UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE PROFILES OF *SALMONELLA* TYPHIMURIUM ISOLATED BETWEEN 1995 AND 2002 FROM ORGANS AND ENVIRONMENTS OF DISEASED POULTRY IN SOUTH AFRICA

BY

JEAN BOSCO NTIVUGURUZWA

A dissertation submitted in partial fulfilment of the requirements for the degree of Master of veterinary science (Veterinary Public Health)

Department of Paraclinical Sciences,

Faculty of Veterinary Science,

University of Pretoria,

Pretoria

2016

DEDICATION

I dedicate this work to my beloved family and friends for their support and for being so patient during my absence.

DECLARATION

I hereby declare that, except the assistance obtained and duly acknowledged, this dissertation represents the original scientific work of the author.

I declare that the findings presented in this dissertation have not been previously submitted for any other degree at this or any other academic institution.

ACKNOWLEDGEMENTS

I strongly acknowledge the Almighty God who protects, provides, inspires, and provides perseverance and wisdom. Glory to The Lord.

I would like to express my heartfelt gratitude to the Department of Paraclinical Science for admitting me into postgraduate studies, and leadership. I am proud to have been a postgraduate student at the University of Pretoria.

Without the help of Dr. Musafiri Karama, this work would not have been a success and I would like to take this opportunity to express my sincere gratitude to him as my supervisor. Your guidance, contribution, expertise and scientific criticism, made completion of this research project possible. I learned a lot from you.

I also want to thank my co-supervisor, Dr. Henriette Van Heerden for encouraging and guiding me through my write up. Your scientific criticism inspired confidence in me and helped shape me into a better person and researcher. You will always be a model of inspiration for me.

I am indebted to Prof. Dayo Facina for his support in Statistics, editing, advices, and encouragements. Your easy approach and humility are good examples to imitate. I am so grateful to Dr. Nenene Qekwana, Dr. Leon, Ms. Ayesha Hassim, Ms. Betty Ledwaba, Dr. Charles Katsande and Dr. Charles Byaruhanga for their advice, support and encouragements. Your kindness will always be engraved in my memory. I would also like to acknowledge:

- Ms. Cindy-Lee du Plessis from Hatfield library for her assistance in reading microfilms;
- The family of John Ishimwe for the great moments spent together;
- All postgraduate students: Dr. John Chipangura, Dr. Oumar, Dr. Benjamin, Dr. Gloria, Dr. Solomon, Dr. Leon, Dr. Alexander Jambalang etc. for your encouragements;
- Ms. Londanani, Ms. Gaia Gobbo and Dr. Libby for your support and encouragements;
- Mr. Johan Gouws, the Head of Bacteriology lab for providing isolates;
- University of Rwanda and the Government of Rwanda, for funding my studies and the hosting country “South Africa” for facilitating this training;
- University of Pretoria and National Research Foundation (NRF) for funding this project;
- Any other person who contributed directly or indirectly for the completion of this work.

Abbreviations

SPIs: *Salmonella* Pathogenicity Islands
SGIs: *Salmonella* Genomic Islands
PFGE: Pulsed Field Gel Electrophoresis
LPS: Lipopolysaccharide
CDC: Centres for Disease Control and Prevention.
DT 104: *Salmonella* Typhimurium Definitive Type 104
NTS: Non-Typhoidal *Salmonella*
HIV: Human Immunodeficient Virus
tRNA: transfer Ribonucleoside Acid
G-C: Guanidine and Cytosine
T3SS: Type III Secretion System
SifA: Salmonella invasion filaments
Sop: Salmonella outer protein
sspH : *Salmonella* secreted proteins
sodC-1: Superoxide dismutase C-1
gtgB: Gifsy two gene B
spv: Salmonella virulence plasmid
pef: plasmid–encoded fimbriae
srgA: SdiA regulated gene
rck: resistance to complement killing
traT: transfer gene
mig5: microphage inducible gene
PSLT: plasmids of *Salmonella* Typhimurium
Inc FIIA: incompatibility group FIIA
DNA: Deoxyribonucleic Acid
ACSSuT: Ampicillin, Chloramphenicol, Streptomycin, Sulphonamides and Tetracycline
mPCR*:* Multiplex Polymerase Chain Reaction
V: virulotype
X: Pulsotype
dNTPs: deoxyribonucleoside triphosphate
IDT: Integrated DNA Technologies
Taq: Thermal Aquaticus
Bp: base pairs
UPGMA: Unweighted Pair Group Method with Averages
F: farm
ST: *Salmonella* Typhimurium
MAP: Mitogen Activated Protein
SCVs*: Salmonella* Containing Vacuoles
rRNA: Ribosomal Ribonucleic Acid
Tm: melting temperature;
[Primer]: final concentration of primer
AM: Ampicillin
AMC: Amoxicillin Clavulanic Acid
CF: Cephalotin
CTX: Cefotaxime
CAZ: Ceftazidime
C: Chloramphenicol
TE: Tetracycline
S10: Streptomycin
GM: Gentamycin
K: Kanamycin
CT: Colistin
CIP: Ciprofloxacin
ENR: Enrofloxacin
SXT: Sulfamethoxazole-Trimethoprim
NA= Nalidixic Acid
S3= Sulphonamides
ATB: Antibiotics

TABLE OF CONTENTS

List of tables, figures, and appendixes

THESIS SUMMARY

MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE PROFILES OF *SALMONELLA* TYPHIMURIUM ISOLATED BETWEEN 1995 AND 2002 FROM ORGANS AND ENVIRONMENTS OF DISEASED POULTRY IN SOUTH AFRICA

MSc Degree of : Jean Bosco Ntivuguruzwa
Supervisor : Dr. Musafiri Karama
Co-supervisor : Dr. Henriette Van Heerden
Department : Paraclinical Sciences

Despite the occurrence of *S.* Typhimurium infections, little is known on the genetic diversity, virulence characteristics and antimicrobial resistance profiles of poultry *S.* Typhimurium in South Africa. Therefore, *S.* Typhimurium (n=141) isolated from organs (n=115) and environments (n=26) of diseased poultry between 1995 and 2002 were screened by PCR for bacteriophages, plasmids and *Salmonella* pathogenicity islands (SPIs) – encoded virulence genes (virulotyping) which are essential for invasion (*invA, sopB, gtgB, sspH1, sopE, spvC,* and *pefA*), survival (*sifA, gipA, sodC1, gtgE, mig5,* and *sspH2*) and serum killing (*rck,* and *srgA*) of the pathogen in the host. Isolates were also characterized by: pulsed field gel electrophoresis (PFGE) for genetic relatedness, and plasmid profiling (n=43). Furthermore, isolates (n=141) were tested for susceptibility to 16 antimicrobials by disk diffusion and further screened by PCR for the carriage of 27 resistance genes, and integrons. Multi-resistant *S.* Typhimurium definitive phage type (DT) 104 were determined by disk diffusion and confirmed by PCR.

All isolates carried SPIs-encoded genes: *invA, sopB,* and *sifA*. Bacteriophages-encoded genes (*sspH2*, *sspH1*, *sodC1*, *gtgB*, and *gtgE)* occurred in more than 74.5% of the isolates expect for *gipA* (57.6%), and *sopE* (19%). The occurrence of plasmid-encoded genes (*pefA*, *mig5*, *rck*,

spvC, and *srgA*) ranged from 48.2% to 74.5%. Two sample t – test showed that virulence genes: *gtgB, spvC, gipA, gtgE, mig5, rck* and *srgA* were more frequent ($p \leq 0.05$) in *S.* Typhimurium isolates from environments. Virulotyping clustered 141 isolates into 59 virulotypes with 97 isolates clustering in 5 predominant virulotypes while 44 were single isolate virulotypes. PFGE grouped 140 isolates into 55 pulsotypes with 66 isolates clustering in 5 major pulsotypes, 51 isolates clustering in small pulsotypes (containing less than 5 isolates) while 33 were single isolate pulsotypes. Ten plasmid profiles ranging from 2kb to 90kb were observed. The most common plasmid profile contained the 90kb plasmid and was observed in 12/43 isolates. Major virulotypes and plasmid profiles corresponded approximately to pulsotypes and clustered isolates recovered from the same farms or during the same period. Virulotyping and PFGE showed identical discriminatory index (D=0.93). Multidrug resistance (resistance to ≥ 2 antimicrobials) was observed in 97.2% of isolates. High levels of resistance phenotypes and their respective resistance genes were observed for: streptomycin (94.3%) conferred by *ant3'la* (60.3%) and *str* (50.4%), sulphonamides (87.2%) conferred by *sul1* (66%) and *sul3* (31.9%), ciprofloxacin (79.4%) conferred by *qnrA* (79.4%), tetracycline (61%) conferred by *tetB* (35.5%) and *tetG* (28.4%), and cefotaxime (55.3%) conferred by *blaSHV* (57.4%). Two sample t – test revealed that isolates from poultry organs were more resistant ($p \leq 0.05$) to ampicillin, amoxicillin clavulanic acid, chloramphenicol, tetracycline and sulfamethoxazole - trimethoprim while isolates collected from poultry environments were more resistant to cephalothin, cefotaxime, ceftazidime, colistin sulphate and nalidixic acid. Using the Kappa statistics, there were agreements ranging from good to perfect between phenotype and genotype. In addition, for every phenotypic resistance recorded, at least one corresponding resistance gene was detected. DT104 strains and class1 integrons were observed in 34.7% and 83% of the isolates respectively. Multi-resistant *S.* Typhimurium (97.2%) also carried SPIs – encoded virulence genes involved in invasion and survival in the host. In addition, more than 50% of resistant isolates to each of the antimicrobials also carried at least 12 virulence genes: *invA, sopB, sifA, sspH2*, *sspH1*, *sodC1*, *gtgB*, *gtgE, pefA*,

