UNIVERSITY OF
ZULULAND

Prevalence, Virulence Factors and Antimicrobial Resistance of *Salmonella* species from Beef in Retail Outlets from KwaZulu-Natal Province, South Africa

MSc (Microbiology) Dissertation

SERISHA NAIDOO

Student number: 201759970

Faculty of Science, Agriculture and Engineering

Department of Biochemistry and Microbiology

University of Zululand

Private Bag X 1001

KwaDlangezwa

3886

Supervisor: Prof E. Madoroba

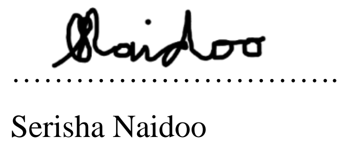
Co-Supervisors: Prof. P. Butaye

Prof. A. K. Basson

December 2021

Declaration

I, Serisha Naidoo, hereby declare that this dissertation is my own original work, with sources having been acknowledged in the text and reference list. To the best of my knowledge, I have acted in accordance with the University's plagiarism policy and have also obtained and complied with an ethical clearance certificate for my research. I further proclaim that this dissertation has not previously undergone submission for another degree at any other institution.



…………………………….	27/01/2022 …………………………………
Serisha Naidoo	Date

Supervisor

…………………………….	…………………………………
Prof. E. Madoroba	Date

Co-supervisors

…………………………….	…………………………………
Prof. A. K. Basson	Date

Dedication

I dedicate this dissertation to my late grandmother, Mavis Ratha Pillay, whose spirit continues to kindle and nurture in me a desire to seek knowledge.

Acknowledgments

I would like to express my utmost gratitude to my supervisor, Prof. Evelyn Madoroba, for her gracious and insightful guidance throughout this project. I also give thanks to my co-supervisors, Prof. A. K. Basson and Prof. P. Butaye, for their in-depth input and assistance. Due acknowledgement goes to the Department of Trade and Industry (DTI) - Technology and Human Resource for Industry Programme (THRIP) for funding this project. The DTI-THRIP grant was awarded to Prof. Evelyn Madoroba through grant number THRIP/22/30/11/2017. I am also grateful to the National Research Foundation (NRF) and Red Meat Research and Development South Africa (RMRDSA) for their contributions toward the project. Thank you to the University of Zululand, for giving me this opportunity, and to the staff and my colleagues at the university. Special mention goes to my parents for their endless support throughout this journey.

Table of Contents

Declaration....1

Dedication....2

Acknowledgements....3

Table of Contents....4

List of Figures....7

List of Tables....8

List of Abbreviations....9

Summary....12

Research Outputs Based on this Dissertation....14

Chapter 1: Introduction....15

1.1. Aim....19

1.2. Objectives....19

1.3. Research Questions....20

1.4. Hypothesis....20

Chapter 2: Literature Review....21

2.1. Background and Classification of *Salmonella* spp.....22

2.1.1. Brief History and Characteristics of *Salmonella*....22

2.1.2. *Salmonella* Taxonomy and Nomenclature....23

2.1.3. Laboratory Diagnosis and Characterization of *Salmonella* spp.....23

2.1.3.1. Detection, Isolation and Identification of *Salmonella* Using Biochemical Tests....23

2.1.3.2. *Salmonella* Serotyping....25

2.1.3.3 Molecular Typing of *Salmonella* Using Pulsed-Field Gel Electrophoresis....26

2.1.3.4. Whole Genome Sequencing (WGS)....27

2.1.4. Clinical Manifestations and Epidemiological Classification..27

2.2. Foodborne Illnesses 28

2.3. Salmonellosis 28

2.4. Virulence of *Salmonella* spp. 30

2.5. The Importance of Beef and Cattle Production in South Africa......................................31

2.5.1. Nutritional Value of Beef...31

2.5.2. Significance of Beef in South Africa 32

2.5.3. Cattle Production in South Africa...32

2.6. Review of *Salmonella* spp. Isolated from Meat and Meat Products in African Countries 34

2.6.1. Prevalence of Non-Typhoid *Salmonella* in Various Meat and Meat Products in Africa36

2.6.2. Prevalence and Characteristics of the Most Frequent *Salmonella* Serovars in Meat and Meat Products in African Countries...51

2.7. Antimicrobial Resistance 56

2.7.1. Mechanisms of Antimicrobial Resistance 56

2.7.2. Review on Antimicrobial Resistance in *Salmonella* spp. from Meat and Products in African Countries 57

2.7.3. The Extent of Antimicrobial Resistance among NTS Recovered from Human Beings in African Countries...60

Concluding Remarks...61

Chapter 3: Materials and Methods 62

3.1. Ethical Approval 63

3.2. Study Area and Design 63

3.3. Sample Size Determination...65

3.4. Reference Strains 66

3.5. Microbiological Analysis...66

3.5.1. Isolation and Identification of *Salmonella* 66
3.5.1.1. Pre-Enrichment and Isolation 66
3.5.1.2. Biochemical Confirmation Tests 67
3.5.1.3. Identification using MALDI-TOF MS and VITEK MS 68
3.5.2. *Salmonella* Serotyping 68
3.6. Antimicrobial Susceptibility Testing 68
3.7. Molecular Characterization of Virulence Genes....69
3.7.1. DNA Extraction 69
3.7.2. PCR Analysis 70
Chapter 4: Results....72
4.1. Microbiological Analysis....73
4.1.1. Detection and Isolation of Presumptive *Salmonella* isolates 73
4.2. *Salmonella* Serotyping 77
4.3. Antimicrobial Susceptibility Testing 77
4.4. Molecular Characterization of Virulence Genes....79
Chapter 5: General Discussion 82
Chapter 6: Conclusion and Recommendations....87
References 89
Appendix....106

List of Figures

Figure 2A: Image illustrating the principle of MALDI-TOF MS 25

Figure 2B: Map outlining countries in Africa where *Salmonella* prevalence studies have been performed in meat/meat products 35

Figure 3A: Map of King Cetshwayo and iLembe districts in KZN province, South Africa, showing the towns where samples were obtained 64

Figure 3B: Flow diagram outlining the study design 65

Figure 4A: Image showing the appearance of pure presumptive *Salmonella* isolate (from ox tripe) on XLD 73

Figure 4B: Image showing the appearance of pure presumptive *Salmonella* isolate on BGA74

Figure 4C: Image showing a typical result for presumptive *Salmonella* after a urea agar test (left) and a TSI agar test (right) 75

Figure 4D: Image showing the Kirby Bauer disk diffusion results for the *S.* Enteritidis isolate against the 10 tested antimicrobials...78

Figure 4E: Image showing the Kirby Bauer disk diffusion results for the *S.* Hadar isolate against the 10 tested antimicrobials 78

Figure 4F: Image showing examples of *inv*A gene amplicons observed on agarose gel 80

Figure 4G: Image showing example of *hil*A gene amplicons observed on agarose gel..........80

Figure 4H: Image showing electrophoresis results for *siv*H gene among the six isolates.......81

Figure 7A: Image showing a sample of ox intestines...108

Figure 7B: Sealed plastic bag containing a 25 g homogenized sample in 225 ml buffered peptone water...108

Figure 7C: Test tubes containing 10 ml each of MKTTn broth (left) and RVS broth (right)109

Figure 7D: Example of a pure presumptive *Salmonella* isolate streaked on nutrient agar....109

List of Tables

Table 1: Total number of serovars identified from the genus *Salmonella*, its two species and six subspecies 17

Table 2.1: *Salmonella* spp. prevalence from selected African countries categorized according to type of meat 37

Table 2.2: *Salmonella* spp. prevalence in diverse unprocessed red meat types.......................40

Table 2.3: *Salmonella* spp. prevalence among different raw unprocessed poultry types 42

Table 2.4: *Salmonella* spp. prevalence among diverse organ meat types...............................43

Table 2.5: *Salmonella* spp. prevalence among different processed meat types.......................44

Table 2.6: *Salmonella* spp. prevalence among diverse ready-to-eat meat types......................45

Table 2.7: Most common serovars isolated according to year and country............................52

Table 2.8: Sources of *S.* Typhimurium and *S.* Enteritidis isolates...55

Table 2.9: Resistance of *S.* Enteritidis and *S.* Typhimurium isolates toward different antimicrobial classes...58

Table 3.1 Disk content of antimicrobials 69

Table 3.2: Primers used to screen for each of eight virulence genes in *Salmonella* spp. using PCR...71

Table 4.1: Prevalence of *Salmonella* in different categories of beef classified according to the towns in which retail outlets were sampled..76

Table 4.2: *Salmonella enterica* subsp. *enterica* serovars confirmed from each positive* beef product 77

Table 4.3: PCR results for the screening of eight virulence genes among six *Salmonella* isolates 79

Table 7.1: Antimicrobial susceptibility results of *Salmonella enterica* subsp. *enterica* serovar (determined by serotyping) isolates against 10 antimicrobials............................110

List of Abbreviations

°C	-	Degrees Celsius
µl	-	Microlitre
AFLP	-	Amplified Fragment Length Polymorphism
*agf*A	-	Aggregative Fimbriae A (gene)
AMR	-	Antimicrobial Resistance
AW	-	Water Activity
BGA	-	Brilliant Green Agar
BLAST	-	Basic Local Alignment Search Tool
bp	-	Base Pair/s
BPW	-	Buffered Peptone Water
BSA	-	Bismuth Sulfite Agar
CDC	-	Centers for Disease Control and Prevention (USA)
CLSI	-	Clinical and Laboratory Standards Institute
DAFF	-	Department of Agriculture, Forestry and Fisheries
DNA	-	Deoxyribose Nucleic Acid
g	-	Grams
h	-	Hour/s
H_2O_2	-	Hydrogen Peroxide
H_2S	-	Hydrogen Sulphide
*hil*A	-	Hyper Invasion Locus A (gene)
HIV	-	Human Immunodeficiency Virus

*inv*A - Invasion A (gene)

ISO - International Organization for Standardization

KZN - KwaZulu-Natal

*lpf*A - Long Polar Fimbrial A (gene)

MALDI-TOF - Matrix-Assisted Laser Desorption Ionization–Time of Flight

MDR - Multi-Drug Resistant

MKTTn - Muller Kauffmann Tetrathionate novobiocin

ml - Millilitres

MS - Mass Spectrometry

NIDDK - National Institute of Diabetes and Digestive and Kidney Diseases (USA)

NTS - Non-Typhoid *Salmonella*

PBPs - Penicillin-binding proteins

PCR - Polymerase Chain Reaction

PFGE - Pulsed-Field Gel Electrophoresis

RAPD - Random Amplification of Polymorphic DNA

RVS - Rappaport-Vassiliadis Soya

*sef*A - *Salmonella* Enteritidis Fimbrial A (gene)

*siv*H - Intimin-like Inverse Autotransporter Protein H (gene)

*sop*E - *Salmonella* Outer Protein E (gene)

SPI - *Salmonella* Pathogenicity Islands

*spv*C - *Salmonella* Plasmid Virulence C (gene)

TSI - Triple Sugar Iron

WGS - Whole Genome Sequencing

WHO	-	World Health Organisation
WKL	-	White Kauffmann Le Minor
XLD	-	Xylose Lysine Deoxycholate
ZOI	-	Zone of Inhibition

Summary

Non-typhoid *Salmonella enterica* subsp. *enterica* serotypes are one of the leading causes of foodborne infections worldwide. Furthermore, the emergence and spread of antimicrobial-resistant *Salmonella* strains is a potential global challenge. This study was aimed at determining the prevalence, serovars, virulence factors and antimicrobial resistance patterns of *Salmonella* recovered from various beef products. Four hundred beef products (n = 169 organ meats, n = 110 raw processed meats, n = 53 raw intact meats and n = 68 ready-to-eat meats) were collected between October 2019 and December 2020 from 25 retail outlets in selected districts of KwaZulu-Natal (KZN) province, South Africa. The International Organization for Standardization (ISO) 6579-1:2017 method was used for microbiological analysis, and presumptive *Salmonella* isolates were confirmed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) and the VITEK system. The *Salmonella* isolates were serotyped according to the White-Kauffmann-Le Minor scheme. The Kirby Bauer disk diffusion method was used to determine antimicrobial resistance profiles of the *Salmonella* isolates against Cefotaxime, Kanamycin, Ampicillin, Amoxicillin, Trimethoprim-Sulfamethoxazole, Ciprofloxacin, Chloramphenicol, Gentamicin, Cefoxitin and Tetracycline. Antimicrobial resistance results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Polymerase chain reaction (PCR) was performed to screen for the presence of *inv*A, *agf*A, *lpf*A, *hil*A, *siv*H, *sef*A, *sop*E and *spv*C virulence genes. *Salmonella* contamination was observed in 1.25% (5/400) of the beef samples. Four serovars: Enteritidis, Hadar, Heidelberg and Stanley were identified. Almost all *Salmonella* isolates were susceptible to all 10 tested antimicrobials except one (*S*. Enteritidis) isolate that was resistant to tetracycline and aminopenicillins. All *Salmonella* isolates carried at least two virulence factors, with *S*. Stanley and *S*. Enteritidis testing positive for six and four of the eight tested virulence genes, respectively. The findings indicate that *Salmonella* prevalence in all beef meat categories of selected KZN retail outlets is low, but should be routinely surveyed to manage the risk associated with virulence factors and to avoid outbreaks. Although antimicrobial resistance was low, the presence of resistant *S*. Enteritidis highlights the need to continually monitor antimicrobial resistance amongst zoonotic pathogens associated with beef in order to contribute to 'One Health'.

Key words: Virulence factors; Antimicrobial resistance; *Salmonella enterica* serovars; Beef and beef products; Prevalence; Food safety

Research Outputs Based on this Dissertation

Parts of this dissertation have been published. The details of the publications are:

1. Book Chapter, cited as

Naidoo S., Basson A.K., Butaye P., Madoroba E. (2021) *Salmonella enterica* Subspecies *enterica* Serotypes Associated with Meat and Meat Products in African Countries: A Review. In: Babalola O.O. (eds) Food Security and Safety. Springer, Cham. https://doi.org/10.1007/978-3-030-50672-8_38.

2. Manuscript, titled

Naidoo, S., Butaye, P., Maliehe, S., Magwedere, K., Basson, A. & Madoroba, E. Virulence Factors and Antimicrobial Resistance among *Salmonella* species from Beef and Beef Products from Selected Retail Outlets in KwaZulu-Natal Province, South Africa. Submitted for peer review.

3. This work has been presented at a symposium with the following details:

Naidoo, S., Butaye, P., Maliehe, S., Magwedere, K., Basson, A. K. & Madoroba, E. (2021). Occurrence, Serotypes and Antimicrobial Resistance of *Salmonella* species from Beef in Retail Outlets from KwaZulu-Natal Province, South Africa. Faculty of Science, Agriculture and Engineering Virtual Symposium.

CHAPTER 1

INTRODUCTION

1. Introduction

Globally, foodborne diseases are a major burden for public health, the food industry and the economy (Ferede, 2014). In 2015, the World Health Organisation (WHO) estimated that, worldwide, 600 million (roughly 1 in 10) people contract foodborne illnesses every year. It was reported that 420 000 people died as a result, mostly from diarrhoeal diseases, and approximately 125 000 of these deaths were associated with children under 5 years old. Developing countries, particularly those in Africa and South East Asia, had the highest reported incidence of foodborne illnesses.

Numerous microorganisms from various sources have been associated with foodborne illnesses, but contaminated raw meat and meat products are among the most significant of those sources. *Salmonella enterica* subsp. *enterica* serotypes are among the leading causes of foodborne illnesses in both developed and developing countries. There is a higher infection rate in developing countries. This is possibly due to poor hygiene standards in food production as well as high rates of Human Immunodeficiency Virus (HIV), malaria, malnutrition and other clinical associations, which lead to more severe disease from a *Salmonella* infection (Gilchrist & Maclennan, 2019). Globally, non-typhoid *Salmonella* (NTS) causes an estimated 80 million foodborne illnesses and 30 000 deaths each year (Keddy, 2016).

Salmonella is a genus of Gram-negative motile bacilli comprising of two species: *Salmonella bongori* and *Salmonella enterica*. The latter is the only species of clinical importance and is separated into six subspecies that are commonly subdivided into serotypes, also known as serovars (Eng *et al.,* 2015). There are approximately 2 659 recorded *Salmonella* serovars (Table 1) (Issenhuth-Jeanjean *et al*., 2014; Ferrari *et al.,* 2019).

Table 1: Total number of serovars identified from the genus *Salmonella*, its two species and six subspecies

Salmonella species	*Salmonella subspecies*	*Number of serovars*
S. enterica	*Enterica*	1586
	Salamae	522
	Arizonae	102
	Diarizonae	338
	Houtenae	79
	Indica	13
Total S. enterica serovars		**2637**
S. bongori		22

Not all serovars have the same clinical importance for humans as compared to animals. In the medical field, only *S. enterica* subsp. *enterica* is of importance. *S. enterica* subsp. *arizonae* is associated with typhoid fever in turkeys, and can cause reduced egg production and hatchability (Gast & Porter, 2019). The remaining subspecies, together with *S. bongori,* are significant in diseases of ectotherms only.

S. enterica subsp. *enterica* serovars can be classified, based on the type of illness they cause, into two main groups, i.e. typhoid and non-typhoid *Salmonella* (Gilchrist & Maclennan, 2019). Typhoid *Salmonella* causes typhoid fever, a distinct and potentially life-threatening systemic infection (National Institute of Communicable Diseases Outbreak Response, 2016). Non-typhoid *Salmonella* serovars, which are the focus of the present study, cause an infection known as salmonellosis, or '*Salmonella* poisoning' in layman's terms, which usually causes self-limiting diarrhoea in immunocompetent individuals (Gilchrist & Maclennan, 2019).

Another classification of *S. enterica* serovars deals with different host specificities (Zghair, 2012). Firstly, there are host-specific serovars, which are restricted to a particular host, and generally cause a typhoid disease. Secondly, there are host-adapted serovars (also named host-restricted serovars), which are usually associated with a particular host, but can occasionally cause infection in other hosts (Ferede, 2014). Finally, there are non-host-specific serovars which are typically

associated with enteric disease and are zoonotic (Jajere, 2019). In poultry, several non-host-specific serovars can be present, with negligible symptoms in the poultry (except in very young chicks), however, they may cause enteritis in humans. Similarly, the most encountered serovar in pigs is *S.* Typhimurium, which occasionally results in diarrhoea and growth retardation in pigs, but its importance lies in its zoonotic aspect. Conversely, in bovines, the host-adapted *S.* Dublin can cause disease in calves, but it is rarely implicated in zoonotic disease (Ferede, 2014; Jajere, 2019).

The predominant cause of non-typhoid *Salmonella* (NTS) infection in humans is animal-derived foods such as meat, eggs and milk. In cattle, the bacteria commonly inhabit the gastrointestinal tract of healthy animals, mainly in the terminal ileum of the intestines, and may contaminate the carcass surface during slaughter (mainly during evisceration and hide removal; Martínez-Chávez *et al.,* 2015). As a result, although *Salmonella* contamination is more frequent in poultry and pork meat, beef has also been closely associated with salmonellosis outbreaks over the years (Martínez-Chávez *et al.,* 2015). Meat handling, processing, transport, storage, distribution and preparation for consumption lead to higher levels of contamination (Ejeta *et al.,* 2004). Specifically, contamination from unsterile equipment, utensils and workers' hands, and cross-contamination between different carcasses and meat types at abattoirs and retail outlets increases the prevalence of *Salmonella* serotypes in meat products (Cabrera-Diaz *et al.,* 2013).

The high incidence of antimicrobial resistance in certain *Salmonella* serotypes has contributed to the pathogen's threat to public health. The extensive use of antimicrobial agents in animal production and clinical practices has contributed to the increasing pervasion of antimicrobial resistance in *Salmonella*, and consequently, to the emergence of multi-drug resistant (MDR) strains (Lu *et al*., 2014). Outbreaks caused by MDR-NTS have been linked to higher rates of hospitalization and treatment failures of patients, and thus higher morbidity and mortality rates over the years (Varma *et al.,* 2005; Jajere, 2019).

In South Africa, the hygiene and quality of meat, and number of units that can be slaughtered are regulated by requirements stated in the Meat Safety Act, 2000 (Act No. 40 Of 2000). Furthermore, the microbiological monitoring of food is regulated by the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972). KwaZulu-Natal province is the second most important cattle producer in South Africa, making it a major contributor to beef supply in the country (Department

of Agriculture Forestry and Fisheries (DAFF), 2017). There is limited knowledge of the prevalence, serovars, antimicrobial resistance and genetic background of *Salmonella*, which could pose a potential health threat to consumers (Mthembu *et al*., 2019).

The aim of this study was to expand the knowledge around the prevalence, serovars, antimicrobial resistance and virulence factors of *Salmonella* isolates recovered from different types of beef and beef products from selected retail outlets in KwaZulu-Natal, South Africa. The sampled beef cuts represent various degrees of processing, including raw intact beef, raw beef organs, raw processed beef and ready to eat beef products, such as biltong and cold meats. This information will provide knowledge on the pathogenicity of *Salmonella* species that are circulating along the food value chain, and that could pose a potential health threat to consumers.

1.1. Aim

The aim of this study was to expand the knowledge around the prevalence, serovars, antimicrobial resistance and virulence factors from *Salmonella* isolates recovered from different types of beef and beef products from selected retail outlets in KwaZulu-Natal, South Africa.

1.2. Objectives

The specific objectives of this study were:

- To isolate and identify *Salmonella* from beef and beef products from retail outlets in KwaZulu-Natal using classical internationally-accepted microbiological techniques and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS).
- To determine the serovars of the confirmed *Salmonella* isolates using classical serotyping techniques.
- To determine the antimicrobial resistance profiles of the isolated *Salmonella* using the Kirby Bauer disk diffusion method.
- To determine the virulence genes of the confirmed *Salmonella* isolates using molecular techniques.

1.3. Research Questions

Detailed knowledge on the extent of *Salmonella* serovars in beef in KZN, South Africa retail outlets is limited. Furthermore, the emergence of multi-drug resistant strains has made *Salmonella* infections more difficult to treat and this needs to be continually monitored. Therefore, the research questions posed by this study were as follows:

- What is the prevalence of *Salmonella* spp. in beef and beef products in KwaZulu-Natal, South Africa?
- Which serovars of *Salmonella* are found in beef and beef products in KwaZulu-Natal, South Africa?
- What is the extent of antimicrobial resistance in *Salmonella* serovars isolated from beef and beef products in KwaZulu-Natal, South Africa?
- What are the virulence genes present in *Salmonella* serovars isolated from beef and beef products in KwaZulu-Natal, South Africa?

1.4. Hypotheses

The hypotheses for this study were:

- A proportion of meat and meat products from retail outlets in selected municipalities of KwaZulu-Natal are contaminated with different *Salmonella* serotypes.
- *Salmonella* isolated from beef and beef products from selected retail outlets in KwaZulu-Natal show diverse antimicrobial resistance patterns.
- *Salmonella* isolated from beef and beef products from selected retail outlets in KwaZulu-Natal province are genetically diverse and carry different combinations of virulence genes.

CHAPTER 2

LITERATURE REVIEW

This chapter is adapted from a publication in the form of a book chapter with the following citation details:

Naidoo S., Basson A.K., Butaye P., Madoroba E. (2021) *Salmonella enterica* Subspecies *enterica* Serotypes Associated with Meat and Meat Products in African Countries: A Review. In: Babalola O.O. (eds) Food Security and Safety. Springer, Cham. https://doi.org/10.1007/978-3-030-50672-8_38

2. Literature Review

2.1. Background and Classification of *Salmonella* spp.

2.1.1. History and Characteristics of *Salmonella* spp.

Although reports of *Salmonella* symptoms have been described since the early 19th century, the bacterium was first observed in 1880 by Karl Joseph Eberth (Monte & Sellera, 2020). Georg Theodor August Gaffky confirmed Eberth's findings in the mid-1880s (Dawoud *et al.,* 2017). The following year, Theobald Smith and Dr. Daniel. E. Salmon isolated '*Salmonella choleraesuis'* (now, *S. enterica* subsp. *enterica* serovar Choleraesuis) from pigs, originally referring to it as 'hog-cholera' (Dawoud *et al.,* 2017). In 1888, *S.* Enteritidis was associated for the first time with a foodborne salmonellosis outbreak (Dawoud *et al.,* 2017). The name *Salmonella* was kept, thereby honouring the work pioneered by Dr Salmon (Ferede, 2014).

Salmonella spp. are Gram-negative non-spore forming, facultative anaerobic bacilli that range from 2 to 3 µm in length and 0.4 to 0.6 µm in width (Ferede, 2014). Optimum growth conditions for most *Salmonella* serovars include temperatures between 35 °C and 37 °C, pH between 6.5 and 7.5 and water activity (aw) between 0.94 and 0.99 (Ferede, 2014). The growth of *Salmonella* is inhibited in temperatures below 7 °C and above 70 °C (Ferede, 2014). The bacterium is also inhibited at pH 3.8 and 0.94 aw (Ferede, 2014).

Salmonella spp. can be isolated selectively, based on biochemical characteristics, as they are non-lactose fermenters that ferment glucose, are unable to hydrolyse urea, and lack cytochrome oxidase (Rahman *et al.,* 2019). Nearly all serovars, with the notable exception of *S*. Typhi, produce H_2S during sugar fermentation, which appear black in Triple Sugar Iron (TSI) agar (Rahman *et al.,* 2019). Non-H_2S producing variants of serovars that are otherwise known to produce H_2S, such as *S.* Typhimurium and *S.* Infantis have also become apparent (Sakano *et al.,* 2013). Most recorded serovars are motile, which can be observed by growth along a stab-line in a motility test (Rahman *et al.,* 2019), with some exceptions (including *S*. Pullorom and *S*. Gallinarum; Ferede, 2014). In most cases, motility is due to flagella, but can be flagella-independent such as in the case in *S.* Typhimurium variants (Park *et al.,* 2015).

