MOLECULAR CHARACTERIZATION OF *SALMONELLA* AND *SALMONELLA* SPECIFIC BACTERIOPHAGES FROM CATTLE AND BEEF IN THE NORTH WEST PROVINCE SOUTH AFRICA

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DECLARATION

I declare that, the dissertation for the Degree Master of Science in Biology at the North West University-Mafikeng Campus hereby submitted, has not been submitted by me for a degree at this or any other university. This is my own work in design and execution and that all material contained herein has been duly acknowledged.

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DEDICATION

I dedicate this work to my family and friends for their unwavering love, support and encouragement.

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ABSTRACT

Salmonella species are currently one of the most widespread foodborne pathogens that claim millions of lives yearly nationwide and therefore they pose severe challenges in humans in developing as well as developed countries. In addition, *Salmonella* infections are difficult to treat due to the emergence and wide spread distribution of antibiotic resistant strains. Treatment failures amplify the need to explore the virulence capabilities of other antibacterial agents such as bacteriophages to serve as alternative antimicrobial agents, especially in resolving the war against resistant bacteria strains. The aim of this study was to isolate, characterise and determine the virulence capabilities of *Salmonella* species and *Salmonella* specific bacteriophages from cattle faeces and raw beef obtained from some supermarkets and butcheries in the North West Province, South Africa against environmental antibiotic resistant isolates.

A total of 300 presumptive *Salmonella* isolates were isolated from 160 samples (cattle faeces and raw beef) using *Salmonella Shigella* agar. Typical pale yellow colonies with black spots on their centres were subjected to both preliminary and confirmatory tests specific for *Salmonella* species. The antibiotic resistance profiles of the *Salmonella* strains were determined against a panel of 12 antimicrobial agents. Multiple antibiotic resistance phenotypes and cluster analysis of antibiotic resistance inhibition zone diameter data was used to determine the phenotypic relationship of isolates from different sources. PCR assays were used to determine the pathogenicity of the isolates in order to assess their impact on consumers. Genetic similarities among isolates from different sampling stations and/or sources were assessed by determining the restriction

fragment length polymorphic (RFLP) patterns of the 16S rRNA gene fragments amplified from isolates in the study. Furthermore, morphological characterisation of *Salmonella* specific bacteriophages was achieved using the double agar layer technique and electron microscopy. The findings of the study suggest a high presence 140 (46%) of *Salmonella* species in both cattle faeces and raw beef obtained from the different locations.

All the isolates were Gram negative rods while a significantly large proportion 291 (97%) were oxidase negative and only 12 (4%) of the isolates produced H_2S. Eight (34.8%) representative isolates were identified as *Salmonella* species. Of the 300 presumptive isolates a large proportion 159 (53%) were positive for *Salmonella* based on agglutination with the polyvalent O antiserum and belonged to groups A-G, while 185 (61.7%) were positive for the polyvalent H antiserum. *Salmonella* species specific 16S rRNA PCR analysis indicated that 128 (42.7%) were positively identified. Despite the fact that only a small proportion 60 (20%) of the isolates were confirmed as *Salmonella* species based on the presence of the *fliC* gene sequence while 80 (26.7%) had the *fli*B gene fragment.

Large proportions (62.4% to 94.3%) of the isolates from both cattle faeces and beef samples were most often resistant to Erythromycin, Rifampicin, Penicillin, Ampicillin and Cephalexin. On the contrary, these isolates showed very little resistance (4.3% to 37.9%) against Cefixime, Nalidixic Acid, Gentamicin, Ciprofloxacin, Tetracycline, Norfloxacin and Chloramphenicol. MAR phenotypes E-RP-PG-CFXM, AP-E-RP-PG-

CFXM, RP-PG-CFXM, AP-CFM-E-RP-PG-CFXM, T-E-RP-PG-CFXM and AP-CFM-T-E-RP-PG-CFXM were dominant among these isolates. On the other hand, phenotypes AP-CFM-NA-CIP-NOR-E-RP-PG, E-RP-PG-CFXM and T-E-RP-PG-CFXM were predominant among isolates obtained from Rustenburg. Despite the fact that phenotype AP-CFM-T-E-RP-PG-CFXM was observed only in 1 isolate obtained from Mafikeng, a cause for concern is that this isolate was resistant to 7 of the 12 antibiotics tested. In addition the AP-CFM-NA-CIP-NOR-E-RP-PG was not only dominant among isolates from Rustenburg but the isolates were resistant to eight different antimicrobial agents. Two major clusters (cluster 1 and cluster 2) that contained four sub-clusters (1A, 1B, 2A and 2B) were obtained. Generally three sub-clusters (1A, 1B and 2A) were mixed since they had isolates from almost all the different sites sampled.

Large proportions 38 (27.1%) and 46 (30.7%) were positive for the *spv*C and *inv*A virulence genes respectively. RFLP patterns of 16S rRNA gene fragments indicated that the band sizes ranged from 50 bp and 572 bp and from 50 bp to 300 bp for *Eco*RI and *Hae*III respectively. The great similarities in the antibiotic resistance profiles of *Salmonella* isolates from the different locations indicated that isolates share similarly antibiotic exposure histories. In addition, the similarities in the RFLP patterns of 16S rRNA gene fragments also revealed that the data may be of great epidemiological importance and therefore be very useful in identifying the source of contamination.

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LIST OF ABBREVIATIONS AND SYMBOLS

ATCC	American Type Culture Collection
API	Analytical Profile Index
bla	Beta-lactamase
Blast	Basic Alignment Search Tool
CTX-M	Cefotaxime München
EDTA	Ethylenediamine-tetraacetic acid
PCR-RFLP	Restriction Fragment Length Polymorphism
ESBL	Extended Spectrum Beta-lactamase
mL	milliliter(s)
mM	millimeter(s)
NCBI	National Center for Biotechnology Information
NCTC	National Collection Type Culture
OXA	Oxacillin
SVH	Sulfhydryl variable
TAE	Tris acetic acid EDTA buffer
TEM	Temoniera
µg	microgram(s)
µm	micrometer(s)
w/v	weight per volume
v/v	volume per volum

DEFINITION OF CONCERPTS

Antibiotic resistance: is the ability of a given bacteria to survive the exposure to a defined concentration of an antimicrobial agent.

Bacteriophage: a virus that is parasitic (reproduces itself) in bacteria.

Cluster analysis: A comparative analysis of typing data collected for a variety of bacterial isolates in order to group them based on the similarity of their data.

Restriction Fragment Length Polymorphism: A DNA fingerprinting that used different restriction enzymes to produce a specific banding pattern to determine genetic similarities of different bacteria strains.

Fingerprint: specific banding pattern displayed by an isolates on application of one or more typing method.

Plasmid: is a circular DNA molecule that can replicate independently from the chromosome and promote lateral transfer among different species of bacteria through the conjugation process.

Polymerase Chain Reaction: is a molecular method that is used to amplify specific regions of DNA many times over using primers.

Phylogeny: the process in which lineage of organisms evolved by separation from common ancestors

Species: collection of bacterial cells which share an overall similar pattern of traits in contrast other bacteria whose pattern differ significantly.

Typing: A phenotypic and/or genetic analysis of bacterial isolates below the species level that is employed to generate strain specific fingerprints that could be used in investigating cross contaminations, transmission patterns and/or sources of infections in humans or consumers of a particular food product.

CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

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1.1 GENERAL INTRODUCTION

Salmonella is a rod-shaped and Gram negative bacteria within the family Enterobacteriaceae (Specimens, 2015). *Salmonella* species and subspecies are found almost everywhere in the environment, hence they easily infect humans and animals (Su and Chiu, 2007). The natural hosts of *Salmonella* species are humans and warm blooded animals, and they reside as normal flora in their hosts gastrointestinal tract (Todar, 2005). However, a number of *Salmonella* species have been associated with disease in humans and these strains are currently known to cause disease especially salmonellosis in humans and animals globally (Huruy *et al.*, 2011; Moreno Switt *et al.*, 2013). *Salmonella* species are therefore considered to be one of the most widespread foodborne pathogens that are responsible for high mortality amongst humans in both developing and developed countries (Graham, 2002; Molla *et al.*, 2003; Zamxaka *et al.*, 2004; Akhtar *et al.*, 2014).

Salmonella species have the potential to cause zoonotic diseases in humans and most outbreaks have been associated with consumption of contaminated food products (Lynch *et al.*, 2009; Friesema *et al.*, 2012). Moreover, infections caused by *Salmonella* species present severe life threatening complications in infants, elderly people, and immune compromised individuals (Gordon *et al.*, 2008; Morpeth *et al.*, 2009). *Salmonella* species may be harboured by various animal species including food producing animals such as cattle, poultry and swine (Giorgio, 2000; Sillankorva *et al.*,

2012). Wild birds and rodents have also been reported to serve as reservoirs for *Salmonella* species although they are currently not considered to be potential source for human infections (Van Der Walt *et al.*, 1997).

The presence of pathogenic *Salmonella* strains in food producing animals suggests that these organisms may be transmitted to the carcass and raw food products if proper hygiene measures are not implemented during preparation and processing of the products (Akhtar *et al.*, 2014). The health risks associated with these pathogens are high in communities where individuals rely on ready to eat food products especially if the food products were contaminated and undercooked (Rocourt *et al.*, 2003). *Salmonella* species are known to cause infections in humans that range from simple diarrhoea, fever, headache, body aches and vomiting (Giorgio, 2000) to more complicated reactive arthritis (Leirisalo-Repo *et al.*, 1997) and focal infections (Cohen *et al.*, 1987; Acheson and Hohmann, 2001).

The world population is growing rapidly and the demand for food including meat is on the rise (Vasil, 1998; Flachowsky *et al.*, 2005). To meet the expectations of consumers food products must be free of pathogenic microorganisms and therefore should be safe (Akhtar *et al.*, 2014). However, increased demand may introduce severe pressure on production processes and therefore provide opportunities for the introduction of pathogenic organisms including *Salmonella* species into the food chain. *Salmonella* species have been reported to cause severe human infections even in countries such as the UK, Canada and the USA that have advanced public health and health care

systems (Majowicz *et al.*, 2010). Despite this, currently there's no documented information on the occurrence and virulence profiles of these pathogens in animals and their corresponding food products in the North West Province in South Africa and therefore this study was designed to generate such base line data.

1.2 PROBLEM STATEMENT

The emergence of antimicrobial resistant *Salmonella* species has become a norm in most developing and developed countries (Threlfall, 2002; Akond *et al.*, 2013). Against this backdrop, human medicine and the food industry are challenged every day by the presence of multiple antibiotic resistant bacteria (White *et al.*, 2002; Delicato *et al.*, 2004). In addition, the use of antimicrobial agents in the treatment of infections in food producing animals or as prophylaxis and growth stimulants have been identified as potential sources that promote the constant development of antimicrobial resistant determinants among bacterial species (Aarestrup, 1999; Forshell and Wierup, 2006). Antimicrobial resistant pathogens may be transmitted to humans through the consumption of contaminated food products, especially if its undercooked (White *et al.*, 2001). Antimicrobial resistant *Salmonella* species are therefore of great public health concern and they are some of the most common foodborne pathogens that pose a severe challenge to human medicine worldwide (Van Den Bogaard and Stobberingh, 2000; White *et al.*, 2009). The presence of drug resistant *Salmonella* strains in food products in a given area may strongly suggest the need to implement strict control measures that will prevent cross contamination with consumers (Lu and Koeris, 2011).

This may not only reduce drug treatment failure rates among humans especially in the given area but may be of great epidemiological significance (Lu and Koeris, 2011).

Given the challenges faced with the management of antimicrobial resistant strains that infect humans through contaminated food products, bacteriophages have shown some potential as alternative biological means for destroying or reducing the level of resistant bacteria strains in food products (Alisky *et al.*, 1998; Garcia *et al.*, 2008). Food safety regulations are designed to ensure that processing plants operate under certain guidelines that ensure that food products are free of pathogenic microbes or that the microbial content in the finished food product is within acceptable limits for it to be considered safe for human consumption. Unfortunately, in some food production facilities standard operating procedures might not be followed strictly by employees at all times and this may pose a serious threat to public health (Delicato *et al.*, 2004).

However, given the constant rise in antibiotic resistant bacteria strains, bacteriophages have also been reported to significantly reduce the level of bacterial contamination in carcasses at both pre and post-slaughter stages in food processing plants such as abattoirs (Hudson *et al.*, 2005). The use of bacteriophages is based on certain advantages they have and these include the fact that they reside in the same environment as their bacterial hosts hence are easy and cheap to isolate (Sillankorva *et al.*, 2012). These advantages provide a cheaper approach that may be more effective in addressing the problems caused by antibiotic resistant bacterial strains that claim millions of human lives globally.

To the best of our knowledge, there is no data in which *Salmonella* species and their specific bacteriophages have been isolated from both their specific animal hosts and their associated raw meat products in South Africa and the North West Province in particular. The present study is therefore designed to isolate, characterize and determine the virulence capabilities of *Salmonella* and *Salmonella* specific bacteriophages from cattle faeces and raw beef in the North West Province, South Africa against environmental antibiotic resistant isolates. Data generated from the current study may be of epidemiological importance. In addition the data may also provide options for biological control of *Salmonella* species in the food production industry.

1.3 RESEARCH AIM AND OBJECTIVES

1.3.1 AIM

The aim of the current study was to isolate, characterise and determine the virulence capabilities of *Salmonella* and *Salmonella* specific bacteriophages from cattle faeces and raw beef obtained from some supermarkets and butcheries in the North West Province, South Africa against environmental antibiotic resistant isolates.

1.3.2 OBJECTIVES

The objectives of the study were to:

- isolate *Salmonella* strains from cattle faecal and raw beef samples

- confirm the identities of *Salmonella* isolates using preliminary (Gram-stain, oxidase test, substrates fermentation, TSI test) and confirmatory (API 20E, serotyping, *Salmonella* specific PCR) identification tests
- determine the antibiotic resistance profiles against a panel of 12 antimicrobial agents
- screen isolates for the presence of *Salmonella* specific virulence genes
- determine the genetic similarities of *Salmonella* isolates using PCR-RFLP
- isolate *Salmonella* specific bacteriophages from cattle faecal samples using both control and environmental strains
- determine the plaque morphology of the bacteriophages
- evaluate the lytic capabilities of the bacteriophages against environmental *Salmonella* isolates.

CHAPTER 2

LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 Background

Salmonella species belong to the family *Enterobacteriaceae* and they are rod shaped, non-spore forming and Gram negative bacteria (Specimens, 2015). Individual cells are most often motile; range from 0.7 to 1.5 µm in diameters and usually possess peritrichous flagella (Fàbrega and Vila, 2013). The genus *Salmonella* is made of two species that include *Salmonella enterica* and *Salmonella bongori* (Brenner *et al.*, 2000; Gillespie and Hawkey, 2006; Su and Chiu, 2007). *Salmonella enterica* is further divided into six subspecies viz *enterica, salamae, diarizonae, arizonae, indica* and *houtenae* and more than 250 serovars (Brenner *et al.*, 2000; Gillespie and Hawkey, 2006; Su and Chiu, 2007). These different *Salmonella* species and subspecies are found almost everywhere in the environment and are well known to infect both humans and animals (Su and Chiu, 2007).

Salmonella strains cause illnesses ranging from mild food poisoning to typhoid fever and paratyphoid fever in humans (Ryan and Ray, 2004). Differences in host preferences and the potential of *Salmonella* species to produce diseases with very specific clinical signs in humans have led to clinical categorisation of these pathogens (Okoro *et al.*, 2012). *Salmonella* species are therefore classified as invasive or non-invasive strains depending on the mechanism in which disease is manifest (Okoro *et al.*,

2012). *Salmonella* infections in humans usually occur through the consumption of contaminated food products but the manifestation of disease greatly depends on host-bacterial interaction and therefore the severity of the disease is based largely on the degree of susceptibility of the host (Gordon *et al.*, 2008; Feasey *et al.*, 2012).

Salmonella species occur as normal flora in the GIT of animals and humans and their presence in the environment may result through uncontrolled release of faeces (Bicudo and Goyal, 2003; Callaway *et al.*, 2005). *Salmonella* species are found almost everywhere in the environment and this increases the chance of food contamination (Sillankorva *et al.*, 2012; Akhtar *et al.*, 2014). It has been reported that *Salmonella* strains can easily contaminate meat products during processing if proper hygiene measures are not fully implemented in the facility (Bicudo and Goyal, 2003; Sillankorva *et al.*, 2012). With this in mind, *Salmonella* species have been isolated from different food products that include raw meat, poultry, seafood, raw eggs, fruits and vegetables and these have been reported to serve as potential sources for human infections (Authority, 2011; Sillankorva *et al.*, 2012). Even though food processing plants have standard hygiene guidelines to follow in order to minimize contamination, it is still difficult to totally eliminate contamination (Rocourt *et al.*, 2003). It is therefore important to constantly monitor the level of contaminants in the raw food products and in food processing plants. This may provide an indication of the health risks associated with the consumption of these products. In addition, there is a need to implement strict control measures to improve food quality and to limit foodborne infections especially in developing countries like South Africa (Sillankorva *et al.*, 2012; Akhtar *et al.*, 2014).

A number of strategies are currently being utilized during food production and preparation in order to reduce contamination and ensure food safety (Gorris, 2005; Biran *et al.*, 2012). However, these control strategies are challenged by changes in human lifestyle, demand and the protocols that guide the international trade of food products (Rocourt *et al.*, 2003). Moreover, the emergence of antibiotic resistant bacteria strains including *Salmonella* species also presents a huge challenge to both the food production industry and the medical profession (Van Den Bogaard and Stobberingh, 2000; White *et al.*, 2009; Lu and Koeris, 2011). There is a need for more effective control strategies to be implemented during food processing or production in order to ensure improved food quality with reduced foodborne infection risks (Holah *et al.*, 2002; Buncic and Sofos, 2012).

Recently, bacteriophages have been considered as potential antimicrobial agents for different bacterial pathogens (Alisky *et al.*, 1998; Callaway *et al.*, 2008; Shin *et al.*, 2012). Bacteriophages have a lot of advantages over conventional antimicrobial agents since they are host specific, self-replicating and self-limiting, low inherent toxicity to their hosts and they are able to prolong the shelf life of food products (Sillankorva *et al.*, 2012). In addition, bacteriophages are easy to isolate since they are usually found in the same environment as their bacterial hosts (Sillankorva *et al.*, 2012) and this makes the approach very cheap (Calci *et al.*, 1998; Hsu *et al.*, 2002; Atterbury *et al.*, 2003). They have been isolated from animal faeces (Calci *et al.*, 1998), raw products (Hsu *et al.*,

2002; Atterbury *et al.*, 2003), fermented products (Suárez *et al.*, 2002), processed products (Kennedy Jr *et al.*, 1986), and seafood (Croci *et al.*, 2000).

Bacteriophages have been used to reduce the level of contamination or cross contamination in the food industry and as an indicator tool to detect pathogenic bacteria (Abuladze *et al.*, 2008; Garcia *et al.*, 2008; Callaway *et al.*, 2010; Sillankorva *et al.*, 2012). The utilization of bacteriophages during the growth stage in animals or prior to slaughter and on the carcass after slaughter have been reported to significantly reduce microbial loads (Hudson *et al.*, 2005; Sillankorva *et al.*, 2012). Moreover, bacteriophages have also been used as biopreservative and biosanitazation agents in the food chain (Greer, 1988; Sillankorva *et al.*, 2012). The capabilities of bacteriophages to kill multi-drug resistant bacteria have attracted a lot of attention worldwide in recent years (Osayande, 2014). This has resulted in more research that focus on the use of bacteriophages as a potential biocontrol agents against resistant bacteria (Osayande, 2014). Any success made may greatly assist in addressing issues of antibiotic resistance that are facing mankind.

2.2. Epidemiology

2.2.1. *Salmonella* species in animals, particularly food producing animals

Salmonella species are able to persist in different food products of animal origin due to the fact that they are naturally found in the GIT of several animal species (Meyer *et al.*, 2010; Miller, 2015). The presence of pathogenic bacteria species including *Salmonella* strains in food producing animals amplifies the need to implement strict control measures that will ensure food safety (Howe, 2015). The growing human population

relies on different agricultural products ranging from meat, milk and vegetables to meet their vital dietary requirements. Based on this a balance between human protein requirements and dietary supply may play a pivotal role in supporting the health and well-being of individuals worldwide (Consultation, 2011).

The pressure created by the high demand of agricultural products increases the chances of compromising the operational standard procedures during processing of food products of animal origin from farm-to-fork, and as a result agricultural products may be contaminated even before they arrive at the retail shops (Hedberg, 2014; Amin and El-Rahman, 2015; Yang *et al.*, 2015). The latter is known to account for the huge increase in foodborne infections worldwide. This magnifies the need for food producing industries to implement strategies that will add to the existing control measures in ensuring better food safety in the food chain (Hedberg, 2014).

Food producing animals can be reared in various ways and for different purposes. Despite this, the ultimate goal is to produce food for human consumption. During the different stages of growth, animals require veterinary attention including food and water so that they can grow properly. During the growth process animals shed different pathogens with faeces (Vidic *et al.*, 2015) . The presence of pathogens including *Salmonella* species in the environment provides opportunities for them to cross contaminate meat and other food products (White *et al.*, 2001; Van *et al.*, 2007; Zarei *et al.*, 2013; Niyonzima *et al.*, 2015). In addition, some studies have shown a direct correlation between pathogenic bacteria that were harboured by animals and their

associated food products, especially if hygiene measures were compromised in any of the processing stages between farm and fork (Van De Venter, 2000).

Several factors are known to contribute to bacterial contamination of animals before and after slaughter (Dewell *et al.*, 2008) which in turn increase the chances of foodborne infections on consumers as well as the risks of disease occurrence (Rostagno, 2009). These factors may include stress during transportation and the waiting time before slaughter (Barham *et al.*, 2002; Beach *et al.*, 2002). These factors are known to promote the uncontrolled release of faeces by animals. In addition, the hides and visceral contents have been reported to play a major role in contaminating carcasses in abattoirs, especially if hygiene measures are not properly implemented during processing of animals (Reicks *et al.*, 2007). On the other hand, contamination of bovine meat can further occur post-slaughter through incorrect storage temperature during distribution or if the hands of employees are contaminated with pathogens (Nel *et al.*, 2004).

Generally, issues relating to meat contamination can only be addressed when all stakeholders that are involved in the processing of food products from farm-to-fork utilize an integrative control measure in the different stages of production (Niyonzima *et al.*, 2015). In addition, the implementation of more systematic control measures may greatly enhance food safety (Nel *et al.*, 2004). Despite all the above considerations, the world is still faced with the challenge of contaminated food products that claim millions of lives every year in both developing and developed countries (Abatcha *et al.*, 2014b). This is due to the fact that either most of the control measures in place are unable to

totally eliminate the pathogens in food products or that they are compromised especially during meat processing (Mead, 1994).

2.2.2. Contamination of food products

Food products are easily contaminated by various foodborne pathogens at different stages of processing and this has been a huge challenge for mankind especially when hygiene measures are not fully implemented (Beuchat, 1996; Dallal, 2009). Foodborne contamination issues cannot be addressed by a country working in isolation and therefore require the active participation of different countries in the world (Van De Venter, 2000). Contaminated food products that are of poor quality must be identified by regulators as they will have been linked to foodborne outbreaks in humans (Lynch *et al.*, 2009; Potter *et al.*, 2012). Thus contaminated food products have been reported to significantly contribute to the dissemination of foodborne pathogens worldwide.

The presence of pathogenic bacteria or biological hazards has resulted in the recalls of agricultural products and therefore operational standards play a major role in the quality of the finished products (Potter *et al.*, 2012). Against this background there is a need to capacitate employees, food producers and suppliers with the skill and abilities that are required in a retail facilities to ensure food safety (Potter *et al.*, 2012). The persistence of foodborne infections worldwide has resulted in the development of different strategies that minimize the chances of contamination during food processing (Hedberg, 2014). In addition, there is a need to use bacterial indicators whose presence may be used as an indirect assessment of the microbial quality of the food product (Kleter and Marvin, 2009).

Contamination of food products can occur at different stages during production and processing and these include packaging and transportation (Van De Venter, 2000). Compromising hygiene measures at any of the production stages will result in contamination and that may increase the risk of human infections (Dallal, 2009). The risks on consumers vary greatly and largely depend on the type of pathogen involved, age as well as immune status of the host (Galanakis *et al.*, 2007; Gordon, 2008; Gordon *et al.*, 2008). It has been reported that infections caused by bacterial pathogens including *Salmonella* are more common in infants, elderly people and immunocompromised individuals (Galanakis *et al.*, 2007; Gordon, 2008; Gordon *et al.*, 2008).