mig5, *spvC*, and *srgA*. A significant number (44.9%) of the DT104 strains that were clustered in the same pulsotype X25 also belonged to virulotype V3a which contained 13 virulence genes: *invA, sopB, sifA, sspH2*, *sspH1*, *sodC1*, *gtgB*, *gtgE, pefA*, *rck, mig5*, *spvC*, and *srgA.* Most of isolates that belonged to the same antimicrobial resistance profile (phenotype and genotype) carried at least 8 common virulence genes.

In conclusion, these data indicate that *S.* Typhimurium isolated from diseased poultry carry virulence genes that are usually incriminated in *Salmonella* human outbreaks. Virulotyping and PFGE showed the same discriminatory index (D=0.93) indicating that virulotyping can be an alternative subtyping method in laboratories where PFGE is not available. *Salmonella* Typhimurium are also genetically diverse since they were recovered from multiple farms and during a period spanning 8 years. Furthermore, isolates were resistant to multiple antimicrobials used in poultry operations (streptomycin, sulphonamides, and tetracycline) and those used to treat human salmonellosis: ciprofloxacin, and cefotaxime. Multidrug resistant isolates carried most of virulence genes. This relationship between virulence and antimicrobial resistance suggests that the adaptation of isolates against antimicrobial effects may induce expression of virulence factors. The increasing incidence of DT104 threatens the public health since DT104 strains are associated with hospitalizations and deaths in humans. *Salmonella* Typhimurium carried mobile genetic elements (bacteriophages, integrons and plasmids) which pose a public hazard as they propagate virulence and resistance genes with emerging new pathogenic bacteria as a result. Therefore, monitoring and surveillance of salmonellosis and prudent antimicrobials use need more efforts to ensure animal health and food safety for consumers in South Africa.

CHAPTER 1: GENERAL INTRODUCTION

South Africa has adopted poultry farming in response to the high demand in animal proteins due to high population growth (54.9 millions) (Thornton 2010, Statistics South Africa. 2014). In 2014, nine hundred sixty (960.4) million broilers (SPA. 2015a), and 24.3 million of layers were produced in South Africa (SPA. 2015b). However, intensive poultry farming is characterized by short production cycle, intense metabolism, high density and poor management which lead to poor hygiene (F. Jones *et al.* 1991, B. A. Jones *et al.* 2013). Poor hygiene creates favourable conditions for the multiplication of foodborne pathogens such as *Salmonella* spp. that can contaminate poultry products and poultry environments (F. Jones *et al.* 1991, B. A. Jones *et al.* 2013). *Salmonella spp*. are common contaminants of poultry environments and among the most frequent causes of foodborne disease outbreaks worldwide (Majowicz *et al.* 2010). *Salmonella* Typhimurium is the most common *Salmonella* serovar that has been incriminated in foodborne outbreaks that were associated with poultry products (CDC. 2013). Humans acquire foodborne salmonellosis mainly by ingestion of contaminated meat and eggs (Fearnley *et al.* 2011).

Infections caused by *S.* Typhimurium are mainly characterized by gastroenteritis including nausea, vomiting, fever, diarrhoea and abdominal cramps (Crum-Cianflone 2008). Furthermore, bacteraemia is common in immune-compromised patients (Keddy *et al.* 2009, E. Threlfall, Ward & Rowe 1998). The severity of *S.* Typhimurium disease in humans or animals depend on the expression of virulence factors (Lopez *et al.* 2012) encoded on the chromosome or mobile genetic elements including plasmids and bacteriophages (Gyles & Boerlin 2014). Chromosomal virulence factors are carried on *Salmonella* pathogenicity islands (SPIs) (Schmidt & Hensel 2004), and bacteriophages (Brussow, Canchaya & Hardt 2004). Furthermore, the emergence and wide dissemination of antimicrobial resistant *S.* Typhimurium variants has been mostly associated with mobile genetic elements including integrons, transposons, plasmids and bacteriophages (Gyles & Boerlin 2014). Salmonellae which are resistant to antimicrobials are difficult to treat and

characterized by long period of hospitalization and death with economic losses as a result (Helms *et al.* 2002).

Although *S.* Typhimurium isolates were isolated from poultry in South Africa (Kidanemariam, Engelbrecht & Picard 2010), data on characteristics of *S.* Typhimurium isolates from South African poultry production systems are scarce. Therefore, the aim of this study is to characterize genetically and phenotypically *S.* Typhimurium isolates from poultry in South Africa. In this study, 141 *S.* Typhimurium isolates were characterized with respect to virulence genes, pulsed field gel electrophoresis (PFGE) profiles, plasmid profiling, and antimicrobial resistance (genetic and phenotypic) patterns. A detailed characterization contribute towards a better understanding of the molecular features of *S.* Typhimurium and determining the potential human health risks associated with these isolates from South Africa.

1.1. Aim and Objectives

The aim of this research was to investigate the molecular subtyping and antimicrobial resistance profiles of clinical *S.* Typhimurium isolated from organs and environments of diseased poultry between 1995 and 2002 in South Africa. The objectives were:

- To characterize *S.* Typhimurium isolates obtained from diagnostic laboratory and isolated between 1995 and 2002 from poultry organs and poultry environments with respect to virulence genes profiles (virulotyping), DNA fingerprinting (PFGE) and plasmid profiling;
- To determine antimicrobial resistance profiles (phenotype and genotype), the prevalence of integrons and DT104 in *S.* Typhimurium isolates from organs and environments of diseased poultry.

CHAPTER 2: LITERATURE REVIEW

2.1. Aetiology and General Epidemiology of *Salmonella* infections

2.1.1. Aetiology and Taxonomy

Salmonellae are facultative anaerobic rod-shaped Gram-negative bacteria (diameter: 0.7 – 1.5 µm and length: 2-5 µm) belonging to the family of Enterobacteriaceae (Ewing 1972). The genus *Salmonella* consists of two main species, *Salmonella enterica* and *Salmonella bongori* (Ewing 1972). *Salmonella enterica* is divided into 6 subspecies: *enterica* (sub I); *salamae* (sub II); *arizonae* (subsp IIIa); *diarizonae* (subspIIIb); *houtenae* (IV); *indica* (VI) (Tindall *et al.* 2005). *Salmonella* serotyping is based on flagellar and O surface antigens that are localized on the bacterial outer membrane. The outer membrane consists of lipopolysaccharide (LPS) which is made of lipid A, polysaccharide (sugar) and somatic antigens (CDC. 2011). The number of somatic antigens (O antigens) is 46 whereas flagellar antigens (H antigens) are 119 (CDC. 2011, Issenhuth-Jeanjean *et al.* 2014). The combination of these surface structures based on their immunologic reactivity, is the basis of *Salmonella* serotyping or serovars classification (CDC. 2011, Issenhuth-Jeanjean *et al.* 2014). Within a serotype, strains may acquire or lose surface antigens and these strains are termed variants. There is a wide range of serotypes within subspecies I and *S.* Typhimurium is among the most serovars infecting animals and humans (CDC. 2011, Issenhuth-Jeanjean *et al.* 2014).