2.1.2. *Salmonella* Taxonomy and Nomenclature

The genus *Salmonella* falls into the taxonomic family of Enterobacteriaceae, which is categorized into the class of Gamma Proteobacteria (Monte & Sellera, 2020). *Salmonella* is separated into two species; which are *S. enterica* and *S. bongori* (Eng *et al.,* 2015). *S. enterica* is found in both endotherms and ectotherms, whereas *S. bongori* is typically limited to ectotherms. *S. enterica* is subdivided into six subspecies, which are characterized by names and Roman numerals, including *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV) and *indica* (VI) (Cheng *et al.*, 2019). *S. bongori* was formerly classified as subspecies V before its re-classification as a separate species (Cheng *et al.*, 2019). Subspecies *enterica* (I) is the most pathogenic of the six subspecies as it accounts for approximately 99% of all *Salmonella* infections in mammals (Eng *et al.,* 2015; Jajere, 2019). Within the subspecies *enterica* (I) there are currently over 2600 recognised serovars (Jajere, 2019).

S. enterica subsp. *enterica* (I) serovars Enteritidis and Typhimurium are currently the 2 most significant *Salmonella* serovars infecting human beings internationally (WHO, 2018). Nomenclature permits these names to be abbreviated into an italicized *S.* for *Salmonella*, followed by the capitalized serovar name. The above mentioned serovars can be written as *S.* Enteritidis and *S.* Typhimurium.

2.1.3. Laboratory Diagnosis and Characterization of *Salmonella* spp.

2.1.3.1. Detection, Isolation and Identification of *Salmonella* Using Biochemical Tests

Salmonella spp. are isolated and identified using the recommended technique described in the International Organization for Standardization (ISO) 6579-1:2017 protocol. The ISO method is sensitive, but also complex and expensive (Ferede, 2014). The protocol involves pre-enriching the sample in buffered peptone water (BPW), and then selectively enriching it in two separate liquid media: Rappaport-Vassiliadis Soya (RVS) broth and Muller Kauffmann tetrathionate novobiocin (MKTTn) broth. The two inoculated media undergo an incubation period, followed by plating on two agar media plates: 1) Xylose Lysine Deoxycholate (XLD), and 2) Brilliant Green agar (BGA) or Bismuth Sulfite agar (BSA). The resulting four plates are then analysed and up to four presumptive colonies are chosen per suspected positive sample, when available. On XLD,

presumptive *Salmonella* are typically observed as bright pink colonies with a black (H_2S) centre. On BGA and BSA, presumptive *Salmonella* appear bright pink and shiny black respectively.

The mentioned ISO protocol also describes biochemical confirmation of *Salmonella*, which involves the inoculation of biochemical media with each presumptive culture, followed by incubation and analysis of the media. For a clear distinction of the biochemical reactions, well characterised positive and negative control strains are also tested. Recommended biochemical tests include the triple sugar iron (TSI) test, for which typical *Salmonella* cultures show an alkaline (red) slant and acidic (yellow) butt with gas formation (observed as bubbles), and in 90% of cases, the agar blackens (Hydrogen Sulphide (H_2S) production) (Ferede, 2014). A Urease test is also recommended, for which urea agar is used and a negative test is expected (colour remains yellow because *Salmonella* spp. do not hydrolyse urea). Additionally, L-Lysine decarboxylation medium can be inoculated. Turbidity and a purple colour are indicative of a positive result, which is seen for most typical *Salmonella* spp. Furthermore, *Salmonella* spp. yield a positive result for a catalase test (seen as production of bubbles as it breaks down hydrogen peroxide into oxygen and water) and a negative oxidase test (due to absence of cytochrome oxidase enzyme). *Salmonella* spp. can also be microscopically distinguished using Gram-staining as they appear as Gram negative bacilli (Ferede, 2014). Biochemical confirmation tests need to be supplemented by more specialised confirmation tests, and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) is an example of a rapid and cost effective method of detection (Dieckman *et al.,* 2008).

The MALDI-TOF MS protocol begins with applying individual colonies onto a MALDI sample target (Dieckman *et al.,* 2008). A matrix solution, typically an aromatic organic acid with a low molecular weight such as ∝-cyano-hydroxy cinnamic acid and 2,5-dihydroxy-benzoic acid is mixed with the sample and allowed to dry (Welker *et al.,* 2002). Following its co-crystallization, the target plate is placed into a high vacuum area of a machine and exposed to a laser beam. This is all controlled by software integrated with the instrument used (Welker *et al.,* 2002; bioMérieux Clinical Diagnostics, 2020). The absorption of photonic energy causes analyte molecules (from the sample) to separate from the matrix and to become subjected to ionization and desorption (Singhal *et al.,* 2015). This is followed by a separation process based on their mass-to-charge ratio (m/z), which is detected by sensors, and a spectrum representing the protein make-up of the sample

is created (Singhal *et al.,* 2015; Figure 2A). Databases such as the Basic Local Alignment Search Tool (BLAST) are consulted to determine final identification (Dieckmann & Malorny, 2011).

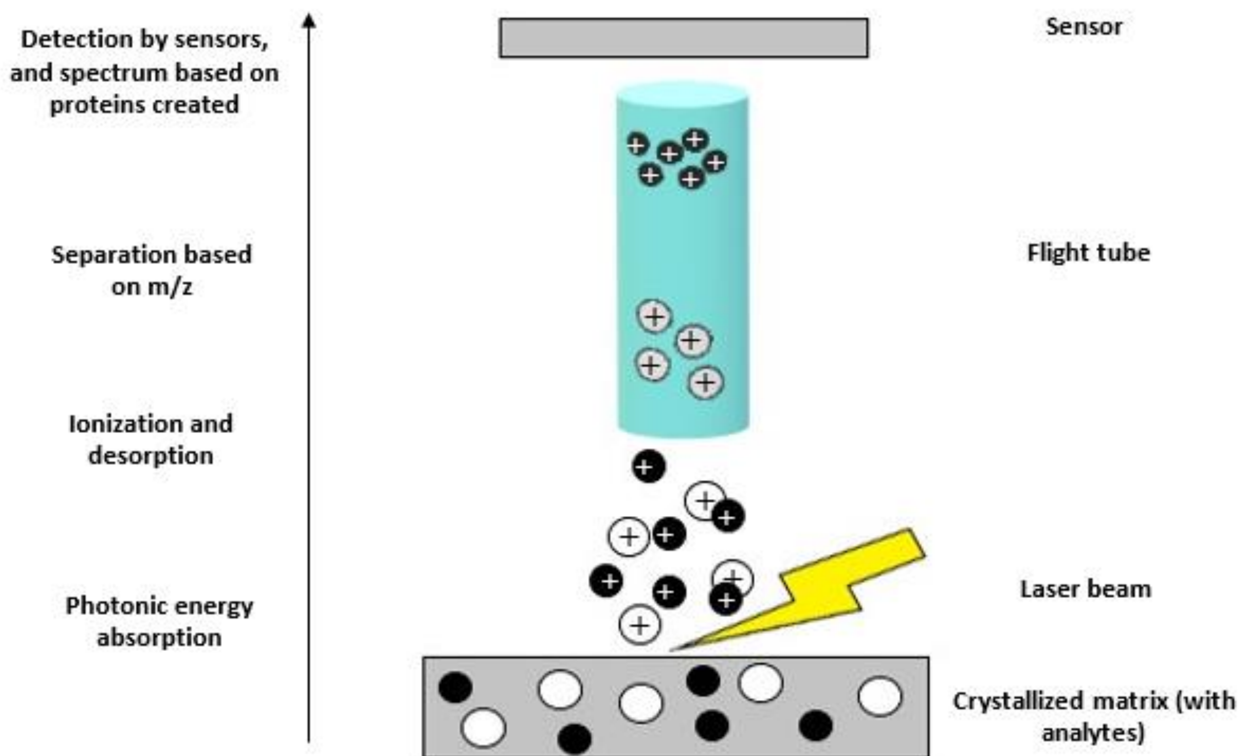


Figure 2A: Image illustrating the principle of MALDI-TOF MS.

2.1.3.2. *Salmonella* Serotyping

Serotyping is still the predominant method of monitoring *Salmonella* infections. Serotyping is typically based on classifying microorganisms from the same subspecies according to antigenic determinants. Using the White-Kauffmann-Le Minor (WKL) classification scheme, which depends specifically on somatic (O) and flagella (H) determinants, antigenic composition translates into the serovar (Wattiau *et al.,* 2011; Jajere, 2019). There are two H determinants, referred to as Phase-I and phase-II motility, that are alternately expressed in most *S. enterica* serovars, (Banerji *et al.,* 2020). The antigenic formula is thus structured as O: H1:H2, with the O antigens and two H antigens each separated by a colon (Banerji *et al.,* 2020). There are also exceptions such as with some variants of *S.* Typhimurium that are monophasic, meaning they only express one flagellin, or *S*. Enteritidis that does not contain a second flagellin (Banerji *et al.,* 2020).

This results in a formula like 1,4,[5],12:i:1,2 for *S*. Typhimurium and 1,9,12:g,m:- for *S*. Enteritidis (Banerji *et al.,* 2020). There are occurrences where different serovars share an antigenic formula, and in those cases additional testing would need to be done for accurate identification.

There have also been molecular tools to determine *Salmonella* serovars. Multiplex Polymerase Chain Reaction (PCR) allows for a more rapid and efficient form of both screening and serotyping as there is no need for obtaining a pure culture. Instead, the serovar is identified directly from the sample being tested, after only an incubation in a pre-enrichment broth (Beaubrun *et al.,* 2017). This method is based on the amplification of serovar-specific target genes, which produce a specific banding pattern for different serovars (Beaubrun *et al.,* 2017). This approach is advantageous in its ability to provide rapid results, as opposed to the conventional method that can take three or more days (Kim *et al.,* 2006). This improves the ability to screen for *Salmonella* serovars in foods/farm feeds and may lead to the prevention of infections and outbreaks in humans and animals (Beaubrun *et al.,* 2017). However, multiplex PCR is limited to being able to determine up to 30 serotypic banding patterns—focusing on the 30 most clinically relevant serovars (Kim *et al.,* 2006). Conventional serotyping has the ability to distinguish between over 2 600 serovars.

Random amplification of polymorphic DNA (RAPD) profiling, which is a variation of PCR, can also be used as an alternative for serotyping; however, not all serovars can be differentiated, thus limiting its application (Khoodoo *et al.,* 2002). Another PCR-based alternative for molecular characterization is amplified fragment length polymorphism (AFLP), which is relatively inexpensive and proven to have a high level of differentiation between *Salmonella* isolates (Wang *et al.,* 2011).

2.1.3.3 Molecular Typing of *Salmonella* Using Pulsed-Field Gel Electrophoresis

Typing beyond the serotype is necessary for epidemiological studies and surveillance purposes (Thong *et al.,* 2011). Molecular approaches such as pulsed-field gel electrophoresis (PFGE) can offer greater insight than more orthodox phenotypic methods of subtyping, such as serotyping and phage typing (Nair *et al.,* 1994). PFGE macro-restriction analysis differentiates bacterial isolates by their genomic DNA pattern, which is referred to as a DNA fingerprint (Wang *et al.,* 2015). For the PFGE protocol, bacterial cells are embedded and lysed within agarose plugs, and the resulting exposed DNA is digested using specific restriction endonucleases (Wang *et al.,* 2015). Once the agarose plugs are inserted into an agarose gel, they are exposed to an oscillating electric field,

which fractionates the DNA fragments, forming a banding pattern on the gel (Wang *et al.,* 2015). This serves to separate larger fragments (up to 10 Mb) (Kaufmann, 1998).

The banding patterns generated by PFGE represent a genetic fingerprint, which can reveal genetic differences among isolates of *Salmonella* serotypes (Thong *et al.,* 2011). Results from PFGE analysis can be used to determine the source of a particular clone and thus facilitate epidemiological studies. However, PFGE is both time and labour intensive, and the technology needed may not be widely available (Champion *et al.,* 2002).

2.1.3.4. Whole Genome Sequencing (WGS)

It is critical to public health to have methods for outbreak detection and the ability to trace the disease transmission path (Ibrahim & Morin, 2018). WGS offers a more streamlined process than other detection and serotyping methods, meaning it is more rapid and it improves discriminatory power (Chattaway *et al.,* 2019). WGS lays out the complete DNA makeup of an organism and is useful in distinguishing isolates across large geographical areas, thus improving the understanding of epidemiology of infections, and contributing to foodborne disease surveillance (Leekitcharoenphon *et al.,* 2014). In-silico data analysis tools are used to predict *Salmonella* serovars from the sequences that are laid out by WGS (Ibrahim & Morin, 2019). Additionally, WGS is pivotal in mutation detection and understanding pathogen genetics, such as with virulence genes and antimicrobial resistance genes (Ibrahim & Morin, 2018). One of the main challenges surrounding WGS is that it requires sequence information to be translated to a universal language for public health intervention (Chattaway *et al.,* 2019).

2.1.4. Clinical Manifestations and Epidemiological Classification

Salmonella spp. can be grouped according to their clinical manifestations, namely typhoid *Salmonella* and non-typhoid *Salmonella* (NTS). Typhoid *Salmonella* infections cause a disease termed enteric fever, which is a systemic disease. This may be in the form of typhoid fever, caused by *S.* Typhi or paratyphoid fever, which is a result of *S.* Paratyphi A, B and C (Eng *et al.,* 2015). Apart from potential abdominal pain and diarrhoea or constipation, enteric fever results in the onset of a specific fever pattern and can also cause rashes, muscle pain, heart difficulties and the enlargement of the liver/ spleen (Eng *et al.,* 2015). NTS infection on the other hand affects mainly

the gastrointestinal tract, causing a disease known as salmonellosis and causes more systemic diseases only in immunocompromised individuals (Eng *et al.,* 2015).

Salmonella can be classified based on host preferences. *Salmonella* spp. that are host restricted, such as *S.* Typhi make up the first group (Ferede, 2014). The second group includes serovars that are host adapted, meaning that although they are associated with one host, they can occasionally cause infection in other hosts (Ferede, 2014). Examples of host adapted serovars include *S.* Dublin in cattle and *S.* Pullorum in avian species. The third group includes the non-host specific serovars, typically associated with enteric disease and zoonosis.

2.2. Foodborne Illnesses

Foodborne illness refers to an infection caused by contaminated food and is a growing public health problem worldwide (National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), 2012). Most foodborne diseases have a rapid onset, only last a short period of time and are self-limiting. However, they can lead to more serious complications such as multi-organ failure and even cancer and mortality (WHO, 2015). The contamination of food can take place during any stage in the food production chain from growth and harvesting or slaughter, to handling, processing and transportation. Food can similarly be contaminated during preparation in restaurants and at home. Common bacteria known to cause foodborne illnesses include *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni*, *Escherichia coli* and *Listeria monocytogenes* (Zhao *et al.,* 2001).

2.3. Salmonellosis

Ingestion of non-typhoid *Salmonella* by mammals leads to an infection termed salmonellosis, which can be symptomatic or asymptomatic (Eng *et al.,* 2015; Jajere, 2019). Susceptible animals include farm animals, birds, reptiles, amphibians, poultry, pigs, horses, cats, dogs, rodents and other small mammals (CDC, 2017). The most frequent mode of transmission in animals is the faecal-oral route because unaffected and asymptomatic animals may contain as many organisms in their faeces and gastrointestinal tract as those affected (CDC, 2017). Animals may also get infected indirectly through contaminated feed and water. *Salmonella* can survive in the environment, without a host, for up to 5 years when protected by organic material (McGuirk & Peek, 2003).

Symptoms of salmonellosis in cattle can manifest as dullness and fever, weakness, drop in milk yield, abdominal pain and severe diarrhoea which may contain blood and mucus (Abbey Veterinary Group, 2021). Extreme cases can result in disease in multiple organs, such as pneumonia, arthritis and meningitis (Holschbach & Peek, 2017). In both adult cows and calves, disease manifestation is dependent upon the virulence of the serovar causing the infection, the dose of the inoculum and the degree of immunity amongst other possible stressors in the host, such as age (Holschbach & Peek, 2017). According to a retrospective study of data gathered between 2006 and 2015, some of the most common serovars isolated from cattle are *S*. Dublin, *S*. Cerro, *S*. Newport, *S*. Montevideo, *S*. Kentucky and *S*. Typhimurium (Holschbach & Peek, 2017; Valenzuela *et al.,* 2017).

S. Dublin is a host-adapted serovar of *Salmonella* and can present itself as diarrhoea, but also causes respiratory illness, most commonly in calves between 2 and 12 weeks old (Abuelo & Cullens, 2020). Infected animals can develop chronic clinical manifestations, which may lead to slower growth rates and shedding of *Salmonella* in their faeces, colostrum and milk throughout life, thus maintaining the pathogen within the herd (Holschbach & Peek, 2017; Abuelo & Cullens, 2020). The asymptomatic shedding of *Salmonella* creates an increased risk for people in contact with the animal, their faeces, milk and meat products. *S*. Typhimurium is of particular concern with regard to human infections as it has been linked to several human foodborne outbreaks in the past (Holschbach & Peek, 2017).

Humans are mainly infected through the consumption of contaminated food (food-borne infection) (Martínez-Chávez *et al.,* 2015). The major food sources are eggs, poultry, pork and beef (Eng *et al.,* 2015). Due to cross contamination, fruits, vegetables, contaminated water and any seafood from contaminated water may also be sources for human infections. Symptoms of salmonellosis in humans typically manifest 12 to 72 hours after exposure to the pathogen in the form of gastroenteritis. This includes diarrhoea, vomiting, abdominal cramps and fever which usually last 4 to 7 days. Infants, elderly and other immunocompromised individuals, such as cancer or HIV-positive patients, are at a higher risk of developing salmonellosis (Eng *et al.,* 2015).

Salmonellosis is diagnosed through laboratory testing of a stool specimen. In severe cases, oral rehydration or intravenous fluids are usually recommended, together with the antimicrobials prescribed to treat the infection. Amoxicillin, ampicillin, ceftriaxone and trimethoprim-

sulfamethoxazole are among the most common antibiotics used to treat *Salmonella* infection (Chatterjee & Steele, 2016). However, most cases do not require any treatment (WHO, 2018).

2.4. Virulence of *Salmonella* spp.

Virulence of *Salmonella* is complex and requires a very precise co-ordination of numerous gene products to act in the right place at the right time (Foley *et al.,* 2013). *In-vitro* and *in-vivo* studies of *S.* Typhimurium have provided a basic understanding of interactions between the bacterial cells and host cells (Marcus *et al.,* 2000). It has been approximated that over 200 virulence genes are required for severe *S.* Typhimurium infection in mice (Marcus *et al.,* 2000). Virulence genes are essential for *Salmonella* pathogenesis, and their corresponding virulence factors each manifest differently within the host (Kshirsagar *et al.,* 2014). Examples include the *invA* gene, which is common among all pathogenic *Salmonella* spp. and encodes virulence factors that invade gut epithelial tissue, whereas the *stn* gene is responsible for the ability to produce toxins that cause gastroenteritis (Kshirsagar *et al.,* 2014).

Encoded virulence factors and determinants comprise, but are not limited to, flagella, capsules, type 3 secretion systems (T3SSs) and adhesion systems including adhesins, invasins, fimbriae, hemagglutinins, exotoxins and endotoxins (Jajere, 2019). These factors allow *Salmonella* to colonize its host by facilitating the adhesion and invasion of enterocytes, followed by suppression of the host's immune system and the pathogen's subsequent intracellular survival in macrophages (Cheng *et al.*, 2019; Jajere, 2019).

Virulence genes are found on plasmids and/or genomic DNA, either as single units or in clusters that form gene cassettes known as *Salmonella* pathogenicity islands (SPIs) (Marcus *et al.,* 2000; Jajere, 2019). Twenty-four SPIs that have been identified, and each typically facilitates a specific virulence phenotype that is expressed at a particular stage of infection (Marcus *et al.,* 2000; Cheng *et al.*, 2019). Certain SPIs are common to many *Salmonella* serovars while others have been associated with only specific serovars, contributing to *Salmonella*'s overall fitness (Cheng *et al.*, 2019). In addition, SPIs can be horizontally transferred to other enteric bacteria, which may result in a usually non-pathogenic microorganism becoming a pathogen (Marcus *et al.,* 2000).

SPI-1 to SPI-5 are the most frequently observed of all SPIs in many different *Salmonella* serovars. SPI-1 specifically is present in all *Salmonella* species and subspecies (Cheng *et al.*, 2019; Jajere,

2019). Described concisely, *S*PI-1 is primarily responsible for invading the enteric cells; SPIs-2 and -3 mainly deal with intracellular survival and growth/replication within the host; SPI-4 also plays a role in adhesion and invasion; and SPI-5 gives rise to enteropathogenic effects within the host (Cheng *et al.*, 2019; Jajere, 2019). Similarly, the rest of the SPIs also have precise and complex roles; can be found in different combinations in different serovars; and may be specific to a *Salmonella* serovar, subspecies or even species (such as SPI-22 to *S. bongori;* Jajere, 2019). However, SPI-1 and SPI-2 remain the most well-studied and well-characterized in terms of their genotypic and phenotypic traits.

2.5. The Importance of Beef and Cattle Production in South Africa

2.5.1. Nutritional Value of Beef

Red meat is regarded as a rich source of protein of a high biological value, as well as a source of fat, essential minerals and vitamins in the body (Bender, 1992). The protein and nutrients contained in red meat, particularly beef, may have a higher bioavailability than other food sources (Wyness, 2016). For example, beef contains haeme iron, which is the most easily absorbed iron in the body (Sheehan, 2017). The protein intake also supplies the body with the amino acids needed. Red meat may also supply zinc to the body, which is required for a strong immune system, wound healing and enzyme activity within cells (Wyness, 2016). It also contributes B vitamins to the body, which have many roles in blood circulation and protein synthesis (Sheehan, 2017). However, these nutrients make beef susceptible to contamination by foodborne pathogens.

The present study analysed a large sample size of beef and beef products, which included different beef meat categories, such as raw intact, raw processed, ready-to-eat and organ meat (cattle tripe, liver, lungs, kidney, heart and spleen) samples that were not analysed in previous studies, thus taking into account the effect that different cuts and levels of processing can have on *Salmonella* contamination. Beef organ meat, also referred to as beef offal, is popular in developing countries with financially poor populations because of its significant nutritional value and inexpensive cost in comparison to other beef cuts (van Heerden & Morey, 2014). Beef organs are high in protein and may be rich sources of other nutrients: liver has a high vitamin A content and the spleen is a rich source of iron (van Heerden & Morey, 2014). Due to the current economic climate in South Africa, there is a significant domestic market for beef organs (van Heerden & Morey, 2014), especially in provinces with large rural populations, like KwaZulu-Natal.

2.5.2. Significance of Beef in South Africa

In a large number of countries, meat makes up a substantial portion of the typical human diet. While meat consumption is predominantly based on availability, price and tradition, it has a high prestige value in many communities (Bender, 1992). In South Africa specifically, cows have historical importance within the Zulu communities in KwaZulu-Natal, from linking families through marriage, symbolising a ruler's power and even as part of ancestral religion (Sithole, 2021). Cattle are therefore widely kept and consumed throughout KZN.

In 2018, South Africa was recognised as having the 16th highest consumption of beef per-capita in the world. Considering the rapid advancements in infrastructure and resulting expansion of supermarkets in peri-urban and rural areas, the beef industry is expected to grow tremendously (Gous, 2018; Kau, 2016). Furthermore, the South African beef industry is considered high calibre owing to the low fat content in the meat (a result of slaughtering young cattle) and high diversification of product types. It is the second fastest growing commodity in the agricultural sector, having had a gross value of R30.6 billion in 2015/2016 (Kau, 2016; Department of Agriculture, Forestry and Fisheries (DAFF), 2017). Beef production is expected to meet the national standards laid out in the Meat Safety Act, 2000 (Act No. 40 of 2000) and the Red Meat Regulations (No. 1072) of 17 September 2004 which are designed to ensure that carcasses are slaughtered and handled hygienically. Furthermore, the Consumer Protection Act (No. 68 of 2008) was implemented to protect consumers against health risks associated with meat products. While this may be true for the commercial sector of the beef industry, an informal (non-commercial) beef sector also exists in South Africa, where cattle are farmed in smallholder farms and sold informally, or produced for household or religious purposes (DAFF, 2017).

2.5.3. Cattle Production in South Africa

The production of food obtained from farming livestock has a major impact on the economy of South Africa. Approximately 84% of South African land is available for farming, but only 11% is suitable for crop cultivation, leaving the majority of land in the country suitable for livestock farming (Red Meat Research and Development South Africa, 2017).

In 2002, the Department of Agriculture, Forestry and Fisheries estimated a total of 13.96 million cattle in South Africa. The Eastern Cape and KwaZulu-Natal are the largest (approx. 3.18 million)

and second largest (an estimated 2.8 million) cattle producing provinces in the country respectively. The Abstract of Agricultural Statistics 2016 states that of the ~ 13.7 million cattle in South Africa in 2014/15, approximately 3.4 million were slaughtered that year. KwaZulu-Natal is therefore a major contributor of beef in the country, making it imperative that abattoirs follow regulations maintaining hygiene as laid out by the Department of Agriculture in the Meat Safety Act, 2000 (Act No. 40 Of 2000).