Despite the fact that foodborne pathogens continuously cause life threatening infections in humans worldwide, there are a lot of challenges that negatively affect the control of these organisms in food products (Graham, 2002; Newell *et al.*, 2010). Despite the fact that there are standard operational procedures in food producing facilities and that new strategies are constantly being developed to limit or minimize foodborne pathogens in the food chain (Fischer *et al.*, 2005), *Salmonella* in particular still claims millions of lives every year in both developing and developed countries (Scallan *et al.*, 2011). This therefore indicates that there is a need to strictly implement the desired operational procedures in personal hygiene on the farms where animals are kept (Potter *et al.*, 2012). This will greatly ensure food safety and limit the burden of human infections.

Salmonella species have been isolated from several food products that include meat from different animals (Maharjan *et al.*, 2006) and this may have serious health implications especially if such products are consumed when they are undercooked. The occurrence of *Salmonella* species in food products are reported to be on the rise worldwide and food products such as eggs and chickens are currently considered to be high risk sources of these pathogens (Forshell and Wierup, 2006).The situation is even worse among African countries in which chicken are not dressed before they are marketed (Rodrigue *et al.*, 1990). Outbreaks of *Salmonella* infections have also been associated with other food products that include fruits and vegetables although the presence of microbial contaminants in these products is usually as a result of contact with other environmental sources (Hanning *et al.*, 2009). *Salmonella* have also been isolated in washed and unwashed beef carcasses in slaughter houses, and this indicates that hygiene measures do not totally eliminate pathogens from food products (Aftab *et al.*, 2012). In addition, *Salmonella* species were detected in retail meat products such as pork, minced beef and mutton that was intended for human consumption (Ejeta *et al.*, 2004; Meyer *et al.*, 2010). However, it has been reported that the level of *Salmonella* contamination in pork was lower during winter than in summer (Meyer *et al.*, 2010).

Generally there is a lack of information regarding the incidence of various foodborne pathogens including *Salmonella* species in humans in many countries, especially in Africa (Van De Venter, 2000). This is mainly due to the fact that most individuals do not report cases to the hospitals or health care personnel and this amplifies the need to

educate individuals in rural areas about the health implications of these pathogens as well as implementing routine screening of diarrhoeal patients for these bacteria species.

2.2.3. Clinical significance of *Salmonella* species

Despite the fact that *Salmonella* species reside in the GIT of various animals, it still remains one of the most important pathogen of both humans and animals (Su and Chiu, 2007). *Salmonella* species are known to most often infect children, elderly people and immunocompromised individuals, especially those with underlying diseases such as diabetes, HIV and AIDS including those who are on immuno suppressants (Acheson and Hohmann, 2001; Fierer and Guiney, 2001). These pathogenic strains pose serious challenges to human medicine throughout the world especially if they harbour multiple drug resistant determinants (Kruger *et al.*, 2004a; Akyala and Alsam, 2015; Girma, 2015). Despite the fact that the challenges caused by these pathogens occur worldwide, the problem is more acute in developing countries (Girma, 2015). Against this background, the findings of a study that was conducted in South Africa indicated that Extended-spectrum Beta-lactamases (ESBL) producing *Salmonella enterica* serotype Isangi is the most predominant diarrhoeal pathogen among children in most South African hospitals (Kruger *et al.*, 2004b). Furthermore, multiple antibiotic resistant *Salmonella* species are reported to persist in animals (Wani *et al.*, 2013; Ahmed *et al.*, 2015), and this increases the chances of them being transmitted to the environment and food products resulting in human infections (Girma, 2015; Negussie *et al.*, 2015).

Given the continued emergence of multiple antibiotic resistant *Salmonella* strains, there has been increased efforts to constantly determine the resistance profiles of circulating strains (Negussie *et al.*, 2015). This is aimed at generating data on the mechanisms of resistance and the development of effective strategies that will provide valid contributions towards the fight against antibiotic resistant strains in general and *Salmonella* in particular (Su and Chiu, 2007). Studies have also revealed that *Salmonella* species isolated from animals are resistant to commonly used antibiotics and there has been a direct relationship between resistant *Salmonella* strains in humans and those originating from animals that received drugs either as growth stimulants or prophylactic treatments (Acheson and Hohmann, 2001). This amplifies that there is a need to regulate the uncontrolled use of antimicrobial agents in animals (Wani *et al.*, 2013; Girma, 2015).

2.3. Pathogenicity

2.3.1. Route of transmission

The commonly known reservoirs for *Salmonella* include poultry and cattle while reptiles such as pet snakes and house flies (*Musca domestica L.*) have also been linked to human *Salmonella* infections (Schröter *et al.*, 2006; Ugbogu *et al.*, 2006; Yates, 2011). Given that *Salmonella* species are transmitted through the fecal-oral route, contact with contaminated food products, especially those obtained from animal species such as meat and eggs poses a great heath risk to consumers. This is even amplified by the fact that *Salmonella* species are zoonotic and have a very low infectious dose in animals.

The movement of animals from one place to the another has been identified as a potential cause for the transmission of *Salmonella* species to healthy animals and humans (Forshell and Wierup, 2006). However, other significantly important routes of transmission include equipment used on farms and in food production and other physical sources, hence these facilitate the spread of this pathogen to cattle and chicken situated in the same geographical location (Langvad *et al.*, 2006; De Vylder *et al.*, 2011). Cross contamination with *Salmonella* species easily occurs in operations such as milking dairy farms and crop cultivation in farmland that have been supplied with manure (Radke *et al.*, 2002). Therefore the prevalence of *Salmonella* species among animals, their associated food products, raw vegetables and water that is intended for human consumption are known to be the leading causes of human infections and its clinical significance in a given area depends largely on the level of exposure to the pathogen (Crump *et al.*, 2002; Forshell and Wierup, 2006; Sadeleer *et al.*, 2009).

2.3.2. Antimicrobial resistance

The use of antimicrobial agents in agriculture contributes largely to the persistence of resistant *Salmonella* species in the environment (Acheson and Hohmann, 2001; Kruger *et al.*, 2004a). Given that antimicrobial resistant strains are easily transmitted to humans through the consumption of contaminated food, particularly those of animal origin (Angulo *et al.*, 2009), some studies have shown that *Salmonella* strains can be resistant to either one or multiple drugs, especially those of clinical importance in humans (Threlfall, 2002; Angulo *et al.*, 2009). Interestingly, the resistance profiles of enteric

pathogens isolated from food producing animals were highly resistant to previously used antimicrobial agents when compared to newly approved drugs that are used in human medicine (Bywater *et al.*, 2004). This strongly supports findings that the emergence of resistant strains depends largely on the misuse of antimicrobial agents in food producing animals and specific resistance determinants can be transmitted to humans through the consumption of contaminated food products (White *et al.*, 2002). Given the challenges faced by the ever increasing resistance among bacterial pathogens coupled with the fact that new resistant strains are evolving every day, there is a need to implement strategies to curb the spread of resistant determinants especially among *Salmonella* species (Newell *et al.*, 2010).

Salmonella species develop resistance to various antimicrobial agents through different mechanisms and these depend on a number of factors that include the antibiotic involved; the procedure that is used to pump the antimicrobial agent out of the cell; the mode in which the cells destroy or modify the antimicrobial agent; strategies used to replace or modify the antimicrobial agent and techniques employed to decreased cell membrane permeability to the antibiotic (Abatcha *et al.*, 2014b). In addition, some pathogenic bacteria develop resistance to antibiotics through gene mutations or through the acquisition of foreign genetic material in the form of plasmids, integrons and transposons (Walsh, 2003; Toleman *et al.*, 2006). Among the various classes of antimicrobial agents used to treat Salmonellosis in both humans and animals, *Salmonella* species have been reported to be commonly resistant to aminoglycosides,

beta-lactams, quinolones, Chloramphenicol, tetracyclines, trimethoprim and sulfonamides (Abatcha *et al.*, 2014b).

Salmonella species have also been reported to harbour one or more genes that are responsible for resistance to several antimicrobial agents and the gene determinants include; *cmlA*, *bla*(TEM), *aadA1*, *tetA*, *dfrA12*, *sul3* and class 1 or 2 integrase resistant genes (Chuanchuen and Padungtod, 2009). Some of these gene determinants have been transmitted to other strains through horizontal gene transfer which explains the need to constantly monitor their occurrence in a given area (Kay *et al.*, 2002; Chuanchuen *et al.*, 2010; Gyles and Boerlin, 2013).

It has also been reported that the increase in salmonellosis globally, is mainly due to the fact that strains associated with disease easily acquired and harboured multiple antibiotic resistance genes (Ma *et al.*, 2007; Lynne *et al.*, 2008; Al-Mazini and Al-Hajaj, 2015). Therefore in the presence of a related antimicrobial agents the resistance genes in the genome of the bacteria may be expressed (Ma *et al.*, 2007). Moreover, the development of resistance determinants in bacterial species may also result from pollution of the soil with faeces of livestock especially breeding stations in a given farm and these genes can be transported through domestic sewage and industrial wastewater to neighboring farms (Xi *et al.*, 2015). This therefore increases the chance of transmitting these antimicrobial resistant determinants to humans. Table 2.1 indicates the different antibiotics used in the study, their mechanisms of action and mechanisms though which bacteria evade destruction against these drugs.

Table 2.1: Antibiotics, mechanisms of action and mechanisms though which bacteria evade destruction

Antibiotic Group	Examples	Target	Active against G-	Resistance mechanisms
Phenicols[a]	Chloramphenicols	Bind to 50S subunit of ribosomes – inhibit protein synthesis	✓	Efflux mechanisms Inactivation by enzymes
Beta- Lactams[b]	Ampicillin	Cell wall synthesis -interfering with seven penicillin binding proteins (PBP)	✓	Penicillin –G impermeable to G Mutation in PBPs. Produce β-Lactamase
Penicillins[c]	Penicillin	Cell wall synthesis -interfering with seven penicillin binding proteins (PBP)	✓	Penicillin –G impermeable to G Mutation in PBPs. Produce β-Lactamase
Aminoglycosides[d]	Gentamicin Streptomycin	Bind to 16S rRNA subunit of 30S ribosomal -lead to misreading and translation inhibition	✓	Aminoglycosides modifying enzymes and ribosomal modification
Tetracyclines[e]	Tetracycline	Bind to 30S subunit of ribosomes -inhibit protein synthesis	✓	Efflux mechanisms 16S mutations
Quinolones[f]	Nalidixic acid	Inhibit DNA gyrase synthesis	✓	Inhibit the microbial enzyme. DNA gyrase and block chromosomal replication
Macrolides[g]	Erythromycin	Bind to 50S subunit of ribosomal -inhibit protein synthesis	✓	Inhibition of extracellular signal regulated kinase 1/2 (ERK1/2) Activate nuclear factor kappa ($NF\text{-}_{\kappa}B$)
Rifamycin[h]	Rifampicin	Bind to bacterial RNA polymerase (RNAP) β-subunit	✓	Inhibit DNA dependent RNA polymerase rpoB gene mutation
Cephems[i]	Cefixime Cephalexin	Cell wall synthesis -interfering with seven penicillin binding proteins (PBP)	✓	Penicillin –G impermeable to G Mutation in PBPs. Produce β-Lactamase
Fluoroquinolones[j]	Ciprofloxacin Norfloxacin	Inhibit synthesis of DNA gyrase and topoisomerase IV enzymes	✓	Inhibit the microbial enzymes DNA gyrase and topoisomerase IV Block chromosomal replication

(Abatcha *et al.*, 2014b)[b,c,i] (Mascaretti, 2003; Gebreyes and Altier, 2002; Guerra *et al.*, 2002)[a,d,e,f] (Kanoh and Rubin, 2010)[g] (Floss and Yu, 2005; Ho *et al.*, 2009)[h] (Hooper, 2001; Higgins *et al.*, 2003)[j]

2.3.3. Microbiological identification of *Salmonella* species

Salmonella are Gram negative, oxidase negative, rod-shaped, non- spore forming bacteria that are predominantly motile due to the presence of peritrichous flagella (Abdullahi, 2010; Specimens, 2015). Despite this, species of *S. pollorum* and *S. gallinarum* are non-motile (Hendriksen, 2003). It is a facultative anaerobic bacterium which grows in temperatures ranging from 6 °C to 46 °C (Matches and Liston, 1968). In addition, *Salmonella* species are unable to ferment lactose (Hendriksen, 2003) and therefore grow optimally in different selective media that include; *Salmonella Shigella* Agar (SSA), Brilliant Green Agar (BGA) and Xylose Lysine Desoxycholate Agar (XLD) (Winn and Koneman, 2006). Although a number of selective media have been used to isolate *Salmonella* species from different types of samples, SSA has produced more reliable results when compared with the others (Gomez *et al.*, 1998), and this explains why it is frequently utilised in studies worldwide.

Biochemical characteristics that are used for presumptive identification of *Salmonella* species include; the ability or inability to hydrolyse the carbohydrates glucose, lactose and sucrose as well as produce H_2S in the Triple Sugar Iron (TSI) agar (Abdullahi, 2010). In addition, serological assays have been used to confirm the identities of *Salmonella* species, especially those with distinct and satisfactory colonial morphologies such as black colonies on SSA and are Gram negative rod shaped cells (Hendriksen, 2003). The identities of *Salmonella* species from food products have been confirmed through the use of O and H specific antigens using antisera (Mohamed and Ibrahim, 2015).

The Analytical profile index (API) 20E is a standardized assay for the identification of *Enterobacteriaceae* and this assay has been shown to be equally sensitive as serotyping (Nucera *et al.*, 2006). However, the sensitivities of these preliminary identification assays depend on a number of factors and are usually time consuming which gives room for high human error. With this in mind it is suggested that preliminary identification assays should be used in combination with more sensitive PCR techniques and therefore PCR assays designed to amplify the *inv*A have been widely utilised as a target for *Salmonella* species (Nucera *et al.*, 2006). However, the sensitivity of PCR assays most often depend on a number of factors such as the origin of the isolates (food and clinical isolates) and the purity of the template DNA (Moraes *et al.*, 2013).

On the contrary, PCR has been reported to be less time consuming, more specific and very sensitive in the identification of *Salmonella* from food products and faecal samples (Malkawi, 2003; Rodulfo *et al.*, 2012; Zahran and El-Behiry, 2014). This therefore explains the rationale behind the choice of methods used to identify *Salmonella* species in the present study. Given the pathogenic nature of *Salmonella* species identification is usually preceded by PCR assays aimed at determining the pathogenicity of the isolates especially in clinical settings and this is achieved through amplification of virulent genes such as the *inv*A, *spv*B and *spv*C (Mirzaie *et al.*, 2010; Mezal *et al.*, 2014). Given that the presence of *Salmonella* species in humans may result through the consumption of contaminated food products and water; contact with domestic animals, especially cattle and person-to-person transmission, there is a need to determine the genetic

relatedness of isolates from different sources from an epidemiological view point. Despite the fact that *Salmonella* species have been typed based on phenotypic data such as Antibiogram, more reliable data that can be used in source tracking especially during outbreaks of infections can be obtained through the application of genotypic typing methods (Wattiau *et al.*, 2011; Abatcha *et al.*, 2014a). Hence, genetic typing methods such as Restriction Fragment Length Polymorphism (RFLP), have been used to determine the genetic similarities or differences among *Salmonella typhimurium* obtained from different sources based on the differences in banding patterns (Sumithra *et al.*, 2014). In the present study RFLP was also used to determine the genetic similarities of Salmonella isolates from cattle dung and beef in the North West Province, South Africa.

2.4. Treatment

Salmonella infections pose a serious health challenge to humans and animals worldwide and the management of *Salmonella* associated infections is achieved through the administration of antibiotics (Van De Venter, 2000; Newell *et al.*, 2010; Scallan *et al.*, 2011; Xi *et al.*, 2015). However, complications caused by *Salmonella* species in both humans and animals, have escalated in recent years due to the emergence of antibiotic resistant strains (Mayrhofer *et al.*, 2004; Newell *et al.*, 2010; Xi *et al.*, 2015; Yang *et al.*, 2015). This is supported by the frequent isolation of multiple drug resistant *Salmonella* strains in human clinical samples (Fierer and Guiney, 2001; Delicato *et al.*, 2004; Srivani *et al.*, 2011), and the presence of antibiotic resistant determinants in strains that colonize patients negatively affects the success of the

treatment processes. Even though *Salmonella* species have been reported to be resistant to several antibiotics, increased susceptibility has been reported against β-lactam antibiotics, Cefotaxime, Ceftriaxone, Imipenem and fluoroquinolones (Kruger *et al.*, 2004a).

Given the challenges faced with the management of antibiotic resistant bacterial strains including those belonging to the genus *Salmonella* (Van De Venter, 2000; Newell *et al.*, 2010; Scallan *et al.*, 2011; Xi *et al.*, 2015), there has been renewed efforts to search for alternative agents particularly bacteriophages that have the potential of destroying these resistant bacterial strains (Alisky *et al.*, 1998; Garcia *et al.*, 2008).

2.5. Prevention

The most effective strategy of preventing bacterial infections in humans is usually directed at the implementation of good hygiene practices especially on the farms where the animals are housed (Tauxe, 1997; Altekruse *et al.*, 1997). In addition, the enforcement of proper personal hygiene as well as the implementation of standard operational procedures in food processing plants are key factors that have a great influence on the microbial quality of the finished food products (Centers for Disease Control Prevention, 2010). Moreover, inadequate or improper cooling or heating during processing, improper handling and fermentation or improper cleaning of working utensils have been shown to increase cross contamination of pathogens to food products (Bryan, 1988).

The food industry is being challenged by the presence of resistant foodborne pathogens every day (Van De Venter, 2000; De Freitas Neto *et al.*, 2010; Evangelopoulou *et al.*, 2014) and as a result there has been several outbreaks due to contaminated food products in the food chain resulting in recalls of certain agricultural products (Torrence and Isaacson, 2008; Lynch *et al.*, 2009; Potter *et al.*, 2012; Bell, 2015). In order to ensure food safety and limit financial or economic loss standard operational procedures must always be implemented in food processing plants especially in developing countries where individuals rarely practice proper personal hygiene (Forshell and Wierup, 2006; Ball *et al.*, 2011). Given these challenges proper cooking of food products especially those of animal origin may greatly reduce the risk of human infections since the consumption of undercooked food products has been identified as a potential source for bacterial infections in humans (Busani *et al.*, 2005; Forshell and Wierup, 2006; Koppel *et al.*, 2014; Motta *et al.*, 2014). This is motivated from the fact that *Salmonella* control measures must be implemented from the farm through to the processing plants (Lynch *et al.*, 2007; Nørrung and Buncic, 2008; Jones, 2011) and any deviation may result to an unsafe food product.

In addition to proper personal hygiene and proper sanitary practices the most effective way of preventing microbial contamination at slaughter houses include the implementation of intervention measures such as decontamination process or treatment of carcasses (Buncic and Sofos, 2012). Traditionally, the occurrence of *Salmonella* species could be controlled through veterinary inspection of stock before and after slaughter, consistent inspection of hygiene measures implementation at all times

throughout the processing stage and constant supervision of the overall meat handling process (Davies *et al.*, 1998). It has been shown that the implementation of different control measures from farm to fork coupled with educational programs on the implications of pathogenic bacteria as well as proper cooking techniques greatly reduced contamination levels and the incidence of foodborne infections (Goldrick, 2003; Stein, 2011).

2.6 Bacteriophages as potential antimicrobial agents in the food industry

Bacteriophages are viruses that infect bacteria and they have been explored for their potential as agents that can be used to control bacterial strains (Sharp, 2001). However, their initial application in therapeutic processes failed due to a lack of knowledge on the scientific processes involved as well as a limitation in technology (Carlton, 1999). However, the emergence of multiple antibiotic resistant (MAR) bacteria strains that are known to cause millions of infections globally and thus claiming millions of lives every year developed renewed interest in bacteriophages and they are currently being exploited for their abilities to serve as promising antimicrobial agents (Wittebole *et al.*, 2014). Despite this renewed interest, to the best of our knowledge this is the first study in the North West Province of South Africa in which Salmonella specific bacteriophages have been isolated from environmental sources and characterised.

CHAPTER 3

METHODS AND MATERIALS

CHAPTER 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Study design, area and sample size

The cross sectional study was conducted at North West University-Mafikeng Campus in the North West Province, South Africa and the samples were all collected from the different districts of the North West Province. The number of samples collected during the current study was determined using the formula outlined below

$$\text{Sample (N)} = \frac{(Z_{1-\alpha/2})^2 P\,(1-P)}{d^2}$$

$Z_{1-\alpha/2}$ = is standard normal variate at 5% type I error ($P< 0.05$) and it is 0.05

P = Expected prevalence in population based on a previous study

d = Absolute error or precision (which is 5%)

For estimating the prevalence of *Salmonella* species, the sample size for the current study was determined using a prevalence of 9.6% obtained in the study by (Mcevoy *et al.*, 2003) in United Kingdom to be the expected prevalence with the 95% confidence level and desired precision of 5% using the formula described by (Charan and Biswas, 2013). Based on this formula, the minimum sample size required was 134. Table 3.1 shows the number and nature of samples collected from different areas.

3.1.1 Ethical clearance

Ethical clearance for this study was sought from the North West University Ethics Committee and an ethics ID (NWU-00066-15-S9) was granted.

3.2 Sample collection

A total of 160 samples (cattle faecal material and raw beef) were collected during the study from different areas in the North West Province (Table 3.1). Meat samples were obtained from retail shops and butcheries in the North West Province, South Africa, while faeces samples were collected from cattle in both commercial and communal farms in the Mafikeng area.

Meat samples were collected in separate sterile plastic containers while faecal samples were obtained directly from the rectum of individual animals using sterile arm length gloves and stored in sterile plastic containers (Cummings *et al.*, 2010). All samples were properly labeled and transported on ice to the laboratory for selective isolation of *Salmonella* species and *Salmonella* specific bacteriophages. Upon arrival in the laboratory, samples were analysed within 24 hours from the time of collection. Table 3.1 shows details of the number of samples to be collected from the different areas.

3.3 Isolation of *Salmonella* species

Two grams of meat and faeces were washed or dissolved in 5 mL (w/v) of *Salmonella* enrichment broth (Merck, KGaA, Darmstadt, Germany) and incubated aerobically at 37

°C for 18 hours.. An aliquot of 50 µL of the pre-enriched *Salmonella* broth for each sample was spread-plated on *Salmonella Shigella* agar and the plates were incubated aerobically at 37 °C for 24 hours. Two potential colonies from each sample were selected based on differences in colonial morphologies and subjected to further purification analysis. Typical pale yellow colonies with black spots on their centers were sub-cultured on *Salmonella Shigella* agar. Pure colonies were retained and used for bacterial identification tests (Miliotis and Bier, 2003; Kruger *et al.*, 2004b).

Table 3.1: Areas / Stations from which beef samples and cattle faeces samples were collected

Sampling Area/Stations	**Source of samples**	**Type of samples**	**Number of samples**
Mafikeng	Beef	Beef=31	31
	Cattle	Faeces=57	57
Rustenburg	Beef	Beef =12	12
Vryburg	Beef	Beef =11	11
Marikana	Beef	Beef=6	6
Brits	Beef	Beef=4	4
Ventersdorp	Beef	Beef =5	5
Potchefstroom	Beef	Beef= 6	6
Carletonville	Beef	Beef =8	8
Lichtenburg	Beef	Beef=5	5
Stella	Beef	Beef=3	3
Boshoek	Beef	Beef=2	2
Total			**150**

3.4 Bacterial identification tests

All isolates were identified using the following criteria:

3.4.1 Cellular morphology

Isolates were Gram stained using standard techniques (Cruikshank *et al.*, 1975). All Gram negative rods were subjected to preliminary and confirmatory biochemical identification tests that are specific for Enterobacteriaceae (Akhtar *et al.*, 2014).

3.4.2 Preliminary identification tests

3.4.2.1 Oxidase tests

This test was performed using the Oxidase Test strips (MB0266A) from Mast Diagnostics (Oxoid Ltd, U.K.) in accordance with the manufacturer's published protocol. Presumptive *Salmonella* isolates were tested for their inability to oxidize the oxidase reagent (Abdullahi, 2010). *Salmonella* species do not possess the cytochrome enzyme as part of their respiratory system and hence they are oxidase-negative (Maddocks *et al.*, 2002).

3.4.2.2 Production of H_2S on the TSI medium

Triple Sugar Iron agar obtained from Merck South Africa was used to determine the ability of presumptive *Salmonella* isolates to breakdown the substrates glucose, lactose and sucrose at concentrations of 0.1, 1.0% and 1.0%, respectively (Abdullahi, 2010).

3.4.3 Confirmatory identification tests

3.4.3.1 Analytical profile index (API) 20E

All presumptive isolates were subjected to the API 20E test (Nucera *et al.*, 2006). The test was performed following the manufacturer's instructions (Bio-Merieux, Marcy l'Etoile, France). The indices obtained were interpreted using the API web software obtained from BioMerieux®, South Africa.

