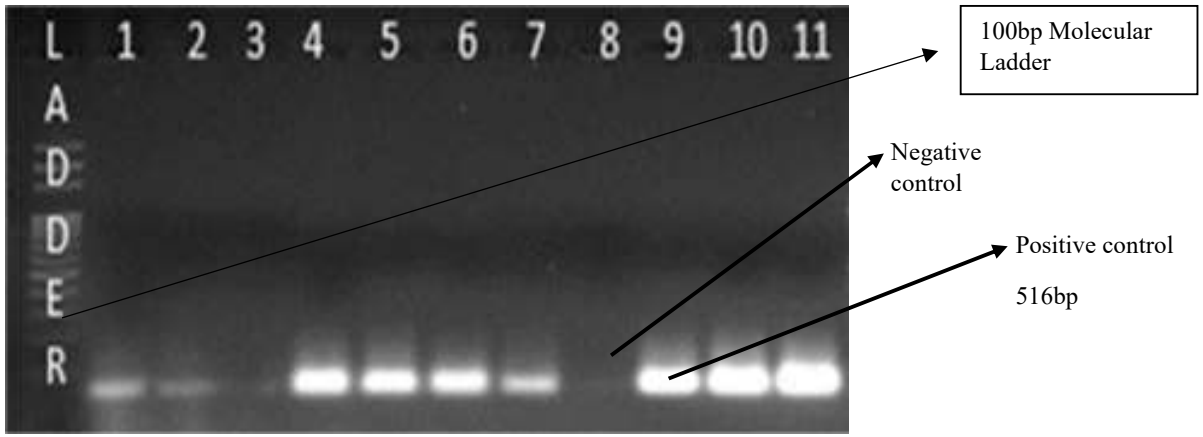

Figure 4. 7: Detection of quinolones genes in *Salmonella enterica* isolates by PCR. Ladder lane contains fast ruler (100bp) LR DNA ladder, lanes 1-2 unspecific bands, lane 3 negative sample, lanes 4-6 positive samples *Salmonella* Typhimurium ATCC 14028, lane 7 non-specific band (inconclusive), lane 8 negative control containing nuclease free water (H20), lane 9 positive control, lanes 10-11 positive samples.

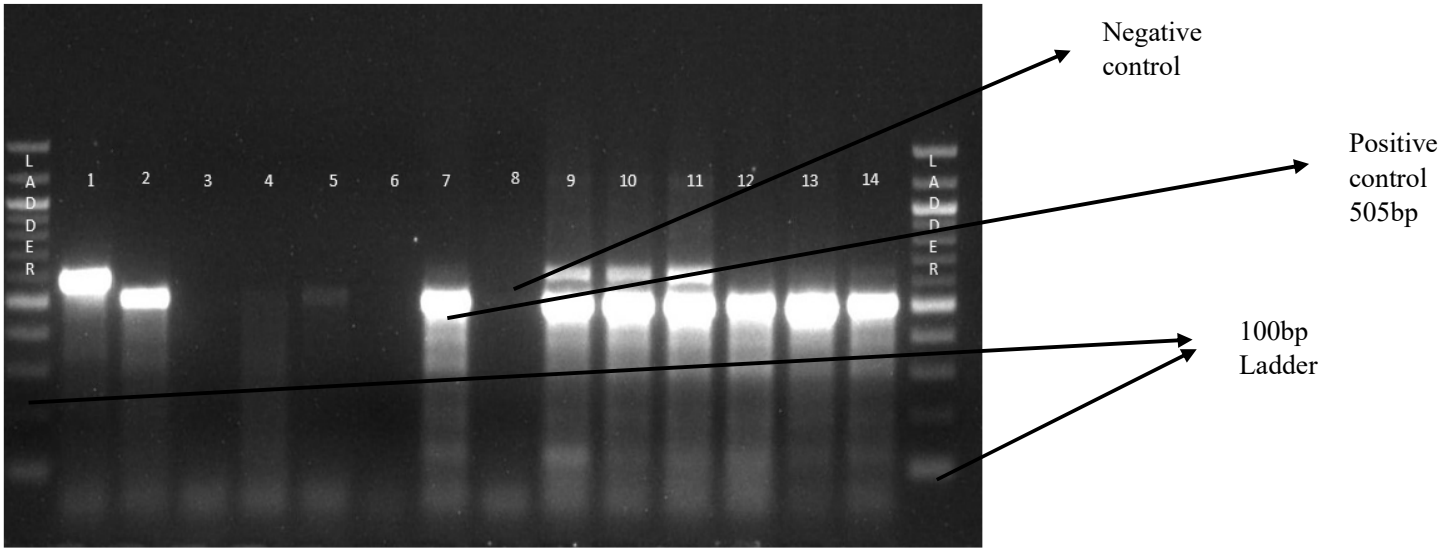

Figure 4. 8: Detection of tetracycline genes in *L. monocytogenes*. Ladder lane contains fast ruler (100bp) LR DNA ladder, lanes 1 non-specific band, lane 2 positive samples, lanes 3-4 negative samples, lane 5 non-specific band, lane 6 negative sample, lane 7 positive control *L. monocytogenes* ATTC 19111, lane 8 negative control containing nuclease free water (H20), lanes 9-14 positive samples.