The purpose of the Meat Safety Act is to promote meat safety by establishing and maintaining crucial national standards in respect to abattoirs, as well as imported and exported meat. According to the aforementioned Act, every specific meat trade has its own requirements to improve the level of hygiene maintained during production of that specific meat. Abattoirs are specifically designed to allow workers to slaughter and dress carcasses hygienically. The hygiene, quality of meat and number of units that can be slaughtered are regulated by requirements stated in the Meat Safety Act, depending on the grade of the red meat abattoir. The three grades present in South Africa are rural, low-throughput and high-throughput red meat abattoirs (Department of Agriculture and Rural Development (DARD), 2016). In the context of cattle, one unit refers to one cow, ox or bull, or two calves (DARD, 2016). These values differ for other animals. Rural abattoirs allow 1–2 units and low-throughput abattoirs allow 3–20 units per day (DARD, 2016). High-throughput abattoirs allow from 21 up to the maximum throughput, which is decided by the provincial executive officer (DARD, 2016). This is selected after considering the size of the lairages, the production rate compared to equipment, and the available facilities.

In addition to organoleptic approaches to meat inspection, the Meat Safety Act also encourages the new targeted risk- and systems-based approaches of Hazard Analysis Critical Control Point (HACCP), International Standards Organization (ISO), Good Manufacturing Practices (GMP), Supplier Quality Assurance (SQA) and Quality Management System (QMS) in promoting adequate food safety management within South Africa. However, it is still the responsibility of the government of South Africa to ensure that the principles of the Act are implemented and that citizens have access to safe food. It is possible that in some South African abattoirs, particularly rural abattoirs, routine meat inspections may not be carried out (Madoroba *et al.,* 2016). This can compromise safe handling practices within the abattoir and thus the safety of the consumers because of foodborne pathogens.

Although studies have been done on the surveillance of pathogens and hygiene practices at farms and abattoirs (Madoroba *et al.,* 2016; Mthembu *et al.,* 2019; Madoroba *et al.,* 2021), there is limited literature covering contamination further on in the beef production chain in South Africa, and particularly in KwaZulu-Natal. There is a possibility that cross-contamination can take place between raw beef products and other meats at butcheries and retail outlets. Furthermore, foodborne-pathogens can be introduced to raw beef during handling, processing and packaging. Studies have documented the frequency of *Salmonella* spp. on carcasses and the escalated levels of contamination after further processing of the meat (Martínez-Chávez *et al.* 2015, Maradiaga *et al.*, 2015).

It has been observed that the contamination of meat with *Salmonella* increases with increasing levels of processing, suggesting that further contamination takes place during the processing of meat (Martínez-Chávez *et al.* 2015). This may include unsanitary handling, grinding and packaging of the meat. Other studies showed that when good hygiene is maintained during processing, the prevalence of *Salmonella* spp. in live cattle is higher than in the meat (Maradiaga *et al.*, 2015). High hygiene standards, together with the HACCP system are part of the European Union (EU) export requirements, allowing countries access to the EU beef market (Shilangale *et al.,* 2015).

Animals, including cattle, may also be slaughtered by subsistence farmers or in homes for peoples' own consumption and religious purposes. In these cases, meat is considered non-commercial as it is not being sold for monetary purposes. Neither meat nor other animal products are allowed to be sold unless slaughtered at an abattoir. The hygiene and safety regulations highlighted by the Meat Safety Act (Act No. 40 of 2000) are therefore not enforced in these situations.

2.6. Review of *Salmonella* spp. Isolated from Meat and Meat Products in African Countries Compared to Other Continents (Published with citation Naidoo *et al.,* 2021)

Research on *Salmonella* prevalence in Africa is not always as detailed as in other countries and few African countries provide *Salmonella* surveillance data in general (Magwedere *et al.,* 2015). *Salmonella* serotyping has not been routinely done, as this is cumbersome and costly, however, these studies are important as they are indicative of overall prevalence and epidemiology. Out of the 54 African countries, relevant prevalence data from only 15 countries were found (Figure 2B). Unlike European countries (The European Food Safety Authority (EFSA) & The European Centre

for Disease Prevention and Control (ECDC), 2018), there is no system in place in Africa to provide a thorough and uniform overview for all African countries.

Figure 2B: Map outlining countries in Africa where *Salmonella* prevalence studies have been performed in meat/meat products

The countries from which *Salmonella* prevalence data was extracted include Tunisia, Tanzania, South Africa, Senegal, Rwanda, Nigeria, Morocco, Madagascar, Libya, Ethiopia, Egypt, Democratic Republic of Congo, Burkina Faso, Botswana and Algeria.

Another problem that exists with studies from African countries is the absence of a standardised method for sampling and isolation. The sample size determination formula according to European Food Safety Authority for harmonising surveillance results of foodborne pathogens is not frequently used in African studies (Madoroba *et al.* 2016). Sampling is, in general, not

epidemiologically substantiated, and is more a convenience sample. Frequently the studies also only cover a part of the country, so the data cannot be regarded as representing overall country prevalence (Kagambega *et al.*, 2011; Mwanyika *et al.,* 2016).

Methods for isolation of *Salmonella* from sampled meat may differ between the studies, though several use various International Organization for Standardization (ISO) protocols, which allow international comparison. Compared to the European surveillance, surveillance in African countries is not performed by official accredited laboratories, but are rather individual researchers' projects (Ferede, 2014). This leads to a very biased overview of the burden caused by *Salmonella* contamination of meat and meat products from Africa. However, it is the only data available, hence it does have value as it gives some idea of the overall serotype prevalence characteristics.

2.6.1. Prevalence of Non-Typhoid *Salmonella* in Various Meat and Meat Products in Africa

In Tables 2.1 to 2.6, there is an overview of surveillances done in Africa. The tables focus on different aspects: the type of meat for all countries in the continent (Table 2.1), and the differences between countries and years for the different types of meat (Tables 2.2 to 2.6). From Table 2.1, the differences between different meats tested in Africa can be seen. It should also be noted that quite some studies do not specify the type of meat, which complicates the analysis of the situation for different meat products. There is also a large difference in samples tested, and as such the precision of the prevalence is not always that good. However, Table 2.1 provides an overview of which animal meats serve as greater reservoirs for *Salmonella* spp. and are more likely to cause infection if proper hygiene standards are not maintained. Tables 2.2 to 2.6 summarise the reported prevalence rates of *Salmonella* in: unprocessed raw/uncooked red meat, unprocessed uncooked poultry meat, uncooked organ meats, processed uncooked meats and meat products that are ready to be eaten, in different African countries and at different time points.

Table 2.1: *Salmonella* spp. prevalence from selected African countries categorized according to type of meat

Raw Meat Type	Total Samples	*Salmonella* +	Prevalence (%)	Reference
Goat	471	135	28.66	Ferede 2014; Musa *et al*. 2017; Mwanyika *et al*. 2016; Niyonzima *et al*. 2018; Smith *et al*. 2016
Turkey	106	30	28.30	Adeyanju *et al*. 2014; Ed-dra *et al*. 2017
Mixture (Merguez)	46	11	23.91	Abbassi-Ghozzi *et al*. 2012; Ed-dra *et al*. 2017
Dog	10	2	20.00	Anejo-Okopi *et al*. 2016
Chicken	3059	598	19.55	Abbassi-Ghozzi *et al*. 2012; Abd-Elghany *et al*. 2015; Abdi *et al*. 2017; Adeyanju *et al*. 2014; Ahmed and Shimamoto 2014; Dione *et al*. 2009; Kagambega *et al*. 2011; Khallaf *et al*. 2014; Ibrahim *et al*. 2014; Moawad *et al*. 2017; Molla *et al*. 2002; Niyonzima *et al*. 2018; Ogu and Akinnibosun 2019; Rabie *et al*. 2012; Samaxa *et al*. 2012; Smith *et al*. 2016; Tarabees *et al*. 2017
Camel	625	104	16.64	Molla *et al*. 2002; Musa *et al*. 2017
Pork	183	29	15.85	Anejo-Okopi *et al*. 2016; Ejeta *et al*. 2004; Niyonzima *et al*. 2018; Samaxa *et al*. 2012; Smith *et al*. 2016
Mutton/ Lamb	249	38	15.26	Abbassi-Ghozzi *et al*. 2012; Ejeta *et al*. 2004; Kagambega *et al*. 2011; Musa *et al*. 2017; Samaxa *et al*. 2012
Beef	5797	778	13.42	Abbassi-Ghozzi *et al*. 2012;

				Ahmed and Shimamoto 2014; Alemu and Zewde, 2012; Anejo-Okopi *et al*. 2016; Cohen *et al*. 2008; Ed-dra *et al*. 2017; Ejeta *et al*. 2004; Gashe and Mpuchane, 2000; Hamad and Saleh 2019; Hiko *et al*. 2015; Ibrahim *et al*. 2014; Kagambega *et al*. 2011; Madoroba *et al*. 2016; Moawad *et al*. 2017; Molla *et al*. 2002; Musa *et al*. 2017; Niyonzima *et al*. 2018; Samaxa *et al*. 2012; Smith *et al*. 2016; Stevens *et al*. 2006; Tafida *et al*. 2013; Wabeto *et al*. 2017
Game	182	11	6.040	A-Mpalang *et al*. 2013
Unspecified Red Meat	348	91	26.15	Anejo-Okopi *et al*. 2016; Azage and Kibret 2017; Ejo *et al*. 2016; Mezali *et al*. 2012
Unspecified Meat	808	110	13.61	Abbassi-Ghozzi *et al*. 2012; Ejo *et al*. 2016; Hamad and Saleh 2019; Mezali *et al*. 2012; Mrema *et al*. 2006; Niyonzima *et al*. 2017
Unspecified Poultry Meat	228	30	13.16	Mezali *et al*. 2012; Missohou *et al*. 2011
Total	12136	1966	16.20	

Salmonella prevalence data from 15 different African countries were found, with a total of 12 136 samples tested between 1997 and 2019. Altogether, 1 966 samples were observed to be *Salmonella* positive, which translates to a prevalence of approximately 16.20% in meat and meat products in African countries. The data in Table 2.1 include a combination of cooked (Table 2.6), uncooked (Table 2.2 to 2.5), unprocessed (Table 2.2 to 2.4) and processed meats (Table 2.5), as it was nearly impossible to divide them as then the numbers would be very small. Cooked meats are, of course, less contaminated due to that treatment and were thus analysed separately. Overall, goat meat and turkey meat samples showed the highest *Salmonella* prevalence, with a little over 28% positive samples. This means that more than one in four samples is positive, making these meats very likely to cause infections. Also, among processed mixed meats the contamination degree is substantial, though it is clear here that there is potential for a substantial cross contamination effect.

Table 2.2: *Salmonella* spp. prevalence in diverse unprocessed red meat types

Meat type	Year	Country	Total samples	*Salmonella* +	Prevalence (%)	Reference
Goat	2013	Nigeria	24	4	16.67	Smith *et al*. 2016
	2013-2014	Ethiopia	249	44	17.67	Ferede 2014
	2014	Nigeria	30	7	23.33	Musa *et al*. 2017
	2015	Tanzania	120	74	61.67	Mwanyika *et al*. 2016
	2017	Rwanda	48	6	12.50	Niyonzima *et al*. 2018
Total			471	135	28.66	
Camel	1997-2002	Ethiopia	238	48	20.17	Molla *et al*. 2002
	2014	Nigeria	30	6	20.00	Musa *et al*. 2017
Total			268	54	20.15	
Beef	1997-1999	Botswana	52	3	5.77	Gashe and Mpuchane, 2000
	1997-2002	Ethiopia	1116	63	5.65	Molla *et al*. 2002
	2004	Senegal	435	275	63.22	Stevens *et al*. 2006
	2006-2007	Ethiopia	186	13	6.99	Alemu and Zewde, 2012
	2006-2008	Tunisia	144	43	29.86	Abbassi-Ghozzi *et al*. 2012
	2008-2009	Burkina Faso	45	0	0.00	Kagambega *et al*. 2011
	2009-2010	Nigeria	370	9	2.43	Tafida *et al*. 2013
	2010	Egypt	480	39	8.13	Ahmed and Shimamoto 2014
	2013	Nigeria	81	18	22.22	Smith *et al*. 2016
	2014	Egypt	90	8	8.89	Ibrahim *et al*. 2014
	2014	Nigeria	30	8	26.67	Musa *et al*. 2017
	2015	South Africa	100	30	30.00	Madoroba *et al*. 2016
	2015-2016	Ethiopia	448	56	12.50	Wabeto *et al*. 2017
	2017	Egypt	90	8	8.89	Moawad *et al*. 2017
	2017	Rwanda	150	35	23.33	Niyonzima *et al*. 2018
Total			3817	608	15.93	

Lamb/ Mutton	2002-2003	Ethiopia	85	12	14.12	Ejeta *et al*. 2004
	2006-2008	Tunisia	33	2	6.06	Abbassi-Ghozzi *et al*. 2012
	2008-2009	Burkina Faso	30	2	6.67	Kagambega *et al*. 2011
	2014	Nigeria	30	11	36.67	Musa *et al*. 2017
Total			178	27	15.17	
Pork	2002-2003	Ethiopia	55	9	16.36	Ejeta *et al*. 2004
	2013	Nigeria	16	1	6.25	Smith *et al*. 2016
	2017	Rwanda	24	4	16.67	Niyonzima *et al*. 2018
Total			95	14	14.74	
Unspecified	2007-2008	Algeria	144	34	23.61	Mezali *et al*. 2012
	2013	Nigeria	124	30	24.19	Anejo-Okopi *et al*. 2016
	2014-2015	Ethiopia	50	6	12.00	Ejo *et al*. 2016
	2015	Ethiopia	30	21	70.00	Azage and Kibret 2017
Total			348	91	26.15	
Overall Total			5177	929	17.94	

Table 2.3: *Salmonella* spp. prevalence among different raw unprocessed poultry types

Meat type	Year	Country	Total Samples	*Salmonella* +	Prevalence (%)	Reference
Turkey	2011	Nigeria	46	16	34.78	Adeyanju *et al*. 2014
Chicken	1997-2002	Ethiopia	452	54	11.95	Molla *et al*. 2002
	2006-2008	Tunisia	60	29	48.33	Abbassi-Ghozzi *et al*. 2012
	2008-2009	Burkina Faso	30	11	36.67	Kagambega *et al*. 2011
	2009	Senegal	285	115	40.35	Dione *et al*. 2009
	2010	Egypt	320	14	4.38	Ahmed and Shimamoto 2014
	2011	Nigeria	106	29	27.36	Adeyanju *et al*. 2014
	2011-2012	Morocco	300	38	12.66	Khallaf *et al*. 2014
	2012	Egypt	50	2	4.00	Rabie *et al*. 2012
	2012	Egypt	100	22	22.00	Abd-Elghany *et al*. 2015
	2013	Nigeria	30	10	33.33	Smith *et al*. 2016
	2014	Egypt	90	7	7.78	Ibrahim *et al*. 2014
	2014-2015	Ethiopia	244	36	14.75	Abdi *et al*. 2017
	2015	Egypt	100	5	5.00	Tarabees *et al*. 2017
	2017	Egypt	90	7	7.78	Moawad *et al*. 2017
	2017	Rwanda	36	8	22.22	Niyonzima *et al*. 2018
	2017-2018	Nigeria	240	40	16.67	Ogu and Akinnibosun 2019
Total			2533	427	16.86	
Unspecified	2007-2008	Senegal	100	7	7.00	Missohou *et al*. 2011
	2007-2008	Algeria	128	23	17.97	Mezali *et al*. 2012
Total			228	30	13.16	
Overall Total			2808	473	16.84	

Table 2.4: *Salmonella* spp. prevalence among diverse organ meat types

Meat type	Year	Country	Total Samples	*Salmonella* +	Prevalence (%)	Reference
Chicken	1997-2002	Ethiopia	312	99	31.73	Molla *et al*. 2002
	2012	Egypt	100	46	46.00	Abd-Elghany *et al*. 2015
Total			412	145	35.19	
Camel	1997-2002	Ethiopia	357	50	14.00	Molla *et al*. 2002
Beef	1997-1999	Botswana	144	14	9.72	Gashe and Mpuchane, 2000
	1997-2002	Ethiopia	370	9	2.43	Molla *et al*. 2002
	2008-2009	Burkina Faso	45	6	13.33	Kagambega *et al*. 2011
Total			559	29	5.19	
Overall Total			1328	224	16.87	

Table 2.5: *Salmonella* spp. prevalence among different processed meat types

Meat type	Year	Country	Total Samples	*Salmonella* +	Prevalence (%)	Reference
Chicken	2008-2009	Botswana	72	20	27.78	Samaxa *et al*. 2012
Merguez	2006-2008	Tunisia	10	0	0.00	Abbassi-Ghozzi *et al*. 2012
	2014-2015	Morocco	36	11	30.56	Ed-dra *et al*. 2017
Total			46	11	23.91	
Turkey	2014-2015	Morocco	60	14	23.33	Ed-dra *et al*. 2017
Pork	2008-2009	Botswana	78	14	17.95	Samaxa *et al*. 2012
Mutton	2008-2009	Botswana	71	11	15.49	Samaxa *et al*. 2012
Beef	1997-1999	Botswana	158	18	11.39	Gashe and Mpuchane, 2000
	1997-2002	Ethiopia	380	46	12.11	Molla *et al*. 2002
	2002-2003	Ethiopia	160	23	14.38	Ejeta *et al*. 2004
	2002-2004	Morocco	250	7	2.80	Cohen *et al*. 2008
	2008-2009	Botswana	79	20	21.67	Samaxa *et al*. 2012
	2014-2015	Morocco	60	9	15.00	Ed-dra *et al*. 2017
	2018	Libya	50	2	4.00	Hamad and Saleh 2019
Total			1137	125	10.99	
Unspecified	2002-2003	Botswana	300	59	19.67	Mrema *et al*. 2006
	2006-2008	Tunisia	56	6	10.71	Abbassi-Ghozzi *et al*. 2012
	2007-2008	Algeria	42	4	9.52	Mezali *et al*. 2012
	2018	Libya	50	3	6.00	Hamad and Saleh 2019
Total			448	72	16.07	
Overall Total			1912	267	13.96	

Table 2.6: *Salmonella* spp. prevalence among diverse ready-to-eat meat types

Meat type	Year	Country	Total Samples	*Salmonella* +	Prevalence (%)	Reference
Dog	2013	Nigeria	10	2	20.00	Anejo-Okopi *et al.* 2016
Chicken	2009	Senegal	42	6	14.29	Dione *et al.* 2009
Pork	2013	Nigeria	10	1	10.00	Anejo-Okopi *et al.* 2016
Game	2009-2010	Democratic Republic of Congo	182	11	6.04	A-Mpalang *et al.* 2013
Beef	2009-2010	Nigeria	65	1	1.54	Tafida *et al.* 2013
	2011-2012	Ethiopia	119	1	0.84	Hiko *et al.* 2015
	2013	Nigeria	100	14	14.00	Anejo-Okopi *et al.* 2016
Total			284	16	5.63	
Unspecified	2014-2015	Ethiopia	60	3	5.00	Ejo *et al.* 2016
	2016	Rwanda	300	35	11.67	Niyonzima *et al.* 2017
Total			360	38	10.56	
Overall Total			888	74	8.33	

Few dogs are consumed in Africa, so obviously the number of samples is low (Table 2.6). Nevertheless, one in five samples tested positive for *Salmonella*. There is limited information on *Salmonella* prevalence in live dogs and the data available are mainly on clinical disease in dogs (Hetsa *et al*. 2013). Raw chicken meat is, in general, considered as having the highest degree of contamination. In developed countries, where chickens are generally slaughtered in large slaughterhouses, the contamination degree is high due to cross contamination during the slaughtering process (Carraturo *et al*. 2016), which is difficult to reduce. In the USA, post-slaughter decontamination with chlorine is practiced to reduce the burden, while in Europe it is reduced mainly through prevention and management at the pre-slaughter process. Vaccination of chickens in combination with elimination of positive breeding stock is used as prevention, while slaughtering negative flocks early in the day and positive flocks later in the day is used as a management measure to avoid cross-contamination between flocks. This, however, necessitates good and fast diagnosis, which costs a lot of money, and is not feasible in resource-poor countries (The Poultry Site, 2011).

Camel meat is typically consumed in North African countries and some parts of Asia. There are thus no comparable data from other continents. There is limited knowledge about *Salmonella* occurrence among live camels, although these bacteria have been recovered from their faecal samples (Molla *et al.* 2004; Ghoneim *et al*. 2017). Only two of the studies I found dealt with camel meat from North African countries, where this meat is consumed. They showed the same prevalence of *Salmonella* in these products. It should be noted however that the studies were around 15 years apart and from quite distant countries, indicating this may be a general situation. Meat production from camels was not common and only recently increased, and the camel slaughtering process is not well documented (Guya and Tolesa 2016). It thus remains unclear what the prevalence and serovars present in these animals are. Nevertheless, over 16% of the meat samples were found positive, similar to pork, mutton and lamb. Beef showed the lowest contamination degree and the highest number of samples tested.

In South Africa, between 2012 and 2014, various samples such as livestock, poultry, abattoir meat, animal food and environmental samples (including coups, slaughterhouses, animal feed and water) tested positive for non-typhoid *Salmonella* (Magwedere *et al.,* 2015). These data were not included in Table 2.1 to 2.6 as prevalence of NTS was not provided according to each source and the tables exclude environmental samples. Over the 3-year period, 180 293 samples were tested and 9 031 (5.0%) contained *Salmonella* spp. A total of 188 serovars were detected with the most common one being *S*. Enteritidis, which constituted 1 944 (21.5%) of the isolates,

and this serotype was found mostly in poultry on farms, poultry meat and poultry houses. This degree of contamination in meat is similar to other parts of the world (EFSA & ECDC, 2018; Laufer *et al.,* 2015).

Game meat, which comprises buffalo, warthog and common duiker showed the lowest contamination degree. The higher contamination degree of livestock is probably due to the fact that livestock is reared in restricted areas, creating a higher density of animals and thus increasing the possibility of transmission of *Salmonella* due to higher contact possibilities. Moreover, game animals are killed individually, reducing the possibility of cross contamination. In the EU, food monitoring data is reported annually. Monitoring results are based on sampling at slaughterhouses, processing plants and retail outlets throughout the different member states and game meat is not evaluated in this way. In 2017, data from 28 member states were evaluated and *Salmonella* prevalence was generally low with the highest being under 5% in broilers, followed by turkey and other poultry, pig and the lowest in bovine (EFSA and ECDC 2018).

Looking at the different types of meat, which are in Table 2.2 to 2.6, they are subdivided into raw unprocessed red meat types, raw unprocessed poultry types, raw organ meat, raw processed meat types and ready-to-eat meat products. This makes it easier to compare contamination levels from different sources, and potentially the effects of processing and cooking different meat types. Red meat types were clustered (beef, mutton, goat, pork and camel) because then unspecified meat types could be included in the Table. The overall prevalence was almost 18%, though with large differences as indicated above. In this review, the earliest data on the prevalence were from Botswana between 1997 and 1999 with a prevalence of almost 6%, but only a little over 50 samples were tested (Gashe & Mpuchane 2000). Most studies were from a later date, mainly after 2008.

Salmonella prevalence in goat meat has been assessed since 2013 and was highest of all (Ferede 2014). While goat is consumed all over Africa, there are data from only four countries—mainly from north and mid-Africa. A very high prevalence was found in Tanzania, (Mwanyika *et al*. 2016). This could be due to the increased goat meat production from 323 000 tons to 449 673 tons during the period 2009–2010, which possibly led to limited veterinary services and thus limited health examinations, vaccinations and prescription of medications.

Diseases left untreated increase the risk of transmission. Other contributing factors may have included the over-crowding of animals, leading to animal-animal contamination and finally

poorer hygiene practices due to capacity limitations for larger productions and cost efficiency (Mwanyika *et al*. 2016). There is no indication of temporal differences in prevalence, though only two studies from the same country were available to assess this (Smith *et al*. 2016; Musa *et al*. 2017). Moreover, one study assessed very few samples (Musa *et al*. 2017). Looking more in detail, there was no indication of differences between meats from large urban and small rural slaughterhouse facilities.

Many studies have been performed on beef over a period of 20 years and in various different countries. This has led to large variations in prevalence, with the highest prevalence of more than 60% in Senegal in 2004, and the lowest in Burkina Faso in 2008–2009 (Stevens *et al*. 2006; Kagambega *et al*. 2011). The factors that contributed to these huge disparities are not clear. It may be related to the prevalence in cattle and to the slaughter practices. In Ethiopia, three studies have been performed between 1997 and 2016, with the highest prevalence in 2015-2016, which may indicate that the situation is not improving (Molla *et al*. 2002; Alemu and Zewde, 2012; Wabeto *et al*. 2017). In Nigeria, three studies were performed, though over a shorter time frame. The highest prevalence was also noted in the last study, which may be indicative of deterioration (Tafida *et al*. 2013; Smith *et al*. 2016; Musa *et al*. 2017). The three studies from Egypt showed little variation over a short period (Ahmed and Shimamoto 2014; Ibrahim *et al*. 2014; Moawad *et al*. 2017). Limited studies have been performed on lamb and mutton, and the *Salmonella* prevalence ranges from approximately 6% to over 36%. The lowest prevalence was in a North African country and the highest in mid-Africa (Abbassi-Ghozzi *et al*. 2012; Musa *et al.* 2017).