3.4.3.2 Serology test

Isolates were analysed for characteristics of *Salmonella* species using the Kauffmann-White Scheme with different monovalent and polyvalent O and H antisera (Popoff *et al.*, 1998) obtained from BioMerieux®, South Africa. All isolates that satisfied the serotyping were further subjected to *Salmonella* specific molecular identification tests.

3.5 Molecular characterisation of *Salmonella* isolates

3.5.1 Extraction of genomic DNA

Genomic and plasmid DNA were extracted from all isolates that were positive for confirmatory identification tests using Zymo Research Genomic DNA™ – Tissue MiniPrep kit (Catalog No. D3050 & D3051-USA) obtained from Inqaba Biotec, South Africa. The protocol was performed according to the manufacturer instructions.

3.5.2 Quantification of genomic DNA extracted

Genomic DNA extracted from *Salmonella* isolates and control strains were quantified using a Nanodrop lite spectrophotometer (Model 1558) obtained from Thermo Scientific, USA.

3.5.3 Molecular identification of *Salmonella* species

3.5.3.1 Amplification of 16S rRNA gene fragments in Salmonella isolates

Salmonella isolates that satisfied both preliminary and confirmatory biochemical tests were further subjected to specific molecular identification assays. PCR analysis was performed using oligonucleotide primer combinations and cycling conditions that appear in Table 3.2. Amplifications were performed using a DNA thermal cycler (C1000 Touch™, BIO-RAD, South Africa).

3.5.3.2 Salmonella specific PCR

All potential *Salmonella* isolates were subjected to species specific PCR assay designed to confirm identities of *Salmonella* species based on successful amplification of the *fliC* and *fliB* specific genes sequences. The primer pairs and PCR conditions that were used to perform the PCR are shown in Table 3.2

Table 3.2: Oxyoligonucleotide primers used in the identification of *Salmonella* species isolated during the study, and the cycling conditions

Primer Name	Sequence (5'-3')	Targeted Genes	Amplicon size (bp)	PCR cycling conditions
16S RNAF 16S RNAR	TGT TGTGGT TAA TAA CCG CA CAC AAA TCCATC TCT GGA	16S RNA	572 bp	94 °C for 4 min, 25 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72° for 1 min 30 sec, followed by 72 °C for 10 min
fliBF fliBR	GGCAACCCGACAGTAACTGGCGATC ATCAACGGTAACTTCATATTTG	*fli*B	2,976 bp	94 °C for 5 min, 35 cycles of 94 °C for 1 min, 47 °C for 1 min, and 72 °C for 1 min, with a final step of 72 °C for 5 min
fliCF fliCR	AAG GAA TTC ATC ATG GCA CAA G GAA GAATTC AAC GCA GTA AAG AGA G	*fli*C	1,448 bp	94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, with extention of 72 °C for 5 min

(Lin and Tsen, 1996; Dauga *et al.*, 1998; Sumithra *et al.*, 2014)

3.5.3.3 PCR assay to detect virulence genes in Salmonella isolates

The pathogenicity of the isolates was determined through the amplification of the *invA* and *spvC* virulence gene fragments (Chiu and Ou, 1996; Amini *et al.*, 2010). Oligonucleotide primer combinations and cycling conditions that appear in Table 3.3 were used for the PCR analysis.

TABLE 3.3: Oligonucleotide primers used for pathogenicity determination of *Salmonella* species isolated during the study, and the cycling conditions utilized in Polymerase chain reaction.

Primer	Sequence (5'-3')	Targeted genes	Amplicon size (bp)	PCR cycling conditions
SPVC-1 SPVC-2	ACTCCTTGCACAACCAAATGCGGA TGTCTTCTGCATTTCGCCACCATCA	*SpvC*	572bp	94 °C for 5 min, 35 cycles of 94 °C for 1 min, 46 °C for 1 min, and 72 °C for 1 min, with a final step of 72 °C for 5 min
INVA-1 INVA-2	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	*InvA*	284 bp	94 °C for 5 min, 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, with a final step of 72 °C for 5 min

(Chiu and Ou, 1996; Ateba and Mochaiwa, 2014)

3.6 Molecular typing method of *Salmonella* isolates

3.6.1 Restriction Fragment Length Polymorphism (RFLP) of 16S rRNA gene fragments

All *Salmonella* isolates were typed using the restriction fragment length polymorphism (RFLP) technique based on standard protocols (Shah and Romick, 1997). Restriction enzymes *Eco*RV and *Hae*III were used for the RFLP analysis (Dauga *et al.*, 1998; Sumithra *et al.*, 2014). Fingerprinting patterns obtained were used to assess the genetic similarities among *Salmonella* isolates obtained from different sources and locations (Shah and Romick, 1997; Fendri *et al.*, 2013).

3.7 Electrophoresis of PCR products

Genomic DNA and PCR products were separated by electrophoresis on a 2% (w/v) agarose gel using a horizontal Pharmacia biotech equipment system (model Hoefer HE 99X; Amersham Pharmacia biotech, Sweden). Each run was conducted at 100 volts for 10 minutes and 80 volts for 1 hour using 1X TAE buffer (40mM Tris, 1mM EDTA and 20mM glacial acetic acid, PH 8.0). Each gel contained either a 100bp or 1kb DNA molecular weight marker (Fermentas, USA), depending on the size of the targeted gene. The gels were stained with ethidium bromide (0.1µg/mL) for 15 minutes and amplicons were visualized under UV light at 420nm wavelength (Sambrook *et al.*, 1989). A ChemiDoc imaging system (Bio-Rad ChemiDoc TMMP imaging system, UK) was used to capture the image using GeneSnap (version 6.08) software and images were analysed in order to determine the relative sizes of the amplicons.

3.8 Antibiotic Susceptibility Test

All confirmed *Salmonella* isolates were subjected to an antibiotic resistance assay against 12 selected antimicrobial agents that appear in Table 3.4 to determine their antibiotic resistance profiles (Abdullahi, 2010; Akond *et al.*, 2013). The antibiotic disc diffusion technique was performed following the Clinical Laboratory Standard Institute guidelines (Wikler, 2007). The antibiotic zone of inhibition diameter data was interpreted using standard reference values (Cockerill *et al.*, 2012) and this was used to classify isolates as susceptible, intermediate resistant or resistant to a particular antibiotic. Percentage antibiotic resistance was calculated and the multiple antibiotic resistant (MAR) phenotypes were generated for isolates that were resistant to three or more antimicrobial agents (Rota *et al.*, 1996).

Table 3.4: Details of the antibiotics that were used in the study

Group	Antibiotic	Abbr.	Disc conc. (µg)	Inhibition zone (mm)		
				R	**I**	**S**
Phenicols	Chloramphenicol	C	30	≤12	13–17	≥18
Beta-Lactams	Ampicillin	AP	10	≤13	14–22	≥23
Penicillins	Penicillin	PG	10	≤28	N/A	≥29
Aminoglycosides	Gentamicin	GM	10	≤ 12	13–14	≥ 15
	Streptomycin	S	10	≤13	14–17	≥18
Cephems	Cephalexin	CFXM	15	≤15	16–20	≥21
	Cefixime	CFM	5	≤15	16–18	≥19
Quinolones	Nalidixic acid	NA	30	≤13	14–18	≥19
Tetracyclines	Tetracycline	T	30	≤14	15–18	≥19
Rifamycin	Rifampicin	P	5	≤16	17–19	≥20
Macrolides	Erythromycin	E	15	≤13	14–22	≥23
Fluoroquinolones	Ciprofloxacin	CIP	5	≤15	16–20	≥21
	Norfloxacin	NOR	10	≤12	13–16	≥17

3.9. Statistical analysis

The percentage antibiotic resistance of *Salmonella* isolates obtained from particular sampling location was determined by dividing the number of isolates resistant to a given antibiotic by the total number of isolates tested. Furthermore, cluster analysis of antibiotic resistance data for *Salmonella* species isolated from the different locations was determined using Wards algorithm and Euclidean distances on Statistica version 12 (Statsoft, US).

3.10 Isolation of *Salmonella* specific bacteriophages and their characterisation

3.10.1. Bacterial strains and growth conditions

Salmonella specific bacteriophages were isolated using environmental *Salmonella* strains whose identities were confirmed using assays indicated in Materials and Methods (Sections 3.4 to 3.5.3.3). In addition *Salmonella enterica* subsp. diarizonae (ATCC 12325) reference strain was also used in bacteriophage isolation. All bacterial strains were grown in tryptic soy broth (TSB) and sub-cultured on tryptic soy agar (TAS). The plates were incubated at 37 °C for 24 hours.

3.10.2 Bacteriophage isolation and purification

Bacteriophages infecting *Salmonella* strains were isolated from faecal samples using a standard protocol (Sambrock and Ressell, 2001; Niu *et al.*, 2012). During phage isolation, 20 g of faecal matter was weighed and dissolved in 60 mL of sterile lambda diluent (10 mM Tris CL, pH 7.5, 8 mM $MgSO_4$). The sample was agitated in a shaking incubator (Model PSIE-SP08) at room temperature for 1 minute and later left at room

temperature for 60 minutes in order to release phage particles into the buffer. An aliquot of 20 mL was extracted from the top layer of each sample, centrifuged at 11,000 × g for 10 minutes, and filtered through a 0,2 µm Acrodisc® disposable syringe filters (Pall Corporation, Ann Arbor, MI, USA). The filtrate were subjected to 20 hours enrichment and used to detect the presence of bacteriophages and single plaques were purified three times (Niu *et al.*, 2012). Phage stock filtrates were prepared using *Salmonella* strains isolated in this study and *Salmonella* reference strains as described previously (Niu *et al.*, 2012). Briefly, purified bacteriophages were mixed thoroughly with 0.9 ml of sterile lambda diluent (10 mM Tris CL, pH 7.5, 8 mM $MgSO_4$) and held at room temperature for 1-2 hours to allow the phage particles to diffuse from the agar. The diluent containing the bacteriophages was centrifuged at 11,000 × g for 10 minutes and filtered through a 0,2 µm Acrodisc® disposable syringe filters (Pall Corporation, Ann Arbor, MI, USA) to remove agar and bacterial cells.

3.10.3 Bacteriophage propagation

An aliquot of 100 µL of each phage filtrate was mixed with 1 mL of a mid-log-phase culture of environmental and control *Salmonella* strains and incubated for 15 minutes at 37 °C to allow for attachment to the host. This was followed by addition of tryptic soy broth amended with $MgSO_4$ at 10 $mmol \cdot L^{-1}$, and further incubated at 37 °C for 4-6 hours with shaking (190 rpm) until complete lysis occurred. The lysates was then centrifuged at 5,250 × g for 20 minutes at 4 °C and filtered through a 0.2 µm SFCA serum filter. Bacteriophage stock lysates were used for phage virulence assay (Niu *et al.*, 2012).

3.10.4 Phage microplate virulence assay

The lytic capabilities of bacteriophages were assessed using a microplate phage virulence assay (Niu *et al.*, 2012). Briefly, high-titre phage stocks (10^9-10^{10} PFU·ml^{-1}) were serially diluted and incubated for 5 hours at 37 °C with overnight cultures of bacterial strains in 96-well microplates. After incubation, wells were examined visually for turbidity due to bacterial growth. For each isolate-phage combination, the highest dilution of phage that resulted in complete lysis (no discernible turbidity) of bacteria was recorded.

CHAPTER 4

RESULTS AND INTERPRETATION

CHAPTER 4

RESULTS AND INTERPRETATION

4. RESULTS AND INTERPRETATION

4.1. Occurrence of *Salmonella species* in cattle and beef using preliminary identification tests

4.1.1 Cellular morphology

A total of one hundred and fifty samples (cattle faecal material and raw beef) were collected from butcheries and retail shops within the North West Province, while faecal samples were collected from cattle in both commercial and communal farms around Mafikeng, North West Province. A total of 300 potential *Salmonella* isolates were obtained based on differences in their colonial morphologies (transparent to translucent colonies with black centers that were non-lactose fermenters). The cellular morphologies of the isolates was determined based on a standard technique (Materials and Methods, Section 3.4.1) and detailed results are shown in Table 4.1. All the isolates were Gram negative rods and therefore satisfied the preliminary identification for *Salmonella* species. The isolates were subjected to further preliminary and confirmatory biochemical identification tests for *Salmonella* (Akhtar *et al.*, 2014).

Table 4.1: Results for preliminary biochemical tests

Sampling Area	No. Tested	Gram-Stain (-rod)	Oxidase (-)	Triple Sugar Iron (TSI)				
				Glucose	Sucrose	Lactose	Gas	H_2S
Mafikeng	NT	176	176	176	176	176	176	176
	NP	176 (100%)	174 (98.8%)	176 (100%)	157 (89.2%)	157 (89.2%)	136 (77.2%)	4 (2.3%)
Rustenburg	NT	24	24	24	24	24	24	24
	NP	24 (100%)	24 (100%)	24 (100%)	23 (95.8%)	23 (95.8%)	17 (70.8%)	2 (8.3%)
Vryburg	NT	22	22	22	22	22	22	22
	NP	22 (100%)	21 (95.5%)	22 (100%)	22 (100%)	22 (100%)	16 (72.7%)	0 (0%)
Marikana	NT	12	12	12	12	12	12	12
	NP	12 (100%)	12 (100%)	12 (100%)	12 (100%)	12 (100%)	11 (91.7%)	0 (0%)
Brits	NT	8	8	8	8	8	8	8
	NP	10 (100%)	6 (75%)	8 (100%)	8 (100%)	8 (100%)	3 (37.5%)	0 (0%)
Ventersdorp	NT	10	10	10	10	10	10	10
	NP	10 (100%)	10 (100%)	10 (100%)	9 (90%)	9 (90%)	8 (80%)	1 (10)
Potchefstroom	NT	12	12	12	12	12	12	12
	NP	12 (100%)	11(91.7%)	12 (100%)	7 (58.3%)	7 (58.3%)	4 (33.3%)	0 (0%)
Carletonville	NT	16	16	16	16	16	16	16
	NP	16 (100%)	14 (87.5%)	16 (100%)	16 (100%)	16 (100%)	7 (43.8%)	0 (0%)
Lichtenberg	NT	10	10	10	10	10	10	10
	NP	10 (100%)	10 (100%)	10 (100%)	7 (70%)	7 (70%)	8 (80%)	5 (50%)
Stella	NT	6	6	6	6	6	6	6
	NP	6 (100%)	5 (83.3%)	6 (100%)	6 (100%)	6 (100%)	4 (66.7%)	0 (0%)
Boshoek	NT	4	4	4	4	4	4	4
	NP	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)	1 (25%)	0 (0%)
TOTAL	**NT**	**300**	**300**	**300**	**300**	**300**	**300**	**300**
	NP	**300 (100%)**	**291 (97%)**	**300 (100%)**	**271 (90.3)**	**271 (90.3)**	**215 (71.7)**	**12 (4%)**

4.1.2 Oxidase test

All 300 isolates were subjected to the oxidase test and results are shown in Table 4.1. A large proportion 291 (97%) of the isolates was negative for the test and did not possess the cytochrome oxidase which is a characteristic of *Salmonella* species.

4.1.3 Production of H_2S on the TSI medium

Presumptive *Salmonella* isolates were further subjected to Triple Sugar Iron agar to assess their ability to produce H_2S in the medium (Abdullahi, 2010). Only 12 (4%) of the isolates produced H_2S and detailed results are shown in Table 4.1.

4.2. Occurrence of *Salmonella species* in cattle faecal and beef samples using confirmatory identification tests

4.2.1 Analytical profile index (API) 20E

A total of 23 randomly selected presumptive *Salmonella* isolates that satisfied the preliminary identification characteristics were further subjected to the analytical profile index (API) 20E assay (Nucera *et al.*, 2006) and the test was performed following the manufacturer's instructions (BioMérieux, France). Eight of the 23 (34.8%) representative isolates were identified as *Salmonella* species and details are shown in Table 4.2.

4.2.2 Serological assay

All 300 presumptive *Salmonella* isolates were serotyped using the Kauffmann-White Scheme with different monovalent and polyvalent somatic (O) and flagella (H) antisera (Popoff *et al.*, 1998). Amongst these isolates 159 (53%) were positive for *Salmonella* species based on agglutination with the polyvalent O antiserum and belonged to groups A-G, while 185 (61,7%) were positive for the polyvalent H antiserum. Detailed results are shown in Table 4.2.

Table 4.2: Results for confirmatory biochemical tests

Sampling Area	**No. Tested**	**Analytical profile index (API) 20E of representative isolates**	**Serology**	
			O antigen Group A-G	**H antigen Phase 1 and 2**
Mafikeng	NT	3	176	**176**
	NP	0 (0%)	107 (60.8%)	**128 (72.7%)**
Rustenburg	NT	5	24	**24**
	NP	2 (40%)	10 (41.7%)	**7 (29.2%)**
Vryburg	NT	2	22	**22**
	NP	0 (0%)	11 (50%)	**15 (68.2%)**
Marikana	NT	1	12	**12**
	NP	1 (100%)	5 (41.7%)	**6 (50%)**
Brits	NT	1	8	**8**
	NP	0 (0%)	4 (50%)	**8 (100%)**
Ventersdorp	NT	2	10	**10**
	NP	1 (50%)	4 (40%)	**2 (20%)**
Potchefstroom	NT	2	12	**12**
	NP	0 (0%)	5 (41.7%)	**1 (8.3%)**
Carletonville	NT	1	16	**16**
	NP	0(0%)	3(18.8)	**3(18.8)**
Lichtenberg	NT	4	10	**10**
	NP	3 (75%)	6 (60%)	**9 (90%)**
Stella	NT	1	6	**6**
	NP	1 (100%)	3 (50%)	**3 (50%)**
Boshoek	NT	1	4	**4**
	NP	0 (0%)	1 (25%)	**3 (75%)**
TOTAL	NT	23	300	**300**
	NP	**8 (34.8%)**	**159 (53%)**	**185 (61.7%)**

4.2.3 Amplification of 16S rRNA gene fragments in *Salmonella* isolates

To avoid any bias all the 300 presumptive *Salmonella* isolates obtained from cattle and beef samples based on colony morphologies were subjected to *Salmonella* specific PCR analysis for the amplification of 16S rRNA gene fragments. The results indicated that 128 (42.7%) of the isolates were positively identified as *Salmonella* species. Detailed results of the number of isolates that were positive from the different samples and areas are shown in Table 4.3. Figure 4.1 shows a representation of a 2% (w/v) agarose gel depicting 16S rRNA gene fragments amplified from isolates and theexpected amplicon size (572 bp) was obtained in the study.

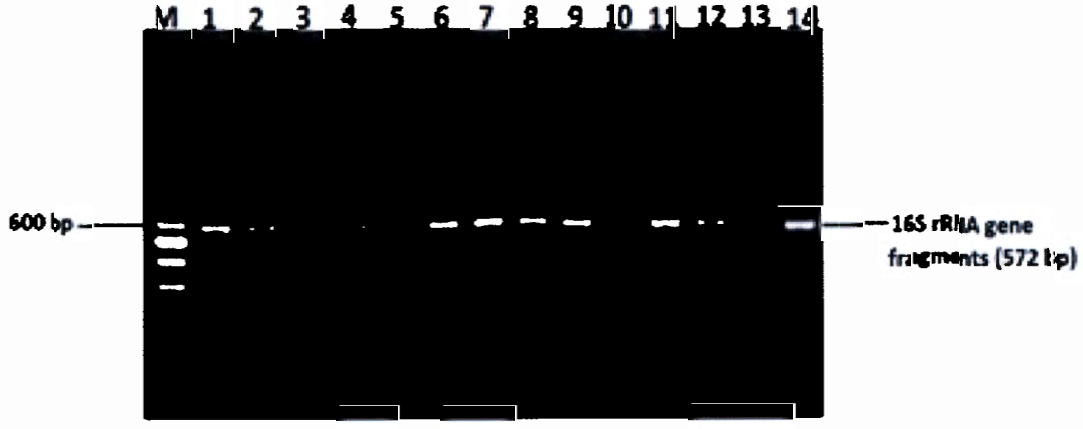

Figure 4.1: Agarose gel electrophoresis of *Salmonella* specific 16S rRNA gene fragments amplified from isolates obtained during the study. Lane M = 100 bp DNA marker; Lane 1 = 16S rRNA gene fragment amplified from *Salmonella enterica* subsp. diarizonae (ATCC 12325); Lanes 2-14 = 16S rRNA gene fragments amplified from isolates obtained from the different sampling sites.

4.2.4 *Salmonella* species specific PCR analysis isolates

4.2.4.1 *Amplification of fliC gene fragments in Salmonella isolates*

All 300 presumptive *Salmonella* isolates were subjected to a species specific PCR analysis, on the basis of successful amplification of *fliC* specific gene sequence. Only a small proportion 60 (20%) of the isolates were confirmed as *Salmonella* and the detailed results are shown on Table 4.3. The expected amplicon size (1448 bp) was obtained and figure 4.2 depicts the 2% agarose (w/v) gel of gene fragments amplified from isolates of different sampling sites within the province.

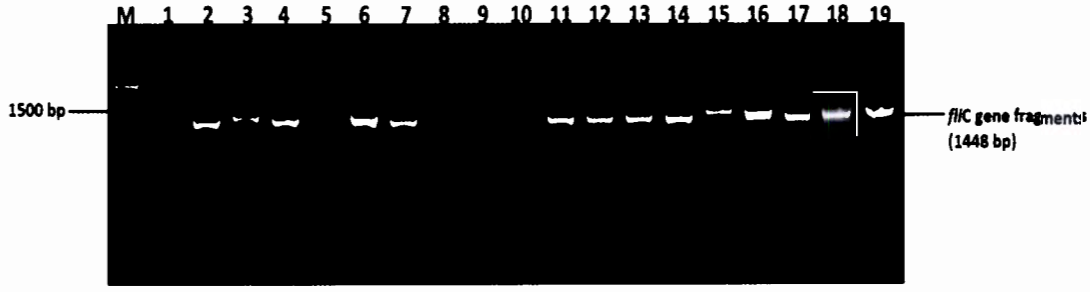

Figure 4.2: Agarose gel electrophoresis depicting *Salmonella* species specific *fliC* gene fragments amplified from isolates obtained during the study. Lane M = 100 bp DNA marker; Lane 1 = 16S rRNA gene fragment amplified from *Salmonella enterica* subsp. diarizonae (ATCC 12325); Lanes 2-19 = *Salmonella* species specific *fliC* gene fragments amplified from isolates in the study

4.2.4.2 *Amplification of fliB gene fragments in Salmonella isolates*

All the isolates were further subjected to a *Salmonella* species specific PCR assay to amplify the *fliB* gene fragments. Eighty (26.7%) of the isolates were positively identified as *Salmonella* species based on the presence of flagellins and detailed results are shown in Table 4.3 Despite the fact that the *fliB* gene was succesfully amplified, it was identitied that the amplicon size was different from what was expected. The size of the amplicons ranged from 700-800 bp and Figure 4.3 depicts a 2% agarose (w/v) gel of *fliB* gene fragments amplified from isolates.

Most *Salmonella* strains exhibit two structural genes (*fliC* and *fliB*) that encode flagellins. However, only one of these structural genes is expressed in the bacterium at any given time which may explain the differences in their occurence.

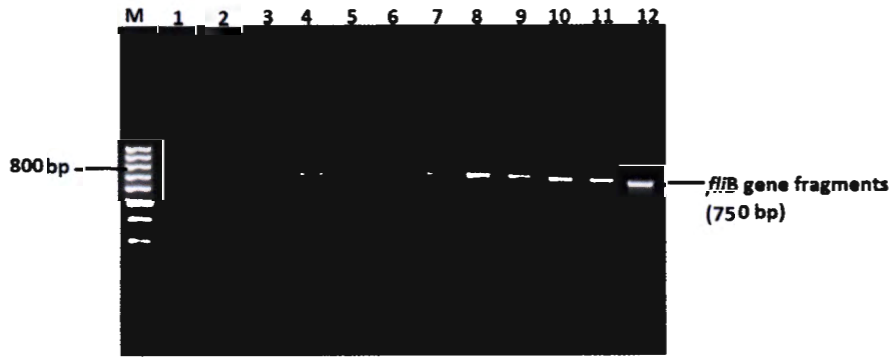

Figure 4.3: Agarose gel electrophoresis depicting *Salmonella* species specific *fliB* gene fragments amplified from isolates obtained during the study. Lane M = 100 bp DNA marker; Lane 1 = *Salmonella* species specific *fliB* gene fragment amplified from *Salmonella enterica* subsp. diarizonae (ATCC 12325); Lanes 2-12 = *Salmonella* species specific *fliB* gene fragments amplified from isolates in the study

Table 4.3: Proportion of isolates that were positive for *Salmonella* specific gene fragments PCR analysis. Superscripts [a], [b] and [c] indicate that the sum of occurrence of gene determinants that encode for flagellins ([b] and [c]) correlates with the detection of *Salmonella* species specific 16S rRNA sequences ([a]) by PCR.