Currently there are more than 2,679 *Salmonella* serotypes with 60% belonging to *Salmonella enterica* subsp *enterica* which cause human diseases (Issenhuth-Jeanjean *et al.* 2014). Serotypes are designated according to the conventions of Kauffman-White scheme established in 2002 (Popoff, Bockemuhl & Gheesling 2003). This scheme is updated every five years and the list of serotypes is available at Centres for Disease Control and Prevention (CDC), Atlanta, USA (Popoff *et al.* 2003). Serological and molecular identification of *S.* Typhimurium

relies on the detection of the constant antigens O4; i: 1, 2 (CDC. 2011, Issenhuth-Jeanjean *et al.* 2014). Each *Salmonella* serotype is determined by a specific antigenic profile which is based on each O and H antigen unique numbers; for example, *S.* Typhimurium belongs to group 4 (in number) or group B (in letter) based on the presence of its constant O4 antigen and two phases of flagellar (H) antigens namely phase-1(H1) and phase-2 termed (H:i) and (H:1, 2) respectively; thus the antigenic formula of *S*. Typhimurium is subspecies I; 4, [5], 12: i: 1, 2 (Issenhuth-Jeanjean *et al.* 2014). Particular antigenic profiles have been mostly associated with particular disease manifestations and host specificity (CDC. 2011, Issenhuth-Jeanjean *et al.* 2014). Therefore, differentiation of *S.* Typhimurium from other *Salmonella* serovars is extremely important in order to understand *Salmonella* epidemiology and for quick diagnosis and prevention.

The evolution of *S.* Typhimurium strains has been characterized by genetic variation resulting in the emergence of new variants and phage types (Brussow *et al.* 2004). Salmonellae can also be classified according to phages types. Phage typing is the classification of bacterial strains of a given serotype based on their susceptibility or resistance to lysis when exposed to a number of known phages (Callow 1959, Anderson *et al.* 1977). For example, the leading prototype is the *S.* Typhimurium Definitive Type (DT) 104 which is multidrug resistant strain that has caused severe outbreaks (Briggs & Fratamico 1999). Phage typing has revealed that there are more than 300 *S.* Typhimurium phage types (Anderson *et al.* 1977, Rabsch *et al.* 2002). Based on the disease manifestations caused by *Salmonella* serotypes, there are two types of salmonellae that affect humans: typhoidal salmonellae that only affect humans and non-typhoidal salmonellae (NTS) which are zoonotic pathogens (Majowicz *et al.* 2010). NTS cause annually 93.7 million of human illness and 155,000 deaths worldwide (Majowicz *et al.* 2010). In Africa, NTS accounts for 2.5 million illnesses and 4100 deaths (Majowicz *et al.* 2010). In South Africa, 16,211 human cases of *Salmonella* were reported from 2003 to 2010 (Crowther-Gibson *et al.* 2011).

2.1.2. General Epidemiology of *Salmonella* Typhimurium

Human infections with NTS serovars are increasingly becoming a threat to human health worldwide with *S.* Enteritidis and *S.* Typhimurium commonly associated with human disease for which poultry are a major source (Fearnley *et al.* 2011, Padungtod & Kaneene 2006). Favourable factors for poultry salmonellosis include: age, serotype and initial dose, stress related to environment and transport, host immunity; presence of antimicrobials and anticoccidials in feeds; survival from low pH of the stomach; competition with the gut microflora; presence of a compatible colonization site; and genetic background of the hosts (Bailey 1988). Chicks are more susceptible to *Salmonella* infection and gut colonization within 96 hrs. from hatching (Bailey 1988). The potential sources of contamination in a poultry farm include: vertical contamination of chicks through infected parents, or horizontal transmission through hatcheries, sexing in contaminated hatcheries, cloacal infection, and transportation equipment's and feeds (Opitz 1992). Environmental factors such as air, litter, and unclean facilities, and vectors such as insects, humans, and rodents are also involved in horizontal transmission (F. Jones *et al.* 1991). It has been suggested that *S.* Typhimurium can persist in soil more than 7 weeks after pig farming (Jensen *et al.* 2006), survive up to 299 days in the pig manure and be killed by the temperature ranging from 57 to 60°C (Baloda, Christensen & Trajcevska 2001). Chicken products (meat and eggs) are the main sources of human salmonellosis (Fearnley *et al.* 2011). However, contact transmission from person to person or animal to person has also been reported (Morpeth, Ramadhani & Crump 2009). In 2008, poultry products were the most frequent vehicles of foodborne *S.* Typhimurium reported in the European Union (EFSA. 2010). In Africa and particularly South Africa the main sources of *Salmonella* are contaminated water, chicken and eggs (Obi *et al.* 2007, van Nierop *et al.* 2005). Immune-compromised patients are the most affected by *Salmonella* infections (Keddy *et al.* 2009).

Salmonella Typhimurium is a major non-typhoidal serovar threatening global public health (E. J. Threlfall 2000). From 1987 to 1999, sixty one per cent of all *Salmonella* that were collected from human outbreaks by the CDC were *S.* Typhimurium (Olsen *et al.* 2001). From 2008 to 2011, 376 human cases of *S.* Typhimurium infections were identified in several parts of the USA (Mettee Zarecki *et al.* 2013). A recent outbreak caused by *S.* Typhimurium affected 356 people from 39 USA states of which 62 were hospitalized and the source of contamination was linked to poultry (CDC. 2013). In 2008, the European Union reported to European CDC 135,335 cases of which 98.6% were confirmed positive for NTS and *S.* Typhimurium was the most frequent pathogen causing 21% of salmonellosis (EFSA. 2010). According to the annual report on foodborne diseases in Australia, the total number of human salmonellosis notified in 2009 was 9,533 and *S.* Typhimurium was the most common serotype identified in all states and territories in Australia accounting for 41% (OzFoodNet Working Group 2010). In Asia, *S.* Typhimurium was the fifth among the 20 most commonly isolated *Salmonella* serotypes (Hendriksen *et al.* 2011).

From 1998 to 2004, *S.* Typhimurium was the most frequent enteric pathogen in Malawi causing 75% of human infections and HIV was the main predisposing factor for acquiring salmonellosis (Feasey *et al.* 2010). Between 2000 and 2001, the serotyping of 407 *Salmonella* isolates from humans revealed that 237 (58.2%) were *S.* Typhimurium in Mozambique (Ruiz *et al.* 2008). In 2006, the total number of human NTS cases was estimated at 563,000; 85,000; 1,488,000; 46,000 and 839,000 respectively for North, Central, East, Southern and West Africa (Majowicz *et al.* 2010). *Salmonella* Typhimurium was the second most isolated *Salmonella* serovar from humans in Central and East Africa (Ekdahl *et al.* 2005).

In South Africa, from 2003 to 2004, 1,318 cases of human NTS salmonellosis were identified and *S.* Typhimurium accounted for 67% (Feasey *et al.* 2010). A large number (3417) of salmonellae were isolated from various animals between 1996 and 2006 from all provinces of South Africa and *S.* Typhimurium was the most frequently identified accounting for 26.6% of 182

serotypes that were detected; and 70. 5% of 26.6% of the *S.* Typhimurium were isolated from poultry and other birds (Kidanemariam *et al.* 2010). Furthermore, in Gauteng province (South Africa) between 2006 and 2007, *S.* Typhimurium isolates were detected in 857 (61%) patients of which 93% were HIV-positive (Keddy *et al.* 2009) whereas 25% of the 317 *Salmonella* isolates from human patients in Eastern Cape province were *S.* Typhimurium (Bisi-Johnson *et al.* 2011). Taken together, these studies indicate that *S.* Typhimurium is an important ubiquitous *Salmonella* serovar that infects a wide range of animals including poultry and humans. Therefore, *S.* Typhimurium deserves particular attention with regards to monitoring and surveillance aiming at eradication or reduction of *S.* Typhimurium infections within poultry industry.

2.2. Molecular Epidemiology of *Salmonella* Typhimurium

Molecular epidemiology is the study of distribution and determinants of infectious diseases that utilizes molecular biology methods (Riley L.W. 2004). The practical goals of molecular biology include identification of the infectious micro-organisms, determination of their physical sources, their biological relationships, and their routes of transmission and those of the genes responsible for their virulence and drug resistance (Levin, Lipsitch & Bonhoeffer 1999). The pathogen's own genetic material against its host's defence mechanisms and its environmental changes may determine the infectious disease distribution itself (Riley L.W. 2004). The study of genetic factors that determine and regulate an organism's specific patterns of transmission should, therefore, be included in the discipline of molecular biology (Riley L.W. 2004). In molecular epidemiology, the target of analysis includes the organism itself and its interactions with the host and the environment in which it resides (Riley L.W. 2004). Molecular epidemiology could provide valuable short- and long terms insight into *S.* Typhimurium surveillance which will help improve control practices to determine the main reservoirs and sources to limit the spread of *S.* Typhimurium.