Salmonella prevalence from pork was typically not determined in the North African countries probably due to religion that does not allow the consumption of pork. Data was obtained from only three countries: namely Ethiopia, Nigeria and Rwanda, and as such little can be said about the data (Ejeta *et al*. 2004; Smith *et al*. 2016; Niyonzima *et al*. 2018) although huge variation was observed. While it is difficult to assess unspecified red meats, it is clear that in Ethiopia there was a challenge, as in this specific study, though on only 30 samples, 70% were positive for *Salmonella* (Azage and Kibret 2017). Other studies in the same country on specified meats showed prevalence rates of between 10% and 20%, which is probably more accurate. Looking at the only study from Algeria, the prevalence is at the higher end for North Africa, with only one Tunisian study on beef, between 2006 and 2008, reporting higher prevalence rates (Abbassi-Ghozzi *et al*. 2012; Mezali *et al*. 2012).

There was only one country found in which unprocessed turkey samples were analysed and the prevalence was high (Adeyanju *et al*. 2014). Studies involving chicken meat were more numerous and were conducted between 1997 and 2018. Highest prevalence rates were seen between 2006 and 2008 in Tunisia (48.33%), 2008–2009 in Burkina Faso (36.6%), and in 2009 in Senegal (40.35%) (Abbassi-Ghozzi *et al*. 2012; Dione *et al*. 2009; Kagambega *et al*. 2011). The increased prevalence in Senegal was explained by the ban on imported chicken meat due to the emergence of the avian flu in sub-Saharan Africa (Dione *et al*. 2009). Nevertheless, there were no strict systematic hygiene measures in Senegal, which may also explain the high prevalence (Dione *et al*. 2009). The high levels in Burkina Faso was probably due to meat-sellers not storing meat at sufficiently low temperatures without protection from flies and lack of hand, tool and surface hygiene (Kagambega *et al.* 2011). As for beef, contamination of meat in Tunisia was amongst the highest reported in Africa, which may indicate generally poor hygiene of the slaughter and handling process of meat and meat products. Looking for possible evolutions, there are several countries from which data from different years have been reported. Two studies from Ethiopia, with a time interval of about 17 years, do not show real differences (Molla *et al*. 2002; Abdi *et al*. 2017). Substantial research has been done in Egypt though only spanning a period of 7 years (Abd-Elghany *et al*. 2015; Ahmed and Shimamoto 2014; Ibrahim *et al*. 2014; Moawad *et al*. 2017; Rabie *et al*. 2012; Tarabees *et al*. 2017). The prevalence rates of *Salmonella* in Egypt were amongst the lowest found in Africa and there is no obvious evolution. Albeit perhaps too presumptive, it looks like the situation in Nigeria showed some improvement (Adeyanju *et al*. 2014; Ogu and Akinnibosun 2019; Smith *et al*. 2016).

Prevalence varied between different types of organ meat. It appeared that organ meat from abdominal organs was more prone to contamination as abdominal organs are often removed from the carcass together with the intestinal package (and content) where *Salmonella* resides. There may also be a large difference between organ meats of different animal species as the smaller organs, like those from poultry, are more prone to getting contaminated. This is reflected in Table 2.4 where *Salmonella* contamination was higher in chicken organs than in organs from other animals. It was also much higher than chicken meat. However, there are only two studies performed on chicken organs and as such the data are not very precise (Molla *et al*. 2002; Abd-Elghany *et al*. 2015).

Regarding the other organ meat, little can be concluded as the number of studies are small, however, for camel and beef organs, a relatively high number of samples were collected in Ethiopia. The reasons for the relatively high contamination of camel organ meat compared to

beef meat is unclear as there is a dearth of knowledge on the prevalence of *Salmonella* in camels. Some provided detailed analysis of the organs that were investigated (Molla *et al*. 2002; Abd-Elghany *et al*. 2015). In the study performed in Egypt, chicken gizzards, in particular, had a high *Salmonella* prevalence of 60% (30/50), almost double that observed in liver (32%:16/50) tissue. In Ethiopia, *Salmonella* prevalence was also highest in gizzard samples with a prevalence of 41.4%, followed by liver (29.7%) and heart (21.2%) samples.

Meat may get contaminated during processing and that is why data was analysed regarding processed and ready-to-eat meat separately as they may differ from the raw unprocessed products. This has been shown in other developing countries and in developed countries (Cabrera-Diaz *et al*. 2013; Carraturo *et al*. 2016; Martínez-Chávez *et al*. 2015). Table 2.5 provides data on processed raw meat (minced meat, sausages etc.) according to meat type and Table 6 provides a summary of findings from ready-to eat meats.

On average, processed chicken had the highest prevalence, followed by turkey meat. This is not surprising as *Salmonella* spp. have been more frequently isolated from poultry meat than any other meat in many studies (Carraturo *et al*. 2016; Akbar and Anal 2013; Yang *et al*. 2010). An investigation undertaken in China between 2007 and 2008 showed high *Salmonella* prevalence in meat, and found over 50% of chicken samples contained *Salmonella* contamination (Yang *et al*. 2010). This was over 20% more than in pork and over 30% more than in lamb and beef, which had the lowest level of contamination. Much lower prevalence rates were reported from Italy, a developed country, but highest contamination was still observed for chicken, followed by turkey, pork, and the lowest in beef (Carraturo *et al*. 2016). The same study in Italy found that over 50% of contaminated meat samples were processed meat samples of mince and sausage.

Merguez sausages had a prevalence of 23.91%, possibly because they contain a mixture of different meat types, including lamb and mutton meat, which had amongst the highest prevalence of *Salmonella*. Also, the extra handling of mixed meats from different sources (different slaughterhouses, with a possible suboptimal temperature control) increased the contamination degree. However, one study in Tunisia found 0% contamination but this is likely because only 10 samples were tested. Like for unprocessed meat, beef had one of the lowest contamination rates.

In ready-to-eat meats, contamination should be as low as possible because there is no further treatment as in the case of unprocessed meats, which are generally cooked at high temperatures

for long times. Dog meat had the highest level of contamination, however this is not a commonly consumed meat in Africa (Anejo-Okopi *et al*. 2016). The dog meat was probably slaughtered and cooked informally, where no hygiene practices were maintained. Data interpretation may be challenging as the treatment processes can be quite different according to the meat types, but it is not surprising that game and beef had relatively low prevalence.

Contamination may be explained by undercooking (which is not common practice in Africa), or post cooking contamination. The latter can be caused by people who are carriers of *Salmonella* or by post cooking addition of contaminated products or use of contaminated cutlery/utensils. Contamination on these products thus represent a direct risk. This may highlight the need for continual surveillance studies to be carried out and possibly regular training regarding hygiene practices during meat production and meal preparations. It would be important for African countries to collaborate on surveillance of foodborne pathogens.

2.6.2. Prevalence and Characteristics of the Most Frequent *Salmonella* Serovars in Meat and Meat Products in African countries

S. enterica subsp. *enterica* (I) contributes to 99% of *Salmonella* infections in mammals (Eng *et al*. 2015). There is however a large difference in the serotypes isolated from the different meats. Table 2.7 shows the most frequently isolated serovars associated with meat and meat products from African countries. The meat types associated with two of the most frequently isolated *Salmonella* serovars (*S.* Typhimurium and *S.* Enteritidis) are shown in Table 2.8.

Table 2.7: Most common serovars isolated according to year and country

Year	Country	Number of isolates isolated in each study												
		Typhimuruim	Enteritidis	Anatum	Braenderup	Bredeney	Dublin	Saintpaul	Kentucky	Muenster	Infantis	Muenchen	Other	Total
1997-2002	Ethiopia	28	8	53	78	-	71	62	-	-	5	12	95	412
2002-2003	Ethiopia	-	-	4	13	-	1	1	-	-	16	-	9	44
2002-2003	Botswana	6	1	7	2	-	-	-	-	-	1	-	42	59
2004	Senegal	-	-	-	-	71	-	-	10	21	-	-	77	179
2006-2007	Ethiopia	6	-	-	-	-	-	-	-	-	5	-	17	28
2006-2008	Tunisia	25	3	-	-	-	-	-	14	-	-	-	38	80
2007-2008	Algeria	5	5	9	-	-	-	-	-	3	1	-	41	64
2008-2009	Burkina Faso	-	-	-	-	1	-	-	-	-	-	-	18	19
2008-2009	Botswana	1	1	6	-	-	-	2	-	-	-	20	35	65
2009-2010	Nigeria	-	-	-	-	-	-	-	2	-	-	-	8	10
2010	Egypt	22	17	-	-	-	-	-	-	-	11	-	3	53
2011-2012	Morocco	4	5	-	-	-	-	2	9	5	-	-	13	33
2012	Egypt	5	7	-	-	-	-	-	-	-	-	-	0	12
2012	Egypt	50	62	8	-	-	-	-	18	14	-	-	14	166
2012	Madagascar	4	-	-	-	-	-	-	-	-	-	-	5	9
2013	Nigeria	-	-	5	-	-	-	-	-	-	-	-	28	33
2014	Egypt	9	2	-	-	-	-	-	1	-	-	-	3	15
2014-2015	Morocco	2	-	1	-	-	-	1	6	1	-	-	23	34
2015	South Africa	0	8	2	-	-	-	-	-	-	-	-	14	24
2015	Egypt	3	2	-	-	-	-	-	-	-	-	-	0	5
2017	Egypt	9	2	-	-	-	-	-	1	-	-	-	3	15
2018	Egypt	-	2	-	-	-	-	-	-	-	-	-	0	2

2018	Libya	2	2	-	-	-	-	-	-	-	-	-	1	5
Total isolates		181	127	95	93	72	72	68	61	44	39	32	487	1371
Proportion (%)		13.20	9.26	6.93	6,78	5.25	5.25	4.96	4.45	3.21	2.84	2.33	35.52	100

(References: Molla *et al*. 2002; Ejeta *et al*. 2004; Mrema *et al*. 2006; Stevens *et al*. 2006; Kagambèga *et al*. 2011; Abbassi-Ghozzi *et al*. 2012; Alemu and Zewde 2012; Mezali *et al*. 2012; Rabie *et al*. 2012; Samaxa *et al*. 2012; Tafida *et al*. 2013; Ahmed and Shimamoto 2014; Ibrahim *et al*. 2014; Khallaf *et al*. 2014; Abd-Elghany *et al*. 2015; Cardinale *et al*. 2015; Madoroba *et al*. 2016; Smith *et al*. 2016; Ed-dra *et al*. 2017; Moawad *et al*. 2017; Tarabees *et al*. 2017 Morshdy *et al*. 2018; Hamad and Saleh 2019)

The most isolated serovar in meat and related products in African countries was *S.* Typhimurium*,* which was found in 189 samples and in 8 countries studied. This represents 13.20% (189/1 371) of all serotyped isolates. *S.* Enteritidis was the second highest occurring serovar, making up 9.26% (127/1 371) of the isolates and was also found in 8 countries. According to WHO (2018), *S.* Typhimurium and *S.* Enteritidis constitute the 2 most significant serovars of *Salmonella* infecting human beings internationally. They are also the most prevalent serovars recovered from meat and related products internationally (Foley *et al*. 2008; Yang *et al*. 2010; Rodpai *et al*. 2013). *S.* Enteritidis is linked mostly to eggs and poultry, whereas *S.* Typhimurium is found mainly in poultry, pork and beef (Thung *et al*. 2016).

Chicken had the highest frequency of both serovars, accounting for over 50% of all *S.* Typhimurium and over 66% of all *S.* Enteritidis isolates (Table 2.8). This is because *S.* Enteritidis is frequently found in living poultry and poultry-rearing environments (Magwedere *et al*. 2015). The colonisation of laying hens' oviducts and ovaries is a major cause of vertical transmission of *S.* Enteritidis and egg contamination (Butaye *et al.,* 2006). In Europe, there are several licensed live attenuated and killed vaccines implemented to control *S.* Enteriditis colonisation (Kilroy *et al.,* 2016; Theuß *et al.,* 2018) and this has led to a major reduction in human infections with this serovar (Kilroy *et al.,* 2016; EFSA & ECDC, 2018).

Table 2.8: Sources of *S*. Typhimurium and *S*. Enteritidis isolates

Meat type	Serotype			
	Typhimurium		**Enteritidis**	
	Isolates	Proportion (%)	Isolates	Proportion (%)
Chicken	95	50.26	84	66.14
Beef	62	32.80	28	22.05
Unspecified Poultry	7	3.70	7	5.51
Unspecified Red Meat	5	2.65	1	0.79
Pork	4	2.12	0	0
Mutton	0	0	2	1.57
Turkey	2	1.06	0	0
Camel	1	0.53	1	0.79
Personnel/ Patients	3	1.59	2	1.57
Unspecified	10	5.29	2	1.57
Total	189	100	127	100

(References: Molla *et al*. 2002; Ejeta *et al*. 2004; Mrema *et al*. 2006; Stevens *et al*. 2006; Kagambèga *et al*. 2011; Abbassi-Ghozzi *et al*. 2012; Alemu and Zewde 2012; Mezali *et al*. 2012; Rabie *et al*. 2012; Samaxa *et al*. 2012; Tafida *et al*. 2013; Ahmed and Shimamoto 2014; Ibrahim *et al*. 2014; Khallaf *et al*. 2014; Abd-Elghany *et al*. 2015; Cardinale *et al*. 2015; Madoroba *et al*. 2016; Smith *et al*. 2016; Ed-dra *et al*. 2017; Moawad *et al*. 2017; Tarabees *et al*. 2017; Morshdy *et al*. 2018; Hamad and Saleh 2019)

A large proportion of both serovars was isolated from beef; however, *S.* Typhimurium was over 10% more prevalent in beef as compared to *S.* Enteritidis. *S.* Typhimurium is associated mostly with cattle and there was a succession of specific phage types that were related to epidemics in cattle in European countries over the years (Butaye *et al*. 2006). *S.* Anatum and *S.* Braenderup were also found in high prevalence. In certain cases, serovars maintain their dominance over a long period of time; however, others may emerge and increase or decrease in prevalence frequently (Molla *et al*. 2002). This is in part due to specific combinations of pathogenicity islands and phages that may lead to an increase in fitness, and thus the ability of specific clones to spread more successfully (Butaye *et al*. 2006).

S. Bredeney had a high prevalence in Senegal, where the study found that although it was frequent at a beef slaughterhouse, it was rarely observed at retail outlets (Stevens *et al*. 2006). Only one slaughterhouse was sourced in this study and may have specifically had an outbreak of this strain—potentially a specific clone with increased fitness. *S.* Dublin and *S.* Saintpaul were isolated at a high frequency in Ethiopia (Molla *et al*. 2002). Ethiopia has the largest livestock population in Africa, which may be a probable cause for the frequent isolation of *S.* Dublin, a serotype common in cattle (UNDP, 2017).

Altogether, 87 *Salmonella enterica* subsp. *enterica* serovars were isolated from African countries. *S.* Muenster, *S.* Kentucky, *S.* Infantis and *S.* Muenchen were also amongst the most frequently observed, and 35.52% of all isolates were made up of other serovars.

2.7. Antimicrobial Resistance

2.7.1. Mechanisms of Antimicrobial Resistance

Antimicrobial Resistance (AMR) is considered a major threat to public health because of it results in high levels of morbidity and mortality, as well as increased hospitalization and treatment costs (Lin *et al.*, 2015). In addition to the possibility of bacteria expressing natural resistance, most pathogenic microorganisms have the ability to acquire resistance toward antimicrobials (Reygaert, 2018). Acquired resistance is usually gained by horizontal gene transfer (such as with transformation, transposition and conjugation) or by its own mutations. Natural resistance can occur as a result of AMR genes naturally occurring within the bacterium (known as induced resistance) or can be intrinsic, meaning it is always expressed by that species (Reygaert, 2018). The mechanisms of antimicrobial resistance are complex but can be condensed into four main strategies.

The first strategy involves inhibition of drug uptake. A common example would be bacteria that lack a cell wall, e.g. *Mycoplasma* spp., which are resistant to antimicrobials that target the cell wall. Modification of the drug's target is another mode of antimicrobial resistance. Penicillin-binding proteins (PBPs), which play a crucial role in peptidoglycan synthesis for the cell wall, is one example of a potential drug-target, commonly for β-lactam antibiotics. Modification of PBPs can make it more difficult for the drug to bind or completely inhibit binding. Thirdly, antimicrobial resistance can involve inactivation of the drugs. Some pathogens have the potential to produce enzymes like β-lactamase that can degrade certain antimicrobials. Another example is the act of transferring a chemical group of the drug by acetylation, phosphorylation or adenylation. The fourth strategy refers to activation of drug influx**.** This is the transport/export of the drug, which is mediated by transport proteins, to eliminate the drug from within the cell (Reygaert, 2018).

2.7.2. Review on Antimicrobial Resistance in *Salmonella* spp. from Meat and Meat Products in African Countries in Comparison to Other Continents (Published with citation Naidoo *et al.,* 2021)

Few studies provided data on both serovar and antimicrobial resistance characteristics of *Salmonella* isolates (Mezali *et al.,* 2012; Samaxa *et al.,* 2012; Alemu and Zewde, 2012; Abbassi-Ghozzi *et al.,* 2012; Ibrahim *et al.,* 2014; Abd-Elghany *et al.,* 2015; Ed-dra *et al.,* 2017; Tarabees *et al.,* 2017; Moawad *et al.,* 2017; Morshdy *et al.,* 2018). Additionally, each study tested isolates against only a limited number of antimicrobial agents. Only one study included a high number of strains, which made it challenging to analyse antimicrobial resistance in *Salmonella* recovered from meats in African countries. This makes it difficult to assess the resistance of *Salmonella* serotypes towards diverse antimicrobial agents from the different meat samples in African countries. However, Table 2.9 provides an overview of the data available on the 2 most prevalent serotypes, which are *S.* Enteritidis and *S.* Typhimurium.

Table 2.9: Resistence of *S.* Enteritidis and *S.* Typhimurium isolates toward different antimicrobial classes

	Antibiotic Class	***S.* Typhimurium**			***S.* Enteritidis**		
		Tested	*Resistant*	*Resistance Percentage (%)*	*Tested*	*Resistant*	*Resistance Percentage (%)*
β-lactams	Penicillins						
	Ampicillin	93	74	79.57	75	60	80
	Amoxicillin-Clavulanic acid	27	9	33.33	9	2	22.22
	3rd Gen Cephalosporins						
	Cefotaxime	18	14	77.78	6	5	83.33
	Ceftriaxone	20	11	55	4	2	50
	Ceftazidime	26	8	30.77	6	2	33.33
	Cefpodoxime	18	12	66.67	4	2	50
	Chloramphenicol	92	47	51.09	77	44	57.14
	Aminoglycosides						
	Streptomycin	92	60	65.22	77	58	75.32
	Neomycin	61	53	86.89	66	52	78.79
	Kanamycin	52	14	26.92	62	4	6.45
	Gentamicin	65	3	4.62	67	2	2.99
	Amikacin	9	1	11.11	3	1	33.33
	Tobramycin	1	1	100	1	1	100
	Quinolones						
	Nalidixic Acid	83	58	69.88	73	64	87.67
	Fluoroquinolones			**36.67***			**24.11***
	Norfloxacin	56	30	53.57	62	20	32.26
	Ciprofloxacin	68	20	29.41	68	12	17.65
	Ofloxacin	3	3	100	2	2	100
	Enrofloxacin	18	2	11.11	4	0	0
	Pefloxacin	5	0	0	5	0	0
	Tetracyclines			**68.42***			**88.61***
	Tetracycline	42	12	28.57	13	6	46.15
	Oxytetracycline	50	50	100	62	60	96.77
	Doxycycline	3	3	100	4	4	100
	Sulphonamides			**87.69***			**86.96***
	Sulphonamides	15	7	46.67	7	4	57.14
	Sulphamethoxazole	50	50	100	62	56	90.32
	Trimethoprim-suplhamethoxazole (cotrimoxazole)	25	6	24	12	1	8.33
	Nitrofurans						
	Furans	5	0	0	5	1	20
	Nitrofurantoin	1	0	0	1	1	100
	Furazolidone	8	1	12.5	2	0	0

*Full cross-resistance

(References: Abbassi-Ghozzi *et al*. 2012; Alemu and Zewde,. 2012; Mezali *et al*. 2012; Samaxa *et al*. 2012; Ibrahim *et al*. 2014; Abd-Elghany *et al*. 2015; Ed-dra *et al*. 2017; Moawad *et al*. 2017; Tarabees *et al*. 2017; Morshdy *et al*. 2018)

Table 2.9 gives a summary of the results from different investigators. It should be noted that results were omitted from antibiotics like erythromycin, penicillin etc. as they are not active on *Salmonella* and should thus not have been tested. Also striking was the high number of intermediately resistant strains, indicating methodological problems. It should be noted that these strains were regarded as resistant by the authors and as such may have caused an overestimation of the prevalence of resistance.

In total, the susceptibility of 93 *S.* Typhimurium isolates and 78 *S.* Enteritidis isolates were assessed, which is hardly representative. An additional issue is that standardized methods were not used for all the tests, and in some cases, outdated guidelines were followed. This is exemplified in some cases by the fact that the bacteria resistance percentages for antimicrobials with full cross resistance was not the same and deviated sometimes substantially. Therefore, the data must be interpreted as an indication of a possibly higher or lower resistance percentage. Still, some very odd things are seen, like the extremely high resistance percentages of *S.* Enteriditis, something that is not reported anywhere else in the world (EFSA & ECDC, 2018; The National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS), 2015). In Europe, the highest prevalence of antimicrobial resistance is found in *S.* Typhimurium (EFSA & ECDC, 2018).

One should treat Table 2.9's information with caution as the 100% resistance against cefuroxime, tobramycin, ofloxacin, oxytetracycline, doxycycline and sulphamethoxazole is merely because there were very few strains tested. High levels of resistance were seen against most of the β-lactams. More than 80% of isolates of both serotypes were resistant to the broad spectrum penicillins. Yet in the USA, this is only about 10% for all strains isolated (NARMS, 2015). Also, resistance against amoxicillin-clavulanic acid, indicative of the presence of certain AmpC and ESBL-enzymes, is alarmingly high; however, it should be noted that this was tested on few strains and interpretation of the results is not always easy. Also resistance against 3rd generation cephalosporins was very high. These data are hard to interpret, but the fact that some percentages are higher than what is seen for penicillins indicates that the data may not always have been reliable. Nevertheless, most of this data comes from Egypt, a country with alarmingly high levels of ESBLs in Enterobacteriaceae of different origins (Moawad *et al.,* 2018), especially the carbapenemases in Enterobacteriaceae from animal origin (Abdullah *et al.,* 2015). Also here, the presence of ESBLs in *S.* Enteriditis is surprisingly high, because in the EU, this is below 1% (EFSA & ECDC, 2018).

Tetracyclines and sulphonamides had amongst the highest resistance levels. Chloramphenicol is an antibiotic that is not used anymore in Europe and the US, however, it is still available in some African countries (The Food and Agriculture Organization (FAO), 2002). Nevertheless, in countries not using this antibiotic resistance can be substantial. Assessing aminoglycoside resistance is not easy due to the diversity of genes involved. Classically, resistance is highest against streptomycin and neomycin, as was the case here also. Amikacin and Tobramycin, two antimicrobials not used in veterinary medicine, had very few isolates tested against it, and resistance percentages are not relevant. Resistance against the quinolone, nalidixic acid, was generally higher than against the fluoroquinolones, which is probably so because they used old or CLSI breakpoints (the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints used in Europe are leading to nearly full cross resistance). Nevertheless, resistance percentages here were also very high for both serotypes and not always consistent.

2.7.3. The Extent of Antimicrobial Resistance among NT*S* Recovered from Human Beings in African Countries

In 2015, the World Health Organisation (WHO) estimated that 600 million (approximately 1 in 10) people contract foodborne illnesses every year worldwide. Third world countries, particularly those in Africa and South East Asia, had the highest incidence of reported foodborne illnesses. It was reported that 420 000 people die annually as a result, mostly from diarrhoeal diseases, and about 125 000 of these deaths were associated with children under 5 years old. Salmonellosis is amongst the most common foodborne illnesses, annually responsible for about 80 million cases and 30 000 deaths worldwide (Keddy, 2016). In most cases where immunocompetent people contract non-typhoid Salmonellosis, there is no need for treatment since it is self-limiting (Feasey *et al.,* 2012). Most clinical isolates of NTS cause gastroenteritis, which may need only support therapy, and not antimicrobial therapy (Feasey *et al.,* 2012).

NTS that are multi-drug resistant have been linked to increased morbidity and mortality rates in children (Kariuki *et al.,* 2006). In Kenya, *Salmonella* was recovered from blood samples of 8.6% of children with febrile illness aged 7 and under. More than 50% and 30% were *S.* Typhimurium and *S.* Enteritidis respectively, and 34.2% of all isolates were multi-drug resistant (Kariuki *et al.,* 2006). Another study between 2013 and 2014 in Ethiopia collected samples from patients of all ages and found *Salmonella* in 6.17% and 40.3% of those isolates

were MDR (Eguale *et al.,* 2015). It has been shown that invasive non-typhoid infections are more prevalent in Africa because of the high prevalence of HIV and infants and children with malaria, anaemia and malnutrition (Uche *et al.,* 2017). The higher level of mortality in Africa and other resource-poor environments are thus probably caused more by poor conditions that people have to live in, rather than the infection itself. The high level of infection is probably also caused by the lack of infrastructure and means to control the infection at the primary level (the animal level) and by the handling of the meat (hygiene but also lack of refrigeration).