Sampling Area	**No. Tested**	**16S rRNA**	***fliC***	***fliB***
Mafikeng	NT	176	176	176
	NP	69(39.2%)	45 (25.6%)	58 (33%)
Rustenburg	NT	24	24	24
	NP	3 (12.5%)	2 (8.3%)	5 (20.8%)
Vryburg	NT	22	22	22
	NP	12 (54.5%)	2 (9.1%)	5 (22.7%)
Marikana	NT	12	12	12
	NP	7 (58.3%)	0 (0%)	1 (8.3%)
Brits	NT	8	8	8
	NP	8 (100%)	1 (12.5%)	1 (12.5%)
Ventersdorp	NT	10	10	10
	NP	6 (60%)	3 (30%)	2 (20%)
Potchefstroom	NT	12	12	12
	NP	5 (41.7%)	1 (8.3%)	3 (25%)
Carletonville	NT	16	16	16
	NP	8 (50%)	4 (25%)	2 (12.5%)
Lichtenberg	NT	10	10	10
	NP	7 (70%)	2 (20%)	1 (10%)
Stella	NT	6	6	6
	NP	2 (33.3%)	0 (0%)	1 (16.7%)
Boshoek	NT	4	4	4
	NP	1(25%)	0 (0%)	1 (25%)
TOTAL	**NT**	**300**	**300**	**300**
	NP	**128(42.7%)[a]**	**60 (20%)[b]**	**80 (26.7%)[c]**

4.3 Phenotypic characterisation of isolates using their antibiotic resistance profiles

4.3.1. Antibiotic disc susceptibility test

All 140 isolates identified as *Salmonella* species based on amplification of *Salmonella* specific genes (16S rRNA, *fli*C and *fli*B) were subjected to antibiotic susceptibility test against a panel of twelve different antimicrobial agents. Detailed results are shown on Tables 4.4A and 4.4B including Figures 4.4A and 4.4B. Large proportions of the isolates from both cattle faecal and beef samples were most often resistant to Erythromycin 120 (83.3%), Rifampicin 129 (92.1%), Penicillin 132 (94.3%), Ampicillin 86 (61.4%), and Cephalexin 101 (72.1%). On the contrary these isolates showed very little resistance (4.3% to 37.9%) against Cefixime, Nalidixic Acid, Gentamicin, Ciprofloxacin, Tetracycline, Norfloxacin and Chloramphenicol.

Table 4.4A: Proportion of isolates from the different stations that were resistant to the antibiotics tested.

Sampling Area	No. Tested	AP(10)	CFM(5)	NA(30)	GM(10)	CIP(5)	T(30)
Mafikeng	NT	16	16	16	16	16	**16**
	NR	9 (56.3%)	4 (25%)	1 (6.3%)	2 (12.5%)	1 (6.3%)	**4 (25%)**
Rustenburg	NT	24	24	24	24	24	**24**
	NR	12 (50%)	13 (54.2%)	9 (37.5%)	0 (0%)	8 (33.3%)	**7 (29.2%)**
Vryburg	NT	22	22	22	22	22	**22**
	NR	15 (68.2%)	8 (36.4%)	3 (13.6%)	0 (0%)	2 (9.1%)	**6 (27.3%)**
Marikana	NT	12	12	12	12	12	**12**
	NR	6 (50%)	4 (33.3%)	4 (33.3%)	4 (33.3%)	6 (50%)	**6 (50%)**
Brits	NT	8	8	8	8	8	**8**
	NR	4 (50%)	0 (0%)	2 (25%)	0 (0%)	0 (0%)	**4 (50%)**
Ventersdorp	NT	10	10	10	10	10	**10**
	NR	8 (80%)	3 (30%)	0 (0%)	0 (0%)	1 (10%)	**4 (40%)**
Potchefstroom	NT	12	12	12	12	12	**12**
	NR	9 (75%)	6 (50%)	1 (8.3%)	0 (0%)	1 (8.3%)	**3 (25%)**
Carletonville	NT	16	16	16	16	16	**16**
	NR	11 (68.8%)	6 (37.5%)	2 (12.5%)	1 (6.3%)	1 (6.3%)	**8 (50%)**
Lichtenberg	NT	10	10	10	10	10	**10**
	NR	7 (70%)	5 (50%)	2 (20%)	0 (0%)	0 (0%)	**6 (60%)**
Stella	NT	6	6	6	6	6	**6**
	NR	4 (66.7%)	2 (33.3%)	3 (50%)	2 (33.3%)	1 (16.7%)	**2 (33.3%)**
Boshoek	NT	4	4	4	4	4	**4**
	NR	1 (25%)	2 (50%)	0 (0%)	0 (0%)	1 (25%)	**0 (0%)**
TOTAL	NT	140	140	140	140	140	**140**
	NR	**86 (61.4%)**	**53 (37.9%)**	**27 (19.2%)**	**9 (6.4%)**	**22(15.7%)**	**48(34.3%)**

AP (Ampicillin), CFM (Cefixime), NA (Nalidixic Acid), GM (Gentamicin), CIP (Ciprofloxacin), T (Tetracycline)
NT= Number Tested, NR= Number Resistant

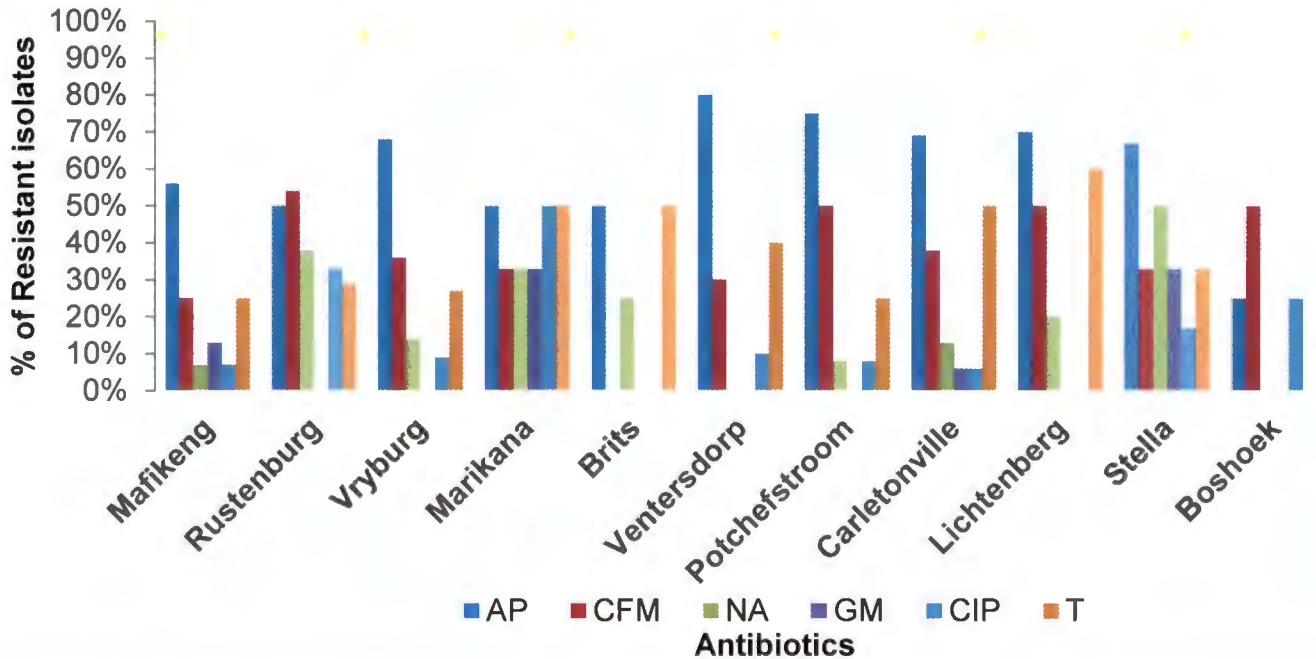

Figure 4.4A Proportion of isolates from the different stations that were resistant to the antibiotics tested: were AP (Ampicillin), CFM (Cefixime), NA (Nalidixic Acid), GM (Gentamicin), CIP (Ciprofloxacin), T (Tetracycline).

Table 4.4B: Proportion of isolates from the different stations that were resistant to the antibiotics tested.

Sampling Area	No. Tested	NOR(10)	E(15)	RP(5)	C(30)	PG(10)	CFXM(30)
Mafikeng	NT	16	16	16	16	16	**16**
	NR	1 (6.3%)	14 (87.5%)	15 (93.8%)	2 (6.3%)	16 (100%)	**13 (81.3%)**
Rustenburg	NT	24	24	24	24	24	**24**
	NR	4 (16.7%)	24 (100%)	24 (100%)	5 (20.8)	24 (100%)	**15 (62.5%)**
Vryburg	NT	22	22	22	22	22	**22**
	NR	0 (0%)	16 (72.7%)	18 (81.8%)	2 (9.1%)	18 (81.8%)	**9 (40.9%)**
Marikana	NT	12	12	12	12	12	**12**
	NR	0 (0%)	10 (83.3)	12 (100%)	12 (100%)	12 (100%)	**7 (58.3%)**
Brits	NT	8	8	8	8	8	**8**
	NR	0 (0%)	8 (100%)	8 (100%)	2 (25%)	8 (100%)	**5 (62.5%)**
Ventersdorp	NT	10	10	10	10	10	**10**
	NR	0 (0%)	8 (80%)	8 (80%)	2 (20%)	8 (80%)	**8 (80%)**
Potchefstroom	NT	12	12	12	12	12	**12**
	NR	1 (8.3%)	10 (83%)	12 (100%)	2 (16.7%)	12 (100%)	**12 (100%)**
Carletonville	NT	16	16	16	16	16	**16**
	NR	0 (0%)	10 (62.5)	12 (75%)	0 (0%)	14 (87.5%)	**14 (87.5%)**
Lichtenberg	NT	10	10	10	10	10	**10**
	NR	0 (0%)	10 (100%)	10 (100%)	2 (20%)	10 (1000%)	**9 (90%)**
Stella	NT	6	6	6	6	6	**6**
	NR	0 (0%)	6 (100%)	6 (100%)	4 (66.7%)	6 (100%)	**6 (100%)**
Boshoek	NT	4	4	4	4	4	**4**
	NR	0 (0%)	4 (100%)	4 (100%)	1 (25%)	4 (100%)	**3 (75%)**
TOTAL	NT	140	140	140	140	140	**140**
	NR	**6 (4%)**	**120 (83.3%)**	**129 (92.1%)**	**34 (24.3%)**	**132 (94.3%)**	**101 (72.1%)**

NOR (Norfloxacin), E (Erythromycin), RP (Rifampicin), C (Chloramphenicol), PG (Penicillin), CFXM (Cephamycin)
NT= Number Tested, NR= Number Resistant

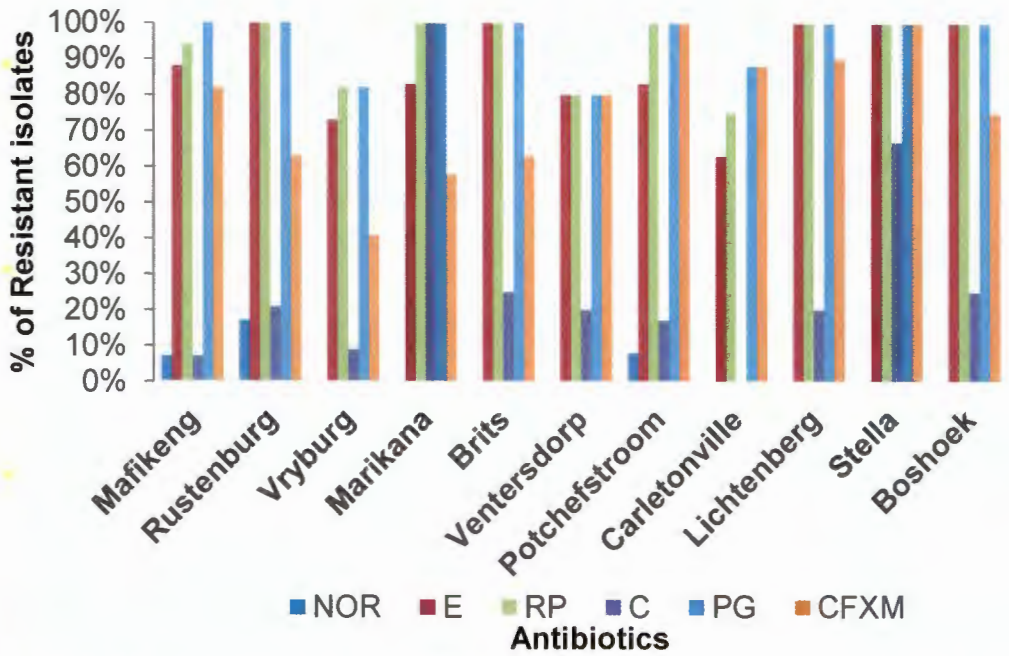

Figure 4.4B Proportion of isolates from the different stations that were resistant to the antibiotics tested: were NOR (Norfloxacin), E (Erythromycin), RP (Rifampicin), C (Chloramphenicol), PG (Penicillin), CFXM (Cephamycin).

4.4 Multiple Antibiotic-Resistant (MAR) Phenotypes of *Salmonella* species isolated from cattle faeces and beef

The multiple antibiotic resistant phenotypes of 140 confirmed *Salmonella* isolates was generated for isolates showing resistance to three or more antibiotics using abbreviations that appear on the discs. The predominant multiple antibiotic-resistant phenotypes observed in isolates from cattle faecal and beef samples obtained from the various areas are shown in Tables 4.5A and 4.5B. Despite the fact that several phenotypes were observed among isolates from Mafikeng MAR phenotypes E-RP-PG-CFXM, AP-E-RP-PG-CFXM, RP-PG-CFXM, AP-CFM-E-RP-PG-CFXM, T-E-RP-PG-CFXM and AP-CFM-T-E-RP-PG-CFXM were dominant among these isolates. On the other hand, phenotypes AP-CFM-NA-CIP-NOR-E-RP-PG, E-RP-PG-CFXM and T-E-RP-PG-CFXM were predominant among isolates obtained from Rustenburg (Tables

4.5A and 4.5B). Despite the fact that phenotype AP-CFM-T-E-RP-PG-CFXM was observed only in 1 isolate obtained from Mafikeng, a cause for concern is that this isolate was resistant to 7 of the 12 antibiotics tested. In addition the AP-CFM-NA-CIP-NOR-E-RP-PG was not only dominant among isolates from Rustenburg but the isolates were resistant to eight different antimicrobial agents. In general the detection of multiple antibiotic resistant *Salmonella* isolates in this study indicates that they may not only negatively affect the treatment of human infections but also serve as reservoirs for the transmission of these resistant determinants to other bacterial strains.

Table 4.5A: Predominant multiple antibiotic-resistant phenotypes for presumptive *Salmonella* isolated from cattle faeces and beef in different sampling areas in the North West Province. Phenotypes were generated using abbreviations that occur in the antibiotic discs

Sample Area	**Phenotype**	**No. Observed**	**% Observed**
Mafikeng (NO=16	E-RP-PG-CFXM	4	25%
	AP-E-RP-PG-CFXM	4	25%
	RP-PG-CFXM	3	18.8%
	AP-CFM-E-RP-PG-CFXM	2	12.5%
	T-E-RP-PG-CFXM	2	12.5%
	AP-CFM-T-E-RP-PG-CFXM	1	6.3%
Rustenburg (NO=24)	AP-CFM-NA-CIP-NOR-E-RP-PG	4	16.7%
	E-RP-PG-CFXM	3	12.5%
	T-E-RP-PG-CFXM	3	12.5%
Vryburg(NO=22)	AP-E-RP-PG	2	9.1%
	E-RP-PG	2	9.1%
	T-E-RP-C-PG	2	9.1%
	RP-PG-CFXM	2	9.1%
	AP-CFM-NA-CIP-E-RP-PG	2	9.1%
	AP-CFM-E-RP-PG	2	9.1%
	AP-E-RP-PG-CFXM	2	9.1%

Table 4.5B: Predominant multiple antibiotic-resistant phenotypes for presumptive *Salmonella* isolated from cattle faeces and beef in different sampling areas in the North West Province. Phenotypes were generated using abbreviations that occur in the antibiotic discs

Sample Area	Phenotype	No. Observed	% Observed
Marikana (NO=12)	AP-CFM-NA-GM-CIPE-RP-PG	2	16.7%
	T-E-RP-C-PG	2	16.7%
	CIP-T-E-RP-PG-CFXM	2	16.7%
Brits (NO=8)	AP-T-E-RP-PG-CFXM	2	25%
	E-RP-PG	2	25%
	E-RP-PG-CFXM	2	25%
Ventersdorp (NO=10)	AP-T-E-RP-PG-CFXM	2	20%
	AP-E-RP-C-PG-CFXM	2	20%
Potchefstroom (NO=12)	AP-CFM-E-RP-C-PG-CFXM	1	8.3
	AP-CFM-RP-PG-CFXM	3	25%
	AP-T-E-RP-PG-CFXM	3	25%
	E-RP-PG-CFXM	2	16.7%
Carletonville (NO=16)	AP-CFM-RP-PG-CFXM	2	12.5%
	AP-CFM-PG-CFXM	2	12.5%
	AP-CFM-NA-T-E-RP-PG-CFXM	2	12.5%
	AP-T-E-RP-PG-CFXM	2	12.5%
	T-E-RP-PG-CFXM	2	12.5%
Lichtenberg (NO=10)	AP-T-E-RP-PG-CFXM	2	20%
	E-RP-PG-CFXM	2	20%
	AP-CFM-NA-T-E-RP-C-PG-CFXM	2	20%
	AP-CFM-E-RP-PG-CFXM	2	20%
Stella (NO=6)	AP-NA-T-E-RP-C-PG-CFXM	2	33.3%
	AP-CFM-NA-GM-E-RP-PG-CFXM	2	33.3%
Boshoek (NO=4)	AP-CFM-CIP-E-RP-C-PG-CFXM	1	25%

4.5 Phenotypic relationship between multiple antibiotic resistant *Salmonella* isolates obtained from cattle faeces and beef based on clustering patterns using the antibiotic inhibition zone diameter data

A total of 78 *Salmonella* isolates from cattle faecal and beef samples obtained from different locations within the North West Province were randomly selected and subjected to cluster analysis using their inhibition zone diameter data. A dendrogram was generated and detailed results are shown in Figure 4.5 . The dendrogram was analysed for associations of isolates from the different sampling sites and results are shown in Table 4.6. Two major clusters (cluster 1 and cluster 2) that contained four sub-clasters (1A, 1B, 2A and 2B) were obtained (Figure 4.5 and Table 4.6). Generally three sub-clusters (1A, 1B and 2A) were mixed since they had isolates from almost all the different sites sampled. Sub-cluster 1A was the largest (N = 37) and this sub-cluster was dominated by *Salmonella* isolates from all the different locations except for Stella and Boshoek. Despite the fact that 3 (8.1%) isolates from cattle faecal samples were present in this sub-cluster, it also possessed the majority 7 (18.9%) of isolates from beef samples obtained from Mafikeng when compared to the other stations. The second largest sub-cluster (sub-cluster 2A) contained 6 (27.3%) of faecal and 3 (13.6%) beef isolates obtained from Mafikeng. With the exception of Ventersdorp, Carletonville, Lichtenburg and Stella this sub-cluster possessed isolates from all the different sampling stations (Table 4.6). This sub-cluster also contained 3 (13.6%) isolates respectively from beef obtained from Rustenburg and Potchefstroom. Isolates from all stations except Boshoek, Potchefstroom, and Marikana were present in sub-cluster 1B while the smallest sub-cluster (sub-cluster 2B) only contained only 2 (50%) of beef

isolates obtained from Mafikeng and Vryburg. The great similarities in the antibiotic resistance profiles of *Salmonella* isolates from the different locations may have resulted from the indiscriminate and frequent use of these antibiotics in animals. It is therefore suggested that studies designed to determine relatedness of different isolates based on clustering of their antibiogram data may provide an understanding of the evolution of newer antibiotic resistant profiles. Furhermore, such data may be of great epidemiological importance and therefore be very useful in identifying the source of contamination.

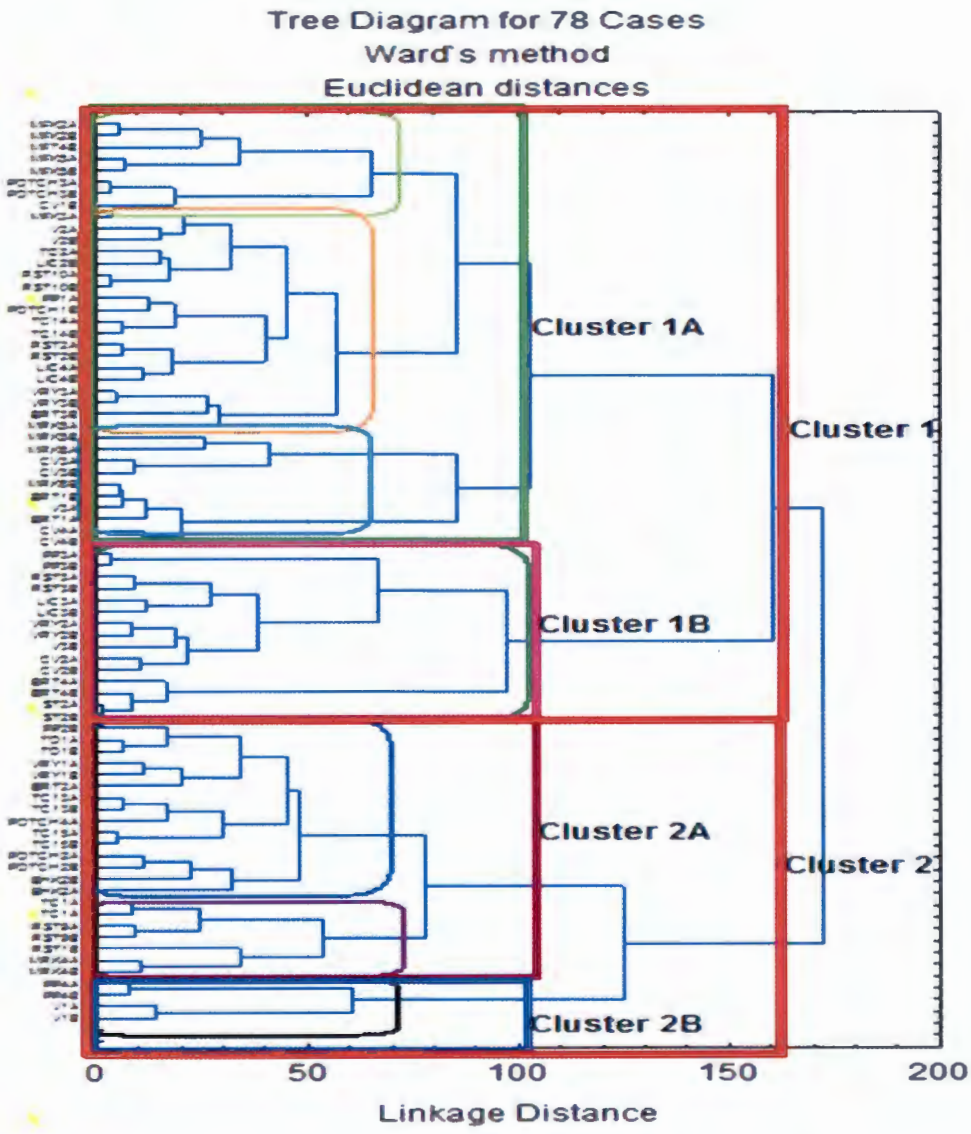

Figure 4.5: Dendrogram showing the relationship between *Salmonella* isolates from cattle faeces and beef obtained in the different locations. Bacterial designation prefixes are based on sampling station origin and sample type. The tree was constructed using Ward's method and Euclidean distances in Statistica, version 10 (Statsoft, US). Designation: MFT=Mafikeng, P=Potchefstroom, CV=Carletonville, V=Ventersdorp, R=Rustenburg, VRY=Vryburg, MRK=Marikana, BRT= Brits, CV=Carletonville, LIC=Lichtenberg, S=Stella, BHK=Boshoek, TC= Mafikeng faecal samples

Table 4.6: The percentage representation of *Salmonella* isolates obtained from different areas within the various clusters

Sampling Area	Sample source	Cluster 1A NT= 37	Cluster 1B NT=15	Cluster 2A NT=22	Cluster2B NT=4
Mafikeng	Beef	7 (18.9%)	2 (13.3)	3 (13.6%)	**2 (50%)**
	Cattle faeces	3 (8.1)	0 (0%)	6 (27.3%)	**0 (0%)**
Rustenburg	Beef	4 (10.8%)	2 (13.3)	3 (13.6%)	**0 (0%)**
Vryburg	Beef	2 (5.4%)	2 (13.3)	2 (9.1%)	**2 (50%)**
Marikana	Beef	4 (10.8%)	0 (0%)	2 (9.1%)	**0 (0%)**
Brits	Beef	3 (8.1%)	2 (13.3)	1 (4.5%)	**0 (0%)**
Ventersdorp	Beef	3 (8.1%)	1 (6.7%)	0 (0%)	**0 (0%)**
Potchefstroom	Beef	3 (8.1%)	0 (0%)	3 (13.6%)	**0 (0%)**
Carletonville	Beef	5 (13.5)	2 (13.3)	0 (0%)	**0 (0%)**
Lichtenberg	Beef	3 (8.1%)	2 (13.3)	0 (0%)	**0 (0%)**
Stella	Beef	0 (0%)	2 (13.3)	0 (0%)	**0 (0%)**
Boshoek	Beef	0 (0%)	0 (0%)	2 (9.1%)	0 (0%)

4.6 PCR assay to detect virulence genes in *Salmonella* isolates

4.6.1 *Detection of spvC virulence gene in Salmonella isolates*

A total of 140 *Salmonella* isolates were subjected to a virulence gene PCR analysis through amplification for *spvC* (Chiu and Ou, 1996; Amini *et al.*, 2010). A large proportion, 46 (30.7%) of the isolates, possessed the *spv*C virulent gene and the composition of isolates from the different sampling stations that were positive for this gene are shown in Table 4.7. Despite the fact that the expected amplicon size was 572 base pairs, fragments obtained from this study were slightly larger (630 bp). Figure 4.6 shows a 2% (w/v) agarose gel depicting the *spvC* gene fragments amplified from isolates.