2.2.1. *Salmonella* Typhimurium virulence factors and pathogenesis

Salmonella Typhimurium virulence factors can be divided into chromosomal, plasmid and bacteriophages-encoded virulence factors (Gyles & Boerlin 2014). Chromosomal virulence genes are mainly encoded on *Salmonella* pathogenicity islands (SPIs) and bacteriophages (Schmidt & Hensel 2004).

2.2.1.1. *Salmonella* Pathogenicity Islands

SPIs are chromosomal loci that contain virulence genes found only in pathogenic strains (Schmidt & Hensel 2004). SPIs loci differ from other chromosomal loci in structure and function and they are mostly located at tRNA insertion sites with a lower guanidine and cytosine content of 37% - 47% than the 52% G-C content of the genome (Schmidt & Hensel 2004). Twenty one SPIs have been identified in salmonellae with only twelve SPIs in *S.* Typhimurium (Sabbagh *et al.* 2010), among them, five are considered major SPIs and only SPI 1 and SPI 2 play a crucial role in virulence (López *et al.* 2012). SPI-1 encodes a type III secretion system: T3SS-1 (Figure 2.1) which secretes invasion effectors such as the invasion factor A (invA) (Sabbagh *et al.* 2010). Some effector proteins including *Salmonella* outer protein (Sop) A, B, D, E, and E_2, *Salmonella* secreted proteins ($SspH_1$) are secreted both by T3SS-1 and bacteriophages, indicated in Table 2.1 (Zhang *et al.* 2002). Effector proteins (effectors) are molecules produced by many Gram negative pathogenic bacteria (Galan 2009). They differ from bacterial toxins in the fact that they require the TTSS “machine” for their translocation from the bacterium to the host cell (Galan 2009). All effector proteins which are delivered by the same molecular machine exert their function collectively in a coordinated way resulting in the functional alteration of target host cells (Zhang *et al.* 2002, Galan 2009). Two SPI-1 effectors alter the host cell recognition mechanisms causing host actin cytoskeleton rearrangement (Schmidt & Hensel 2004). This structural change leads to membrane ruffling which favours cell penetration by effector proteins (Ly & Casanova 2007). SPI-

2 encodes the T3SS-2 which is essential for the secretion of virulent proteins: SifA, and SspH2 (Schmidt & Hensel 2004). SPI-2-effectors are involved in bacterial replication (Drecktrah *et al.* 2008) and contribute to intestinal disease and inflammation (Schmidt & Hensel 2004).The T3SS-5 carried by SPI5 secretes an effector (SopB) involved in the rearrangement of the host cell cytoskeleton (Figure 2.2). This effector is also secreted by T3SS-1 and T3SS-2 respectively (Knodler *et al.* 2002).

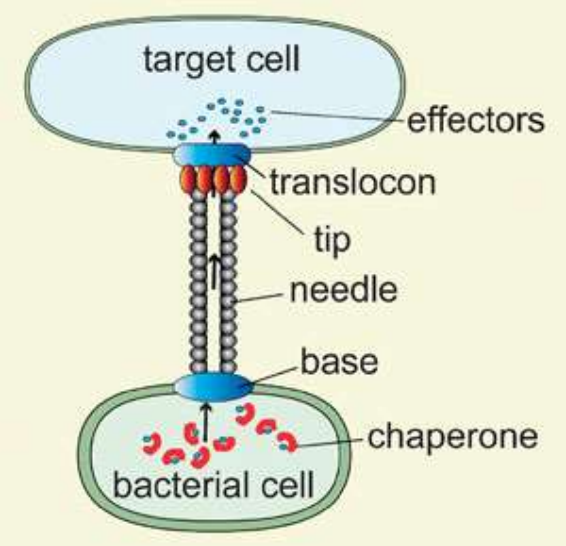


Figure 2.1. Structure of type III secretion system infecting host cells: TTSS is a syringe with a needle used to inject effector proteins into the host cell (Wang *et al.* 2008)

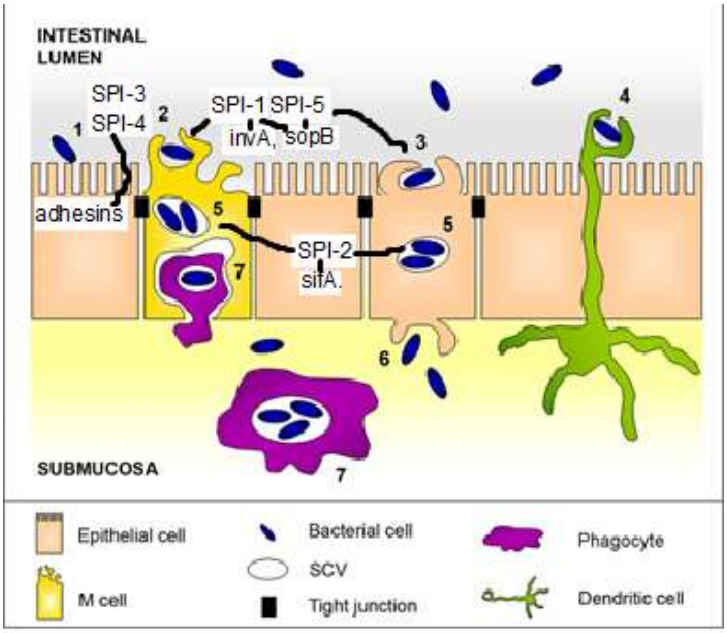


Figure 2.2. The model of *Salmonella* Typhimurium pathogenesis. (1) Adhesion of *Salmonella* cell to intestinal epithelium cells by means of adhesins of *Salmonella* pathogenicity Island: SPI-3 and *Salmonella* pathogenicity island: SPI-4. (2 and 3) Invasion and internalization mediated by virulence factors of SPI-1 and SPI-5 (the invasion factor A: invA, *Salmonella* outer protein: sopB). (4) Uptake of *Salmonella* by dendritic cell of the payer's patches. (5) Replication and survival of *Salmonella* inside the *Salmonella* containing-vacuoles (SCV) by means of SPI-2 effectors such as *Salmonella* induced filaments (sifA). (6) Release of *Salmonella* in the sub-mucosa via transcytosis. 7. Engulfment of *Salmonella* within phagocytes and possibility of dissemination into the blood stream. Adapted and modified from (Fàbrega & Vila 2013).

2.2.1.2. Role of bacteriophages in *Salmonella* virulence

Previous studies have demonstrated that *S.* Typhimurium carries temperate bacteriophages as lysogens which encode several virulence genes (Brussow *et al.* 2004, Figueroa-Bossi *et al.* 2001).The role played by these prophages - encoded genes in the *Salmonella* pathogenesis is summarized in Table 2.1. *Salmonella* strains cured of the prophage Gisfy-2 have been shown to have significantly reduced virulence in mouse experiments

(Figueroa-Bossi & Bossi 1999). The Gisfy-2 effector proteins sodC-1, gtgE, gtgB are responsible for systemic infection in mice (Figueroa-Bossi & Bossi 1999, Ehrbar & Hardt 2005). Furthermore, the Gisfy-1 found in some strains of *S.* Typhimurium has been associated with virulence reduction in mice in the absence of Gisfy-2 (Figueroa-Bossi *et al.* 2001). The Gisfy two gene (*gtgB)* is responsible for the polymerization of the actin cytoskeleton of the host resulting in the permeability and introduction of effector proteins into the host cytoplasm (Miao & Miller 1999). The SodC-1 protein catalyses the hydrolysis of peroxide into hydrogen peroxide (DeGroote *et al.* 1997) thereby avoiding the formation of a toxic peroxinitrite resulting from the reaction of peroxide and nitric acid which are both produced by macrophages (DeGroote *et al.* 1997). This is one of the defence mechanisms by which salmonellae survive in macrophages (DeGroote *et al.* 1997). The gene *sopE* is responsible for interference of the host cellular signalling pathways followed by membrane ruffling and invasion of intestinal cells (Friebel *et al.* 2001, Clark *et al.* 2011), *sopE* contributes also in bacterial replication within *Salmonella*-containing vacuoles (Vonaesch *et al.* 2014).

Table 2.1. Bacteriophages - encoded virulence genes of *Salmonella enterica.* Adapted and modified from (Silva C., Wiesner M., Calva E. 2012).