Concluding Remarks

Salmonella infections are more frequent and more severe in developing than developed countries, which is in part due to poor hygiene standards during animal slaughtering and food production. The South African beef industry is expected to expand and beef organs are among the most popular beef cut in financially poor populations because of its significant nutritional value and inexpensive cost in comparison to other beef cuts. Beef organ meats are more prone to contamination because they are usually removed together with the intestinal package, a natural reservoir for *Salmonella* spp. and other pathogens, during the slaughter process. Previous South African studies did not place emphasis on the effect that different beef cuts and levels of processing can have on the prevalence of *Salmonella* contamination. Furthermore, the prevalence of *Salmonella* in beef organs has not previously been determined in KZN, South Africa. In parallel with other continents, the serovars most isolated in meat and related products in African countries are *S.* Typhimurium and *S.* Enteritidis. Antimicrobial resistance, a result of the extensive use of antimicrobials in animal production, veterinary and clinical treatments, has worsened the threat of salmonellosis. This has resulted in increasing morbidity and mortality rates over the years, and needs to be continually monitored. Virulence genes play an important role in *Salmonella* pathogenesis and need to be well understood and surveyed for epidemiological purposes.

CHAPTER 3

MATERIALS AND METHODS

This chapter has been submitted for publication in the form of a manuscript with the following citation details:

Naidoo, S., Butaye, P., Maliehe, S., Magwedere, K., Basson, A. & Madoroba, E. Virulence Factors and Antimicrobial Resistance among *Salmonella* species from Beef and Beef Products from Selected Retail Outlets in KwaZulu-Natal Province, South Africa. Submitted to: Applied Sciences, MDPI.

3. Materials and Methods

3.1. Ethical Approval

Ethical approval for this study was obtained from the University of Zululand with approval reference number UZREC171110-030 PGM 2019/109.

3.2. Study Area and Design

A cross-sectional study was undertaken within four local municipalities in King Cetshwayo district and three local municipalities within iLembe district in KwaZulu-Natal (KZN) province, South Africa (Figure 3A). A total of 400 samples were obtained from 25 facilities (retail stores and butcheries) of different sizes across King Cetshwayo and Ilembe between October 2019 and December 2020. The samples included raw intact beef cuts (n = 53), raw beef organ meats such as tripe, liver, lung and spleen (n = 169) (Appendix – Figure 7A), raw processed beef products (n = 110) and ready-to-eat beef products (n = 68). All sample sources were coded in order to maintain strict confidentiality. The samples were packed on ice in insulated boxes, followed by transportation to the Department of Biochemistry and Microbiology at University of Zululand. The schematic flow diagram in Figure 3B outlines the study design, including the sample collection and methods used.

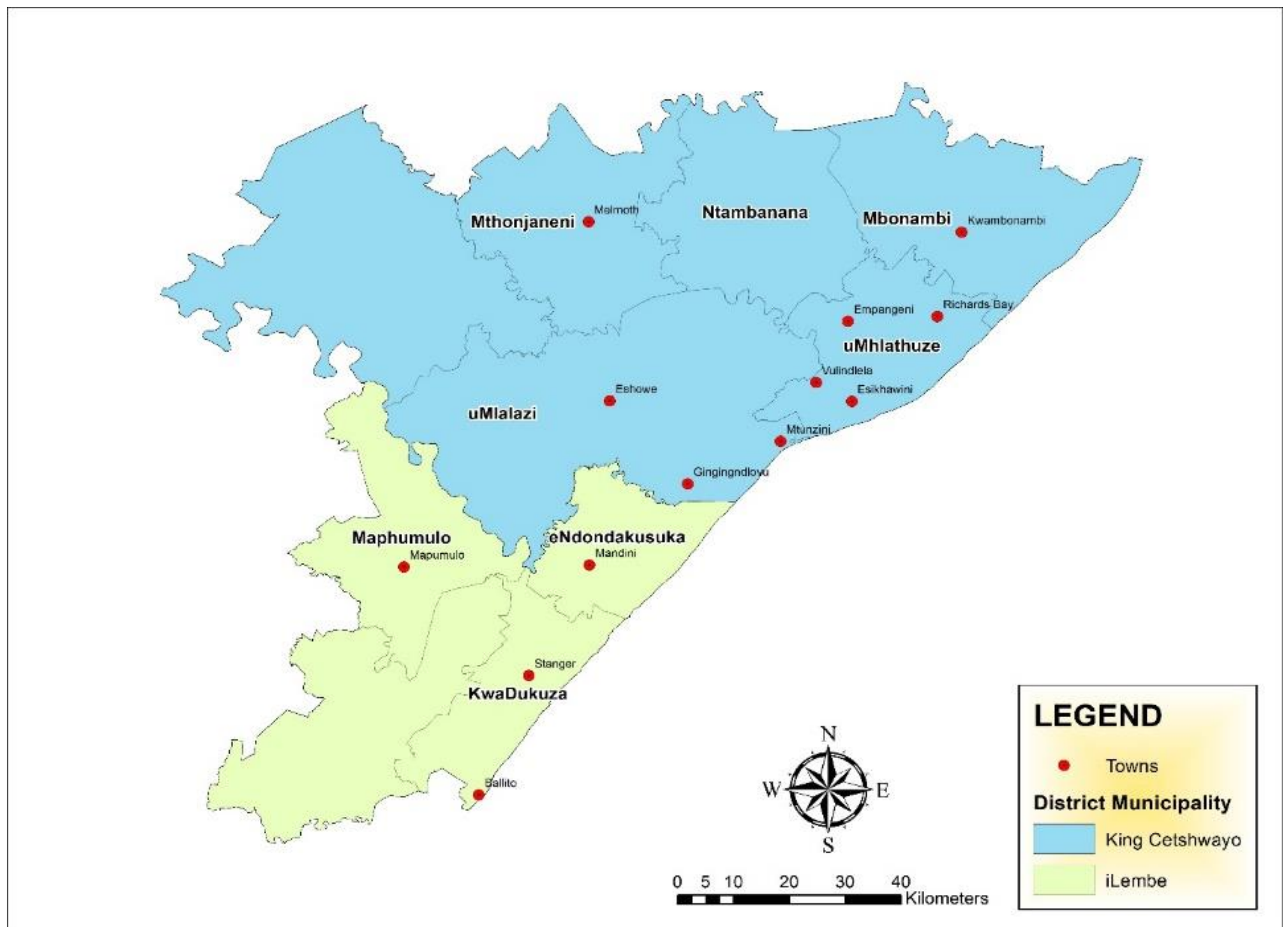


Figure 3A: Map of King Cetshwayo and iLembe districts in KZN province, South Africa, showing the towns where samples were obtained

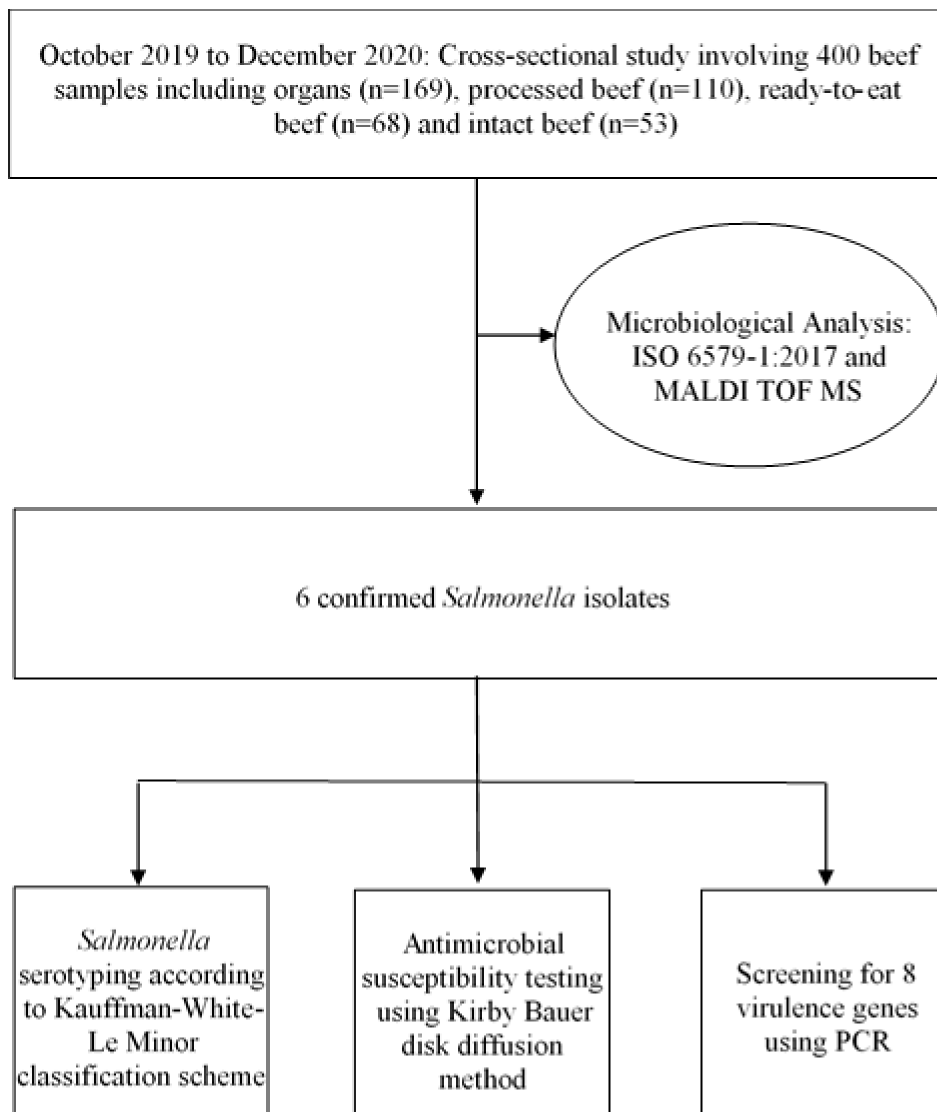


Figure 3B: Flow diagram outlining the study design

3.3. Sample Size Determination

The sample size was determined using an equation for cross-sectional studies (Charan & Biswas, 2013; Käsbohrer *et al*. 2010):

$$n = \frac{Z_{\left(1-\frac{\propto}{2}\right)}{}^{2} p(1-p)}{d^2}$$

Where n = sample size, $Z_{\left(1-\frac{\propto}{2}\right)}$= standard normal variate (at 5% type 1 error, yielding 1.96);

p = the expected proportion in the population based on previous studies and;

d = absolute error.

There was no official record for the expected proportion of *Salmonella* in South African beef samples from retail outlets, hence an estimated prevalence of 50% was used. The sample size was calculated with an absolute error of 5% to allow for a slight variation in samples.

$$n = \frac{1.96^2 \text{ x } 0.5(1 - 0.5)}{0.05^2}$$

$$n = 384.16 \approx 384$$

Using these values, the estimated minimum sample size needed was calculated to be 384. A sample size of 400 was used in this study.

3.4. Reference strains

To ensure validity of results in this study, the following reference strains were included for all experiments: *S.* Typhimurium ATCC® 13076™, *E. coli* ATCC 25922 and field strains that are positive or negative for the required analysis.

3.5 Microbiological Analysis

3.5.1. Isolation and identification of *Salmonella*

Detection, isolation and preliminary identification of *Salmonella* spp. was based on the International Organization for Standardization (ISO) 6579-1:2017 (Microbiology of the food chain—Horizontal method for the detection, enumeration and serotyping of *Salmonella*).

3.5.1.1. Pre-enrichment and Isolation

For pre-enrichment, each sample was weighed to obtain a mass of 25 g and homogenised in 225 ml buffered peptone water (Appendix – Figure 7B), followed by incubation for 18 h ± 2 h at 37 °C ± 2°C. For selective enrichment, 1 ml and 0.1 ml of pre-enriched broths were inoculated into 10 ml of Muller Kauffmann tetrathionate novobiocin (MKTTn) broth and of Rappaport-Vassiliadis Soya (RVS) broths, respectively (Appendix – Figure 7C). The inoculated MKTTn and RVS broths were incubated for 24 h ± 2 h at 37 °C ± 2 °C and 41.5 °C ±2 °C, respectively. The broths were streaked onto Brilliant Green Agar (BGA) and Xylose Lysine Deoxycholate (XLD) agar, followed by incubation at 37 °C ± 2 °C for 24 h ± 2h. Presumptive *Salmonella* colonies on XLD plates were identified by a bright pink colour with/ without a black centre. Presumptive *Salmonella* colonies appeared bright pink on BGA plates. When available, up to 4 isolated colonies were picked per suspected positive sample. Pure cultures were streaked onto nutrient agar before further steps (Appendix – Figure 7D).

3.5.1.2. Biochemical Confirmation Tests

Triple sugar iron (TSI) and urea agar tests were conducted in accordance with the ISO 6579-1:2017 method. A pure suspended culture of each presumptive isolate was streaked onto the surface of urea agar and TSI agar slant, and stabbed to the butt of the TSI agar. The agars were incubated at 37 °C for 24 h ± 3 h. Typical *Salmonella* was observed with a negative (yellow) urea test result (positive result is pink and therefore not *Salmonella* spp.); and the TSI slant as red with a yellow butt, bubble formation and blackening of the agar (from H_2S production). Isolates that did not produce H_2S were also considered as possible non-H_2S producing *Salmonella* spp.

Presumptive isolates also underwent pre-emptive screening using the Gram stain method described by Tripathi & Sapra (2021), oxidase (American Society for Microbiology, 2010) and catalase tests (American Society for Microbiology, 2010). The Gram stain protocol began with the preparation of a slide smear whereby a drop of each suspended pure culture was placed on a microscope slide, spread using a sterile inoculation loop, air-dried and gently heat-fixed over a flame. Crystal violet was poured over the fixed cultures, kept for 1 minute and rinsed off with sterile water. Grams Iodine was then added for 1 minute to fix the dye and rinsed off with sterile water again. Next, 95% alcohol was added as a solvent treatment for 20 seconds and washed off with sterile water. Lastly, the smear was counter-stained with safranin for a minute and rinsed with sterile water. After allowing time to air dry and blotting out excess water, the slide could be microscopically examined.

The filter paper test method was employed for the oxidase test. Filter paper cards were soaked with 1% Kovác's reagent and allowed to dry. A single isolated colony from each fresh pure *Salmonella* culture was spread onto a filter paper with a sterile inoculation loop. The filter paper cards were then observed for colour change. Positive tests are observed when the colour changes to a dark purple in 10 seconds (or it can be delayed by up to 90 seconds). If colour change is not observed, the isolate is oxidase negative, as is the case with *Salmonella* spp.

The catalase test was performed using the slide (drop) method. Microscope slides were placed into sterile petri dishes and a sterile inoculation loop was used to transfer a fresh pure isolated colony onto it. A sterile Pasteur pipette was then used to drop a single drop of 3% hydrogen peroxide (H_2O_2) onto the microorganism on the slide. The mixture could then be observed for an immediate bubbling effect, which is indicative of a positive result. *Salmonella* spp. yield a positive catalase test result. The absence of bubbles is indicative of a negative test result.

All presumptive *Salmonella* were preserved and stored in sterile Eppendorf tubes containing sterile nutrient broth, supplemented with glycerol, to a final concentration of 30% until required for further analysis.

3.5.1.3. *Salmonella* Identification using MALDI-TOF MS and VITEK MS

Presumptive *Salmonella* isolates were identified using MALDI-TOF MS technology (Dieckman *et al.,* 2008) and VITEK technology (bioMérieux Clinical Diagnostics, 2020).

3.5.2. Serotyping

Six *Salmonella* isolates that were confirmed using MALDI-TOF MS and VITEK technology were sub-cultured and serotyped according to the Kauffman-White-Le Minor classification scheme (Grimont & Weill, 2007) as previously described by Madoroba *et al.,* 2016. Briefly, *Salmonella* colonies were suspended in sterile physiological saline and evaluated for auto-agglutination, prior to serotyping using 'O' and 'H' antisera.

For O-typing, loopfuls of a saline solution were placed on glass slides, and pure cultures of each isolate were individually added to each drop, using an inoculation loop (Huq *et al.,* 2012). Polyvalent O-antisera were added and mixed, and observed after a minute (Huq *et al.,* 2012). If the mixture remained homogenous, it was considered negative. If clumping was observed, the test was positive and further typing was done using monovalent antisera to get specific results.

For H-typing, pure bacterial cultures were taken from the edge of inoculated swarm agar, followed by overnight incubation at 37 °C ± 2 °C. The culture was suspended in sterile saline and mixed with H-antisera, to identify for both H-phase 1 and H-phase 2, in the same manner as O-typing.

3.6. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility patterns of each *Salmonella* isolate were determined using the Kirby Bauer disk diffusion method (American Society for Microbiology, 2016). Briefly, the optical density (OD) of bacterial suspensions at exponential growth stage were determined at 625 nm using a spectrophotometer (Merck Spectroquant Pharo, Darmstadt, Germany) and adjusted to 0.08–0.13. The suspensions were compared to 0.5 McFarland turbidity standard. The standardized *Salmonella* suspensions were inoculated onto Mueller Hinton (MH) agar using sterile cotton swabs. The plates were left to stand at room temperature for 5 minutes to

allow them to dry prior to the insertion of antimicrobial disks. The ten antimicrobial disks (Table 3.1; amoxicillin, ampicillin, cefoxitin, cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, tetracycline and trimethoprim sulfamethoxazole) were placed onto the MH plates, followed by incubation according to the time and temperature recommended by the CLSI guidelines for each antimicrobial (CLSI, 2017). The inhibition zones were measured using callipers and results were recorded as isolates being either susceptible, intermediate or resistant to each drug, based on the Clinical Laboratory Standard guidelines (CLSI, 2017).

Table 3.1 Disk content of antimicrobials

Antimicrobial agent	**Abbreviation**	**Disk content (µg)**
Amoxicillin	A	25
Ampicillin	AP	10
Cefoxitin	FOX	30
Cefotaxime	CTX	30
Chloramphenicol	C	30
Ciprofloxacin	CIP	5
Gentamicin	GM	10
Kanamycin	K	30
Tetracycline	TET	30
Trimethoprim sulfamethoxazole	TS	25

3.7. Molecular Characterisation of Virulence Genes

The *Salmonella* isolates were subjected to Polymerase Chain Reaction (PCR) to screen for the presence of 8 virulence genes (Inqaba Biotech, Pretoria, South Africa). Virulence genes *inv*A, *hil*A and *siv*H play a role in the invasion of epithelial cells (Siddiky *et al*. 2021). Genes *agf*A, *ipf*A and *sef*A are responsible for the fimbriae of *Salmonella* spp. which are necessary for adhesion (Siddiky *et al*. 2021). The *sop*E gene codes for an effector protein, and *spv*C is plasmid-mediated (Siddiky *et al*. 2021).

3.7.1. DNA Extraction

The Quick-DNA™ Miniprep Kit (California, USA) was used for extraction of DNA from the confirmed *Salmonella* isolates in accordance with the manufacturer's instructions. Briefly, each isolate that was initially suspended in 400 µl of RNase-free water and frozen, was thawed at room temperature, after which 1.6 ml of genomic lysis buffer with added beta-mercaptoethanol (0.5%) was added to each suspension and vortexed for 6 seconds to achieve a 4:1 dilution. After 10 minutes, the mixtures were each added to a Zymo-Spin™ Column in a collection tube and centrifuged for 1 minute, after which the collection tubes with the flow through were discarded. All centrifugation steps were performed at 13 000 rpm. The Zymo-Spin™ Columns were each added to a new collection tube, followed by addition of 200 µl of DNA pre-wash buffer to the spin columns and centrifugation for 1 minute. This was followed by the addition of 500 µl of g-DNA wash buffer to the spin columns and another 1-minute centrifugation. The spin columns were then inserted into clean microcentrifuge tubes and 60 µl of DNA elution buffer was added to each, incubated for 5 minutes at room temperature and then centrifuged for 30 seconds. The eluted DNA in the microcentrifuge tubes were then stored at -80 °C till PCR was performed.

3.7.2. PCR Analysis

The *Salmonella* isolates were subjected to Polymerase Chain Reaction (PCR) to screen for the presence of the following 8 virulence genes: invasion A (*inv*A), aggregative fimbriae A (*agf*A), long polar fimbrial A (*lpf*A), hyper invasion locus A (*hil*A), intimin-like inverse autotransporter protein H (*siv*H), *Salmonella* Enteritidis Fimbrial A (*sef*A), *Salmonella* outer protein E (*sop*E), and *Salmonella* plasmid virulence C (*spv*C) as described by Siddiky *et al*. 2021. Forward and reverse primer sequences for each gene are shown in Table 3.2.

Table 3.2: Primers used to screen for each of eight virulence genes in *Salmonella* spp. using PCR

Target Gene	Sequence	Amplicon size (bp)
***inv*A**	F-GTGAAATTATCGCCACGTTCGGGCAA R-TCATCGCACCGTCAAAGGAACC	284
***agf*A**	F-TCCACAATGGGGCGGCGGCG R-CCTGACGCACCATTACGCTG	350
***Ipf*A**	F-CTTTCGCTGCTGAATCTGGT R-CAGTGTTAACAGAAACCAGT	250
***hil*A**	F-CTGCCGCAGTGTTAAGGATA R-CTGTCGCCTTAATCGCATGT	497
***siv*H**	F-GTATGCGAACAAGCGTAACAC R-CAGAATGCGAATCCTTCGCAC	763
***sef*A**	F-GATACTGCTGAACGTAGAAGG R-GCGTAAATCAGCATCTGCAGTAGC	488
***sop*E**	F-GGATGCCTTCTGATGTTGACTGG R-ACACACTTTCACCGAGGAAGCG	398
***spv*C**	F-CCCAAACCCATACTTACTCTG R-CGGAAATACCATCTACAAATA	669

The 20 µl PCR master mix consisted of 10 µl NEB OneTaq 2X MasterMix with Standard Buffer (Catalogue No. M0482S), 1 µl Genomic DNA (10–30 ng/µl), 7 µl Nuclease free water (Catalogue No. E476) and 1 µl of each primer (10 µM forward and 10 µM reverse). The mixtures were subjected to amplification in a thermocycler machine and underwent the following conditions: initial denaturation at 94 °C for 5 minutes, 35 cycles each of denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds, elongation at 68 °C for 1 minute and a final extension at 68 °C for 10 minutes. The amplicons were then held at 4 °C.

3.7.3 Agarose Gel Electrophoresis

The PCR amplicons were subjected to agarose gel electrophoresis using 1.5% ethidium bromide stained agarose gel at 3 V/cm for approximately 1 hour. A 50 bp DNA ladder was used to estimate the size of the amplicons. Gels were viewed and documented under ultraviolet light using a VILBER (Marne La Vallée, France) gel documentation system.

CHAPTER 4

RESULTS

This chapter has been submitted for publication in the form of a manuscript with the following citation details:

Naidoo, S., Butaye, P., Maliehe, S., Magwedere, K., Basson, A. & Madoroba, E. Virulence Factors and Antimicrobial Resistance among *Salmonella* species from Beef and Beef Products from Selected Retail Outlets in KwaZulu-Natal Province, South Africa. Submitted to: Applied Sciences, MDPI.

4. Results

4.1. Microbiological Analysis

4.1.1. Detection and Isolation of Presumptive *Salmonella* isolates

Based on macroscopic appearance, 200 isolated colonies that appeared either pink on BGA (Figure 4B), or pink/pink with black centres on XLD (Figure 4A) were considered as presumptive *Salmonella* isolates (ISO 6579-1, 2017). XLD agar are selective and differential media; *Salmonella* are differentiated by their ability to degrade lysine with the enzyme lysine decarboxylase, causing an increased (alkaline) pH and resultant red colour, due to the phenol red indicator (HiMedia, 2019). An H_2S indicator system, formed by sodium thiosulfate and ferric ammonium citrate, adds to differentiation as it leads to the formation of black centres in the presence of H_2S (HiMedia, 2019). The 'brilliant green' component inhibits most Gram positive and Gram negative bacteria, including *Salmonella* Typhi on BGA (HiMedia, 2015). As a result, *Salmonella* spp. that are not inhibited cause the phenol red indicator to turn the agar a pink/red colour.

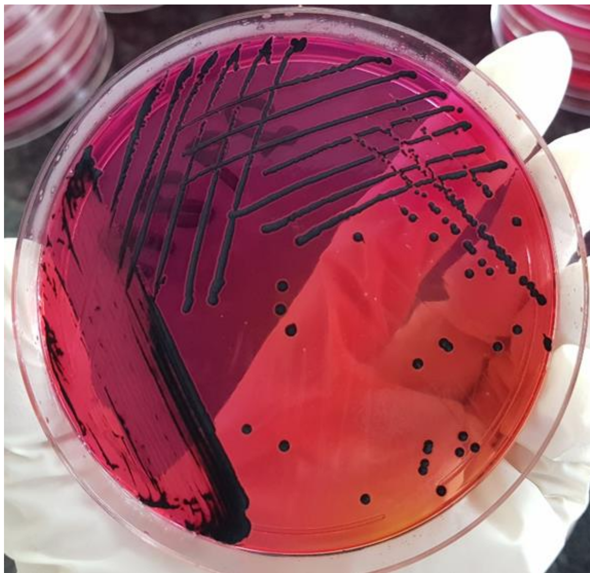


Figure 4A: Image showing the appearance of pure presumptive *Salmonella* isolate (from ox tripe) on XLD.