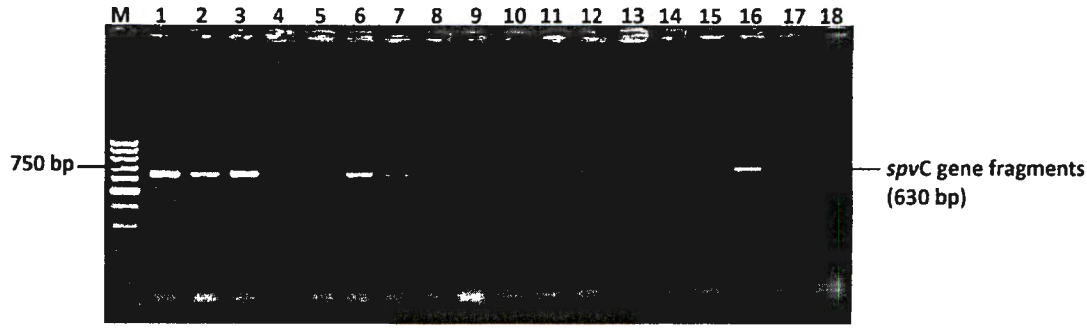

Figure 4.6: Agarose gel electrophoresis depicting *Salmonella* species specific for the *spv*C virulence gene fragments amplified from isolates obtained during the study. Lane M = 100 bp DNA marker; Lane 1 = *spv*C gene fragment amplified from *Salmonella enterica* subsp. diarizonae (ATCC 12325); Lanes 2-18 = *Salmonella* species specific *spv*C virulent gene fragments amplified from isolates obtained in the study.

4.6.2 Detection of *inv*A virulence gene in *Salmonella* isolates

A total of 140 *Salmonella* isolates were screened for the *inv*A virulence gene and a large proportion 38 (27.1%) were carrying this gene fragment (Table 4.7. Amplicons obtained in this study were 380 bp and Figure 4.7 shows a representation of a 2% (w/v) agarose gel depicting the *inv*A gene fragments amplified from isolates.

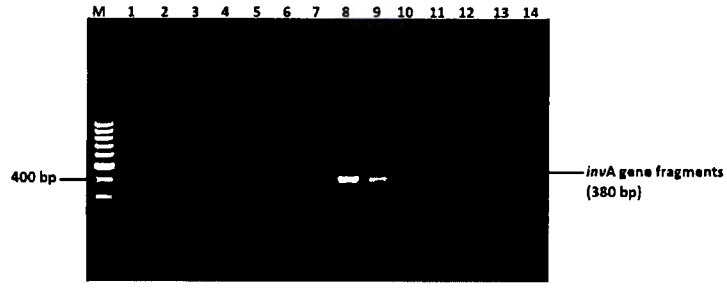

Figure 4.7: Agarose gel electrophoresis depicting *Salmonella* species specific *invA* virulent gene fragments amplified from isolates obtained during the study. Lane M = 100 bp DNA marker; Lane 1 = *invA* gene fragment amplified from *Salmonella enterica* subsp. diarizonae (ATCC 12325); Lanes 2-14 = *Salmonella* species specific virulent *invA* gene fragments amplified from isolates in the study

Table 4.7: Proportion of isolates that were positive for *Salmonella* virulent gene PCR analysis

Sampling Area	**No. Tested**	***SpvC***	***invA***
Mafikeng	NT	16	16
	NP	4 (25%)	6 (37.5%)
Rustenburg	NT	24	24
	NP	5 (20.8%)	3 (12.5%)
Vryburg	NT	22	22
	NP	10 (45.5%)	7 (31.8%)
Marikana	NT	12	12
	NP	3 (25%)	5 (41.7%)
Brits	NT	8	8
	NP	4 (50%)	4 (50%)
Ventersdorp	NT	10	10
	NP	4 (40%)	3 (30%)
Potchefstroom	NT	12	12
	NP	5 (41.7%)	1 (8.3%)
Carletonville	NT	16	16
	NP	4 (25%)	2 (12.5%)
Lichtenberg	NT	10	10
	NP	4 (40%)	3 (30%)
Stella	NT	6	6
	NP	2 (33.3%)	2 (33.3%)
Boshoek	NT	4	4
	NP	1 (25%)	2 (50%)
TOTAL	NT	140	140
	NP	**46 (30.7%)**	**38 (27.1%)**

4.7 Restriction fragment length polymorphism (RFLP) analysis of *Salmonella* 16S rRNA gene from isolates of various locations

A total of 128 *Salmonella* isolates obtained in the study through 16S rRNA gene fragments amplification were subjected to restriction fragment length polymorphism (RFLP) in order to determine their genetic similarities and differencies. The enzymes *EcoRI* and *HaeIII* were used to digest *Salmonella* specific 16S rRNA gene fragments amplified from the isolates. The banding patterns obtained for *Eco*RI and *Hae*III are shown in Figures 4.8 and 4.9 respectively. Despite the fact that the majority of *Salmonella* isolates produced similar banding patterns that consited of four fragments for both *Eco*RI and *Hae*III enzymes , the fragment differed in terms of size. For *Eco*RI the sizes ranged from 50 bp to 572 bp while fragments obtained with *Hae*III ranged from 50 bp to 300 bp.

Despite that most of the isolates yielded the same banding patterns of matching fragment sizes based on the different enzymes used, there was a smaller percentage of isolates that were not diggested by both *EcoRI* and *HaeIII* enzymes. Irrespective of the sampling site, the banding patterns observed in the majority of the *Salmonella* isolates were matching in terms of fragments and sizes depending on the enzyme involved. Moreover, the banding patterns of *Salmonella* isolates from cattle samples matched those of meat samples obtained from various areas within the North West Province.

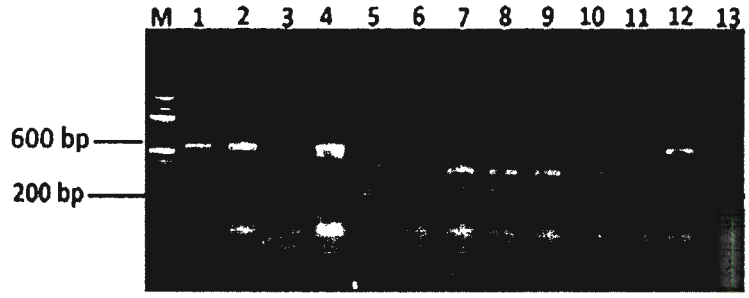

Figure 4.8: Restriction fragment length polymorphism (RFLP) patterns of representative *Salmonella* isolates from the different sources. Restriction enzyme *EcoRI* digestion of 16S rRNA gene. Lane M = 100 bp DNA marker; Lanes 1-14 = represantative *Salmonella* species band patterns observed in most of the *Salmonella* isolates.

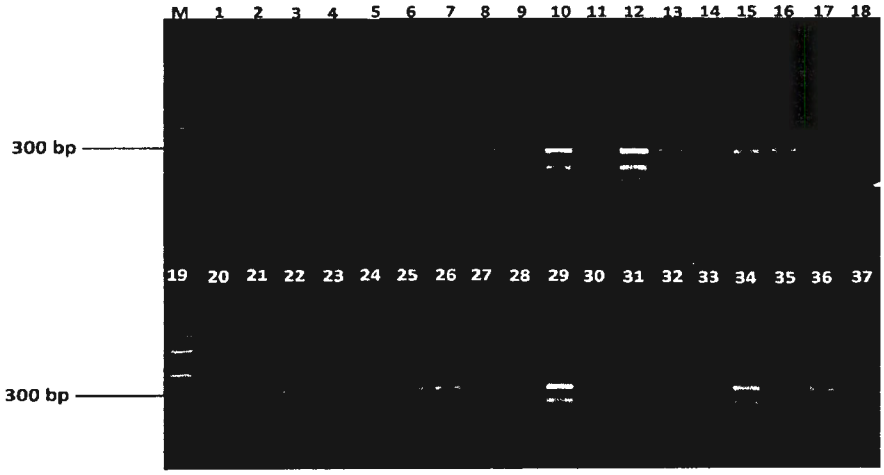

Figure 4.9: Restriction fragment length polymorphism (RFLP) patterns of representative *Salmonella* isolates from the different sources. Restriction enzyme *HaeIII* digestion of 16S rRNA gene. Lane M = 100 bp DNA marker; Lanes 1-37 = represantative *Salmonella* species band patterns observed in most of the *Salmonella* isolates obtained from both faeces and meat products.

4.8 Isolation of *Salmonella* specific bacteriophages and characterisation

4.8.1 *Plaque morphology*

A total of 10 randomly selected environmental antibiotic resistant *Salmonella* isolates whose identities had been confirmed by both biochemical and molecular assays and a control strain were used to isolate bacteriophages from faecal samples. All the strains were infected by bacteriophages and produced visible plaques and detailed results are shown in Figure 4.10 and 4.12. Despite the fact that plaques produced on environmental isolates were small and showed high similarities in their sizes, those obtained when using *Salmonella enterica* subsp. diarizonae (ATCC 12325) reference strain were much bigger.

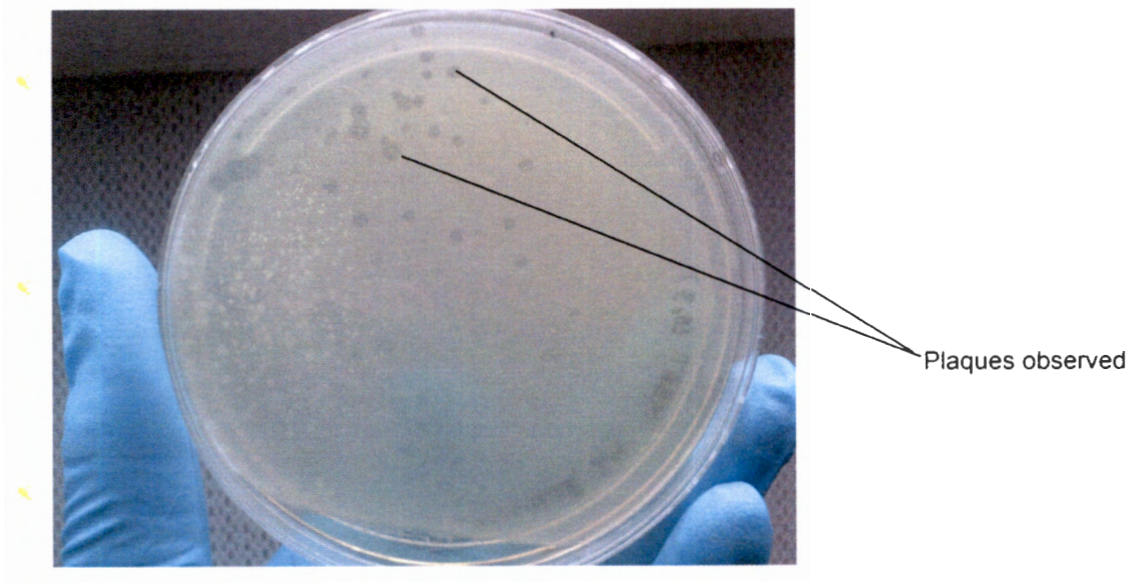

Figure 4.10: Double plaque soft agar assay of *Salmonella enterica* subsp. diarizonae (ATCC 12325) reference strain. The image depicts the plaques morphology of *Salmonella* species specific phage observed during plaque assay.

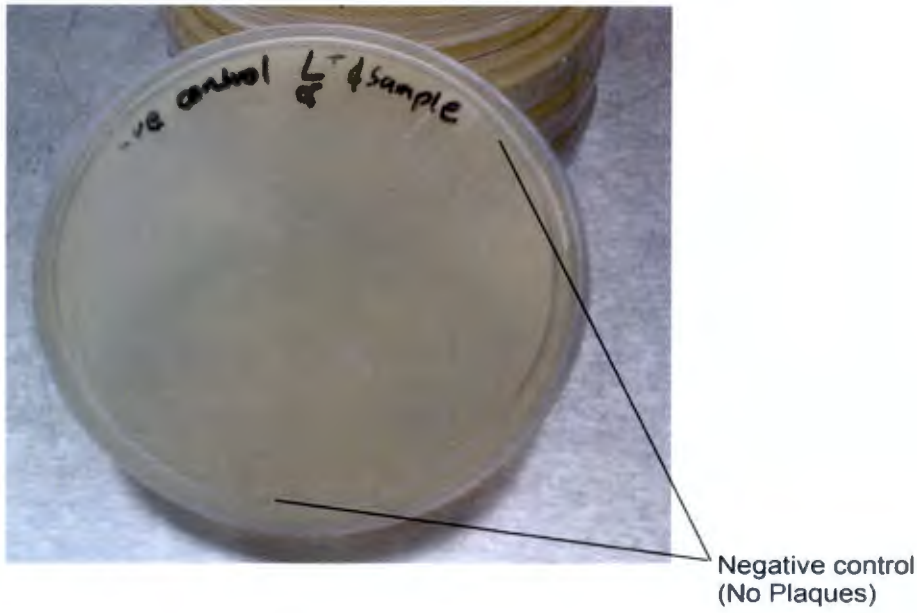

Figure 4.11: Double plaque soft agar assay of negative control. The image depicts the absence of plaques on the plate observed during plaque assay.

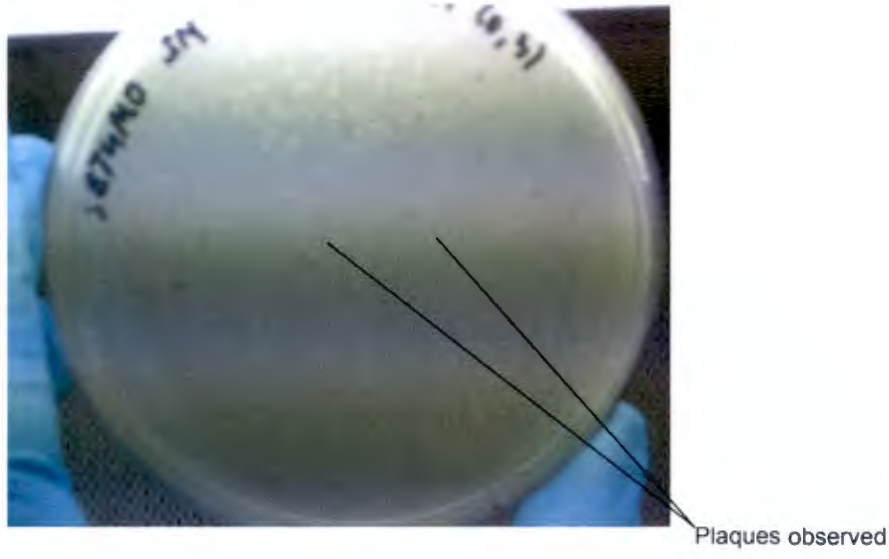

Figure 4.12: Double plaque soft agar assay of multiple antimicrobial *Salmonella* isolates obtained from different locations within the North West Province. The image depicts the plaques of *Salmonella* species specific phages observed during plaque assay.

4.8.2 *Phage microplate virulence assay*

The virulence potential of ten isolated bacteriophages were screened to assess their ability to lyse multiple antibiotic resistant *Salmonella* strains using microplate virulence assay. None of the isolates was able to lyse any of the *Salmonella* strains tested (Figure 4.14. Phage titers can influence or greatly affect the virulance abilities of bacteriophages and also the bacteriophage/host (target) bacteria ratio is very crutial in the success or effectiveness of a phage (Ly-Chatain, 2014). This may therefore explain the reasons for the observation.

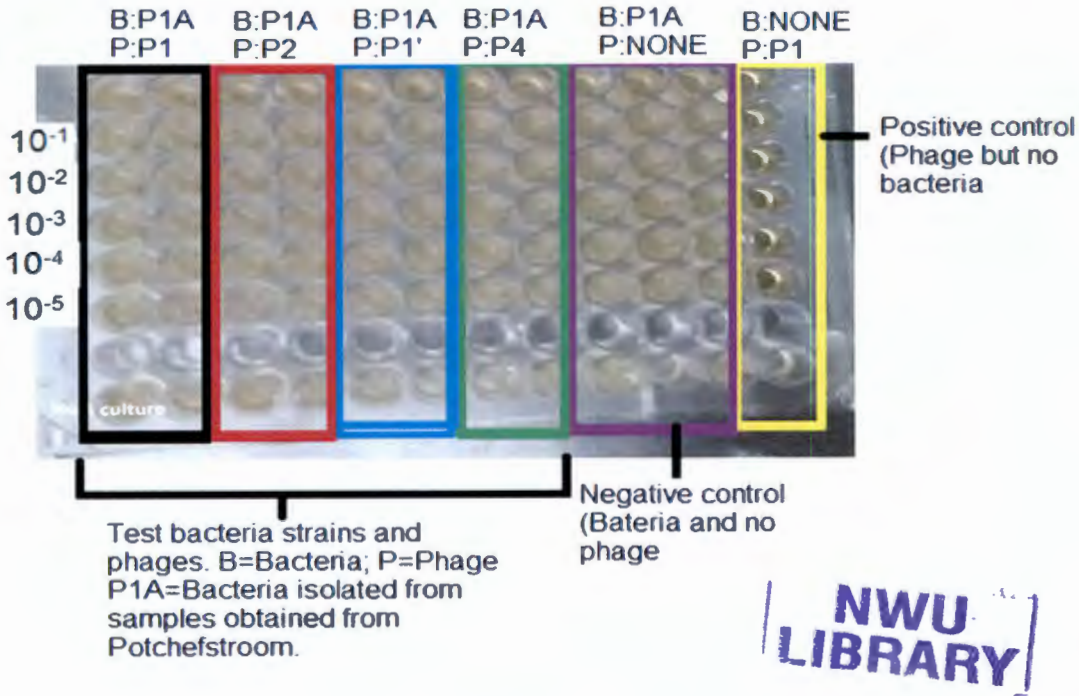

Figure 4.13: Phage microplate virulence assay of environmental *Salmonella* isolates obtained in different locations within the North West Province. The picture represents the results observed for all the tested bacteriophages and *Salmonella* isolates as well as the positive *Salmonella* strain. P1(POTCH 1A) = phage 1 and *Salmonella* isolate obtained from Potchefstroom, NC= negative control, PC= postive control and host culture= *Salmonella* isolate.

CHAPTER 5

DISCUSSION

CHAPTER 5

DISCUSSION

5.1. GENERAL DISCUSSION

The primary aim of this study was to isolate *Salmonella* species from cattle faecal material and raw beef samples obtained from some supermarkets and butcheries in the North West Province, South Africa. A total of 300 presumptive isolates satisfied the morphological characteristics of *Salmonella* species and a large proportions 291 (97%) of the isolates did not possess the cytochrome enzyme that forms part of bacterial respiratory system and therefore were oxidase-negative (Maddocks *et al.*, 2002). This preliminary finding were similar to those of a previous report which indicated that *Salmonella* species are usually oxidase negative (Maharjan *et al.*, 2006), despite the fact that a small proportion 9 (3%) of isolates have been reported to possess the enzyme (Maharjan *et al.*, 2006).

In addition, a large proportion, 90.3% to 100%, of these presumptive isolates were able to ferment glucose, sucrose and lactose in the TSI medium. *Salmonella* species are known not to lactose and this is currently used to morphologically differentiate *Salmonella* (non-lactose fermenters) from *E. coli* (lactose fermenters) on MacConkey agar which is a differential medium for *Enterobacteriaceae*. However, a previous report highlighted that inability to ferment lactose and sucrose cannot be used to discriminate *Salmonella* from other bacterial strains as this could present misleading data (Gonzalez, 1966). This explains the need to supplement these tests with more reliable genetic assays.

Despite the fact that a large proportion 215 (71.7%) of the isolates in this study produced gas on Triple Sugar Iron (TSI) agar and this phenotypic characteristic is typical for *Salmonella* species, only a small proportion 12 (4%) produced hydrogen sulfide (H_2S) on the agar. These results are in accordance with a report by Dube (1983) in which some *Salmonella* species produced gas from fermentation of carbohydrates but without any hydrogen sulfide activity on TSI agar. In addition, it has been reported that *Salmonella* species may not produce hydrogen sulfide gas as a result of mutation in the bacterial genome (Barrett and Clark, 1987). Moreover, *Salmonella* species from food samples have also been reported to be negative for H_2S production (Sperber and Deibel, 1969). These findings as well as those previously documented indicate that *Salmonella* species may react differently to some biochemical assays.

The high unreliability of preliminary biochemical tests suggests that presumptive identification of bacteria species including isolates belonging to the genus *Salmonella* need to be supplemented with specific confirmatory tests in order to obtain true positive results and vice versa (Gonzalez, 1966). Against this background, all the presumptive *Salmonella* isolates were further subjected to confirmatory biochemical tests that included the analytical profile index (API) 20E test (Nucera *et al.*, 2006) and *Salmonella* specific serological assays (Popoff *et al.*, 1998). A total of 23 representative isolates selected randomly were analysed using API 20E and among those only 8 (34.8%) we positively identified as *Salmonella* species. These findings are in line with a study that was conducted in Iraq to determine the presence of *Salmonella* species in poultry meat (Saeed *et al.*, 2011). The findings of the study indicated that not all *Salmonella* species

can be identified through using API 20E and therefore some strains will require other more sensitive assays such as molecular genetic tests.

Serological assays are generally considered to be less labour intensive techniques with very high sensitivity and specificity and these assays were also employed for identification of all 300 *Salmonella* isolates (Gruenewald *et al.*, 1990). A large proportion 185 (61.7%) of the isolates were positively identified as *Salmonella* species through the use of the "H" antigen phases 1 and 2. In addition, a significantly large proportion 159 (53%) of the isolates were also identified as *Salmonella* species using *Salmonella* specific groups A to G "O" antigen. The results obtained using the serological assay were in accordance with those of a previous report (Roy *et al.*, 2002). Furthermore, it has also been reported that serological assays that are used to identify *Salmonella* species may fail to detect some species in certain instances therefore giving false negative results (Abouzeed *et al.*, 2000).

It is well known that PCR remains the gold standard tool for the identification of bacterial isolates and this is based on its high sensitivity and specificity (Malkawi, 2003; Rodulfo *et al.*, 2012; Zahran and El-Behiry, 2014). All 300 presumptive *Salmonella* isolates were further subjected to *Salmonella* specific PCR analysis that was designed to target the 16S rRNA, *fli*C and *fli*B gene fragments. These genes are present in all *Salmonella* species and are therefore known to be housekeeping genes. A total of 128 (42.7%) isolates were positively identified as *Salmonella* species through amplification of 16S rRNA gene fragment and similar observations have been reported (Jadidi *et al.*, 2012). It is suggested that the 16S rRNA gene segment is highly conserved in *Salmonella*

species despite the host species and geographical location from which it was isolated and is routinely used for the identification of *Salmonella* species (Sumithra *et al.*, 2014). Among the different locations sampled, large proportions of the isolates from beef samples obtained from Brits 8 (100%), Lichtenberg 7 (70%), Ventersdorp 6 (60%), Marikana 7 (58.3%) and Vryburg 12 (54.5%) were positive for the 16S rRNA gene fragment. From these results it can be deduced that the level of *Salmonella* contamination was high among raw beef obtained from these sampling sites and the consumption of these food products when undercooked may result in serious public health complications in individuals in the area (Dallal, 2009).