Phage's	**Genes**	**Protein**	**Function in virulence**
Gifsy-1	*gipA*	Type III effector	Growth and survival in Payer's Patches (Stanley, Ellermeier & Slauch 2000)
Gifsy-2	*SseI (gtgB)*	Type III effector	Involved in invasion (Miao *et al.* 2003)
	sodC-1	Superoxide dismutase	Intracellular survival through catalysis of the hydrolysis of peroxide (DeGroote *et al.* 1997)
	gtgE	Type III effector	Prevents the entry of the host antimicrobial factors into *Salmonella*-containing vacuoles (Kohler *et al.* 2014)
Gifsy-3	*sspH1*	Type III effector	Involved in invasion (affects the IL-8 production (Haraga & Miller 2003)

Remnant phage	*sspH2*	Type III effector	Modulation of innate immunity (Bhavsar *et al.* 2013)
SopEΦ	*sopE*	Type III effector	Involved in invasion especially intestinal inflammation (Friebel *et al.* 2001)

2.2.1.3. Role of plasmids in *Salmonella* virulence

Salmonella harbours a 90 kb virulence plasmid which encodes *Salmonella* virulence plasmid (*spv*) genes which are found in 88% of *S.* Typhimurium (Helmuth *et al.* 1985). Four structural genes *spv ABCD* have been identified on the *spv* plasmid (Gulig *et al.* 1993, El-Gedaily, Paesold & Krause 1997). The *spv* genes are responsible for intra-macrophage survival and three genes *spvR, spvB* and *spvC* of the spv region have been shown to increase virulence in the host (Guiney & Fierer 2011). The *spvR* gene is essential for the regulation of operon transcription while the *spvB* translocated by TTSS-2 inhibits the actin polymerization of the macrophages and softens its cytoskeleton leading to cell death by apoptosis (Guiney & Fierer 2011). Virulence plasmids are present in few serovars of *S. enterica* subspecies I and are mostly found in strains associated with non-typhoid bacteraemia via multiplication in the reticulo-endothelial system (Guiney & Fierer 2011, Fierer *et al.* 1992). Other loci located on the spv region include *pef* (plasmid–encoded fimbriae) and *srg*A (serum resistance gene) which hosts *rck* (resistance to complement killing) gene (Rychlik, Gregorova & Hradecka 2006). The *pef* locus mainly harbours four genes, namely *pef BACDI* (Baumler, Tsolis & Heffron 1996). This locus is responsible for attachment and adhesion of the bacterium to the small intestine (Baumler *et al.* 1996). Three genes including *traT* (transfer gene), *rsk* (regulation of serum killing) and *rck* (resistance to complement killing) have been found to induce serum resistance in *S.* Typhimurium (Guiney & Fierer 2011). The plasmids of *S.* Typhimurium (PSLT) are classified into incompatibility (inc.) groups and encode plasmid virulence genes (Rychlik *et al.* 2006) (Table 2.2).

Table 2.2. Plasmids - encoded virulence genes of *Salmonella* Typhimurium (Guiney & Fierer 2011, Rychlik *et al.* 2006, Carattoli 2003).

Plasmids	Genes	Function in virulence
PSLT038-39 (Inc FIIA)	*spv C and spv B*	Intra-macrophage survival, multiplication, polymerization of actin cytoskeleton and apoptosis
PSLT018 (Inc FIB)	*pef A*	Attachment and invasion of epithelial cells
PSLT (Inc FIB)	*rck*	Resistance to complement killing
PSLT (Inc FIB)	*srgA*	Serum resistance gene
PSLT(Inc FIB)	*mig5*	Intra-macrophage survival by hydrolysis of dioxide carbon

2.2.2. Molecular subtyping of *Salmonella* Typhimurium

Salmonella subtyping involves identifying the lowest form of "strains or subtypes" deriving from a common parent "*Salmonella* serotype" and these subtypes are indistinguishable from each other by molecular tests and thus they are called genetically related strains (Pagotto *et al.* 2005). The molecular subtyping method approved by PulseNet (CDC) for subtyping (DNA fingerprinting) of foodborne pathogens including *Salmonella* isolates is PFGE (Swaminathan *et al.* 2001, Gerner-Smidt *et al.* 2006). The preference of PFGE among other molecular subtyping methods consists of its discriminatory power, usefulness in the intraserovars and clonal differentiation of *Salmonella* from different environments, reproducibility and epidemiologic concordance in case of outbreak investigations (Swaminathan *et al.* 2001, Gerner-Smidt *et al.* 2006). In addition, PulseNet has chosen PFGE as a suitable tool for molecular surveillance in outbreaks of foodborne pathogens (Swaminathan *et al.* 2001, Gerner-Smidt *et al.* 2006). In the USA, data collected from PulseNet participant laboratories or from epidemiologic investigations (animals) are analysed by PFGE for the confirmation of their relatedness with clinical isolates from human patients of various locations (Swaminathan *et al.* 2001, Gerner-Smidt *et al.* 2006). Furthermore, due to the wide distribution

of food products and intensive human movements, PFGE is able to link the clinical isolates with the presumed source (animal or food) (Swaminathan *et al.* 2001, Gerner-Smidt *et al.* 2006).

Since its application in outbreak investigation in 1993, PFGE has been used to distinguish outbreak strains worldwide (Swaminathan *et al.* 2001). (Sandt *et al.* 2013)) using PFGE showed that 99% of *Salmonella* isolates from humans in Pennsylvania (USA) were closely related to *Salmonella* isolates from chickens and poultry meat. In Thailand, PFGE has been used to distinguish *S.* Typhimurium from monophasic *S.* Typhimurium (Huoy *et al.* 2014). (Leekitcharoenphon *et al.* 2013) showed that human *S.* Typhimurium ST313 isolated in the Republic Democratic of Congo shared the same PFGE clusters with those isolated in Nigeria. In Burkinafaso, (Kagambega *et al.* 2013) using PFGE technique found that *S.* Typhimurium strains isolated from poultry were closely related to human isolates. (Kariuki *et al.* 2006) using PFGE reported the genetic relatedness of *S.* Typhimurium strains isolated from children and their parents, and those from their close environment including animals in Kenya. (Ruiz *et al.* 2008) isolated from children two isolates of DT104 which shared identical PFGE pattern in Mozambique. (Akinyemi *et al.* 2010) demonstrated that 68.8% of *S.* Typhimurium isolates from animals and the environment presented the same PFGE cluster as the *S.* Typhimurium isolates from humans in Nigeria. These studies show the role played by PFGE in molecular epidemiology by tracking *S.* Typhimurium outbreaks.

In South Africa, PFGE has been mostly used to differentiate *Salmonella* isolates from humans (Keddy *et al.* 2009) and only a single recent study showed the relatedness of human and wild birds *S.* Enteritis strains (Smith *et al.* 2014). However, there are no other studies using PFGE to subtype *Salmonella* isolates from animals or link animal isolates to human infections. In developing countries, national coordinated epidemiological surveillance systems that use PFGE are non-existent. Therefore, PFGE would improve the tracking and control of *S.* Typhimurium infections in developing countries.

2.2.3. Antimicrobial resistance in bacteria

The ability of bacteria to withstand the killing or inhibition effects of antimicrobial agents is known as antimicrobial resistance (Morar & Wright 2010). Genes encoding antimicrobial resistance in bacteria are localized on both chromosomal elements including *Salmonella* genomic islands, integrons, and plasmids (Silva C., Wiesner M., Calva E. 2012). Recently, sequencing data revealed that there are more than 20,000 resistance genes belonging to 400 different bacteria (Liu & Pop 2009).

2.2.3.1. Origins of antimicrobial resistance

Antimicrobial resistance shows diverse origins (Schwarz, Kehrenberg & Walsh 2001, J. Davies & Davies 2010). There are three ways for the development of antimicrobial resistance (Schwarz *et al.* 2001). First of all, bacterial species which produce antimicrobials carry genes encoding resistance against their own antimicrobials as self-defence mechanism and these bacteria are naturally resistant and spread their resistance genes to other non-pathogenic bacteria either by transformation, transduction or conjugation (Schwarz *et al.* 2001, J. Davies & Davies 2010, Wright 2007). For example, the naturally resistant species harbouring beta lactamase genes have been identified before the discovery of antimicrobials and when these genes are spread across species and genus, they may undergo mutations in the new hosts and acquire an unrelated structure which doesn't change their functionality (Wright 2007, B. G. Hall & Barlow 2003). Efflux proteins associated with tetracycline resistance evolved by this way (Schwarz *et al.* 2001, Roberts 1996). The second way for bacteria to acquire resistance consists of the target modification site by single-step (streptomycin resistance) or multiple-steps (fluoroquinolones resistance) (Schwarz *et al.* 2001). The third way is that resistance genes in bacteria emerge from activation or stepwise mutation of bacterial proto-resistance genes which are often putative antimicrobial resistance elements that may play other cellular functions or have

little or no antimicrobial resistance capacity (Morar & Wright 2010). These genes are able to evolve into resistance elements when exposed to selection pressures such as sub-inhibitory concentrations of antibiotics (Morar & Wright 2010). Genes producing acetyl -, adenyl -, or phosphor - transferases enzymes that inactivate aminoglycosides and chloramphenicol compounds have been evolved this way (Schwarz *et al.* 2001, J. Davies 1994). In addition, uncontrolled waste disposal containing antimicrobials contribute to selection pressure of environmental bacteria (J. Davies & Davies 2010). The bacteria surviving from this selection pressure acquire resistance genes which may be located on transmissible mobile genetic elements such as plasmids (J. Davies & Davies 2010). Therefore, soil bacteria are considered reservoirs of resistance genes (Dantas *et al.* 2008). Furthermore, anthropogenic activity including the underuse, overuse and misuse of antimicrobials contribute also to the selection pressure of microflora; then surviving bacteria which acquire resistance genes are later shed in external environment where they contribute to the dissemination of antimicrobial resistance genes (J. Davies & Davies 2010). Finally, the mobile genetic elements (phages, genomic islands, integrons and plasmids) play an important role in lateral/horizontal transfer of resistance genes thereby spreading widely antimicrobial resistance to various bacteria (Gyles & Boerlin 2014, Brussow *et al.* 2004, Silva C., Wiesner M., Calva E. 2012).