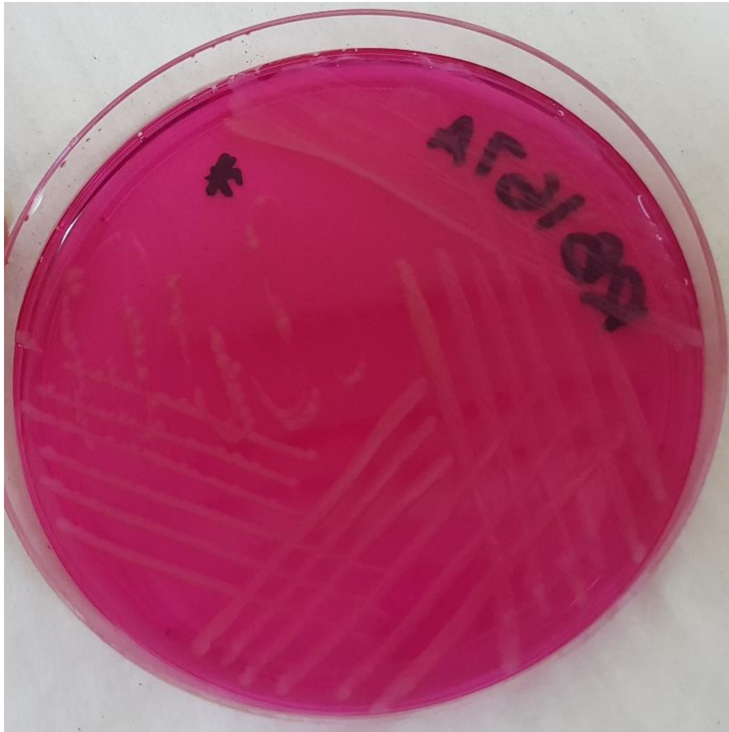


Figure 4B: Image showing the appearance of pure presumptive *Salmonella* isolate on BGA

Presumptive isolates underwent TSI and urea agar tests as described by ISO 6579-1:2017. Typical *Salmonella* could be observed by a yellow butt (due to acidity), a red (alkaline) slant, gas formation in the form of bubbles and in most cases, H_2S production seen as a blackening of the agar (Figure 4C), in the TSI agar tests. *Salmonella* produce negative urea agar tests, and this was observed by the media remaining yellow since *Salmonella* do not hydrolyse urea (Figure 4C).

Figure 4C: Image showing a typical result for presumptive *Salmonella* after a urea agar test (left) and a TSI agar test (right)

Gram negative rods that were oxidase negative and catalase positive were analysed using MALDI-TOF MS. Based on MALDI TOF MS, the average prevalence of *Salmonella* spp. in beef and beef products was 1.25% (n = 5/400). The samples were mainly of gastrointestinal origin. From these five samples, six isolates were obtained. Table 4.1 shows the prevalence of *Salmonella* spp. from 400 diverse types of meat from beef in King Cetshwayo and Ilembe district municipalities in KwaZulu-Natal, South Africa. The names of the retail outlets have not been disclosed in order to maintain strict confidentiality. MALDI-TOF revealed that, on average, the prevalence of *Salmonella* spp. from beef and beef products was 1.25% (n = 5/400).

Table 4.1: Prevalence of *Salmonella* in different categories of beef classified according to the towns in which retail outlets were sampled

Beef Category	Percentage (%) of *Salmonella* positive samples (n)	Towns												
		Empangeni	**Eshowe**	**Esikhaleni**	**Gingindlovu**	**Kwambonambi**	**Melmoth**	**Mtunzini**	**Richards Bay**	**Vulindlela**	**Ballito**	**Maphumulo**	**Mandeni**	**KwaDukuza**
Raw organs	2.37 (4/169)	0 (0/20)	0 (0/31)	0 (0/4)	30 (3/10)	0 (0/3)	2.70 (1/37)	0 (0/4)	0 (0/3)	0 (0/4)	-	0 (0/8)	0 (0/17)	-
Raw intact beef cuts	1.89 (1/53)	0 (0/5)	0 (0/5)	0 (0/4)	0 (0/2)	-	0 (0/5)	0 (0/1)	0 (0/7)	0 (0/3)	0 (0/3)	0 (0/3)	6.67 (1/15)	-
Raw processed	0 (0/110)	0 (0/12)	0 (0/10)	0 (0/5)	0 (0/8)	0 (0/3)	0 (0/6)	0 (0/3)	0 (0/9)	0 (0/1)	0 (0/4)	-	0 (0/29)	0 (0/20)
Ready-to-eat products	0 (0/68)	-	-	-	-	0 (0/31)	-	0 (0/7)	0 (0/11)	-	0 (0/12)	-	0 (0/2)	0 (0/33)
Total samples	1.50 (5/400)	0 (0/37)	0 (0/46)	0 0/13)	15 (3/20)	0 (0/37)	2.08 (1/48)	0 (0/15)	0 (0/30)	0 (0/8)	0 (0/19)	0 (0/11)	1.59 (1/63)	1.89 (1/53)

- no samples were collected in that category

4.2. Serotyping

Serotyping determined four different serovars among the six confirmed *Salmonella* isolates from five different beef samples (Table 4.2). Drug resistance was observed for only *S.* Enteritidis.

Table 4.2: *Salmonella enterica* subsp. *enterica* serovars confirmed from each positive* beef product

Beef Meat Product	Serovar
Ox tripe (Intestines)	Stanley
	Stanley
Ox bible (Omasum)	Heidelberg
Ox tripe (Intestines)	Hadar
Kidney	Enteritidis
Rib	Stanley

*positive refers to samples contaminated with *Salmonella* spp.; Ox bible is also known as omasum (part of the 3rd stomach chamber)

4.3. Antimicrobial Susceptibility Testing

Based on the parameters listed in the CLSI guidelines, zones of inhibition were measured and analysed to determine if the six isolates were resistant, intermediate or susceptible to Amoxicillin, Ampicillin, Cefoxitin, Cefotaxime, Chloramphenicol, Ciprofloxacin, Gentamicin, Kanamycin, Tetracycline and Trimethoprim sulfamethoxazole. The *S.* Enteritidis isolate displayed resistance against tetracycline and aminopenicillins (ampicillin and amoxicillin), showing no zones of inhibition (ZOI) against the three antimicrobials at all. The remaining isolates were susceptible to all tested antimicrobials. The full set of results can be seen in the appendix – Table 7.1.

Figure 4D: Image showing the Kirby Bauer disk diffusion results for the *S.* Enteritidis isolate against the 10 tested antimicrobials

Resistance (no ZOI) were seen against ampicillin, amoxicillin (left) and tetracycline (right).

Figure 4E: Image showing the Kirby Bauer disk diffusion results for the *S.* Hadar isolate against the 10 tested antimicrobials

S. Hadar displayed susceptibility against all 10 antimicrobials.

4.4. Molecular Characterization of Virulence Genes

Diverse virulence profiles were observed among the confirmed *Salmonella* serovars (Table 4.3). *Salmonella* isolates tested positive to at least two virulence genes with some serovars testing positive for up to six out of the eight virulence factors (Table 4.3). None of the isolates had the same combination of virulence genes. Virulence gene agfA was the most prevalent, having been found across all six isolates; and both *inv*A and *hil*A were found in all but one isolate. One of the three isolates of *S.* Stanley contained six of the virulence genes, while the one S. Heidelberg isolate had the least (2/8). None of the isolates were positive for the presence of *spv*C.

Table 4.3: PCR results for the screening of eight virulence genes among six *Salmonella* isolates

Sample type	**Serovar**	**Virulence Gene**							
		*inv*A	*agf*A	*lpf*A	*hil*A	*siv*H	*sef*A	*sop*E	*spv*C
Beef Intestines	Stanley	+	+	-	+	+	-	-	-
Beef Intestines	Stanley	+	+	-	+	-	-	-	-
Beef tripe	Heidelberg	-	+	-	+	-	-	-	-
Beef intestines	Hadar	+	+	+	+	+	-	-	-
Beef kidney	Enteritidis	+	+	+	-	-	+	-	-
Beef rib	Stanley	+	+	+	+	-	+	+	-

+ Refers to positive PCR test for the target virulence gene; - Refers to negative PCR test for the target virulence gene.

Figures 4F to 4H show examples of amplicons from the PCR test run by agarose gel electrophoresis. The base pair (bp) length of the amplicons can be seen in accordance with the labelled DNA ladders.

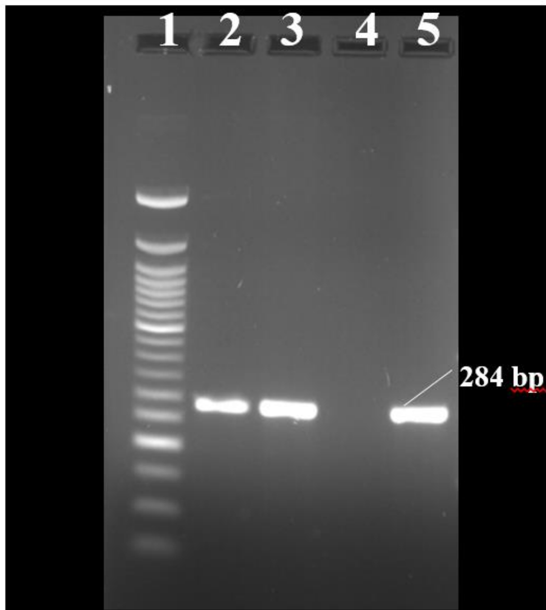


Figure 4F: Image showing examples of *inv*A gene amplicons observed on agarose gel

Lane 1 contains the 50 bp DNA ladder and lanes 2–5 had PCR amplicons run. Lane 4 shows a negative result for *inv*A (*S.* Heidelberg) whereas lanes 2,3 (Both *S.* Stanley isolates) and 5 (*S.* Hadar) were positive. The *inv*A gene is 284 bp in length.

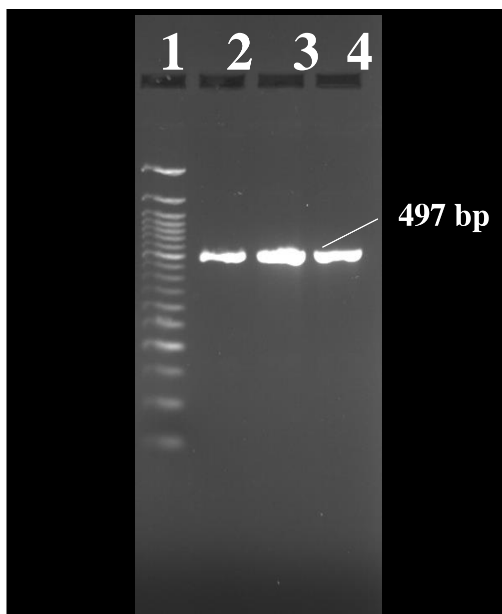


Figure 4G: Image showing example of *hil*A gene amplicons observed on agarose gel

Lane 1 contains the 50 bp DNA ladder and lanes 2–4 are indicative of positive results for the *hil*A gene (497 bp). Lanes 2 and 3 are *S.* Stanley isolates and lane 4 is *S.* Hadar.

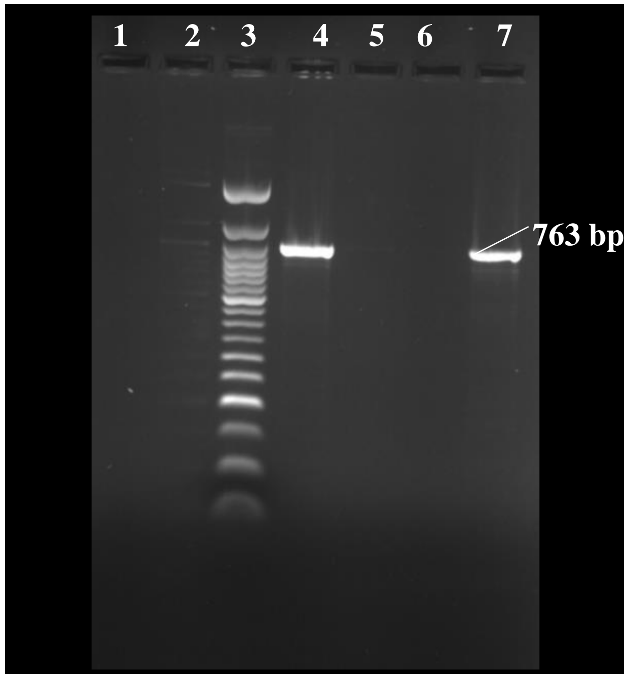


Figure 4H: Image showing electrophoresis results for *siv*H gene among the six isolates

Lane 3 contained the 50 bp DNA ladder and the remainder of the lanes had PCR amplicons run. Lanes 1 (*S.* Stanley), 2 (*S.* Stanley), 5 (*S.* Heidelberg) and 6 (*S.* Enteritidis) were negative whereas lanes 4 (*S.* Stanley) and 7 (*S.* Hadar) were positive for the 763 bp *siv*H gene.

CHAPTER 5

GENERAL DISCUSSION

This chapter has been submitted for publication in the form of a manuscript with the following citation details:

Naidoo, S., Butaye, P., Maliehe, S., Magwedere, K., Basson, A. & Madoroba, E. Virulence Factors and Antimicrobial Resistance among *Salmonella* species from Beef and Beef Products from Selected Retail Outlets in KwaZulu-Natal Province, South Africa. Submitted to: Applied Sciences, MDPI.

5. Discussion

The overall low prevalence of *Salmonella* (1.25%) in this study was similar to other studies in in KwaZulu-Natal (Madoroba *et al.,* 2021), where abattoirs and meat from different animal species were also involved but organ meat was excluded. In this study, the majority of the *Salmonella* originated from organ meat, and thus the former study probably underrepresented its prevalence in beef products. The total prevalence found in the former study was 3.2%, with a lower prevalence for beef meats in KwaZulu-Natal and in the Western Cape compared to other provinces (Madoroba *et al.,* 2021). In other African countries like Botswana, a higher prevalence was found, though these studies were completed some time ago, and the epidemiological situation might have changed (Gashe & Mpuchane, 2000) since then. The low prevalence found in this study may be due to the fact that samples were not obtained from informal butcheries. A study carried out in Senegal found *Salmonella* contamination in 63% of total beef samples tested, 43% (101/236) in slaughterhouses and 87% (174/199) in retail outlets, with 97% of the tested retail outlets having at least one positive sample (Stevens *et al.,* 2006). The study highlighted reasons for the high contamination levels as incorrect storage temperatures, poor hygiene of workers and equipment, and unwashed cutting tables, which could have been reservoirs for *Salmonella* to spread to other equipment through flies or direct contact (Stevens *et al.,* 2006). The presence of *Salmonella* spp. in retail meat samples, even in low amounts, creates the basis for potential spread. Regular surveillance should be carried out in both the formal and informal beef sector in South Africa to ensure conditions do not lead to outbreaks.

This is the first study on beef organ meats in KwaZulu-Natal, and they have proven to be the most contaminated of beef products, probably due to intestinal contamination and being part of the gut. However, tripe and intestines are usually thoroughly cooked, hence the chances of *Salmonella* survival are reduced. Even so, tripe and intestines may cross contaminate different surfaces and utensils, which may result in infection. A higher prevalence in such organ meat compared to other beef cuts has been shown in Burkina Faso and Botswana (Kagambéga *et al.* 2011, Gashe & Mpuchane (2000). Ready-to-eat meat samples, which included sample types like cold meats, biltong (dried, cured meat) and smoked meats did not contain *Salmonella*, which is not surprising because of the different processes applied to them.

The rib meat sample that tested positive for *Salmonella* in this study could have been contaminated during one of the many stages of beef processing due to the complex nature of meat contamination (Madoroba *et al*., 2016). Probably, contamination of rib pieces occurred during cutting and packaging, as large quantities may have been cut using the same equipment and on the same surfaces before being placed into their individual packages.

Surprisingly, the current investigation did not yield *Salmonella* from raw processed products. Sausages are generally produced in bulk, and can contain ground meat of various different cuts of beef, hence our findings may indicate that strict hygiene processes were implemented during the production process, and the cuts used were not contaminated.

From the six *Salmonella* isolates, four different serovars were identified. *S.* Stanley (n = 3) was the most prevalent serotype and *S.* Hadar, *S.* Heidelberg and *S.* Enteritidis, were each represented by a single isolate. A recent review on serotypes in different African countries found that the most isolated serovars from meat and meat products, including beef were *S.* Typhimurium and *S.* Enteritidis (Naidoo *et al.,* 2021). In a previous South African study undertaken between 2011 and 2012, the most common *Salmonella* serovar found on cattle carcasses and hides was *S.* Enteritidis (Madoroba *et al.,* 2016). While *S.* Heidelberg was also found (Madoroba *et al.,* 2016), *S.* Stanley and *S.* Hadar were rather uncommon in Africa (Naidoo *et al.*, 2021).

All *Salmonella* isolates were susceptible to cefoxitin, cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, kanamycin and trimethoprim-sulfamethoxazole. However, it is important to note that first and second generation cephalosporins (cefoxitin and cefotaxime) and aminoglycosides (gentamicin and kanamycin) that appear effective *in-vitro*, may be ineffective in clinical treatment and it can thus not be reported that the tested *Salmonella* isolates are susceptible. Widespread resistance against tetracyclines may arise as a result of selection pressure caused by the use of these agents in clinical and veterinary treatment, prophylaxis and as growth promoters for livestock (Chopra & Roberts, 2001). Previous studies in South Africa found a high level of resistance to tetracyclines and ampicillin in *Salmonella* from cattle meats (Madoroba *et al.*, 2016; Mthembu *et al.,* 2019). Also in other African countries, resistance against these antibiotics in *Salmonella* was highest (Naidoo *et al.*, 2021). However, the small number of 6 *Salmonella* isolates from this study does not provide a good overview of the potential threat of multi-drug resistance in *Salmonella* in KZN.

Virulence genes are crucial for *Salmonella* pathogenesis and they manifest differently within different hosts (Kshirsagar *et al.,* 2014). The six *Salmonella* isolates were screened for the presence of eight virulence genes, namely, *inv*A, *agf*A, *lpf*A, *hil*A, *siv*H, *sef*A, *sop*E, and *spv*C using PCR. The *inv*A gene, responsible for the invasion of epithelial cells at the onset of infection, is typically present in all *Salmonella* spp. and is therefore used as a target for rapid identification using PCR (Siddiky *et al.,* 2021). However, in the present study, the *inv*A gene was not observed in one of the six isolates (*S.* Heidelberg). Although *inv*A-negative *Salmonella* are considered to be rare, they have been identified in other studies (Malorny *et al*., 2003; Rahn *et al*. 1992). Nevertheless, *invA negative* strains may use other mechanisms for invasion (Galán & Curtiss, 1989; Galán &Curtiss, 1991).

The *inv*A-negative *S.* Heidelberg isolate was positive for *hil*A, another virulence gene associated with invasion. The *hil*A gene is responsible for encoding an OmpR/ToxR transcriptional regulator, which activates the expression of invasion genes (Thung *et al.,* 2018). The *hil*A gene was found in 83% (5/6) of the isolates in this study, which supports findings from another study that isolated *Salmonella* serovars from beef in Malaysia with a frequency of 82.16% (19/23) of the *hil*A gene, regardless of serovar (Thung *et al.,* 2018).

The virulence gene that was common to all six isolates was *agf*A, which encodes fimbriae that increase adhesion of the bacterium to intestinal cells, and is also involved in biofilm formation (Siddiky *et al.,* 2021). Other genes were variably present, but *spv*C, a plasmid mediated gene involved in vertical transmission (Siddiky *et al.,* 2021) was not present in any of the six isolates. Unlike the present study, the frequency of the *spv*C gene was high (92%) in a study that screened *S.* Enteritidis isolated from chickens. That study did not include varied serovars, but like in this work, *spv*C was the least prevalent virulence gene compared to *agf*A, *sef*A, *inv*A, *hil*A, *sop*E and *siv*H. (Borges *et al.,* 2013).

All isolates, including the 3 *S.* Stanley isolates, had a different virulence profile, indicating a different potential for infection of animals and humans (Cheng *et al*., 2019). Interestingly, studies have shown that certain genes in *Salmonella* spp. have different frequencies based on the host they were isolated from, as is the case with a 100% frequency of *S.* Enteritidis in humans and 90% in cattle (Amini *et al.,* 2010; Borges *et al.,* 2013). This is indicative that virulence in S. Enteritidis can vary based on plasmid and chromosomal factors according to the genetic make-up of individual isolates (Borges et al., 2013). Additionally, Moussa *et al.*

(2016) observed a significant difference between the frequency of *spv*C in *S.* Enteritidis isolates (92%) and *S.* Typhimurium (28%), a serovar which was not found in the present study.

Salmonella spp. can gain and lose virulence genes based on host and environmental adaptations (Borges et al., 2013). It is important to understand the mechanisms involved, and the genetic traits aiding this ability. Understanding the characteristics associated with virulence factors in *Salmonella* spp. will aid in developing strategies to reduce virulence and its threat to public health.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6. Conclusion and Recommendations

In this study, we determined the prevalence, serovars, antimicrobial resistance profiles and selected virulence factors of *Salmonella* spp. in raw intact beef, raw processed beef, ready-to-eat beef products and beef organs from selected retail outlets and butcheries in KZN, South Africa. Overall, a low prevalence (1.25%) of *Salmonella* spp. was observed and the isolated serovars were Enteritidis, Hadar, Heidelberg and Stanley. Beef organs had the highest degree of contamination, hence there is need to be vigilant when processing these meat types. For this reason, it is important that regular surveillance be done on food pathogens in meat products to ensure than prevalence remains low and therefore reduces the risk of possible outbreaks. Drug resistance was observed in *S.* Enteritidis, which is one of the most common serovars associated with salmonellosis. The presence of virulence genes is crucial in *Salmonella* pathogenicity and it is important to have an understanding of the virulence profiles as this contributes to epidemiological knowledge about the potential severity of infections.

Future studies should include a larger sample size taking the informal sector into consideration. It is important to undertake surveillance of food pathogens in meat products to ensure that prevalence remains low, and therefore reduces the risk of outbreaks that can be a result of improper hygiene in food preparation settings. Multi-drug resistance remains a global threat and needs to be continually monitored. It is recommended that there are systems in place to obtain a thorough and uniform overview of foodborne pathogens like *Salmonella* spp. in all South African provinces, and ideally, in all African countries like the systems on other continents. Furthermore, the informal meat sector should be included in regular monitoring for the presence of foodborne pathogens.

Future projects should also extend sampling to abattoirs to get a better evaluation of the source of *Salmonella* contamination and what changes can be implemented to avoid cross contamination between different meat types and cuts during the slaughtering process. This should include water, food, personnel, equipment and environment samples, but on a national scale.

References

A-Mpalang, R. K., A-Mpalang, M. K., Kaut, C. M., Boreux, R., Melin, P., Ni Bitiang, F. K. m. A., Daube, G., & De Mol, P. (2013). Bacteriological assessment of smoked game meat in Lubumbashi, D.R.C. *Qualité bactériologique de la viande boucanée de gibier à Lubumbashi, R.D.C., 17*(3),441–449.

Abbassi-Ghozzi, I., Jaouani, A., Hammami, S., Martinez-Urtaza, J., Boudabous, A., & Gtari, M. J. P. B. (2012). Molecular analysis and antimicrobial resistance of *Salmonella* isolates recovered from raw meat marketed in the area of "Grand Tunis". *Tunisia, 60*(5), 49–54.

Abdallah, H. M., Reuland, E. A., Wintermans, B. B., al Naiemi, N., Koek, A., Abdelwahab, A. M., Ammar, A. M., Mohamed, A. A., & Vandenbroucke-Grauls, C. M. J. E. (2015). Extended-Spectrum β-Lactamases and/or carbapenemases-producing Enterobacteriaceae isolated from retail chicken meat in Zagazig, Egypt. *PLoS One, 10*(8), e0136052.

Abd-Elghany, S. M., Sallam, K. I., Abd-Elkhalek, A., & Tamura, T. (2015). Occurrence, genetic characterization and antimicrobial resistance of Salmonella isolated from chicken meat and giblets. *Epidemiology & Infection, 143*(5), 997–1003.

Abdi, R. D., Mengstie, F., Beyi, A. F., Beyene, T., Waktole, H., Mammo, B., Ayanda, D., & Abunna, F. (2017). Determination of the sources and antimicrobial resistance patterns of *Salmonella* isolated from the poultry industry in Southern Ethiopia. *BMC Infectious Diseases, 17*, 1–12.

Abuelo, A. & Cullens, F. (2020). *Salmonella* Dublin in dairy Calves. Michigan State University. [online] Available at: https://www.canr.msu.edu/news/salmonella-dublin-in-dairy-calves [Accessed 22 April 2021].

Abbey Veterinary Group (2021). Cattle Disease and Conditions. Abbey-vetgroup.co.uk. [online] Available at: https://www.abbey-vetgroup.co.uk/Cattle_Diseases_and_Conditions.html [Accessed 8 December 2021].

Adeyanju, G. T., & Ishola, O. (2014). *Salmonella* and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *SpringerPlus, 3*(1), 139.

Ahmed, A. M., & Shimamoto, T. (2014). Isolation and molecular characterization of *Salmonella enterica*, *Escherichia coli* O157:H7 and Shigella spp. from meat and dairy products in Egypt. *International Journal of Food Microbiology, 168–169*, 57–62.

Akbar, A. & Anal A, K. (2013) Prevalence and antibiogram study of *Salmonella* and *Staphylococcus aureus* in poultry meat. *Asian Pac J Trop Biomed, 3*(2), 163–8.

Alemu, S., & Zewde, B. (2012). Prevalence and antimicrobial resistance profiles of *Salmonella enterica* serovars isolated from slaughtered cattle in Bahir Dar, Ethiopia. *Tropical Animal Health & Production, 44*(3), 595–600. https://doi.org/10.1007/s11250-011-9941-y.

American Society of Microbiology (2010). *Catalase Test Protocol.* ASM.org [online] Available at: https://asm.org/getattachment/72a871fc-ba92-4128-a194-6f1bab5c3ab7/Catalase-Test-Protocol.pdf [Accessed 11 December 2021].

American Society of Microbiology (2010). *Oxidase Test Protocol.* ASM.org [online] Available at: https://asm.org/getattachment/00ce8639-8e76-4acb-8591-0f7b22a347c6/oxidase-test-protocol-3229.pdf [Accessed 11 December 2021].