Despite the fact that a large proportion of *Salmonella* isolates was positively identified through amplification of *Salmonella* specific 16S rRNA gene fragments, small proportions 60 (20%) and 80 (26.7%) possessed the *fli*C and *fli*B *Salmonella* specific gene fragments respectively. Despite the fact that the number of isolates that was positively identified as *Salmonella* species based on amplification of the 16S rRNA, *fliC* and *fliB* gene fragments varied, the sum of occurrence of gene determinants that encode for flagellins (*fliC* and *fliB*) correlates with the detection of *Salmonella* species based on the 16S rRNA specific PCR analysis. This is supported by the fact that most *Salmonella* strains exhibit two structural genes (*fliC* and *fliB*) that encode flagellins and only one of these structural genes is expressed in the bacterium at any given time (Paiva *et al.*, 2009). This may therefore explain the differences in their occurrence of both the *fliC* and *fliB* genes in the isolates that were screened.

Food products especially beef have been reported to cause foodborne infections worldwide and *Salmonella* species have been isolated from beef products in many countries (Ejeta *et al.*, 2004; Meyer *et al.*, 2010; Adesiji *et al.*, 2011; Aftab *et al.*, 2012). As such *Salmonella* species are of great public health concern both to humans and food quality regulating authorities (Maharjan *et al.*, 2006). Despite the fact that infants, immuno-compromised individuals and elderly people are the most vulnerable, bacterial foodborne infections including those caused by *Salmonella* species (Gordon *et al.*, 2008; Morpeth *et al.*, 2009), and the presence of these pathogens in food products is a cause of concern. This is even amplified by the fact that *Salmonella* species are known to claim millions of human lives yearly in both developing and developed countries (Graham, 2002; Molla *et al.*, 2003; Zamxaka *et al.*, 2004; Akhtar *et al.*, 2014). Despite the fact that there is a relative acceptable number of bacterial cells which when present in a food product still renders it safe for consumption by public health standards the presence of these pathogens in food products including beef, still remains a public health concern. This is motivated by the fact that *Salmonella* species have a very low infectious dose and this explains why it poses serious challenges in both developing and developed countries. It is also suggested that food products particularly those of animal origin be properly cooked to reduce the level of contamination before they are consumed. This will in turn reduce the occurrence and burden of human infections.

Another objective of the present study was to determine the antibiotic resistance profiles of *Salmonella* isolates against a panel of 12 antimicrobial agents. Large proportions of the *Salmonella* isolates were resistant to Erythromycin 120 (83.3%), Rifampicin 129 (92.1%), Penicillin 132 (94.3%), Ampicillin 86 (61.4%), and Cephalexin 101 (72.1%).

These results are in agreement with several studies which indicated that *Salmonella* species isolated from food and clinical samples were resistant to these drugs (Threlfall, 2002; Angulo *et al.*, 2009; Abatcha *et al.*, 2014b). It is reported that resistance of food borne pathogens to antimicrobial agents usually results from the misuse or over use of antimicrobial agents in food producing animals either as treatment options or growth stimulants (Hao *et al.*, 2014). Despite the benefits to both farmers and animals on using amtimicrobial agents in animals, this still remains a complex and controversial issue since it is still not clear if the development and presence of antimicrobial resistance determinants is outweighed by these benefits (Marshall and Levy, 2011; Hao *et al.*, 2014). However, the detection of multiple antibiotic resistant *Salmonella* strains was a cause for concern.

Among the antibiotics tested, Norfloxacin showed the least resistance 6 (4.3%) against a large proportion of the *Salmonella* isolates from the different sampling locations. This might have been due to the fact that this drug is not used in animal or human medicine in the area. Interestingly, a large proportion of isolates 129 (92.1%) were resistant to Rifampicin (Table 4.4B). However, this drug is not used in food producing animals but in companion animals in the area, and it is used concurrently with other drugs such as erythromycin to limit resistance of pathogens and this may account for the high resistance observed. Similarly, a large proportion 120 (83.3%) of these isolates were resistant to erythromycin. These results are similar to those of a previous report in which high resistance to erythromycin was detected among *E. coli* O157 strains isolated from food producing animals in the area (Ateba and Bezuidenhout, 2008).

Tetracyclines and Fluoroquinolones are commonly used in the treatment of disease in livestock in most developing countries (Adesokan *et al.*, 2015). The results obtained in the current study showed that 48 (34.3%) of the isolates were resistant to tetracycline while only 6 (4.3%) were resistant to Norfloxacin. However, resistance of *Salmonella* isolates to tetracyclines may be linked to the fact that tetracycline is generally used in animal medicine and as a growth promoter (Ateba and Bezuidenhout, 2008). Despite the fact that tetracycline is a schedule 4 product which requires a prescription, it is easily accessible over the counter and this may account for high resistance to the drug in the area. Similarly, a large proportion 132 (94.3%) of the isolates were resistant to penicillin and this drug is commonly used as a first line of defense against infectious agents in both animal and human medicine in the area.

In general, a very large proportion 134 (95.7%) of the *Salmonella* isolates were resistant to three or more antibiotics belonging to different classes and were termed multiple antibiotic resistant strains. These findings are in agreement with previous reports in which *Salmonella* species from food products were resistant to one or more of the antimicrobial agents tested (Threlfall, 2002; Angulo *et al.*, 2009). The high prevalence of multiple antibiotic resistance (MAR) *Salmonella* that was observed in this study indicate that these pathogens may not only negatively affect the treatment of human infections in the area but also serve as reservoirs for the transmission of resistant determinants to other bacterial strains. This is based on the fact that food products, particularly those of animal origin have been reported to be the main source of resistant bacterial strains that

pose a huge challenge to human medicine even in countries with more advanced health care facilities (White *et al.*, 2002). Against this background, it is of great importance to implement strategies that will significantly reduce the occurrence of antibiotic resistant foodborne pathogens, especially *Salmonella* species in a given area (Newell *et al.*, 2010).

It has been suggested that continuous surveillance of food products to monitor the occurrence of antibiotic resistant bacterial species that are generally associated with infections in humans and animals is of huge epidemiological importance (Cardinale *et al.*, 2005). In the current study, cluster analysis of antibiotic inhibition zone diameter data of multiple antibiotic resistant *Salmonella* isolates obtained from cattle faecal material and beef was used to determine relatedness of isolates from the different sources. Large proportions 37(48.7%) of the *Salmonella* isolates from cattle faecal material and raw beef obtained from different locations clustered together. The similarities in the antibiotic resistance profiles of the isolates was responsible for the clustering patterns observed and this indicated that animals may be harboring multiple antibiotic resistant strains that are transmitted to their associated food products such as beef.

Given the public health risks associated with the presence of resistant *Salmonella* strains in animals and food products it is of great importance to ensure that proper farm management techniques as well as appropriate standard operating measures are implemented in both the farms and abattoirs (Kagambèga *et al.*, 2013). This is based on

the premise that agricultural food products of animal origin that have been exposed to the uncontrolled usage of antimicrobial agents are known to significantly contribute to the emergence of antibiotic resistant strains that pose serious challenges to both veterinary and human medicine (White *et al.*, 2002; Marshall and Levy, 2011; Hao *et al.*, 2014)

Another objective of the study was to determine the pathogenicity of the isolates through amplification of *Salmonella* specific virulence gene fragments obtained from both cattle faecal material and beef samples. Large proportions 46 (30.7%) and 38 (27.1%) of the isolates possessed the *spv*C and *inv*A genes indicating that they are able to cause health complications in susceptible individuals. Similar observations have been reported in several studies that were designed to determine the pathogenic capabilities of *Salmonella* species isolated from food products, particularly beef (Abouzeed *et al.*, 2000; Meyer *et al.*, 2010; Ateba and Mochaiwa, 2014; Amin and El-Rahman, 2015). Despite the fact that the amplicon sizes were not as expected, the sizes of these gene fragments among the environmental and positive control *Salmonella* strains were consistent.

The high prevalence of *inv*A and *spv*C genes in large proportions of *Salmonella* species isolated from different locations within the North West Province indicates that beef products sold at retail supermarkets and butcheries could serve as potential sources for the transmission of disease to humans. The *inv*A gene segment is present in the genetic locus of *Salmonella* species and this gene determinant is responsible for the

ability of these pathogens to invade cells of the intestinal epithelium (Galan *et al.*, 1992). Moreover, expression of genes that encode the *inv*A segment is known to initiate internalization of cells of *Salmonella* organisms that is required for invasion of deeper tissues during human infections (El-Feky *et al.*, 2014). However, the detection of the *inv*A gene in 38 (27.1%) of the *Salmonella* strains obtained from Brits, Vryburg, Mafikeng, Ventersdorp, Lichtenberg, Stella and Boshoek was a cause for great concern.

In general, 46 (30.7%) of the *Salmonella* isolates that carried the *spv*C gene also possessed the *inv*A gene while only a small proportion 8 (5.7%) of those that were positive for the *inv*A gene fragment did not harbour the *spv*C gene. These findings are in accordance with a report involving *Salmonella* isolated from animals and humans in Iraq (Amini *et al.*, 2010). However, the high prevalence of the *spv*C gene fragments in large proportions of the *Salmonella* isolates may be due to the fact that it is plasmid encoded and can easily be transferred through conjugation, transformation and transduction among bacterial species (Chuanchuen *et al.*, 2010).

Despite the fact that the mechanisms through which the two structural (spv) genes such as *spv*C enhence bacterial virulence is still not well understood, it is documented that the *spv*C gene segement is translocated into the host cell during an infection through the *Salmonella* pathogenicity island-2 type-three secretion system (Guiney and Fierer, 2011). In the present study, a large proportion of the *Salmonella* isolates obtained from cattle faecal matter and beef haboured both *inv*A and *spv*C virulent genes. The findings are similar to previous reports (Abouzeed *et al.*, 2000; Amini *et al.*, 2010; Smith *et al.*,

2015) and this may suggest that beef products can pose grave health risks, particularly to consumers.

Another objective of the study was to determine the genetic similarities of *Salmonella* isolates using PCR-RFLP. It is suggested that subtyping techniques such as RFLP combined with phenotypic properties are very instrumental in determining the genetic relatedness of *Salmonella* isolates from different sources. This could be used to significantly determine the source of contamination during outbreaks of infection (Abatcha *et al.*, 2014a) and it is recommended for epidemiological studies (Wattiau *et al.*, 2011). Most of the *Salmonella* species isolated from cattle faecal material and raw beef produced similar banding patterns for both *Eco*RI and *Hae*III enzymes. Despite this, the bands obtained for these enzymes differed significantly with respect to their sizes and similar observations have been reported (Sumithra *et al.*, 2014). The findings suggest that the great genetic similarities among *Salmonella* isolates obtained from different sources of cattle faecal matter and raw beef indicate the need to implement proper strategies that will limit cross contamination.

A further objective of the study was to isolate and determine the morphology of *Salmonella* specific bacteriophages from cattle faecal material. The emergence of multiple antibiotic resistant (MAR) bacterial strains including *Salmonella* has resulted in treatment failures to several human diseases that claim millions of lives every year globally (Abatcha *et al.*, 2014b). This has recently developed renewed interest in bacteriophages and they are currently being exploited for their abilities to serve as

promising antimicrobial agents (Wittebole *et al.*, 2014). In the current study all ten different randomly selected *Salmonella* isolates obtained from different locations were successful infected with bacteriophages from feacal samples and plaques were detected using the double agar technique as shown in Figure 4.10 and 4.12. These findings are in line with a previous report in which *Salmonella* and *Escherichia coli* specific bacteriophages were detected in animal faeces (Callaway *et al.*, 2008; Callaway *et al.*, 2010). In addition, phages in the faeces also infected the *Salmonella* reference strain (Figure 4.10).

CHAPTER 6

CONCLUSION AND RECOMENDATIONS

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The main aim of this study was to detect *Salmonella* species from cattle faecalmaterial and raw beef products obtained from various supermarkets and butcheries within the North West Province. The results obtained indicated that *Salmonella* species were positively indentified and therefore their presence in raw beef in particular may present serious health challenges to humans in the area. This is motivated by the fact that foodborne pathogens including *Salmonella* are reported to claim millions of human lives worldwide and these infections occur even in countries with both more advanced public health systems and health care facilites. This therefore amplifies the need to implement strict control measures that will greatly reduce cross contamination.

In this study, a large proportion of the isolates were resistant to three or more antibiotics and were termed MAR strains. In addition the MAR profiles of *Salmonella* isolates from different sources as well as sampling stations indicate the need to control the usage of antimicrobial agents, especially in the agricultural sector. This is based on the notion that the uncontrolled usage of certain antimicrobial agents either as growth stimulant or prophylactic treatment in animals significantly contributes to the development of resistant bacterial strains which results in treatment failures especially in human medicine.

The presence of virulence gene fragments in a large proportion of the *Salmonella* isolates indicated that cattle and beef may serve as potential sources for the transmission of pathogenic *Salmonella* to humans in the area. Therefore, the consumption of undercooked beef pose serious health risks to individuals especially infants, the elderly and those who are immunocompromised. Moreover, the very low infectious dose of *Salmonella* species amplifies the need to enforce strict control measures in the farms, abattoirs and retail shops. However, proper cooking of meat products by subjecting them to appropriate temperatures that inactivate the cells cannot be overlooked.

Salmonella specific bacteriophages were detected in cattle faecal material and their morphologies were determined. It was also determined that *Salmonella* specific bacteriophages isolated from cattle faeces were capable of infecting multiple antibiotic resistant (MAR) *Salmonella* strains based on the double agar plaque assay. *Salmonella* specific phages did not reveal any significant virulence capabilities. However, it is suggested that more studies designed to critically dertermine the virulence potential as well as fully chracterise these *Salmonella* specific phages be performed. This will provide options for new biological products that may be very useful in the control of *Salmonella* isolates on farms and food industries.

REFERENCES

Aarestrup, F. M. 1999. Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. *International Journal of Antimicrobial Agents,* 12, 279-285.

Abatcha, M., Zakaria, Z., Dauda, M., Dhaliwal, K. 2014a. Typing of *Salmonella* Species: A Mini-Review. *Journal of Natural Sciences Research,* 4, 13-17.

Abatcha, M., Zakaria, Z., Kaur, D., Thong, K. 2014b. Review Article: A trends of *Salmonella* and antibiotic resistance. *Advances in Life Science and Technology,* 17, 9-21.

Abdullahi, M. 2010. Incidence and antimicrobial susceptibility pattern of *Salmonella* species in children attending some hospitals in kano metropolis, kano state–Nigeria. *Bayero Journal of Pure and Applied Sciences,* 3.

Abouzeed, Y. M., Hariharan, H., Poppe, C., Kibenge, F. S. 2000. Characterization of *Salmonella* isolates from beef cattle, broiler chickens and human sources on Prince Edward Island. *Comparative immunology, microbiology and infectious diseases,* 23, 253-266.

Abuladze, T., Li, M., Menetrez, M. Y., Dean, T., Senecal, A., Sulakvelidze, A. 2008. Bacteriophages reduce experimental contamination of hard surfaces, tomato, spinach, broccoli, and ground beef by *Escherichia coli O157: H7. Applied and Environmental Microbiology,* 74, 6230-6238.

Acheson, D., Hohmann, E. L. 2001. Nontyphoidal salmonellosis. *Clinical Infectious Diseases,* 32, 263-269.

Adesiji, Y., Alli, O., Adekanle, M., Jolayemi, J. 2011. Prevalence of *Arcobacter, Escherichia coli, Staphylococcus aureus* and *Salmonella* species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. *Sierra Leone Journal of Biomedical Research,* 3, 8-12.

Adesokan, H. K., Akanbi, I. O., Akanbi, I. M., Obaweda, R. A. 2015. Pattern of antimicrobial usage in livestock animals in south-western Nigeria: the need for alternative plans: original research. *ONDERSTEPOORT Journal of Veterinary Research,* 82, 1-6.

Aftab, M., Rahman, A., Qureshi, M., Akhter, S., Sadique, U., Sajid, A., Zaman, S. 2012. Level of *Salmonella* in beef of slaughtered cattle at Peshawar. *J Animal Plant Sci,* 22, 24-27.

Ahmed, T., Mahbub-E-Elahi, A., Islam, M. J., Hasan, M. K. 2015. Isolation and Identification of Bacteria from the Faecal Samples of Diarrhoeic Calf and Their Antibiogram Study. *Journal of Chemical, Biological and Physical Sciences (JCBPS),* 5, 2671.

Akhtar, S., Sarker, M. R., Hossain, A. 2014. Microbiological food safety: a dilemma of developing societies. *Critical Reviews in Microbiology,* 40, 348-359.

Akond, M. A., Shirin, M., Alam, S., Hassan, S., Rahman, M. M., Hoq, M. 2013. Frequency of drug resistant *Salmonella* spp. isolated from poultry samples in Bangladesh. *Stamford Journal of Microbiology,* 2, 15-19.

Akyala, A. I., Alsam, S. 2015. Extended Spectrum Beta Lactamase Producing Strains of *Salmonella* species-A Systematic Review. *Journal of Microbiology Research,* 5, 57-70.

Al-Mazini, M. A., Al-Hajaj, M. A. 2015. Detetion of Antibiotics Resistance Genes in Multidrug Resistant of *Salmonella Enterica* Serovar Typhi. *International Journal of Innovations in Engineering and Technology (IJIET)* 5.

Alisky, J., Iczkowski, K., Rapoport, A., Troitsky, N. 1998. Bacteriophages show promise as antimicrobial agents. *Journal of Infection,* 36, 5-15.

Altekruse, S., Cohen, M., Swerdlow, D. 1997. Emerging foodborne diseases. *Emerging infectious diseases,* 3, 285.

Amin, H. S., El-Rahman, A. 2015. Molecular characterization of *Salmonella enterica* isolated from chicken meat and its products by multiplex PCR. *Alexandria Journal of Veterinary Sciences,* 46, 155-160.

Amini, K., Salehi, T. Z., Nikbakht, G., Ranjbar, R., Amini, J., Ashrafganjooei, S. B. 2010. Molecular detection of *invA* and *spv* virulence genes in *Salmonella enteritidis* isolated from human and animals in Iran. *African Journal of Microbiology Research,* 4, 2202-2210.

Angulo, F. J., Collignon, P., Powers, J. H., Chiller, T. M., Aidara-Kane, A., Aarestrup, F. M. 2009. World Health Organization ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies for the use of antimicrobials in food production animals. *Clinical infectious diseases,* 49, 132-141.

Ateba, C. N., Bezuidenhout, C. C. 2008. Characterisation of *Escherichia coli* O157 strains from humans, cattle and pigs in the North-West Province, South Africa. *International journal of food microbiology,* 128, 181-188.

Ateba, C. N., Mochaiwa, B. 2014. Use of *inv*A Gene Specific PCR Analysis for the Detection of Virulent *Salmonella* Species in Beef Products in the North West Province, South Africa. *Journal of Food and Nutrition Research,* 2, 294-300.

Atterbury, R. J., Connerton, P. L., Dodd, C. E., Rees, C. E., Connerton, I. F. 2003. Application of host-specific bacteriophages to the surface of chicken skin leads to a reduction in recovery of *Campylobacter jejuni*. *Applied and environmental microbiology,* 69, 6302-6306.

Authority, E. 2011. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. *EFSa Journal,* 9, 2090.

Ball, M., Magowan, E., Taylor, M., Bagdonaite, G., Madden, R. 2011. A review of current knowledge on *Salmonella* control on-farm and within the processing plant relevant to the Northern Ireland pig industry. *Agri-Food Biosci Inst,* 1-47.

Barham, A., Barham, B., Johnson, A., Allen, D., Blanton, J., Miller, M. 2002. Effects of the transportation of beef cattle from the feedyard to the packing plant on prevalence levels of *Escherichia coli O157* and *Salmonella* spp. *Journal of Food Protection®,* 65, 280-283.

Barrett, E. L., Clark, M. A. 1987. Tetrathionate reduction and production of hydrogen sulfide from thiosulfate. *Microbiological reviews,* 51, 192.

Beach, J. C., Murano, E. A., Acuff, G. R. 2002. Prevalence of *Salmonella* and *Campylobacter* in beef cattle from transport to slaughter. *Journal of Food Protection®,* 65, 1687-1693.

Bell, R. 2015. Outbreak of *Salmonella Newport* Infections Linked to Cucumbers—United States, 2014. *Centers for Disease Control Prevention,* 64, 144-147.

Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection®,* 59, 204-216.

Bicudo, J., Goyal, S. 2003. Pathogens and manure management systems: A review. *Environmental Technology,* 24, 115-130.

Biran, A., Curtis, V., Gautam, O. P., Greenland, K., Islam, S., Schmidt, W. P., Sijbesma, C., Sumpter, C., Torondel, B. 2012. Background Paper on Measuring WASH and Food Hygiene Practices–Definition of Goals to be Tackled Post 2015 by the Joint Monitoring Programme. Citeseer.

Brenner, F., Villar, R., Angulo, F., Tauxe, R., Swaminathan, B. 2000. *Salmonella* nomenclature. *Journal of clinical microbiology,* 38, 2465-2467.

Bryan, F. L. 1988. Risks of practices, procedures and processes that lead to outbreaks of foodborne diseases. *Journal of Food Protection®,* 51, 663-673.

Buncic, S., Sofos, J. 2012. Interventions to control *Salmonella* contamination during poultry, cattle and pig slaughter. *Food research international,* 45, 641-655.

Busani, L., Cigliano, A., Taioli, E., Caligiuri, V., Chiavacci, L., Di Bella, C., Battisti, A., Duranti, A., Gianfranceschi, M., Nardella, M. 2005. Prevalence of *Salmonella enterica* and *Listeria monocytogenes* contamination in foods of animal origin in Italy. *Journal of Food Protection®,* 68, 1729-1733.

Bywater, R., Deluyker, H., Deroover, E., De Jong, A., Marion, H., Mcconville, M., Rowan, T., Shryock, T., Shuster, D., Thomas, V. 2004. A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. *Journal of Antimicrobial Chemotherapy,* 54, 744-754.

Calci, K. R., Burkhardt, W., Watkins, W. D., Rippey, S. R. 1998. Occurrence of male-specific bacteriophage in feral and domestic animal wastes, human feces, and human-associated wastewaters. *Applied and Environmental Microbiology,* 64, 5027-5029.

Callaway, T., Keen, J., Edrington, T., Baumgard, L., Spicer, L., Fonda, E., Griswold, K., Overton, T., Vanamburgh, M., Anderson, R. 2005. Fecal prevalence and diversity of *Salmonella* species in lactating dairy cattle in four states. *Journal of Dairy Science,* 88, 3603-3608.

Callaway, T. R., Edrington, T. S., Brabban, A., Kutter, E., Karriker, L., Stahl, C., Wagstrom, E., Anderson, R. C., Genovese, K., Mcreynolds, J. 2010. Occurrence of *Salmonella*-specific bacteriophages in swine feces collected from commercial farms. *Foodborne pathogens and disease,* 7, 851-856.

Callaway, T. R., Edrington, T. S., Brabban, A. D., Anderson, R. C., Rossman, M. L., Engler, M. J., Carr, M. A., Genovese, K. J., Keen, J. E., Looper, M. L. 2008. Bacteriophage isolated from feedlot cattle can reduce Escherichia coli O157: H7 populations in ruminant gastrointestinal tracts. *Foodborne pathogens and disease,* 5, 183-191.

Cardinale, E., Perrier Gros-Claude, J., Rivoal, K., Rose, V., Tall, F., Mead, G., Salvat, G. 2005. Epidemiological analysis of *Salmonella enterica* ssp. enterica serovars Hadar, Brancaster and Enteritidis from humans and broiler chickens in Senegal using pulsed-field gel electrophoresis and antibiotic susceptibility. *Journal of applied microbiology,* 99, 968-977.

Carlton, R. M. 1999. Phage therapy: past history and future prospects. *Archivum Immunologiae Et Therapiae Experimentalis-English Edition,* 47, 267-274.

Centers for Disease Control Prevention, C. 2010. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-10 states, 2009. *MMWR. Morbidity and mortality weekly report,* 59, 418.

Charan, J., Biswas, T. 2013. How to calculate sample size for different study designs in medical research? *Indian Journal of Psychological Medicine,* 35, 121.

Chiu, C.-H., Ou, J. T. 1996. Rapid identification of *Salmonella* serovars in feces by specific detection of virulence genes, invA and spvC, by an enrichment broth culture-multiplex PCR combination assay. *Journal of Clinical Microbiology,* 34, 2619-2622.

Chuanchuen, R., Ajariyakhajorn, K., Koowatananukul, C., Wannaprasat, W., Khemtong, S., Samngamnim, S. 2010. Antimicrobial resistance and virulence genes in *Salmonella enterica* isolates from dairy cows. *Foodborne pathogens and disease,* 7, 63-69.

Chuanchuen, R., Padungtod, P. 2009. Antimicrobial resistance genes in *Salmonella enterica* isolates from poultry and swine in Thailand. *Journal of Veterinary Medical Science,* 71, 1349-1355.