2.2.3.2. Mechanisms of antimicrobial resistance

The mechanism by which bacteria resist antimicrobials consists of enzymatic inactivation resulting in changes that affect bacterial cell permeability, drug activity site modification, destruction or inhibition and energetic rejection of antimicrobials by efflux pumps located on bacterial outer membrane (Table 2.3) (Morar & Wright 2010, Schwarz *et al.* 2001).

Table 2.3. Modes of action and resistance mechanisms of commonly used antibiotics in bacteria (Morar & Wright 2010)

Antibiotic class	Examples	Target	Mode(s) of resistance
β-Lactams	Penicillins (ampicillin), cephalosporins (cephamycin), penems (meropenem), monobactams (aztreonam)	Peptidoglycan biosynthesis	Hydrolysis, efflux, altered target
Aminoglycosides	Gentamycin, streptomycin, spectinomycin	Translation	Phosphorylation, acetylation, nucleotidylation, efflux, altered target
Tetracyclines	Minocycline, tigecycline	Translation	Monooxygenation, efflux, altered target
Macrolides	Erythromycin, azithromycin	Translation	Hydrolysis, glycosylation, Phosphorylation, efflux, altered target
Lincosamides	Clindamycin	Translation	Nucleotidylation, efflux, altered target
Phenicols	Chloramphenicol	Translation	Acetylation, efflux, altered target
Quinolones	Ciprofloxacin	DNA replication	Acetylation, efflux, altered target
Pyrimidines	Trimethoprim	C1 metabolism	efflux, altered target
Sulphonamides	Sulfamethoxazole	C1 metabolism	efflux, altered target
Cationic peptides	Colistin	Cell membrane	altered target, efflux

Molecular basis of antimicrobial resistance requires mostly the expression of antimicrobial resistance genes, cross-resistance between heavy metals / antiseptics and antimicrobial resistance.

2.2.3.2.1. Expression of antimicrobial resistance genes

There are more than 75 genes encoding resistance to β-lactams (ampicillin, amoxicillin-clavulanic acid, cephalothin, ceftazidime and cefotaxime etc..) through inactivation of the β-lactams ring. The most identified β-lactamases genes include *blaTEM-1, blaSHV-1, blaCMY-1* and *blaPSE-1* (Livermore 1995). *bla* stands for β-lactamase, *TEM* stands of the name of a patient (Temoniera)

from whom resistant bacteria were isolated while *SHV* refers to as sulfhydryl variable due to chemical properties of *blaSHV-1* (Matthew 1979). *CMY* stands for Cephamycins while *PSE* stands for Pseudomonas-specific enzyme (Matthew 1979, Matthew 1979). Resistance to chloramphenicol is mostly mediated by chloramphenicol acetyltransferases (*cat1* and *cmlA*), and chloramphenicol/ florfenicol associated resistance genes: *flo,* or *floR.* While *cat1* inactivates the drug (W. V. Shaw 1983), *cmlA* and *flo* are associated with energetic efflux pumps (Bissonnette *et al.* 1991). More than 29 *tet* genes have been associated with tetracycline resistance (Chopra & Roberts 2001). The most *tet* genes include *tetA, tetB, tetC,* and *tetG* whose mechanism of resistance consists of energy dependent efflux pumps (Roberts 1996). Aminoglycosides resistance is mainly associated with enzymatic inactivation by acetyltransferases (*aac*), adenylytransferases (*ant* or *aad*) and phosphotransferases *(aph)* (K. J. Shaw *et al.* 1993). There are more than 22 acetyltransferase (*aac*) genes (K. J. Shaw *et al.* 1993). The gene *ant (3')-Ia* have been associated with resistance to streptomycin and spectinomycin (Hollingshead & Vapnek 1985) while the gene *str* confers resistance to streptomycin (K. J. Shaw *et al.* 1993). Resistance to gentamycin has been mostly associated with *aadB* and *aac3'-IVa* genes while *aph* genes are mostly associated with resistance to kanamycin (K. J. Shaw *et al.* 1993). Resistance to quinolone and fluoroquinolone antimicrobials (ciprofloxacin, enrofloxacin, nalidixic acid etc…) can be multifactorial via one or a combination of the target-site (topoisomerase) modifications (Cavaco & Aarestrup 2009), production of multidrug-resistance (MDR) efflux pumps, or target protection by plasmid mediated – quinolones resistance (QMQR) such as *qnrA* gene (Tran, Jacoby & Hooper 2005). The *qnrA* gene produces a pentapeptide protein which binds to DNA – gyrase and topoisomerase IV to prevent the contact of the antimicrobial (ciprofloxacin, enrofloxacin, nalidixic acid etc…) with the target enzyme (Tran *et al.* 2005). Resistance to trimethoprim is usually associated with dihydrofolate reductase (Dhfr) enzyme, which catalyses the reduction of dihydrofolate to tetrahydrofolate in prokaryotic and eukaryotic cells (Huovinen *et al.* 1995). *Dhfr* genes are frequently located on mobile genetic elements such as plasmids, transposons,

cassettes and more than 15 *dhfrs* genes are recognized to confer resistance to trimethoprim in gram negative bacteria (Huovinen *et al.* 1995). Sulphonamides resistance genes (*sul1, sul2 and sul3*) produce dihydropteroic acid synthetase which binds to the target structure of the antimicrobial agent (Schwarz *et al.* 2001). These genes are located on mobile genetic elements such as plasmids, transposons, integrons and gene cassettes (Schwarz *et al.* 2001). The determination of antimicrobial resistance genes among *S.* Typhimurium isolates from poultry will explain the resistance mechanisms used by bacterium to adapt to different environments.

2.2.3.2.2. Cross-resistance between heavy metals and antimicrobial resistance

Metal contamination plays a big role in the co-selection and propagation of antibiotic resistance via co-resistance (existence of different resistance determinants on the same genetic element) and cross-resistance: the same genetic determinant responsible for resistance to antimicrobials and metals (Baker-Austin *et al.* 2006). Furthermore, mercury and organomercury operon which harbours *mer A, mer B, mer C, mer D, mer R* and *mer T* genes were found located on plasmids which often carry resistance determinants to antimicrobials and other metals such as arsenate, lead, cadmium and bismuth ions (Kondo, Ishikawa & Nakahara 1974). Mercury and organomercurial resistant bacteria were discovered from mercury-contaminated soil in Japan (Tezuka & Tonomura 1978). A strong relationship between antimicrobial resistance and resistance to metals has been reported in many enteric bacterial investigations including *Escherichia coli*, *Salmonella spp*, *Pseudomonas* (Schottel *et al.* 1974). A study of 787 *Pseudomonas aeruginosa* isolates showed that 99.8% were metal resistant with 75.1% resistant to mercury but only 53.2% of mercury resistant isolates were also resistant to antimicrobial agents (Furukawa, Suzuki & Tonomura 1969).