American Society of Microbiology (2016). ASM.org [online] Available at: https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf [Accessed 11 November 2021].

Amini, K., Salehi, T. Z., Nikbakht, G., Ranjbar, R., Amini, J. & Ashrafganjooei, S. B. (2010). Molecular detection of *inv*A and *spv* virulence genes in *Salmonella* Enteritidis isolated from human and animals in Iran. *Afr. J. Microbiol. Res. 4(21):*2 202-2210.

Anejo-Okopi, A., Adeniyi, D., Audu, O., Okojokwu, O., Zumbes, J., Okechalu, J., Augustine, B., Ali, M., Akindigh, T., & Lar, M. (2016). *Molecular detection of virulent Salmonella Strains in commercially sold meat in Jos Metropolis, North-Central Nigeria*.

Azage, M., & Kibret, M. (2017). The bacteriological quality, safety, and antibiogram of *Salmonella* isolates from fresh meat in retail shops of Bahir Dar City, Ethiopia. *International Journal of Food Science, 2017*.

Banerji, S., Simon, S., Tille, A., Fruth, A., & Flieger, A. (2020). Genome-based *Salmonella* serotyping as the new gold standard. *Scientific Reports*, *10*(1).

Beaubrun, J., Ewing, L., Dudley, K., Benhamed, F., Wang, H., & Hanes, D. (2017). Evaluation of a multiplex PCR method to serotype *Salmonella* in animal feeds pre-enrichment broth cultures. *Methodsx*, *4*, 335-345.

Bender, A. (1992). Meat and meat products in human nutrition in developing countries. *Food and Agriculture Organisation.* [online] Available at: http://www.fao.org/docrep/t0562e/t0562e05.htm [Accessed 2 March 2018].

bioMérieux Clinical Diagnostics (2020). VITEK® MS. [online] Available at: https://www.biomerieux-diagnostics.com/vitekr-ms-0 [Accessed 20 April 2021].

Borges, K. A., Furian, T. Q., Borsoi, A., Moraes, H. L. S., Salle, C. T. P. & Nascimento, V. P. (2013). Detection of virulence-associated genes in *Salmonella* Enteritidis isolates from chicken in Southern Brazil. *Pesquisa Veterinária Brasileira, 33(12):* 1416-1422.

Butaye, P., Michael, G. B., Schwarz, S., Barrett, T. J., Brisabois, A., & White, D. G. J. M. (2006). The clonal spread of multidrug-resistant non-typhi *Salmonella* serotypes. *Microbes and Infection, 8*(7), 1891–1897.

Cabrera-Diaz, E., Barbosa-Cardenas, C. M., Perez-Montano, J. A., Gonzalez-Aguilar, D., Pacheco-Gallardo, C., & Barba, J. (2013). Occurrence, serotype diversity, and antimicrobial resistance of *Salmonella* in ground beef at retail stores in Jalisco State, Mexico. *Journal of Food Protection, 76*(12), 2004–2010.

Cardinale, E., Abat, C., Bénédicte, C., Vincent, P., Michel, R., & Muriel, M. (2015). *Salmonella* and *Campylobacter* contamination of ready-to-eat street-vended pork meat dishes in Antananarivo, Madagascar: A risk for the consumers? *Journal of Foodborne Pathogens, 12*(3), 197–202.

Carraturo, F., Gargiulo, G., Giorgio, A., Aliberti, F., & Guida, M. (2016). Prevalence, distribution, and diversity of *Salmonella* spp. in meat samples collected from Italian slaughterhouses. *Journal of Food Science, 81*(10), 2545–2551.

CDC (2017) Salmonella infection. *Centres for disease control and prevention.* [online] Available at: https://www.cdc.gov/healthypets/diseases/salmonella.html [Accessed 28 February 2018].

Champion, O. L., Best, E. L., & Frost, J. A. (2002). Comparison of pulsed-field gel electrophoresis and amplified fragment length polymorphism techniques for investigating outbreaks of enteritis due to campylobacters. *Journal of clinical microbiology*, *40*(6), 2263–2265.

Charan, J. & Biswas, C (2013). How to Calculate Sample Size for Different Study Designs in Medical Research? *Indian Journal of Psychological Medicine*, *35(2),* 121-126.

Chattaway, M., Dallman, T., Larkin, L., Nair, S., McCormick, J., & Mikhail, A. et al. (2019). The Transformation of Reference Microbiology Methods and Surveillance for *Salmonella* with the Use of Whole Genome Sequencing in England and Wales. *Frontiers in Public Health*, *7*.

Chatterjee, A. & Steele, R. W. (2016). Pediatric *Salmonella* Infection Medication. *Medscape.* [online] Available at: https://emedicine.medscape.com/article/968672-medication [Accessed 1 March 2018].

Cheng, R. A., Eade, C. R., & Wiedmann, M. (2019). Embracing diversity: Differences in virulence mechanisms, disease severity, and host adaptations contribute to the success of nontyphoidal *Salmonella* as a foodborne pathogen. *Frontiers in Microbiology, 10*(1368).

Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and molecular biology reviews: MMBR*, *65*(2), 232–260.

CLSI (2017). Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplemnet M100. Wayne, PA: Clinical and Laboratory Standards Institute, 2017.

Cohen, N., Filliol, I., Karraouan, B., Badri, S., Carle, I., Ennaji, H., Bouchrif, B., Hassa, M., & Karib, H. (2008). Microbial quality control of raw ground beef and fresh sausage in Casablanca (Morocco). *Journal of Environmental Health, 71*(4), 51–55.

DAFF (2017). A Profile of the South African Beef Market Value Chain. Department of Agriculture, Forestry and Fisheries. [online] Available at: https://www.nda.agric.za/doaDev/sideMenu/Marketing/Annual%20Publications/Commodity%20Profiles/Beef%20Market%20Value%20Chain%20Profile%202017.pdf [Accessed 23 April 2021].

DAFF (2017). Trends in the Agricultural Sector. Department of Agriculture, Forestry and Fisheries. [online] daff.gov.za. Available at https://www.daff.gov.za/Daffweb3/Portals/0/Statistics%20and%20Economic%20Analysis/Statistical%20Information/Trends%20in%20the%20Agricultural%20Sector%202017.pdf [Accessed 22 January 2020].

Dawoud, T. M., Shi, Z., Kwon, Y. M. & Ricke, S. C. (2017). Overview of Salmonellosis and Food-borne *Salmonella*: Historical and Current Perspectives. Chapter 7. *Elsevier Inc.*

Department of Agriculture and Rural Development (DARD) (2016). Red Meat Abattoir Handout. kzndard.gov.za [online] Available at: https://www.kzndard.gov.za/images/Documents/AnimalHealth/AbattoirHandouts/RED-MEAT-HANDOUT-2016.pdf. [Accessed 8 December 2021].

Dieckmann, R., Helmuth, R., Erhard, M & Malorny, B. (2008). Rapid Classification and Identification of *Salmonellae* at the Species and Subspecies Levels by Whole-Cell Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. *Applied and Environmental Microbiology*, *74(24),* 7767-7778.

Dieckmann, R. & Malorny, B (2011). Rapid Screening of Epidemiologically Important *Salmonella enterica* subsp. *enterica* Serovars by Whole-Cell Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. *Applied and Environmental Microbiology*, *77(12),* 4136–4146.

Dione, M. M., Ieven, M., Garin, B., Marcotty, T., & Geerts, S. (2009). Prevalence and antimicrobial resistance of *Salmonella* isolated from broiler farms, chicken carcasses, and street-vended restaurants in Casamance, Senegal. *Journal of Food Protection, 72*(11), 2423–2427.

Ed-dra, A., Filali, F. R., Karraouan, B., El Allaoui, A., Aboulkacem, A., & Bouchrif, B. (2017). Prevalence, molecular and antimicrobial resistance of *Salmonella* isolated from sausages in Meknes, Morocco. *Microbial Pathogenesis, 105*, 340–345.

EFSA and ECDC (European Food Safety Authority and European Center for Disease Prevention and Control). (2018). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA Journal, 16*(12), 5500, 262.

Eguale, T., Gebreyes, W. A., Asrat, D., Alemayehu, H., Gunn, J. S., & Engidawork, E. (2015). Non-typhoidal *Salmonella* serotypes, antimicrobial resistance and co-infection with parasites among patients with diarrhea and other gastrointestinal complaints in Addis Ababa, Ethiopia. *BMC Infectious Diseases, 15*(1), 497.

Ejeta, G., Molla, B., Alemayehu, D., & Muckle, C. J. R. (2004). *Salmonella* serotypes isolated from minced meat beef, mutton and pork in Addis Ababa, Ethiopia. *Revue de Médecine Vétérinaire, 155*(11), 547–551.

Ejo, M., Garedew, L., Alebachew, Z., & Worku, W. (2016). Prevalence and antimicrobial resistance of *Salmonella* isolated from animal-origin food items in Gondar, Ethiopia. *BioMed Research International, 2016*, 1–8.

Eng, S., Pusparajah, P., Mutalib, N. A., Ser, H., Chan, K., & Lee, L. (2015). *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science, 8*(3), 284–293.

FAO (2002). *FAO Urges Countries to Discontinue the Use of Chloramphenicol in Animal Production*. [online] Available at: http://www.fao.org/asiapacific/news/detail-events/en/c/47419/ [Accessed 13 August 2019].

Feasey, N. A., Dougan, G., Kingsley, R. A., Heyderman, R. S., & Gordon, M. A. (2012). Invasive non-typhoidal *Salmonella* disease: An emerging and neglected tropical disease in Africa. *The Lancet, 379*(9835), 2489–2499.

Ferrari, R. G., Rosario, D. K. A., Cunha-Neto, A., Mano, S. B., Figueiredo, E. E. S., Conte-Junior, C. A., (2019). Worldwide epidemiology of *Salmonella* serovars in animal-based foods: a metaanalysis. *Appl Environ Microbiol 85(14),* e00591-19.

Ferede, B. (2014). *Isolation, identification, antimicrobial susceptibility test and public awareness of Salmonella on raw goat meat at Dire Dawa Municipal Abattoir, eastern Ethiopia*. Addis Ababa University.

Foley, S., Lynne, A., & Nayak, R. J. (2008). *Salmonella* challenges: Prevalence in swine and poultry and potential pathogenicity of such isolates. *Journal of Animal Science, 86*, 149–162.

Foley, S. L., Johnson, T. J., Ricke, S. C., Nayak, R., & Danzeisen, J. (2013). *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. *Microbiology and molecular biology reviews: MMBR*, *77*(4), 582–607.

Galán, J. E., & Curtiss, R., 3rd (1989). Cloning and molecular characterization of genes whose products allow *Salmonella* Typhimurium to penetrate tissue culture cells. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(16), 6383–6387.

Galán, J. E., & Curtiss, R., 3rd (1991). Distribution of the invA, -B, -C, and -D genes of *Salmonella* Typhimurium among other Salmonella serovars: invA mutants of *Salmonella* Typhi are deficient for entry into mammalian cells. *Infection and immunity*, *59*(9), 2901–2908.

Gashe, B. A., & Mpuchane, S. (2000). Prevalence of *Salmonellae* on beef products at butcheries and their antibiotic resistance profiles. *Journal of Food Science (Wiley-Blackwell), 65*(5), 880–883.

Gast, R. K., & Porter, R. E. (2019). *Salmonella* Infections. *Diseases of Poultry*, 717–753.

Ghoneim, N., Abdel-Moein, K., & Zaher, H. (2017). Camel as a transboundary vector for emerging exotic *Salmonella* serovars. *Pathogens and Global Health, 111*(3), 143–147.

Gilchrist, J. J. & Maclennan, C. A. (2019). Invasive Nontyphoidal *Salmonella* Disease in Africa. *EcoSal Plus*, *8*(2).

Gous, N. (2018). South Africans love meat, but how do we stack up globally? TimesLive. [online] Available at: https://www.timeslive.co.za/news/south-africa/2018-12-10-south-africans-love-meat-but-how-do-we-stack-up-globally/ [Accessed 23 April 2021].

Grimont, P. A. D. & Weill, F. X. (2007) Antigenic Formulae of the *Salmonella* Serovars. 9th edition, World Health Organization Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur, Paris.

Guya, M., & Tolesa, G. (2016). Camel slaughtering practices and meat production in Eastern Ethiopia. *Science, Technology and Arts Research Journal, 4*, 123.

Hamad, R., & Saleh, A. (2019). Incidence of some food poisoning bacteria in raw meat products with molecular detection of *Salmonella* in Al Beida City, Libya. *Journal for Veterinary Sciences, 61*(2).

Hetsa, B., Ateba, T., Moroane, T., Nyirenda, M., Gopane, R., & Ateba, C. (2013). Detection of antibiotic resistant Enterobacteriaceae from dogs in North West University (South Africa) animal health hospital. *Journal of Microbiology Research, 7*(43), 5004–5010.

Hiko, A., Ameni, G., Langkabel, N. & Fries, R. (2015). Microbiological load and zoonotic agents in beef mortadella from Addis Ababa city supermarkets. *J Food Prot, 78*(5), 1043–5.

Himedia (2015). Technical data - Brilliant Green Agar Medium MU016 [online] Available at: https://himedialabs.com/TD/MU016.pdf [Accessed 10 December 2021]

Himedia (2019). Technical data - Xylose-Lysine Deoxycholate Agar (XLD Agar) M031 [online] Available at: https://himedialabs.com/TD/M031.pdf [Accessed 9 December 2021].

Holschbach, C. L., & Peek, S. F., (2018). *Salmonella* in Dairy Cattle. *Veterinary Clinics of North America: Food Animal Practice*, 34(1), 133-154.

Huq, A., Haley, B. J., Taviani, E., Chen, A., Hasan, N. A., & Colwell, R. R. (2012). Detection, isolation, and identification of Vibrio cholerae from the environment. *Current protocols in microbiology*, Unit6A5.

Ibrahim, A. A., El-Diasty, M. M., Mahmoud, M. M., & Nada, H. S. (2014). *Molecular characterization of multidrug-resistant Salmonella species from meat in delta area, Egypt*.

Ibrahim, G., & Morin, P. (2018). *Salmonella* Serotyping Using Whole Genome Sequencing. *Frontiers In Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.02993.

Issenhuth-Jeanjean, S., Roggentin, P., Mikoleit, M., Guibourdenche, M., de Pinna, E., Nair, S., Fields, P. I., & Weill, F. X. (2014). Supplement 2008-2010 (no. 48) to the White-Kauffmann-Le Minor scheme. *Research in microbiology*, *165*(7), 526–530.

Jajere, S. M. (2019). A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance, *Veterinary World, 12*(4), 504–521.

Kagambèga, A., Haukka, K., Siitonen, A., Traoré, A. S., & Barro, N. (2011). Prevalence of *Salmonella* enterica and the hygienic indicator *Escherichia coli* in raw meat at markets in Ouagadougou, Burkina Faso. *Journal of Food Protection, 74*(9), 1547–1551. https://doi.org/10.4315/0362-028X.JFP-11-124.

Kariuki, S., Revathi, G., Kariuki, N., Kiiru, J., Mwituria, J., Muyodi, J., Githinji, J. W., Kagendo, D., Munyalo, A., & Hart, C. A. (2006). Invasive multidrug-resistant non-typhoidal *Salmonella* infections in Africa: Zoonotic or anthroponotic transmission? *Journal of Medical Microbiology, 55*(5), 585–591.

Käsbohrer, A., Bernd-Alois, T., Appel, B. & Fetsch, A. (2010). Development of harmonised survey methods for food-borne pathogens in foodstuffs in the European Union. European Food Safety Authority, *7(11).*

Kau, J. S. (2016). The Growth Prospects of the South African Beef Industry in an Uncertain Socio-Economic Environment. Annual Beef Bulletin, Department of Agriculture, Forestry and Fisheries. Available at: https://www.arc.agric.za/Economic%20News%20articles/The%20Growth%20Prospects%20of%20the%20SA%20Beef%20Industry%20(ARC%20Annual%20Beef%20Bulletin,%202016,%20pp%2022-24).pdf [Accessed 23 April 2021].

Kaufmann, M. E. (1998). Pulsed-Field Gel Electrophoresis. *Molecular Bacteriology,* 15: 33–50.

Keddy, K. H., Dwarika, S., Crowther, P., Perovic, O., Wadula, J. & Hoosen, A. (2009). Genotypic and demographic characterization of invasive isolates of *Salmonella Typhimurium* in HIV co-infected patients in South Africa. *J Infect Dev Ctries., 3,* 585–592.

Keddy, K. (2016). Non typhoidal *Salmonella* and the African growth and opportunity act. *Communicable Diseases Surveillance Bulletin, 14*(2).

Khallaf, M., Ameur, N., Terta, M., Lakranbi, M., Senouci, S., & Ennaji, M. (2014). Prevalence and antibiotic-resistance of *Salmonella* isolated from chicken meat marketed in Rabat, Morocco. *International Journal of Innovation Applied Studies, 6*(4), 1123.

Khoodoo, M. H. R., Issack, M. I., & Jaufeerally-Fakim, Y. (2002). Serotyping and RAPD profiles of *Salmonella enterica* isolates from Mauritius. *Letters In Applied Microbiology*, *35*(2), 146-152. https://doi.org/10.1046/j.1472-765x.2002.01151.x.

Kilroy, S., Raspoet, R., Haesebrouck, F., Ducatelle, R., & Van Immerseel, F. (2016). Prevention of egg contamination by *Salmonella* Enteritidis after oral vaccination of laying hens with *Salmonella* Enteritidis *ΔtolC* and *ΔacrABacrEFmdtABC* mutants. *Veterinary Research, 47*, 82.

Kim, S., Frye, J., Hu, J., Fedorka-Cray, P., Gautom, R., & Boyle, D. (2006). Multiplex PCR-Based Method for Identification of Common Clinical Serotypes of Salmonella enterica subsp. enterica. *Journal of Clinical Microbiology*, *44*(10), 3608-3615. https://doi.org/10.1128/jcm.00701-06.

Kshirsagar, D. P., Singh, S., Brahmbhatt, M. N. & Nayak, J. B. (2014). Isolation and Molecular Characterization of Virulence-Associated Genes of *Salmonella* from Buffalo Meat Samples in Western Region of India. *Israel Journal of Veterinary Medicine,* 69(4).

Laufer, A. S., Grass, J., Holt, K., Whichard, J. M., Griffin, P. M., & Gould, L. H. (2015). Outbreaks of *Salmonella* infections attributed to beef – United States, 1973–2011. *Epidemiology and Infection, 143*(9), 2003–2013.

Leekitcharoenphon, P., Nielsen, E. M., Kaas, R. S., Lund, O., and Aarestrup, F. M. (2014). Evaluation of whole genome sequencing for outbreak detection of *Salmonella enterica*. *PLoS ONE* 9:e87991.

Lin, J., Nishino, K., Roberts, M., Tolmasky, M., Aminov, R., & Zhang, L. (2015). Mechanisms of antibiotic resistance. *Frontiers In Microbiology*, *6*. https://doi.org/10.3389/fmicb.2015.00034.

Lu, Y., Zhao, H., Sun, J., Liu, Y., Zhou, X., Beier, R. C., Wu, G., & Hou, X. (2014). Characterization of multidrug-resistant *Salmonella enterica* serovars Indiana and Enteritidis from chickens in Eastern China. *PLoS One, 9*(5).

Madoroba, E., Kapeta, D., & Gelaw, A. K. (2016). *Salmonella* contamination, serovars and antimicrobial resistance profiles of cattle slaughtered in South Africa. *Onderstepoort Journal of Veterinary Research, 83*(1).

Madoroba, E., Magwedere, K., Chaora, N. S., Matle, I., Muchadeyi, F., Mathole, M. A. & Pierneef, R. (2021). Microbial Communities of Meat and Meat Products: An Exploratory Analysis of the Product Quality and Safety at Selected Enterprises in South Africa. *Microorganisms*, 9, 507.

Magwedere, K., Rauff, D., De Klerk, G., Keddy, K. H., & Dziva, F. J. (2015). Incidence of nontyphoidal *Salmonella* in food-producing animals, animal feed, and the associated environment in South Africa, 2012–2014. *Clinical Infectious Diseases, 61*(4), 283–289.

Malorny, B., Hoorfar, J., Bunge, C., & Helmuth, R. (2003). Multicenter validation of the analytical accuracy of *Salmonella* PCR: towards an international standard. *Applied and environmental microbiology*, *69*(1), 290–296.

Maradiaga, M., Miller, M. F., Thompson, L., Pond, A., Gragg, S. E., Echeverry, A., Garcia, L. G., Loneragan, G. H. & Brashears, M. M. (2015). *Salmonella* in Beef and Produce from Honduras. *Journal of Food Protection*, 78(3): 498-502.

Marcus, S. L., Brumell, J. H., Pfeifer, C. G., & Finlay, B. B. (2000). *Salmonella* pathogenicity islands: big virulence in small packages. *Microbes and Infection*, *2*(2), 145-156.

Martínez-Chávez, L., Cabrera-Diaz, E., Pérez-Montaño, J. A., Garay-Martínez, L. E., Varela-Hernández, J. J., Castillo, A., Lucia, L., Ávila-Novoa, M. G., Cardona-López, M. D., Gutiérrez-González, P., & Martínez-Gonzáles, N. E. (2015). Quantitative distribution of *Salmonella spp.* and *Escherichia coli* on beef carcasses and raw beef at retail establishments. *International Journal of Food Microbiology, 210*, 149–155.

McGuirk, S. M., & Peek, S. (2003). Salmonellosis in cattle: A review. *American Association of Bovine Practitioners.* [online] Available at: https://www.vetmed.wisc.edu/dms/fapm/fapmtools/7health/Salmorev.pdf. [Accessed 4 Mar 2018].

Mezali, L., & Hamdi, T. M. (2012). Prevalence and antimicrobial resistance of *Salmonella* isolated from meat and meat products in Algiers (Algeria). *Foodborne Pathogen Disease, 9*(6), 522–529.

Missohou, A., Mbodj, M., Zanga, D., Niang, S., Sylla, K., Seydi, M., Cissé, O., & Seck, S. (2011). Analysis of microbiological and chemical quality of poultry meat in the vicinity of the Mbeubeuss landfill in Malika (Senegal). *Tropical Animal Health & Production, 43*(5), 983–988.

Moawad, A. A., Hotzel, H., Awad, O., Tomaso, H., Neubauer, H., Hafez, H. M., & El-Adawy, H. (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 Pathogens, 9*(1), 57.

Moawad, A. A., Hotzel, H., Neubauer, H., Ehricht, R., Monecke, S., Tomaso, H., Hafez, H. M., Roesler, U., & El-Adawy, H. (2018). Antimicrobial resistance in Enterobacteriaceae from healthy broilers in Egypt: Emergence of colistin-resistant and extended-spectrum β-lactamase producing *Escherichia coli*. *Gut Pathogens, 10*(1), 39.

Molla, B., Alemayehu, D., & Salah, W. (2002). *Sources and distribution of Salmonella serotypes isolated from food animals, slaughterhouse personnel and retail meat products in Ethiopia*. 22, 88.93.

Molla, B., Mohammed, A., & Salah, W. (2004). *Salmonella* prevalence and distribution of serotypes in apparently healthy slaughtered camels (*Camelus dromedarius*) in Eastern Ethiopia. *Tropical Animal Health and Production, 36*(5), 451–458.

Monte, D. & Sellera, F. P. (2020). *Salmonella*. *Emerging Infectious Diseases*, *26*(12), 2955.

Morshdy, A. E. M., Darwish, W. S., El-Dien, W. M. S., & Khalifa, S. (2018). Prevalence of multidrug-resistant *Staphylococcus aureus* and *Salmonella* Enteritidis in meat products retailed in Zagazig City, Egypt. *Veterinary Medicine In-between Health Economy, 55*.

Moussa, I. M., Aleslamboly, Y. S., Al-Arfaj, A. A., Hessain, A. M., Gouda, A. S. & Kamal, R. M. (2013). Molecular characterization of *Salmonella* virulence genes isolated from different sources relevant to human health. *J. Food Agrc. Environ. 11(2):* 197-201.

Mrema, N., Mpuchane, S., & Gashe, B. A. (2006). Prevalence of *Salmonella* in raw minced meat, raw fresh sausages and raw burger patties from retail outlets in Gaborone, Botswana. *Food Control, 17*(3), 207–212.

Mthembu, T. P., Zishiri, O. T., & El Zowalaty, M. E. (2019). Molecular Detection of Multidrug-Resistant *Salmonella* Isolated From Livestock Production Systems In South Africa. *Infection and drug resistance*, *12*, 3537–3548.

Musa, Z., Onyilokwu, S. A., Jauro, S., Yakubu, C., & Musa, J. (2017). Occurrence of *Salmonella* in ruminants and camel meat in Maiduguri, Nigeria and their antibiotic resistant pattern. *Journal of Advanced Veterinary Animal Research, 4*(3), 227–233.

Mwanyika, G. O., Buza, J., Rugumisa, B. T., Luanda, C., Murutu, R., Lyimo, B., Subbiah, M., & Call, D. R. (2016). Recovery and prevalence of antibiotic-resistant *Salmonella* from fresh goat meat in Arusha, Tanzania. *African Journal of Microbiology Research, 10*(32), 1315–1321.

Naidoo S., Basson A.K., Butaye P., Madoroba E. (2021) *Salmonella enterica* Subspecies *enterica* Serotypes Associated with Meat and Meat Products in African Countries: A Review. In: Babalola O.O. (eds) Food Security and Safety. Springer, Cham.