Cockerill, F. R., Clinical, Institute, L. S. 2012. Performance standards for antimicrobial susceptibility testing: twenty-second informational supplement;provides updated tables for M02-A11 and M07-A9. *Clinical Laboratory Standards Institute,* 32, 1-185.

Cohen, J. I., Bartlett, J. A., Corey, G. R. 1987. Extra-intestinal manifestations of *Salmonella* infections. *Medicine,* 66, 349-388.

Consultation, R. 2011. Dietary protein quality evaluation in human nutrition. *Rome, Italy: Food and Agriculture Organization of The United Nations.*

Croci, L., De Medici, D., Scalfaro, C., Fiore, A., Divizia, M., Donia, D., Cosentino, A., Moretti, P., Costantini, G. 2000. Determination of enteroviruses, hepatitis A virus, bacteriophages and *Escherichia coli* in Adriatic Sea mussels. *Journal of Applied Microbiology,* 88, 293-298.

Cruikshank, R., Duguid, J. P., Marmoin, B. P., Swain, R. H. 1975. *Medical Microbiology, 12th Ed.*

Crump, J. A., Griffin, P. M., Angulo, F. J. 2002. Bacterial contamination of animal feed and its relationship to human foodborne illness. *Clinical Infectious Diseases,* 35, 859-865.

Cummings, K. J., Warnick, L. D., Elton, M., Gröhn, Y. T., Mcdonough, P. L., Siler, J. D. 2010. The effect of clinical outbreaks of salmonellosis on the prevalence of fecal *Salmonella* shedding among dairy cattle in New York. *Foodborne Pathogens and Disease,* 7, 815-823.

Dallal, M. S. 2009. Incidence of *Salmonella* serovars and its antimicrobial pattern in barbecued meat and ground beef burgers in Tehran. *Iranian Journal of Microbiology,* 1, 37-41.

Dauga, C., Zabrovskaia, A., Grimont, P. A. 1998. Restriction fragment length polymorphism analysis of some flagellin genes of *Salmonella enterica*. *Journal of Clinical Microbiology,* 36, 2835-2843.

Davies, A. R., Board, R., Board, R. 1998. *Microbiology of Meat and Poultry*, Springer Science & Business Media.

De Freitas Neto, O., Penha Filho, R., Barrow, P., Berchieri Junior, A. 2010. Sources of human non-typhoid salmonellosis: a review. *Revista Brasileira de Ciência Avícola,* 12, 01-11.

De Vylder, J., Dewulf, J., Van Hoorebeke, S., Pasmans, F., Haesebrouck, F., Ducatelle, R., Van Immerseel, F. 2011. Horizontal transmission of *Salmonella Enteritidis* in groups of experimentally infected laying hens housed in different housing systems. *Poultry science,* 90, 1391-1396.

Delicato, E. R., Mikcha, J. M. G., Fernandes, S. A., Pelayo, J. S. 2004. Resistance profile to antimicrobials of *Salmonella* spp. isolated from human infections. *Brazilian Archives of Biology and Technology,* 47, 193-197.

Dewell, G., Simpson, C., Dewell, R., Hyatt, D., Belk, K., Scanga, J., Morley, P., Grandin, T., Smith, G., Dargatz, D. 2008. Impact of transportation and lairage on hide contamination with *Escherichia coli O157* in finished beef cattle. *Journal of Food Protection®,* 71, 1114-1118.

Dube, S. 1983. Outbreak of food poisoning caused by lactose-fermenting *Salmonella tuebingen*. *Journal of clinical microbiology,* 17, 698-699.

Ejeta, G., Molla, B., Alemayehu, D., Muckle, C. 2004. *Salmonella* serotypes isolated from minced meat beef, mutton and pork in Addis Ababa, Ethiopia. *Revue de médecine vétérinaire,* 155, 547.

El-Feky, M. A., Hassan, M. A., Mohamed, W. A., Ibrahim, N. H., Rashwan, R. S. 2014. Detection of InvA Gene in Non Typhoidal *Salmonellae* Isolated from Food Products and Clinical Cases. *Egyptian Journal of Medical Microbiology,* 23.

Evangelopoulou, G., Kritas, S., Govaris, A., Burriel, A. R. 2014. Pork meat as a potential source of *Salmonella enterica* subsp. Arizonae infection in humans. *Journal of clinical microbiology,* 52, 741-744.

Fàbrega, A., Vila, J. 2013. *Salmonella enterica* serovar Typhimurium skills to succeed in the host: virulence and regulation. *Clinical microbiology reviews,* 26, 308-341.

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, 2489-2499.

Fendri, I., Hassena, A. B., Grosset, N., Barkallah, M., Khannous, L., Chuat, V., Gautier, M., Gdoura, R. 2013. Genetic diversity of food-isolated *Salmonella* strains through pulsed field gel electrophoresis (PFGE) and enterobacterial repetitive intergenic consensus (ERIC-PCR). *PloS one,* 8, e81315.

Fierer, J., Guiney, D. G. 2001. Diverse virulence traits underlying different clinical outcomes of *Salmonella* infection. *Journal of Clinical Investigation,* 107, 775.

Fischer, A. R., De Jong, A. E., De Jonge, R., Frewer, L. J., Nauta, M. J. 2005. Improving food safety in the domestic environment: The need for a transdisciplinary approach. *Risk analysis,* 25, 503-517.

Flachowsky, G., Chesson, A., Aulrich, K. 2005. Animal nutrition with feeds from genetically modified plants. *Archives of Animal Nutrition,* 59, 1-40.

Floss, H. G., Yu, T.-W. 2005. Rifamycin mode of action, resistance, and biosynthesis. *Chemical reviews,* 105, 621-632.

Forshell, L. a. P., Wierup, M. 2006. *Salmonella* contamination: a significant challenge to the global marketing of animal food products. *Scientific and Technical Review of the Office International des Epizooties (Paris),* 25, 541-554.

Friesema, I., De Jong, A., Fitz James, I., Heck, M., Van Den Kerkhof, J., Notermans, D., Van Pelt, W., Hofhuis, A. 2012. Outbreak of *Salmonella Thompson* in the Netherlands since July 2012. *Euro Surveill,* 17, 20303.

Galan, J. E., Ginocchio, C., Costeas, P. 1992. Molecular and functional characterization of the *Salmonella* invasion gene *inv*A: homology of *Inv*A to members of a new protein family. *Journal of bacteriology,* 174, 4338-4349.

Galanakis, E., Bitsori, M., Maraki, S., Giannakopoulou, C., Samonis, G., Tselentis, Y. 2007. Invasive non-typhoidal salmonellosis in immunocompetent infants and children. *International journal of infectious diseases,* 11, 36-39.

Garcia, P., Martinez, B., Obeso, J., Rodriguez, A. 2008. Bacteriophages and their application in food safety. *Letters in Applied Microbiology,* 47, 479-485.

Gebreyes, W. A., Altier, C. 2002. Molecular characterization of multidrug-resistant *Salmonella enterica* subsp. enterica serovar Typhimurium isolates from swine. *Journal of Clinical Microbiology,* 40, 2813-2822.

Gillespie, S., Hawkey, P. M. 2006. *Principles and practice of clinical bacteriology,* John Wiley & Sons.

Giorgio, D. 2000. Role of antibiotic therapy on long-term germ excretion in faeces and digestive symptoms after *Salmonella* infection. *Alimentary Pharmacology and Therapeutics,* 14, 1127-1131.

Girma, G. 2015. Prevalence, Antibiogram and Growth Potential of *Salmonella* and *Shigella* in Ethiopia: Implications for Public Health. A Review. *Advances in Life Science and Technology,* 33, 74-87.

Goldrick, B. A. 2003. Foodborne Diseases: More efforts needed to meet the Healthy People 2010 objectives. *AJN The American Journal of Nursing,* 103, 105-106.

Gomez, J. R., Salinas, I. L., Salmerón, J. P., Cordoba, E. S., Campos, L. M. 1998. Evaluation of methods for isolation of *Salmonella* species using modified semisolid Rappaport-Vassiliadis medium and *Salmonella-Shigella* agar. *European Journal of Clinical Microbiology and Infectious Diseases,* 17, 791-793.

Gonzalez, A. 1966. Lactose-fermenting *Salmonella*. *Journal of bacteriology,* 91, 1661.

Gordon, M. A. 2008. *Salmonella* infections in immunocompromised adults. *Journal of Infection,* 56, 413-422.

Gordon, M. A., Graham, S. M., Walsh, A. L., Wilson, L., Phiri, A., Molyneux, E., Zijlstra, E. E., Heyderman, R. S., Hart, C. A., Molyneux, M. E. 2008. Epidemics of invasive *Salmonella enterica* serovar enteritidis and *S. enterica* Serovar typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clinical Infectious Diseases,* 46, 963-969.

Gorris, L. G. 2005. Food safety objective: an integral part of food chain management. *Food Control,* 16, 801-809.

Graham, S. M. 2002. Salmonellosis in children in developing and developed countries and populations. *Current Opinion in Infectious Diseases,* 15, 507-512.

Greer, G. G. 1988. Effects of phage concentration, bacterial density, and temperature on phage control of beef spoilage. *Journal of Food Science,* 53, 1226-1227.

Gruenewald, R., Dixon, D., Brun, M., Yappow, S., Henderson, R., Douglas, J., Backer, M. 1990. Identification of *Salmonella* somatic and flagellar antigens by modified serological methods. *Applied and environmental microbiology,* 56, 24-30.

Guerra, B., Soto, S., Helmuth, R., Mendoza, M. C. 2002. Characterization of a self-transferable plasmid from *Salmonella enterica* serotype Typhimurium clinical isolates carrying two integron-borne gene cassettes together with virulence and drug resistance genes. *Antimicrobial agents and chemotherapy,* 46, 2977-2981.

Guiney, D. G., Fierer, J. 2011. The role of the spv genes in *Salmonella* pathogenesis. *Salmonella host-pathogen interactions*, 65.

Gyles, C., Boerlin, P. 2013. Horizontally transferred genetic elements and their role in pathogenesis of bacterial disease. *Veterinary Pathology Online*, 0300985813511131.

Hanning, I. B., Nutt, J., Ricke, S. C. 2009. Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. *Foodborne Pathogens and Disease,* 6, 635-648.

Hao, H., Cheng, G., Iqbal, Z., Ai, X., Hussain, H. I., Huang, L., Dai, M., Wang, Y., Liu, Z., Yuan, Z. 2014. Benefits and risks of antimicrobial use in food-producing animals. *Frontiers in microbiology,* 5.

Hedberg, C. W. 2014. Potential Impacts of a Zero Tolerance Policy for *Salmonella* on Raw Meat and Poultry.

Hendriksen, R. 2003. A global *Salmonella* surveillance and laboratory support project of the World Health Organization: Laboratory Protocols Level 1 Training Course Identification of Salmonella 4 th Ed. April 2003.

Higgins, P., Fluit, A., Schmitz, F. 2003. Fluoroquinolones: structure and target sites. *Current drug targets,* 4, 181-190.

Ho, M. X., Hudson, B. P., Das, K., Arnold, E., Ebright, R. H. 2009. Structures of RNA polymerase–antibiotic complexes. *Current opinion in structural biology,* 19, 715-723.

Holah, J., Taylor, J., Dawson, D., Hall, K. 2002. Biocide use in the food industry and the disinfectant resistance of persistent strains of Listeria monocytogenes and *Escherichia coli. Journal of Applied Microbiology,* 92, 111S-120S.

Hooper, D. C. 2001. Mechanisms of action of antimicrobials: focus on fluoroquinolones. *Clinical Infectious Diseases,* 32, S9-S15.

Howe, K. M. 2015. *Investigation of microbial aspects related to Salmonella as a food pathogen: bioluminescent reporting system and mechanisms for host invasion.* MISSISSIPPI STATE UNIVERSITY.

Hsu, F.-C., Shieh, Y.-S. C., Sobsey, M. 2002. Enteric bacteriophages as potential fecal indicators in ground beef and poultry meat. *Journal of Food Protection®,* 65, 93-99.

Hudson, J., Billington, C., Carey-Smith, G., Greening, G. 2005. Bacteriophages as biocontrol agents in food. *Journal of Food Protection®,* 68, 426-437.

Huruy, K., Kassu, A., Mulu, A., Worku, N., Fetene, T., Gebretsadik, S., Biadglegne, F., Belyhun, Y., Muche, A., Gelaw, A. 2011. Intestinal parasitosis and shigellosis among diarrheal patients in Gondar teaching hospital, northwest Ethiopia. *BMC Research Notes,* 4, 472.

Jadidi, A., Hosseni, S. D., Homayounimehr, A., Hamidi, A., Ghani, S., Rafiee, B. 2012. Simple and rapid detection of *Salmonella sp.* from cattle feces using polymerase chain reaction (PCR) in Iran. *Afr J Microbiol Res,* 6, 5210-5214.

Jones, F. 2011. A review of practical *Salmonella* control measures in animal feed. *The Journal of Applied Poultry Research,* 20, 102-113.

Kagambèga, A., Lienemann, T., Aulu, L., Traoré, A. S., Barro, N., Siitonen, A., Haukka, K. 2013. Prevalence and characterization of *Salmonella enterica* from the feces of cattle, poultry, swine and hedgehogs in Burkina Faso and their comparison to human Salmonella isolates. *BMC microbiology,* 13, 253.

Kanoh, S., Rubin, B. K. 2010. Mechanisms of action and clinical application of macrolides as immunomodulatory medications. *Clinical Microbiology Reviews,* 23, 590-615.

Kay, E., Vogel, T. M., Bertolla, F., Nalin, R., Simonet, P. 2002. In situ transfer of antibiotic resistance genes from transgenic (transplastomic) tobacco plants to bacteria. *Applied and environmental microbiology,* 68, 3345-3351.

Kennedy Jr, J., Wei, C., Oblinger, J. 1986. Distribution of coliphages in various foods. *Journal of Food Protection (USA).*

Kleter, G. A., Marvin, H. J. 2009. Indicators of emerging hazards and risks to food safety. *Food and Chemical Toxicology,* 47, 1022-1039.

Koppel, K., Suwonsichon, S., Chitra, U., Lee, J., Chambers Iv, E. 2014. Eggs and poultry purchase, storage, and preparation practices of consumers in selected Asian countries. *Foods,* 3, 110-127.

Kruger, T., Szabo, D., Keddy, K. H., Deeley, K., Marsh, J. W., Hujer, A. M., Bonomo, R. A., Paterson, D. L. 2004a. The global problem of salmonellosis and the emergence of multiple-drug resistance. *Antimicrobial Agents and Chemotherapy,* 4263-4270.

Kruger, T., Szabo, D., Keddy, K. H., Deeley, K., Marsh, J. W., Hujer, A. M., Bonomo, R. A., Paterson, D. L. 2004b. Infections with nontyphoidal *Salmonella* species producing TEM-63 or a novel TEM enzyme, TEM-131, in South Africa. *Antimicrobial Agents and Chemotherapy,* 48, 4263-4270.

Langvad, B., Skov, M., Rattenborg, E., Olsen, J. E., Baggesen, D. L. 2006. Transmission routes of *Salmonella Typhimurium* DT 104 between 14 cattle and pig herds in Denmark demonstrated by molecular fingerprinting. *Journal of applied microbiology,* 101, 883-890.

Leirisalo-Repo, M., Helenius, P., Hannu, T., Lehtinen, A., Kreula, J., Taavitsainen, M., Koskimies, S. 1997. Long term prognosis of reactive *Salmonella* arthritis. *Annals of The Rheumatic Diseases,* 56, 516-520.

Lin, C., Tsen, H. 1996. Use of two 16S DNA targeted oligonucleotides as PCR primers for the specific detection of *Salmonella* in foods. *Journal of applied bacteriology,* 80, 659-666.

Lu, T. K., Koeris, M. S. 2011. The next generation of bacteriophage therapy. *Current Opinion in Microbiology,* 14, 524-531.

Ly-Chatain, M. H. 2014. The factors affecting effectiveness of treatment in phages therapy. *Frontiers in microbiology,* 5.

Lynch, M., Tauxe, R., Hedberg, C. 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology and Infection,* 137, 307-315.

Lynch, P. B., Leonard, N., Egan, J., Kozlowski, M., Mannion, C. 2007. Development of on-farm control measures for the reduction of *Salmonellosis* in slaughter pigs. Teagasc, Oak Park, Carlow, Ireland.

Lynne, A. M., Rhodes-Clark, B. S., Bliven, K., Zhao, S., Foley, S. L. 2008. Antimicrobial resistance genes associated with *Salmonella enterica* serovar Newport isolates from food animals. *Antimicrobial agents and chemotherapy,* 52, 353-356.

Ma, M., Wang, H., Yu, Y., Zhang, D., Liu, S. 2007. Detection of antimicrobial resistance genes of pathogenic *Salmonella* from swine with DNA microarray. *Journal of Veterinary Diagnostic Investigation,* 19, 161-167.

Maddocks, S., Olma, T., Chen, S. 2002. Comparison of CHROMagar *Salmonella* medium and xylose-lysine-desoxycholate and *Salmonella*-Shigella agars for isolation of *Salmonella* strains from stool samples. *Journal of clinical microbiology,* 40, 2999-3003.

Maharjan, M., Joshi, V., Joshi, D. D., Manandhar, P. 2006. Prevalence of *Salmonella* species in various raw meat samples of a local market in Kathmandu. *Annals of the New York academy of sciences,* 1081, 249-256.

Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'brien, S. J., Jones, T. F., Fazil, A., Hoekstra, R. M. 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clinical Infectious Diseases,* 50, 882-889.

Malkawi, H. I. 2003. Molecular identification of *Salmonella* isolates from poultry and meat productsin Irbid City, Jordan. *World Journal of Microbiology and Biotechnology,* 19, 455-459.

Marshall, B. M., Levy, S. B. 2011. Food animals and antimicrobials: impacts on human health. *Clinical microbiology reviews,* 24, 718-733.

Mascaretti, O. 2003. Inhibitors of the 30S ribosomal subunit aminoglycosides and tetracyclines. *Bacteria Versus Antimicrobial Agents: An Integrated Approach. Washington, DC: ASM Press, American Society of Microbiology*, 229-246.

Matches, J. R., Liston, J. 1968. Low temperature growth of *Salmonella. Journal of Food Science,* 33, 641-645.

Mayrhofer, S., Paulsen, P., Smulders, F. J., Hilbert, F. 2004. Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. *International journal of food microbiology,* 97, 23-29.

Mcevoy, J., Doherty, A., Sheridan, J., Blair, I., Mcdowell, D. 2003. The prevalence of *Salmonella spp.* in bovine faecal, rumen and carcass samples at a commercial abattoir. *Journal of Applied Microbiology,* 94, 693-700.

Mead, G. C. 1994. Microbiological hazards from red meat and their control. *British Food Journal,* 96, 33-36.

Meyer, C., Thiel, S., Ullrich, U., Stolle, A. 2010. *Salmonella* in raw meat and by-products from pork and beef. *Journal of Food Protection®,* 73, 1780-1784.

Mezal, E. H., Sabol, A., Khan, M. A., Ali, N., Stefanova, R., Khan, A. A. 2014. Isolation and molecular characterization of *Salmonella enterica* serovar Enteritidis from poultry house and clinical samples during 2010. *Food microbiology,* 38, 67-74.

Miliotis, M. D., Bier, J. W. 2003. *International handbook of foodborne pathogens*, CRC Press.

Miller, I. 2015. *Anne Hardy, Salmonella Infections, Networks of Knowledge and Public Health in Britain 1880–1975 (Oxford: Oxford University Press, 2015), pp. x+ 249,£ 60, hardback, ISBN: 978-0-19-870497-3.*

Mirzaie, S., Hassanzadeh, M., Ashrafi, I. 2010. Identification and characterization of *Salmonella* isolates from captured house sparrows. *Turk J Vet Anim Sci,* 34, 181-186.

Mohamed, W. S., Ibrahim, M. A. 2015. Isolation of *Salmonella* Species from Buffalo Calves Meat And Preliminary Evaluation of its Irradiated Vaccine. *Assiut Veterinary Mededicine Journal,* 61, 19-23.

Molla, B., Alemayehu, D., Salah, W. 2003. Sources and distribution of *Salmonella* serotypes isolated from food animals, slaughterhouse personnel and retail meat products in Ethiopia: 1997-2002. *Ethiopian Journal of Health Development,* 17, 63-70.

Moraes, P. M., Perin, L. M., Silva Júnior, A., Nero, L. A. 2013. Comparison of phenotypic and molecular tests to identify lactic acid bacteria. *Brazilian Journal of Microbiology,* 44, 109-112.

Moreno Switt, A. I., Den Bakker, H. C., Vongkamjan, K., Hoelzer, K., Warnick, L. D., Cummings, K. J., Wiedmann, M. 2013. *Salmonella* bacteriophage diversity reflects host diversity on dairy farms. *Food Microbiology,* 36, 275-285.

Morpeth, S. C., Ramadhani, H. O., Crump, J. A. 2009. Invasive non-typhi *Salmonella* disease in Africa. *Clinical Infectious Diseases,* 49, 606-611.

Motta, S. P. O. D., Flint, S., Perry, P., Noble, A. 2014. Consumer contribution to food contamination in Brazil: modelling the food safety risk in the home. *Brazilian Journal of Food Technology,* 17, 154-165.

Negussie, A., Mulugeta, G., Bedru, A., Ali, I., Shimeles, D., Lema, T., Aseffa, A. 2015. Bacteriological Profile and Antimicrobial Susceptibility Pattern of Blood Culture Isolates among Septicemia Suspected Children in Selected Hospitals Addis Ababa, Ethiopia. *International Journal of Biological & Medical Research,* 6, 4709-4717.

Nel, S., Lues, J., Buys, E., Venter, P. 2004. Bacterial populations associated with meat from the deboning room of a high throughput red meat abattoir. *Meat science,* 66, 667-674.

Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F. 2010. Food-borne diseases—the challenges of 20years ago still persist while new ones continue to emerge. *International journal of food microbiology,* 139, S3-S15.

Niu, Y. D., Stanford, K., Ackermann, H.-W., Mcallister, T. A. 2012. Characterization of 4 T1-like lytic bacteriophages that lyse Shiga-toxin *Escherichia coli* O157: H7. *Canadian Journal of Microbiology,* 58, 923-927.

Niyonzima, E., Ongol, M. P., Kimonyo, A., Sindic, M. 2015. Risk Factors and Control Measures for Bacterial Contamination in the Bovine Meat Chain: A Review on *Salmonella* and Pathogenic *E. coli*. *Journal of Food Research,* 4, p98.

Nørrung, B., Buncic, S. 2008. Microbial safety of meat in the European Union. *Meat Science,* 78, 14-24.

Nucera, D. M., Maddox, C. W., Hoien-Dalen, P., Weigel, R. M. 2006. Comparison of API 20E and invA PCR for identification of *Salmonella enterica* isolates from swine production units. *Journal of Clinical Microbiology,* 44, 3388-3390.

Okoro, C. K., Kingsley, R. A., Connor, T. R., Harris, S. R., Parry, C. M., Al-Mashhadani, M. N., Kariuki, S., Msefula, C. L., Gordon, M. A., De Pinna, E. 2012. Intracontinental spread of human invasive *Salmonella Typhimurium* pathovariants in sub-Saharan Africa. *Nature genetics,* 44, 1215-1221.

Osayande, J. 2014. Pathogen Traffickers: Disease causing pathogens do not have legs. *Nature 510 doi:10.1038/510015a*, 15–16.

Paiva, J., Cavallini, J., Silva, M., Almeida, M., Ângela, H., Berchieri Junior, A. 2009. Molecular differentiation of *Salmonella Gallinarum* and *Salmonella Pullorum* by RFLP of fliC gene from Brazilian isolates. *Revista Brasileira de Ciência Avícola,* 11, 271-275.

Popoff, M., Bockemühl, J., Brenner, F. 1998. Supplement 1997 (no. 41) to the Kauffmann-White scheme. *Research in Microbiology,* 149, 601-604.

Potter, A., Murray, J., Lawson, B., Graham, S. 2012. Trends in product recalls within the agri-food industry: Empirical evidence from the USA, UK and the Republic of Ireland. *Trends in Food Science & Technology,* 28, 77-86.

Radke, B. R., Mcfall, M., Radostits, S. M. 2002. *Salmonella* Muenster infection in a dairy herd. *The Canadian Veterinary Journal,* 43, 443.

Reicks, A., Brashears, M., Adams, K., Brooks, J., Blanton, J., Miller, M. 2007. Impact of transportation of feedlot cattle to the harvest facility on the prevalence of *Escherichia coli O157: H7, Salmonella*, and total aerobic microorganisms on hides. *Journal of Food Protection®,* 70, 17-21.

Rocourt, J., Moy, G., Vierk, K., Schlundt, J. 2003. The present state of foodborne disease in OECD countries. *Geneva: WHO,* 1.