2.2.3.2.3. Cross-resistance between antiseptics and antimicrobial resistance

The first genetic determinant of resistance to antiseptics was quaternary ammonium compounds (*qac*) genes which mediate resistance to quaternary ammonium compounds (QACs), intercalating dyes (Acriflavine, ethidium), biguanidines (chlorhexidine), diamidines (propamidine, hexamidine), and guanylhydrazones (Jaglic & Cervinkova 2012). The gene *qacE* and its attenuated variant *qacEΔ1* are the most frequently spread in Gram - negative bacteria (Kucken, Feucht & Kaulfers 2000). The mechanism of resistance conferred by *qac* genes has been associated with energy - dependent efflux pump of outer membrane (Rouch *et al.* 1990). In Gram-negative bacteria, *qac* genes are plasmid-mediated class 1 integrons together with resistance genes such as *dfrA* for trimethoprim, *blaZ* for β-lactams, and *aacA-aphD* for aminoglycosides (Zhao *et al.* 2012). It has been also demonstrated that *qacC* confers resistance to a number of β-lactams (Fuentes *et al.* 2005). Furthermore, a non-specific cross resistance between antiseptics and antimicrobials has been reported and the mechanism responsible for this cross-resistance was associated with changes in the outer membrane (Russell 2000). In addition, n*or* genes encoding for fluoroquinolones resistance were also found to encode resistance to antiseptics and to share the same substrates with *qac* genes (DeMarco *et al.* 2007).

2.2.3.3. Role of plasmids in antimicrobial resistance

Plasmids play an important role in virulence (Fierer *et al.* 1992) and resistance genes dissemination (Carattoli 2003). Based on their function, three kinds of well-studied plasmids have been distinguished in *S. enterica* namely virulence plasmids, high molecular weight plasmids and low molecular weight plasmids (Rychlik *et al.* 2006). Resistance genes are mainly encoded on high molecular weight plasmids while the role played by low molecular weight plasmids in antimicrobial resistance is less known (Rychlik *et al.* 2006). High molecular weight plasmids which are frequently conjugative contribute to the dissemination of resistance genes in bacterial

populations via conjugation (Carattoli 2003). During bacterial conjugation which consists of direct cell to cell contact, the donor conjugative plasmid produces a pilus which connects the donor cell to the recipient cell. Nuclease breaks down the DNA of the conjugative plasmid and one strand of DNA is transferred to the recipient cell (Kaiser G and Suchman E 2013) (Figure 2.3). Plasmids have been found to harbour genes that encode resistance to beta-lactams and cephalosporins such as ampicillin and apramycin (Kruger *et al.* 2004). The following antimicrobial resistance-encoding genes have been identified on plasmids: resistance genes to beta-lactams (*blaTEM, blaCTX-M, blaSHV-12* and *blaCMY-2)*; streptomycin *(strA/B)*; gentamicin *(aad4),* chloramphenicol *(cat);* tetracycline's *(tet A/B/C),* sulphonamides *(sul 2)* and quinolones *(qnrA1)* resistance genes reviewed from (Schwarz *et al.* 2001).

2.2.3.4. Role of bacteriophages in antimicrobial resistance

Temperate prophages that are embedded in the chromosome of *S.* Typhimurium also play an important role in the transmission of antimicrobial resistance genes to other bacteria through transduction (Brabban, Hite & Callaway 2005, Schmieger & Schicklmaier 1999). In the presence of a stimulus, temperate prophages are released and infect surrounding bacteria (microflora) by incorporating their DNA which may contain antimicrobial resistance genes, into the host bacterial genome (Brabban *et al.* 2005). A recent study has shown that the exposure of *S.* Typhimurium to carbadox, an antimicrobial growth promoter used in dogs, induces prophages which transfer resistance genes to the host bacteria (Bearson *et al.* 2014).

2.2.3.5. Role of integrons and transposons in antimicrobial resistance

Other chromosome-encoded antimicrobial resistance genes are found in SGI which harbour integrons (Boyd *et al.* 2001). Integrons are genetic elements composed of integrase gene, a promoter and a primary recombination site (Cambray, Guerout & Mazel 2010, R. M. Hall & Collis 1995). Integrons capture environmental gene cassettes, incorporate them into specific

recombination site and convert them into functional genes that are expressed (Cambray *et al.* 2010, R. M. Hall & Collis 1995). Most of these gene cassettes carry antimicrobial resistance genes (Cambray *et al.* 2010, R. M. Hall & Collis 1995) (Figure 2.3). There are five classes of integrons in Enterobacteriaceae but only three are well documented (Cambray *et al.* 2010) and type 1 integrons was found in 97% in *S.* Typhimurium (Lindstedt *et al.* 2003). Some integrons are associated with transposons which are responsible for the transfer of resistance genes from one bacterium to the host bacterial chromosome (Silva C., Wiesner M., Calva E. 2012). Transposons are fragments of DNA which are movable within bacterial cells and between bacteria and are also mostly incorporated in conjugative plasmids (Gyles & Boerlin 2014). A typical example of SGI carrying integrons that have been shown to encode antimicrobial resistance is found in some *S.* Typhimurium Definitive Type 104 (DT 104) (Boyd *et al.* 2001). *Salmonella* Typhimurium DT 104 SGI harbours class 1 integron in its antimicrobial resistance cluster (Boyd *et al.* 2001). This multi-drug resistance (MDR) gene cluster is essential for ACSSuT resistance – type: ampicillin, chloramphenicol / florfenicol, streptomycin / spectinomycin, sulphonamides, and tetracycline (Boyd *et al.* 2001). The determination of integrons among *S.* Typhimurium isolates from poultry organs and poultry environments in South Africa will contribute to understanding the origin and dissemination of resistance genes in bacteria species in South Africa.

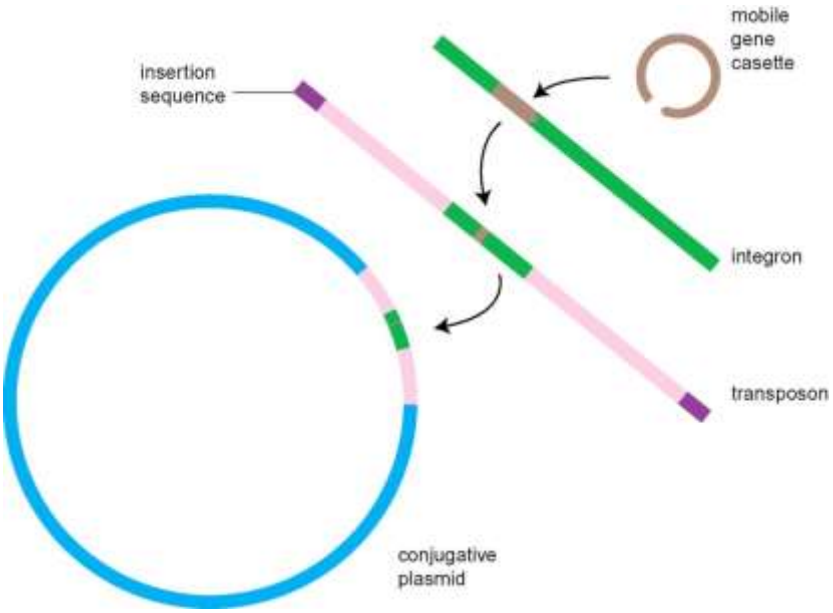


Figure 2.3. Role of mobile genetic elements in the propagation of antimicrobial resistance. Integrons capture and incorporate genes cassettes into their specific recombination site. Integrons are also mostly inserted into transposons which in turn are inserted into conjugative plasmids. Plasmids transfer resistance genes to other bacteria through conjugation (Norman, Hansen & Sorensen 2009).

2.2.3.6. *Salmonella* Typhimurium DT104 and antimicrobial resistance

Salmonella Typhimurium DT104 has multidrug properties and remains a public health concern (E. J. Threlfall 2000). Resistance genes that are harboured by DT104 include: *pse-1, flo/ floR, aadA2, sul1,* and *tetG* which respectively encode resistance to ampicillin, chloremphenicol, streptomycin, sulphonamides and tetracycline (ACSSuT) (Briggs & Fratamico 1999, Boyd *et al.* 2001). DT104 is known to be more virulent than other non-DT104 *Salmonella* as its infections are characterized by high hospitalization rates in humans (Helms *et al.* 2002). DT104 has been isolated from humans in different parts of the globe including the United Kingdom and different European countries (Mindlin *et al.* 2013), the USA (Cody *et al.* 1999), and Canada (Farzan *et al.* 2008). Between December 2000 and January 2001, two cases of DT104 associated illness

(diarrhoea, fever and anaemia) were detected at Monhica district hospital in Mozambique (Ruiz *et al.* 2008). These DT104 isolates were resistant to ampicillin, chloramphenicol and tetracycline; one DT104 isolate showed an intermediate resistance to amoxicillin- clavulanic acid (Ruiz *et al.* 2008). In 2007, a pentaresistant *S.* Typhimurium DT 104 (ACSSuT) which was also resistant to ciprofloxacin, nitrofurantoin and cephalotin, was isolated from humans in Ethiopia (Molla *et al.* 2007). In South Africa, previous studies have shown that *S.* Typhimurium isolates from humans displayed two resistance types: ACSSuNa (14%) and ACSSuTNa (13%) which is characteristic of D104 (Keddy *et al.* 2009). The main sources of human infections caused by multi-drug resistant DT104 include mainly the ingestion of poultry products (Wall *et al.* 1994) and the multidrug resistant DT104 was isolated from gulls, exotic birds and in poultry (Evans & Davies 1996). Therefore, the identification of DT104 isolates from poultry in South Africa will help to understand the sources of DT104 isolates and of antimicrobial resistance in human salmonellosis and their transmission routes.