Nair, S., Poh, C. L., Lim, Y. S., Tay, L. & Goh, K. T. (1994) Genome fingerprinting of *Salmonella* Typhi by pulsed-field gel electrophoresis for subtyping common phage types. *Epidemiol Infect.,* 113(3): 391-402.

NARMS. (2015). 2014–2015 retail meat interim report. [online] *FDA U.S. Food and Drug Administration*. [online] Available at: https://wayback.archive-it.org/7993/20190208023629/https://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm498038.htm. [Accessed 15 Aug 2019].

National Institute of Communicable Diseases Outbreak Response (2016). *Salmonella* bacteria and salmonellosis. [online] nicd.ac.za. Available at https://www.nicd.ac.za/assets/files/Salmonellosis_20160114_final%20(1).pdf [Accessed 24 August 2021].

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (2012). Foodborne illnesses. *National Institutes of Health.* [online] Available at: https://www.niddk.nih.gov/health-information/digestive-diseases/foodborne-illnesses [Accessed 2 March 2018].

Niyonzima, E., Ongol, M. P., Brostaux, Y., Koulagenko, N. K., Daube, G., Kimonyo, A., & Sindic, M. (2017). Consumption patterns, bacteriological quality and risk factors for *Salmonella* contamination in meat-based meals consumed outside the home in Kigali, Rwanda. *Journal of Food Control, 73*, 546–554.

Niyonzima, E., Ongol, M. P., Brostaux, Y., Korsak, N., Daube, G., Kimonyo, A., & Sindic, M. (2018). Meat retail conditions within the establishments of Kigali city (Rwanda): Bacteriological quality and risk factors for *Salmonella* occurrence. *Tropical Animal Health & Production, 50*(3), 537–546. https://doi.org/10.1007/s11250-017-1466-6.

Ogu, G. I. & Akinnibosun, F. (2019). Occurrence of *Salmonella* in raw chicken meat from retail equipment and environments in southern Nigeria open markets. *Notulae Scientia Biologicae, 11*(2), 175–182.

Park, S. Y., Pontes, M. H., & Groisman, E. A. (2015). Flagella-independent surface motility in *Salmonella enterica* serovar Typhimurium. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(6), 1850–1855.

Rabie, N. S., Khalifa, N. O., Radwan, M. E., & Afify, J. S. (2012). Epidemiological and molecular studies of *Salmonella* isolates from chicken, chicken meat and human in Toukh, Egypt. *Global Vet, 8*(2), 128–132.

Rahn, K., De Grandis, S. A., Clarke, R. C., McEwen, S. A., Galán, J. E., Ginocchio, C., Curtiss, R., 3rd, & Gyles, C. L. (1992). Amplification of an *inv*A gene sequence of *Salmonella* Typhimurium by polymerase chain reaction as a specific method of detection of *Salmonella*. *Molecular and cellular probes*, *6(4),* 271–279.

Rahman, A., Haque, A., Ahmad, T., Mahmud, S., Sohana, S., & Hossain, R. et al. (2019). Isolation, Identification, and Antibiotic Sensitivity Pattern of *Salmonella* spp. from Locally Isolated Egg Samples. *American Journal of Pure And Applied Biosciences*, 1-11.

Reygaert, W. C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology, 4(3),* 482–501.

RMRDSA (2017). Red Meat Industry: Overview of the Industry. *Red Meat Research and Development South Africa*. [online] Available at: http://www.rmrdsa.co.za/REDMEATINDUSTRY/OverviewoftheIndustry.aspx [Accessed 3 March 2018].

Rodpai, E., Moongkarndi, P., Tungrugsasut, W., Phisannoradej, R., & Kanarat, S. (2013). Comparison of multiplex polymerase chain reaction and immunoassay to detect *Salmonella spp.*, *S.* Typhimurium, and *S.* Enteritidis in Thai chicken meat. *Science Asia, 39*(2), 150–159.

Sakano, C., Kuroda, M., Sekizuka, T., Ishioka, T., Morita, Y., & Ryo, A. (2013). Genetic Analysis of Non-Hydrogen Sulfide-Producing *Salmonella enterica* Serovar Typhimurium and *S. enterica* Serovar Infantis Isolates in Japan. *Journal Of Clinical Microbiology*, *51*(1), 328-330.

Samaxa, R. G., Matsheka, M. I., Mpoloka, S. W., & Gashe, B. A. (2012). Prevalence and antimicrobial susceptibility of *Salmonella* isolated from a variety of raw meat sausages in Gaborone (Botswana) retail stores. *Journal of Food Protection, 75*(4), 637–642.

Sheehan, J. (2017). Why Is Eating Meat Important in Your Diet. *Livestrong*. [online] Available at: https://www.livestrong.com/article/441314-why-is-eating-meat-important-in-your-diet/ [Accessed 2 March 2018].

Shilangale, R. P., Kaaya, G. P. & Chimwamurombe, P. M. (2015). Prevalence and Characterization of *Salmonella* Isolated from Beef in Namibia. *European Journal of Nutrition & Food Safety, 5(4),* 267-274.

Siddiky, N. A., Sarker, M. S., Khan, M., 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.

Singhal, N., Kumar, M., Kanaujia, P. K., & Virdi, J. S. (2015). MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Frontiers in microbiology*, *6*, 791.

Sithole, J. (2021). Zulu culture and cattle symbolism. Southafrica.net [online] Available at: https://www.southafrica.net/za/en/travel/article/zulu-culture-and-cattle-symbolism. [Accessed 8 December 2021].

Smith, S., Braun, S., Akintimehin, F., Fesobi, T., Bamidele, M., & Coker, A. (2016). Serogenotyping and antimicrobial susceptibility testing of *Salmonella* spp. isolated from retail meat samples in Lagos, Nigeria. *Molecular and Cellular Probes, 30*(4), 189–194.

Stevens, A., Kaboré, Y., Perrier-Gros-Claude, J.-D., Millemann, Y., Brisabois, A., Catteau, M., Cavin, J. F., & Dufour, B. (2006). Prevalence and antibiotic-resistance of *Salmonella* isolated from

beef sampled from the slaughterhouse and from retailers in Dakar (Senegal). *International Journal of Food Microbiology, 110*(2), 178–186.

Tafida, S., Kabir, J., Kwaga, J., Bello, M., Umoh, V., Yakubu, S., Nok, A. J., & Hendriksen, R. (2013). Occurrence of *Salmonella* in retail beef and related meat products in Zaria, Nigeria. *Journal of Food Control, 32*(1), 119–124.

Tarabees, R., Elsayed, M. S., Shawish, R., Basiouni, S., & Shehata, A. A. (2017). Isolation and characterization of *Salmonella* Enteritidis and *Salmonella* Typhimurium from chicken meat in Egypt. *The Journal of Infection in Developing Countries, 11*(04), 314–319.

The Poultry Site. (2011). *Strategies to control Salmonella in poultry*. [online] Thepoultrysite.com. Available at: https://thepoultrysite.com/articles/strategies-to-control-salmonella-in-poultry. [Accessed 5 Aug 2019].

Theuß, T., Woitow, G., Bulang, M., & Springer, S. (2018). Demonstration of the efficacy of a *Salmonella* Enteritidis live vaccine for chickens according to the current European Pharmacopoeia Monograph. *Heliyon, 4*(12).

Thong, K. L., Lai, W. L. & Dhanoa, A. (2011). Antimicrobial susceptibility and pulsed – Field Gel Electrophoretic analysis of *Salmonella* in a tertiary hospital in northern Malaysia. *Journal of Infection and Public Health*, 4(2): 65-72.

Thung, T. Y., Mahyudin, N. A., Basri, D. F., Wan Mohamed Radzi, C. W. J., Nakaguchi, Y., Nishibuchi, M., & Radu, S. (2016). Prevalence and antibiotic resistance of *Salmonella* Enteritidis and *Salmonella* Typhimurium in raw chicken meat at retail markets in Malaysia. *Poultry Science, 95*(8), 1888–1893.

Thung, T., Radu, S., Mahyudin, N., Rukayadi, Y., Zakaria, Z., & Mazlan, N. et al. (2018). Prevalence, Virulence Genes and Antimicrobial Resistance Profiles of *Salmonella* Serovars from Retail Beef in Selangor, Malaysia. *Frontiers In Microbiology*, *8*.

Tripathi, N. & Sapra, A. (2021). Gram Staining. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available at: https://www.ncbi.nlm.nih.gov/books/NBK562156/.

Uche, I. V., MacLennan, C. A., & Saul, A. (2017). A systematic review of the incidence, risk factors and case fatality rates of invasive nontyphoidal *Salmonella* (iNTS) disease in Africa (1966 to 2014). *PLoS Neglected Tropical Diseases, 11*(1).

UNDP. (2017). *Transforming Ethiopia's livestock sector.* [online] UNDP in Ethiopia. [online] Available at: https://www.et.undp.org/content/ethiopia/en/home/presscenter/articles/2017/05/12/08/transforming-ethiopia-s-livestock-sector-.html. [Accessed 13 Aug 2019].

Valenzuela, J. R., Sethi, A. K., Aulik, N. A.., & Poulsen, K. P. (2017). Antimicrobial resistance patterns of bovine *Salmonella enterica* isolates submitted to the Wisconsin Veterinary Diagnostic Laboratory: 2006–2015. *Journal of Dairy Science*, *100*(2), 1319-1330.

van Heerden, S. M. & Morey, L. (2014). Nutrient content of South African C2 beef offal. *Food Measure, 8*, 249-258.

Varma, J. K., Greene, K. D., Ovitt, J., Barrett, T. J., Medalla, F., & Angulo, F. J. (2005). Hospitalization and Antimicrobial Resistance in *Salmonella* Outbreaks, 1984–2002. *Emerging Infectious Diseases, 11*(6), 943–946.

Wabeto, W., Abraham, Y., & Anjulo, A. A. (2017). Detection and identification of antimicrobial-resistant *Salmonella* in raw beef at Wolaita Sodo municipal abattoir, Southern Ethiopia. *Journal of Health, Population & Nutrition, 36*, 1–7.

Wang, B., Wang, C., McKean, J. D., Logue, C. M., Gebreyes, W. A., Tivendale, K. A., & O'Connor, A. M. (2011). *Salmonella enterica* in Swine Production: Assessing the Association between Amplified Fragment Length Polymorphism and Epidemiological Units of Concern. *Applied And Environmental Microbiology*, *77*(22), 8080-8087.

Wang, X., Jordan, I., & Mayer, L. (2015). A Phylogenetic Perspective on Molecular Epidemiology. *Molecular Medical Microbiology*, 517-536.

Wattiau, P., Boland, C. & Bertrand, S. (2011). Methodologies for *Salmonella enterica* subsp. *enterica* subtyping: gold standards and alternatives. *Appl Environ Microbiol,* 77: 7877–7885.

Welker, M., Fastner, J., Erhard, M. & von Döhren, H. (2002). Applications of MALDI-TOF MS Analysis in Cyanotoxin Research. *Environmental toxicology, 17(4),* 367-74.

Wyness, L. (2016). The role of red meat in the diet: Nutrition and health benefits. *Proceedings of the Nutrition Society,* 75(3), 227-232.

WHO (2015). WHO's first ever global estimates of foodborne diseases find children under 5 account for almost one third of deaths. *World Health Organisation*. [online] Available at:

http://www.who.int/mediacentre/news/releases/2015/foodborne-disease-estimates/en/ [Accessed 1 March 2018].

WHO (2018). *Salmonella (Non-Typhoidal)*. [online] Available at: https://www.who.int/newsroom/fact-sheets/detail/salmonella-(non-typhoidal) [Accessed 13 August 2019].

Yang, B., Qu, D., Zhang, X., Shen, J., Cui, S., Shi, Y., Xi, M., Sheng, M., Zhi, S., & Meng, J. (2010). Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China. *International Journal of Food Microbiology, 141*(1–2), 63–72.

Zghair, Z. R. (2012). Histopathological study of *Salmonella* Typhimurium infection in laboratory mice by using the light and electron microscope. *Kufa Journal for Veterinary Medical Sciences*, *3(1).*

Zhao, C., Ge, B., De Villena, J., Sudler, R., Yeh, E., Zhao, S. & Meng, J. (2001) Prevalence of *Campylobacter spp.*, *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, DC, area. *Applied and environmental microbiology*, 67(12), 5431-5436.

Appendix

7.1. Ethical clearance letter

UNIVERSITY OF ZULULAND
RESEARCH ETHICS COMMITTEE
(Reg No: UZREC 171110-030)

RESEARCH & INNOVATION
Website: http://www.unizulu.ac.za
Private Bag X1001
KwaDlangezwa 3886
Tel: 035 902 6324/6374
Email: MangeleS@unizulu.ac.za/
MkwanaziMM@unizulu.ac.za

ETHICAL CLEARANCE CERTIFICATE

Certificate Number	UZREC 171110-030 PGM 2019/109							
Project Title	Prevalence, Virulence Factors and Antimicrobial Resistance of Salmonella species from Beef in Retail Outlets from KwaZulu-Natal							
Principal Researcher/ Investigator	S Naidoo							
Supervisor and Co-supervisor	Prof E Madoroba				Prof A.K Basson Prof P Butaye			
Department	Agriculture							
Faculty	Science and Agriculture							
Type of Risk	Low Risk – Desktop, fieldwork, or laboratory							
Nature of Project	Honours/4th Year		Master's	x	Doctoral		Departmental	

The University of Zululand's Research Ethics Committee (UZREC) hereby gives ethical renewal approval in respect of the undertakings contained in the above-mentioned project. This approval is extended for another 1 year. The Researcher may therefore continue with data collection as from the date of this Certificate, using the certificate number indicated above.

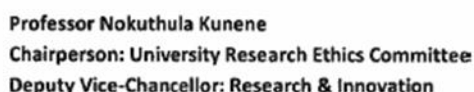
Special conditions:

(1) This certificate is valid for 1 year from the date of issue.

(2) Principal researcher must provide an annual report to the UZREC in the prescribed format [due date- 12 October 2022]

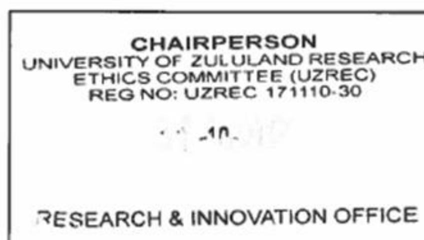
(3) The UZREC must be informed immediately of any material change in the conditions or undertakings mentioned in the documents that were presented to the meeting.

The UZREC wishes the researcher well in conducting research.



Professor Nokuthula Kunene
Chairperson: University Research Ethics Committee
Deputy Vice-Chancellor: Research & Innovation

12 October 2021



7. 2 Book Chapter, cited as:

Naidoo S., Basson A.K., Butaye P., Madoroba E. (2021) *Salmonella enterica* Subspecies *enterica* Serotypes Associated with Meat and Meat Products in African Countries: A Review. In: Babalola O.O. (eds) Food Security and Safety. Springer, Cham. https://doi.org/10.1007/978-3-030-50672-8_38

Chapter 38
Salmonella enterica Subspecies *enterica* Serotypes Associated with Meat and Meat Products in African Countries: A Review

Serisha Naidoo, Albertus Kotze Basson, Patrick Butaye, and Evelyn Madoroba

Abstract Non-typhoid *Salmonella enterica* subspecies *enterica* serotypes are causative agents of foodborne infections in developed and developing countries. The incidence of non-typhoid salmonellosis is reported to be highest in African countries. Non-typhoid *Salmonella* infections in humans are mainly reported in children, elderly and immunocompromised individuals, as they can cause more serious infections in those groups. Virulence factors present in the serotypes are vital in the pathogenesis of each serotype in their ability of causing salmonellosis. The predominant origin of non-typhoid *Salmonella* infection is animal-derived foods such as undercooked meat, eggs and raw milk. Antimicrobial resistance among *Salmonella* is of global concern. The epidemiology of *Salmonella* antimicrobial resistance is complicated, serotype dependent and is yet to be entirely understood. This review discussed the prevalence and traits of non-typhoid *Salmonella* serotypes from meat and related meat products in African countries compared to the situation in the other continents, and evaluated the extent of resistance against antimicrobials by non-typhoid *Salmonella* recovered from humans in African countries in comparison to the rest of the world. We also highlighted gaps in theoretical and practical knowledge of *Salmonella enterica* and proposed possible future research areas.

Keywords *Salmonella* · Africa · Prevalence · Serotypes · Antimicrobial resistance · Meat and meat products

S. Naidoo · A. K. Basson · E. Madoroba (✉)
Department of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa, South Africa
e-mail: MadorobaE@unizulu.ac.za

P. Butaye
Department of Biomedical Sciences, Ross University School of Veterinary Medicine, Basseterre, ST Kitts and Nevis

Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan, Merelbeke, Belgium

© Springer Nature Switzerland AG 2021
O. O. Babalola (ed.), *Food Security and Safety*,
https://doi.org/10.1007/978-3-030-50672-8_38

7.3. Examples of types of samples that were analysed in this study

Figure 7A: Image showing a sample of ox intestines

Figure 7B: Sealed plastic bag containing a 25 g homogenized sample in 225 ml buffered peptone water.

7.4. Examples of media that were used for analysis

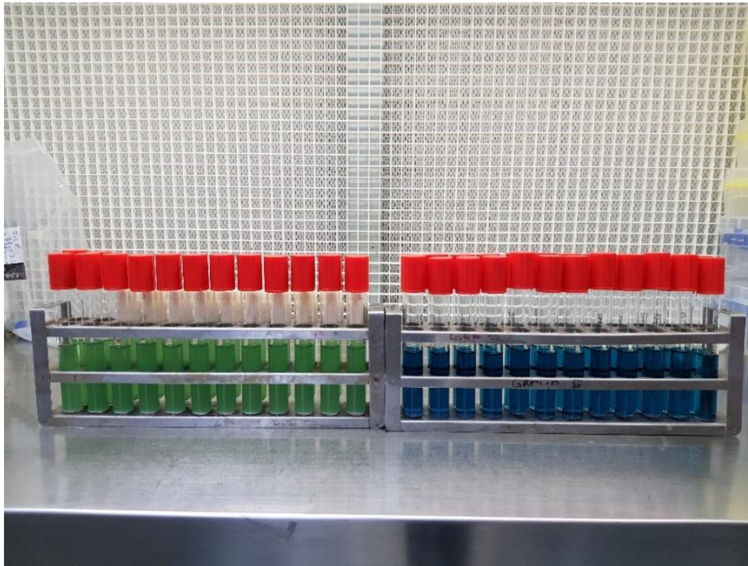


Figure 7C: Test tubes containing 10 ml each of MKTTn broth (left) and RVS broth (right)

7.5. Example of purified *Salmonella* isolates

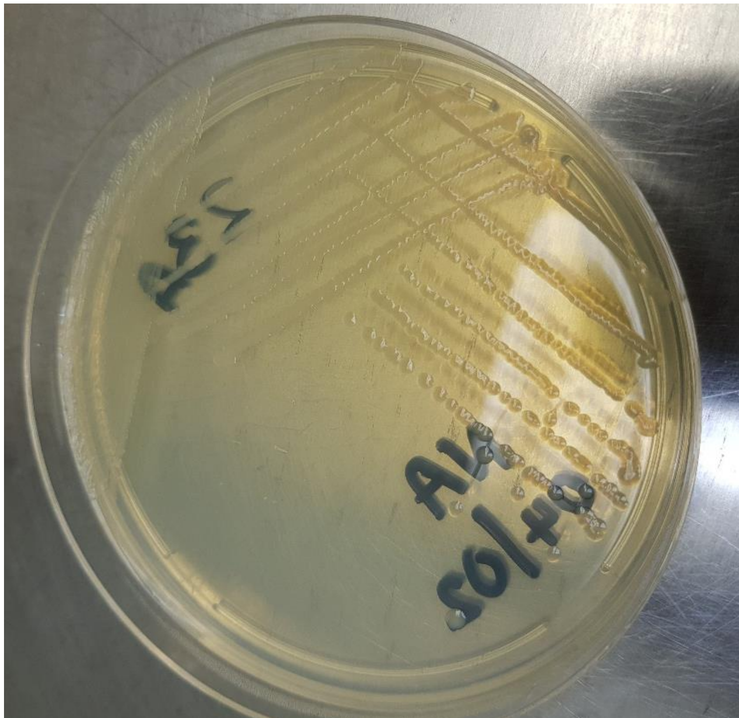


Figure 7D: Example of a pure presumptive *Salmonella* isolate streaked on nutrient agar

Table 7.1: Antimicrobial susceptibility results of *Salmonella enterica* subsp. *enterica* serovar (determined by serotyping) isolates against 10 antimicrobials

Sample code	**Serovar**	**Antimicrobial Susceptibility**									
		A	AP	FOX	CTX	C	CIP	GM	K	TET	TS
151A	**Stanley**	S	S	S*	S*	S	S	S*	S*	S	S
151B	**Stanley**	S	S	S*	S*	S	S	S*	S*	S	S
152A	**Heidelberg**	S	S	S*	S*	S	S	S*	S*	S	S
164C	**Hadar**	S	S	S*	S*	S	S	S*	S*	S	S
237A	**Enteritidis**	R	R	S*	S*	S	S	S*	S*	R	S
331A	**Stanley**	S	S	S*	S*	S	S	S*	S*	S	S

* Isolates appear as susceptible *in-vitro* but may be resistant *in-vivo*

Constitution and preparation of reagents and media

Buffered peptone water

Supplier: HiMedia Laboratories

Reference: M614-500g

Ingredients (g/litre):

Proteose peptone	10.00
Sodium chloride	5.00
Disodium hydrogen phosphate	3.50
Potassium dihydrogen phosphate	1.50

Final pH (at 25 °C) 7.2 ± 0.2

Preparation: Suspend 20.0 g in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense into suitable bottles as required. Sterilize by autoclaving at 121 °C for 15 minutes.

Rappaport Vassiliadis Soya (RVS) broth

Supplier: HiMedia Laboratories

Reference: M1491-500G

Ingredients (g/litre):

Soya peptone	4.50
Sodium chloride	8.00
Potassium dihydrogen phosphate	0.60
Dipotassium hydrogen phosphate	0.40
Magnesium chloride hexahydrate	29.00
Malachite green	0.036

Final pH (at 25 °C) 5.2 ± 0.2

Preparation: Suspend 27.11 g of dehydrated medium in 1000 ml purified/distilled water. Heat gently if necessary to dissolve the medium completely. Dispense as desired into tubes and sterilize by autoclaving at 115 °C for 15 minutes.

Mueller Kauffman Tetrathionate Novobiocin (MKTTn) broth base

Supplier: HiMedia Laboratories

Reference: M1496I-500G

Ingredients (g/litre):

Peptone	4.30
Tryptone	8.60
Bile	4.75
Sodium chloride	2.60
Calcium carbonate	38.70
Sodium thiosulphate pentahydrate	47.80
Brilliant green	0.0095

Final pH (at 25 °C) 8.2 ± 0.2

Preparation: Suspend 89.42 g (equivalent weight of dehydrated medium per litre) in 1000 ml purified/ distilled water. Heat the medium just to boiling. DO NOT AUTOCLAVE. Cool to 45–50 °C and just before use aseptically add rehydrated contents of 1 vial of MKTT Novobiocin Supplement (FD203) and 20 ml of iodine-iodide solution (20 g iodine and 25 g potassium iodide in 100 ml sterile distilled water). Mix well to disperse calcium carbonate uniformly before dispensing in sterile tubes.

MKTT Novobiocin Supplement

Supplier: HiMedia Laboratories

Reference: FD203

Ingredients (g/litre):	(1 vial)
Novobiocin	0.040

Preparation: Rehydrate contents of 1 vial aseptically with 5 ml of sterile distilled water and aseptically add to sterile, cooled (45–50 ºC) Mueller Kauffman Tetrathionate Novobiocin Broth Base (M1496I). Mix well and dispense as desired.

Xylose-Lysine Deoxycholate (XLD) agar

Supplier: HiMedia Laboratories

Reference: M031-500G

Ingredients (g/litre):

Yeast extract	3.00
L-Lysine	5.00
Lactose	7.50
Sucrose	7.50
Xylose	3.50
Sodium chloride	5.00
Sodium deoxycholate	2.50
Sodium thiosulphate	6.80
Ferric ammonium citrate	0.80
Phenol red	0.08
Agar	15.00

Final pH (at 25 °C) 7.4 ± 0.2

Preparation: Suspend 56.68 g in 1000 ml purified / distilled water. Heat with frequent agitation until the medium boils. DO NOT AUTOCLAVE OR OVERHEAT. Transfer immediately to a water bath at 50 °C. After cooling, pour into sterile Petri plates. It is advisable not to prepare large volumes that will require prolonged heating, thereby producing precipitate.

Brilliant green agar (BGA) base, modified

Supplier: HiMedia Laboratories

Reference: M016-500G

Ingredients (g/litre):

Proteose peptone	10.00
Yeast extract	3.00
Lactose	10.00
Saccharose (Sucrose)	10.00
Sodium chloride	5.00
Phenol red	0.08
Brilliant green	0.0125

Final pH (at 25°C) 6.9 ± 0.2

Preparation: Suspend 29.0 g in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121 °C) for 15 minutes. AVOID OVERHEATING. Cool to 45 50 °C.

Nutrient agar

Supplier: HiMedia Laboratories

Reference: M001-500G

Ingredients (g/litre):

Peptone	5.00
Sodium chloride	5.00
HM peptone B	1.50
Yeast extract	1.50

Agar	15.00

Final pH (at 25 °C) 7.4 ± 0.2

Preparation: Suspend 28.0 g in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121 °C) for 15 minutes. Cool to 45–50 °C. If desired, the medium can be enriched with 5–10% blood or other biological fluids. Mix well and pour into sterile Petri plates