Rodrigue, D., Tauxe, R., Rowe, B. 1990. International increase in *Salmonella enteritidis*: a new pandemic? *Epidemiology and infection,* 105, 21-27.

Rodulfo, H., Donato, M. D., Luiggi, J., Michelli, E., Millán, A., Michelli, M. 2012. Molecular characterization of *Salmonella* strains in individuals with acute diarrhea syndrome in the State of Sucre, Venezuela. *Revista da Sociedade Brasileira de Medicina Tropical,* 45, 329-333.

Rostagno, M. H. 2009. Can stress in farm animals increase food safety risk? *Foodborne pathogens and disease,* 6, 767-776.

Rota, C., Yanguela, J., Blanco, D., Carramilana, J. J., Arino, A., Herrera, A. 1996. High prevalence of multiple resistance to antibiotics in 144 Listeria isolates from Spanish dairy and meat products. *Journal of Food Protection®,* 59, 938-943.

Roy, P., Dhillon, A., Lauerman, L. H., Schaberg, D., Bandli, D., Johnson, S. 2002. Results of *Salmonella* isolation from poultry products, poultry, poultry environment, and other characteristics. *Avian diseases,* 46, 17-24.

Ryan, K., Ray, C. 2004. Sherris medical microbiology: an introduction to infectious diseases. 4th. McGraw Hall USA.

Sadeleer, L. D., Dewulf, J., Zutter, L. D., Stede, Y., Ribbens, S., Busser, E. D., Quoilin, S., Houf, K., Delhalle, L., Grijspeerdt, K. 2009. A qualitative risk assessment for human salmonellosis due to the consumption of fresh pork in Belgium. *Vlaams Diergeneeskundig Tijdschrift,* 78, 34-43.

Saeed, A. A., Hasoon, M. F., Mohammed, M. H. 2011. Isolation and Molecular Identification of *Salmonella typhimurium* from Chicken Meat in Iraq.

Sambrock, J., Ressell, D. W. 2001. *Molecular cloning: a laboratory manual 3rd ed,* Cold spring Harbor , N.Y. USA.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M.-A., Roy, S. L., Jones, J. L., Griffin, P. M. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis,* 17.

Schröter, M., Speicher, A., Hofmann, J., Roggentin, P. 2006. Analysis of the transmission of *Salmonella* spp. through generations of pet snakes. *Environmental microbiology,* 8, 556-559.

Shah, S., Romick, T. 1997. Subspecies differentiation of *Salmonella* by PCR-RFLP of the ribosomal operon using universal primers. *Letters in Applied Microbiology,* 25, 54-57.

Sharp, R. 2001. Bacteriophages: biology and history. *Journal of Chemical Technology and Biotechnology,* 76, 667-672.

Shin, H., Lee, J.-H., Kim, H., Choi, Y., Heu, S., Ryu, S. 2012. Receptor diversity and host interaction of bacteriophages infecting *Salmonella enterica* serovar Typhimurium. *PloS one,* 7, e43392.

Sillankorva, S. M., Oliveira, H., Azeredo, J. 2012. Bacteriophages and their role in food safety. *International Journal of Microbiology,* 2012.

Smith, S. I., Fowora, M. A., Atiba, A., Anejo-Okopi, J., Fingesi, T., Adamu, M. E., Omonigbehin, E. A., Ugo-Ijeh, M. I., Bamidele, M., Odeigah, P. 2015. Molecular detection of some virulence genes in *Salmonella spp* isolated from food samples in Lagos, Nigeria. *Animal and Veterinary Sciences,* 3 22-27.

Specimens, A. 2015. UK Standards for Microbiology Investigations. 1-23.

Sperber, W., Deibel, R. 1969. Accelerated procedure for *Salmonella* detection in dried foods and feeds involving only broth cultures and serological reactions. *Applied microbiology,* 17, 533-539.

Srivani, R., Gopal Muthu, Arumugam Suresh, Sumathy, G. 2011. Studies On Antimicrobial Susceptibility Pattern Of *Salmonella* Isolates From Chennai, India 2.

Stein, R. A. 2011. Bacterial Infections of Humans: Epidemiology and Control. *JAMA,* 305, 1488-1489.

Su, L., Chiu, C. 2007. *Salmonella*: clinical importance and evolution of nomenclature. *Chang Gung medical journal,* 30, 210.

Suárez, V., Quiberoni, A., Binetti, A., Reinheimer, J. 2002. Thermophilic lactic acid bacteria phages isolated from Argentinian dairy industries. *Journal of Food Protection®,* 65, 1597-1604.

Sumithra, T. G., Chaturvedi, V. K., Gupta, P. K., Rai, A., Chougule, S., Joseph, B., Cherian, S., Patel, C. L. 2014. PCR-RFLP analysis of fliC, fimH and 16S rRNA genes in *Salmonella Typhimurium* isolates of varied origin. *Annals of Microbiology,* 64, 177-183.

Tauxe, R. V. 1997. Emerging foodborne diseases: an evolving public health challenge. *Emerging infectious diseases,* 3, 425.

Threlfall, E. J. 2002. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food-and water-borne infections. *FEMS Microbiology Reviews,* 26, 141-148.

Todar, K. 2005. *Salmonella* and salmonellosis. *Todar's Online Textbook of Bacteriology.*

Toleman, M. A., Bennett, P. M., Walsh, T. R. 2006. Common regions eg orf513 and antibiotic resistance: IS91-like elements evolving complex class 1 integrons. *Journal of Antimicrobial Chemotherapy,* 58, 1-6.

Torrence, M. E., Isaacson, R. E. 2008. *Microbial food safety in animal agriculture: current topics*, John Wiley & Sons.

Ugbogu, O., Nwachukwu, N., Ogbuagu, U. 2006. Isolation of *Salmonella* and *Shigella* species from house flies (Musca domestica l.) in Uturu, Nigeria. *African Journal of Biotechnology,* 5.

Van De Venter, T. 2000. Emerging food-borne diseases: a global responsibility. *Food Nutrition and Agriculture*, 4-13.

Van Den Bogaard, A. E., Stobberingh, E. E. 2000. Epidemiology of resistance to antibiotics: links between animals and humans. *International Journal of Antimicrobial Agents,* 14, 327-335.

Van Der Walt, M. L., Huchzermeyer, F. W., Steyn, H. C. 1997. *Salmonella* isolated from crocodiles and other reptiles during the period 1985-1994 in South Africa.

Van, T. T. H., Moutafis, G., Istivan, T., Tran, L. T., Coloe, P. J. 2007. Detection of *Salmonella* spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. *Applied and environmental microbiology,* 73, 6885-6890.

Vasil, I. K. 1998. Biotechnology and food security for the 21st century: a real-world perspective. *Nature Biotechnology,* 16, 399-400.

Vidic, V., Prica, N., Vidic, B., Bugarski, D., Milanov, D., Savic, S. 2015. Influence of Intensive Animal Breeding to the Appearance of Infectious Diseases (Zoonoses).

Walsh, C. 2003. *Antibiotics*, American Society of Microbiology.

Wani, S., Hussain, I., Beg, S., Rather, M., Kabli, Z., Mir, M., Nishikawa, Y. 2013. Diarrhoeagenic *Escherichia coli* and *Salmonellae* in calves and lambs in Kashmir: absence, prevalence and antibiogram. *Rev. sci. tech. Off. int. Epiz,* 32, 3.

Wattiau, P., Boland, C., Bertrand, S. 2011. Methodologies for *Salmonella enterica* subsp. enterica subtyping: gold standards and alternatives. *Applied and environmental microbiology,* 77, 7877-7885.

White, D. G., Mcdermott, P. F., Jaykus, L., Wang, H., Schlesinger, L. 2009. Antimicrobial resistance in food-borne pathogens. *Food-Borne Microbes: Shaping The Host Ecosystem*, 231-265.

White, D. G., Zhao, S., Simjee, S., Wagner, D. D., Mcdermott, P. F. 2002. Antimicrobial resistance of foodborne pathogens. *Microbes and Infection,* 4, 405-412.

White, D. G., Zhao, S., Sudler, R., Ayers, S., Friedman, S., Chen, S., Mcdermott, P. F., Mcdermott, S., Wagner, D. D., Meng, J. 2001. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. *New England Journal of Medicine,* 345, 1147-1154.

Wikler, M. A. 2007. *Performance standards for antimicrobial susceptibility testing: seventeenth informational supplement*, Clinical and Laboratory Standards Institute.

Winn, W. C., Koneman, E. W. 2006. *Koneman's color atlas and textbook of diagnostic microbiology*, Lippincott williams & wilkins.

Wittebole, X., De Roock, S., Opal, S. M. 2014. A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. *Virulence,* 5, 226-235.

Xi, X., Zhang, J., Kwok, L., Huo, D., Feng, S., Zhang, H., Sun, T. 2015. Microbial Pollution Tracking of Dairy Farm with a Combined PCR-DGGE and qPCR Approach. *Current microbiology,* 71, 678-686.

Yang, X., Wu, Q., Zhang, J., Huang, J., Guo, W., Cai, S. 2015. Prevalence and Characterization of Monophasic *Salmonella* Serovar 1, 4,[5], 12: i:-of Food Origin in China. *PloS one,* 10, e0137967.

Yates, A. 2011. *Salmonella* (non-typhoidal). *AGENTS OF FOODBORNE ILLNESS*, 31.

Zahran, R., El-Behiry, A. 2014. Prevalence, molecular identification and virulence attributes of *Salmonella* serovars isolated from feces of diarrheic cow and buffalo-calves. *Int. J. Curr. Microbiol. App. Sci,* 3, 9-27.

Zamxaka, M., Pironcheva, G., Muyima, N. 2004. Microbiological and physico-chemical assessment of the quality of domestic water sources in selected rural communities of the Eastern Cape Province, South Africa. *Water Sa,* 30, 333-340.

Zarei, M., Basiri, N., Jamnejad, A., Eskandari, M. H. 2013. Prevalence of *Escherichia coliO157*: H7, Listeria monocytogenesand *Salmonella* spp. in Beef, Buffalo and Lamb Using Multiplex PCR.

APPENDICES

APPENDIX A

Table 1A: Antibiogram data of isolates derived from the inhibition zone data

Sample ID	AP	CFM	NA	GM	CIP	T	NOR	E	RP	C	PG	CFXM
MFK2A	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Susceptible
MFK2B	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Susceptible
MFT4B	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Intermediate	Resistant	Susceptible	Resistant	Susceptible
MFK5A	Resistant	Susceptible	Intermediate	Susceptible	Intermediate	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
MFK5B	Resistant	Resistant	Intermediate	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
PP1A	Intermediate	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
PP2B	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
PP3A	Resistant	Resistant	Susceptible	Resistant	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
PP3B	Resistant	Resistant	Susceptible	Resistant	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
PP4A	Intermediate	Intermediate	Susceptible	Resistant	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Susceptible	Resistant	Resistant
PP4B	Intermediate	Intermediate	Susceptible	Resistant	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Susceptible	Resistant	Resistant

TO1A	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
TO1B	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
TC1A	Resistant	Resistant	Resistant	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
TC1A	Resistant	Resistant	Resistant	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
TC2A	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant
TC13A	Intermediate	Susceptible	Intermediate	Susceptible	Intermediate	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
TC13B	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
TC14A	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
TC14B	Resistant	Resistant	Susceptible	Intermediate	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
TC15A	Resistant	Intermediate	Resistant	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
TC15B	Resistant	Intermediate	Resistant	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
RST4A	Susceptible	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
RST4B	Intermediate	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant
RST5A	Resistant	Resistant	Resistant	Susceptible	Resistant	Susceptible	Resistant	Resistant	Resistant	Susceptible	Resistant	Intermediate

RST5B	Resistant	Resistant	Resistant	Susceptible	Resistant	Intermediate	Resistant	Resistant	Resistant	Intermediate	Resistant	Resistant
RST6A	Resistant	Resistant	Resistant	Susceptible	Resistant	Susceptible	Intermediate	Resistant	Resistant	Susceptible	Resistant	Susceptible
RST6B	Resistant	Resistant	Resistant	Susceptible	Resistant	Susceptible	Resistant	Resistant	Resistant	Susceptible	Resistant	Intermediate
RST7A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
RST7B	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
RST8A	Intermediate	Resistant	Intermediate	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Intermediate	Resistant	Resistant
RST8B	Intermediate	Susceptible	Resistant	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
RST9A	Resistant	Intermediate	Resistant	Susceptible	Resistant	Intermediate	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
RST9B	Resistant	Resistant	Resistant	Susceptible	Resistant	Intermediate	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
RST10A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
RST10B	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
RST11A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Intermediate	Resistant	Intermediate
RST11B	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Intermediate	Resistant	Resistant
RST12A	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant

RST12B	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
VRY1A	Resistant	Susceptible	Intermediate	Susceptible	Intermediate	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
VRY1B	Resistant	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
VRY2A	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Intermediate	Resistant	Resistant
VRY2B	Resistant	Resistant	Intermediate	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Intermediate	Resistant	Resistant
VRY3A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
VRY3B	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
VRY4A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Resistant	Resistant	Susceptible
VRY4B	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Resistant	Resistant	Susceptible
VRY5A	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant
VRY5B	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant
VRY6A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant
VRY6B	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant
VRY7A	Resistant	Resistant	Resistant	Susceptible	Resistant	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate

VRY7B	Resistant	Resistant	Resistant	Susceptible	Resistant	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
VRY8A	Resistant	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
VRY8B	Resistant	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
VRY9A	Resistant	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
VRY9B	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible
VRY10A	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible
VRY10B	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
VRY11A	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Resistant	Intermediate	Resistant	Resistant
VRY11B	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Resistant	Intermediate	Resistant	Resistant
MRK1A	Resistant	Resistant	Resistant	Resistant	Resistant	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
MRK1B	Resistant	Resistant	Resistant	Resistant	Resistant	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
MRK2A	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant
MRK2B	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant
MRK3A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Resistant	Resistant	Susceptible

MRK3B	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Resistant	Resistant	Susceptible
MRK4A	Resistant	Resistant	Resistant	Resistant	Resistant	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
MRK4B	Resistant	Resistant	Resistant	Resistant	Resistant	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
MRK5A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
MRK5B	Intermediate	Intermediate	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
MRK6A	Susceptible	Intermediate	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
MRK6B	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
BRT1A	Resistant	Susceptible	Intermediate	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
BRT1B	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
BRT2A	Susceptible	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
BRT2B	Susceptible	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
BRT3A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Intermediate	Resistant	Intermediate
BRT3B	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Intermediate	Resistant	Resistant
BRT4A	Resistant	Susceptible	Resistant	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant

BRT4B	Resistant	Susceptible	Resistant	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Resistant	Resistant	Intermediate
V1A	Resistant	Resistant	Susceptible	Susceptible	Intermediate	Susceptible	Susceptible	Intermediate	Intermediate	Susceptible	Susceptible	Susceptible
V1B	Resistant	Resistant	Susceptible	Susceptible	Resistant	Susceptible	Susceptible	Intermediate	Intermediate	Susceptible	Susceptible	Intermediate
V2A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
V2B	Intermediate	Resistant	Intermediate	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
V3A	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
V3B	Resistant	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
V4A	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant
V4B	Resistant	Intermediate	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant
V5A	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
V5B	Resistant	Susceptible	Resistant	Intermediate	Intermediate	Intermediate	Susceptible	Resistant	Resistant	Intermediate	Resistant	Resistant
POTCH1A	Intermediate	Resistant	Resistant	Susceptible	Resistant	Intermediate	Resistant	Resistant	Resistant	Susceptible	Resistant	Resistant
POTCH1B	Intermediate	Resistant	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
POTCH2A	Resistant	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant

POTCH2B	Resistant	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant
POTCH3A	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Intermediate	Resistant	Susceptible	Resistant	Resistant
POTCH3B	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Intermediate	Resistant	Susceptible	Resistant	Resistant
POTCH4A	Resistant	Intermediate	Intermediate	Susceptible	Intermediate	Intermediate	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
POTCH4B	Resistant	Intermediate	Intermediate	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Intermediate	Resistant	Resistant
POTCH5A	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
POTCH5B	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
POTCH6A	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
POTCH6B	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
CV1A	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Intermediate	Resistant	Susceptible	Resistant	Resistant
CV1B	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Intermediate	Susceptible	Intermediate	Resistant	Susceptible	Resistant	Resistant
CV2A	Resistant	Resistant	Resistant	Intermediate	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
CV2B	Resistant	Resistant	Resistant	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
CV3A	Susceptible	Susceptible	Susceptible	Intermediate	Resistant	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant

CV3B	Susceptible	Susceptible	Susceptible	Resistant	Intermediate	Resistant	Intermediate	Resistant	Resistant	Susceptible	Resistant	Resistant
CV4A	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
CV4B	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
CV5A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
CV5B	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
CV6A	Resistant	Resistant	Susceptible	Susceptible	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant
CV6B	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant
CV7A	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
CV7B	Resistant	Susceptible	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
CV8A	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible
CV8B	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible
LIC1A	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
LIC1B	Resistant	Resistant	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
LIC2A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant

LIC2B	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
LIC3A	Resistant	Resistant	Resistant	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant
LIC3B	Resistant	Resistant	Resistant	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant
LIC4A	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Intermediate	Resistant	Intermediate
LIC4B	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Intermediate	Resistant	Resistant
LIC5A	Resistant	Resistant	Intermediate	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant
LIC5B	Resistant	Resistant	Intermediate	Intermediate	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Intermediate	Resistant	Resistant
ST1A	Susceptible	Susceptible	Intermediate	Susceptible	Intermediate	Susceptible	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant
ST1B	Susceptible	Susceptible	Intermediate	Susceptible	Resistant	Susceptible	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant
ST2A	Resistant	Susceptible	Resistant	Susceptible	Susceptible	Resistant	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant
ST2B	Resistant	Susceptible	Resistant	Susceptible	Intermediate	Resistant	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant
ST3A	Resistant	Resistant	Resistant	Resistant	Susceptible	Susceptible	Intermediate	Resistant	Resistant	Susceptible	Resistant	Resistant
ST3B	Resistant	Resistant	Intermediate	Resistant	Susceptible	Susceptible	Resistant	Resistant	Resistant	Susceptible	Resistant	Resistant
BHK1A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Resistant

usceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Susceptible	Resistant	Intermediate
usceptible	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible	Resistant	Resistant	Intermediate	Resistant	Resistant
esistant	Resistant	Susceptible	Susceptible	Resistant	Susceptible	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant

APPENDIX 2A

Appendix 2A: Multi Antibiotic Resistance (MAR) phenotypes of *Salmonella* isolates derived from cattle faeces and beef samples obtained from Mafikeng.

Sample area	Phenotype	Sample area	Phenotype
Mafikeng	T-E-RP-C-PG-CFXM	**Mafikeng cont...**	AP-CFM-T-E-RP-PG-CFXM
	T-E-RP-PG		AP-NA-E-RP-PG-CFXM
	AP-CFM-T-E-RP-PG		AP-NOR-E-RP-C-PG-CFXM
	T-E-RP-PG-CFXM		NA-E-RP-PG-CFXM
	AP-T-E-RP-PG		AP-CFM-GM-E-RP-PG-CFXM
	AP-T-RP-PG		AP-PG-CFXM
	AP-CFM-E-RP-PG-CFXM		CFM-E-RP-PG
	AP-CFM-RP-PG		AP-GM-T-RP-PG-CFXM
	AP-E-RP-PG		AP-CFM-E-PG-CFXM
	AP-CFM-GM-T-NOR-E-RP-C-PG-CFXM		AP-CFM-T-E-RP-C-PG-CFXM
	GM-E-RP-PG		E-PG-CFXM
	NA-T-E-RP-PG		E-C-PG-CFXM
	GM-PG-CFXM		E-PG-CFXM
	AP-NOR-E-RP-C-PG		PG-CFXM
	GM-E-RP-PG-CFXM		AP-CFM-E-C-PG-CFXM
	AP-RP-C-PG		RP-PG-CFXM
	E-RP-PG		AP-T-E-RP-PG-CFXM
	AP-E-RP-PG-CFXM		AP-CFM-NA-E-RP-PG-CFXM
	E-RP-PG-CFXM		GM-T-E-RP-C-PG-CFXM
	E-RP-C-PG-CFXM		AP-NA-GM-T-E-RP-C-PG-CFXM
	AP-RP-C-PG-CFXM		AP-CFM-CIP-T-E-RP-PG
	CFM-E-RP-PG-CFXM		AP-CIP-NOR-E-RP-PG-CFXM
	AP-CFM-E-RP-PG		
	GM-E-PG-CFXM		

Appendix 3A: Multi Antibiotic Resistance (MAR) phenotypes of *Salmonella* isolates derived from cattle faeces and beef samples obtained from Vryburg, Rustenburg, Ventersdorp and Marikana.

Sample area	Phenotype	Sample area	Phenotype
Vryburg	AP-E-RP-PG	Rustenburg	AP-CFM-NA-CIP-E-RP-PG
	AP-T-E-RP-PG-CFXM		AP-CFM-NA-CIP-NOR-E-RP-PG
	AP-CFM-T-E-RP-PG-CFXM		T-E-RP-PG
	E-RP-PG		CFM-T-E-RP-PG
	T-E-RP-C-PG		AP-E-RP-C-PG-CFXM
	AP-CFXM		AP-CFM-E-RP-C-PG-CFXM
	AP-CFM-CFXM		E-RP-PG-CFXM
	RP-PG-CFXM		CFM-E-RP-C-PG-CFXM
	AP-CFM-NA-CIP-E-RP-PG		AP-CFM-NA-CIP-NOR-E-RP-PG-CFXM
	AP-CFM-E-RP-PG		AP-CFM-NA-CIP-E-RP-PG
	AP-CFM-NA-E-RP-PG-CFXM		T-E-RP-PG-CFXM
	AP-T		CFM-T-E-RP-PG-CFXM
	AP-CFM-T		NA-E-RP-PG
	E-RP-PF		AP-NA-CIP-E-RP-PG-CFXM
	AP-E-RP-PG-CFXM		AP-CFM-NA-CIP-E-RP-PG-CFXM
			AP-CFM-E-RP-PG-CFXM
Ventersdorp	AP-CFM		
	AP-CFM-CIP	Marikana	AP-CFM-NA-GM-CIPE-RP-PG
	T-E-RP-PG-CFXM		RP-PG-CFXM
	CFM-T-E-RP-PG-CFXM		AP-RP-PG-CFXM
	AP-T-E-RP-PG-CFXM		T-E-RP-C-PG
	AP-E-RP-C-PG-CFXM		AP-CFM-NA-GM-CIP-E-RP-PG-CFXM
	AP-E-RP-PG-CFXM		AP-CFM-NA-GM-CIP-E-RP-PG
	AP-NA-E-RP-PG-CFXM		T-E-RP-PG-CFXM
			CIP-T-E-RP-PG-CFXM
			AP-T-E-RP-PG-CFXM

Appendix 4A: Multi Antibiotic Resistance (MAR) phenotypes of *Salmonella* isolates derived from cattle faeces and beef samples obtained from Potchefstroom, Carletonville, Lichtenberg, Stella, Boshoek and Brits.

Sample area	Phenotype	Sample area	Phenotype
Potchefstroom	CFM-NA-CIP-NOR-E-RP-PG-CFXM	Carletonville	AP-CFM-RP-PG-CFXM
	CFM-E-RP-PG-CFXM		AP-CFM-NA-T-E-RP-PG-CFXM
	AP-CFM-E-RP-C-PG-CFXM		CIP-T-E-RP-PG-CFXM
	AP-CFM-RP-PG-CFXM		GM-T-E-RP-PG-CFXM
	AP-E-RP-PG-CFXM		AP-T-E-RP-PG-CFXM
	AP-T-E-RP-PG-CFXM		T-E-RP-PG-CFXM
	E-RP-PG-CFXM		AP-CFM-PG-CFXM
			E-RP-PG-CFXM
Lichtenberg	AP-T-E-RP-PG-CFXM 2		AP-E-RP-PG-CFXM
	AP-CFM-T-E-RP-PG-CFXM		
	E-RP-PG-CFXM	Stella	E-RP-C-PG-CFXM
	AP-CFM-NA-T-E-RP-C-PG-CFXM 2		CIP-E-RP-C-PG-CFXM
	T-E-RP-PG		AP-NA-T-E-RP-C-PG-CFXM
	AP-CFM-E-RP-PG-CFXM		AP-CFM-NA-GM-E-RP-PG-CFXM
			E-RP-PG-CFXM
Boshoek	E-RP-PG-CFXM		
	E-RP-PG	Brits	AP-T-E-RP-PG-CFXM
	CFM-E-RP-PG-CFXM		E-RP-PG
	AP-CFM-CIP-E-RP-C-PG-CFXM		E-RP-PG-CFXM
			AP-NA-T-E-RP-C-PG-CFXM
			AP-NA-T-E-RP-C-PG