2.2.3.7. Antimicrobial use in agriculture and antimicrobial resistance

Another major factor that might be playing an important role in the development of antimicrobial resistance worldwide is the wide use of antimicrobial growth promoters in Agriculture in some countries (Aarestrup *et al.* 1998). In South Africa, a large number of antibiotics are approved for therapy as well as for growth promotion in animals and the families of the most used antibiotics include the macrolides, pleuromutilines, tetracyclines, sulphonamides and penicillins (Eagar H.A. 2008). The main growth promoters used in South Africa include tetracyclines, ionophores, macrolides, lincosamides and pleuromotulins, quinoxalines, polypeptides, streptogramins, glycolipids, oligosaccharides phosphonic acids and polymeric compound (Eagar H.A. 2008).

2.2.3.8. Antimicrobial resistance of *Salmonella* isolates in South Africa

In South Africa, *Salmonella* isolates affecting poultry and humans have been found to be resistant to oxytetracycline, sulfamethoxazole - trimethoprim, ciprofloxacin and nalidixic acid, amoxicillin, amoxicillin - clavulanic acid, and ampicillin (β-lactams), chloramphenicol (phenicols), kanamycin, streptomycin and gentamicin (Keddy *et al.* 2009, Govender *et al.* 2009, Manie *et al.* 1998, Gouws & Brozel 2000). Antimicrobial resistance to fluoroquinolones and extended spectrum β-lactams that are alternatively used in human treatment is also becoming a very important public health concern (Keddy *et al.* 2009, Govender *et al.* 2009). For example, *S.* Typhimurium has been resistant to ciprofloxacin, and ampicillin (Govender *et al.* 2009). Resistance to chloramphenicol (Keddy *et al.* 2009) is also a concern as this antimicrobial has been found to be efficient in the treatment of human infections.

2.2.3.9. The South African Antimicrobial Resistance Strategy Framework

Interestingly, South Africa is currently aware of the situation of antimicrobial resistance and has adopted some strategies to tackle this public health concern. The bodies that have been set in response to the increasing antimicrobial resistance include: The National Veterinary Surveillance and Monitoring for Resistance to Antimicrobial Drugs (SANVAD) in 2003, The Global Antibiotic Resistance Partnership-South Africa (GARP-SA) in 2011, Federation of Infectious Diseases Societies of Southern Africa (FIDSSA) and South African Antimicrobial Stewardship Program (SAAP) which include practitioners in various domains (Moyane, Jideani & Aiyegoro 2013). The South African Antimicrobial Resistance Strategy Framework highlighted the main pillars as follows: enhance antimicrobial resistance surveillance, antimicrobial stewardship and improved infection prevention and control. The plans include: strengthening the existing systems and educating workforce and public, stimulating local research development into therapeutics, diagnostics and preventive measures. However, the sectors of animal productions and animal

health need a particular attention since the animals are considered reservoirs of several infectious diseases and salmonellosis in particular (Brink A. 2014).

2.3 References

1. AARESTRUP, F.M., BAGER, F., JENSEN, N.E., MADSEN, M., MEYLING, A. & WEGENER, H.C. 1998. Surveillance of antimicrobial resistance in bacteria isolated from food animals to antimicrobial growth promoters and related therapeutic agents in Denmark. *APMIS : Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, 106:606-622
2. AKINYEMI, K., O., PHILIPP, W., BEYER, W. & BOHM, R. 2010. Application of phage typing and pulsed-field gel electrophoresis to analyse *Salmonella enterica* isolates from a suspected outbreak in Lagos, Nigeria. *Journal of Infection in Developing Countries*, 4:828-833
3. ANDERSON, E.S., WARD, L.R., SAXE, M.J. & DE SA, J.D. 1977. Bacteriophage-typing designations of *Salmonella* Typhimurium. *The Journal of Hygiene*, 78:297-300
4. BAILEY, J.S. 1988. Integrated Colonization Control of *Salmonella* in Poultry. *Poultry science*, 67:928-932
5. BAKER-AUSTIN, C., WRIGHT, M.S., STEPANAUSKAS, R. & MCARTHUR, J.V. 2006. Co-selection of antibiotic and metal resistance. *Trends in microbiology*, 14:176-182
6. BALODA, S.B., CHRISTENSEN, L. & TRAJCEVSKA, S. 2001. Persistence of a *Salmonella enterica* serovar Typhimurium DT12 clone in a piggery and in agricultural soil amended with *Salmonella*-contaminated slurry. *Applied and Environmental Microbiology*, 67:2859-2862
7. BAUMLER, A.J., TSOLIS, R.M. & HEFFRON, F. 1996. Contribution of fimbrial operons to attachment to and invasion of epithelial cell lines by *Salmonella* Typhimurium. *Infection and Immunity*, 64:1862-1865
8. BEARSON, B.L., ALLEN, H.K., BRUNELLE, B.W., LEE, I.S., CASJENS, S.R. & STANTON, T.B. 2014. The agricultural antibiotic carbadox induces phage-mediated gene transfer in *Salmonella*. *Frontiers in Microbiology*, 5:52
9. BHAVSAR, A.P., BROWN, N.F., STOEPEL, J., WIERMER, M., MARTIN, D.D.O., HSU, K.J., IMAMI, K., ROSS, C.J., HAYDEN, M.R., FOSTER, L.J., LI, X., HIETER, P. & IMAMI, K. 2013. The *Salmonella* Type III Effector SspH2 Specifically Exploits the NLR Co-chaperone Activity of SGT1 to Subvert Immunity. *Plos Pathogens*, 9:e1003518
10. BISI-JOHNSON, M.A., OBI, C.L., VASAIKAR, S.D., BABA, K.A. & HATTORI, T. 2011. Molecular basis of virulence in clinical isolates of *Escherichia coli* and *Salmonella* species from a tertiary hospital in the Eastern Cape, South Africa. *Gut pathogens*, 3:9
11. BISSONNETTE, L., CHAMPETIER, S., BUISSON, J.P. & ROY, P.H. 1991. Characterization of the Nonenzymatic Chloramphenicol Resistance (*Cmla*) Gene of the In4 Integron of Tn1696 - Similarity of the Product to Transmembrane Transport Proteins. *Journal of Bacteriology*, 173:4493-4502
12. BOYD, D., PETERS, G.A., CLOECKAERT, A., BOUMEDINE, K.S., CHASLUS-DANCLA, E., IMBERECHTS, E. & MULVEY, M.R. 2001. Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella*

enterica serovar Typhimurium DT104 and its identification in phage type DT120 and serovar Agona*. Journal of Bacteriology*, 183:5725-5732

13. BRABBAN, A.D., HITE, E. & CALLAWAY, T.R. 2005. Evolution of foodborne pathogens via temperate bacteriophage-mediated gene transfer. *Foodborne Pathogens and Disease*, 2:287-303
14. BRIGGS, C.E. & FRATAMICO, P.M. 1999. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella* Typhimurium DT104*. Antimicrobial Agents and Chemotherapy*, 43:846-849
15. BRINK A. 2014. *The South African Antimicrobial Resistance Strategy Framework*, http://www.fidssa.co.za/Content/Documents/01_The_South_African_Antimicrobial_Resistance_Strategy_Framework_Adrian_Brink.pdf ed. Johannesburg, South Africa: Ampath National Laboratory Services, Milpark Hospital, Johannesburg
16. BRUSSOW, H., CANCHAYA, C. & HARDT, W. 2004. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiology and Molecular Biology Reviews*, 68:560-602tabeofontents
17. CALLOW, B.R. 1959. A new phage-typing scheme for *Salmonella* Typhimurium. *The Journal of Hygiene*, 57:346-359
18. CAMBRAY, G., GUEROUT, A.M. & MAZEL, D. 2010. Integrons. *Annual Review of Genetics*, 44:141-166
19. CARATTOLI, A. 2003. Plasmid-mediated antimicrobial resistance in *Salmonella enterica*. *Current Issues in Molecular Biology*, 5:113-122
20. CAVACO, L.M. & AARESTRUP, F.M. 2009. Evaluation of quinolones for use in detection of determinants of acquired quinolone resistance, including the new transmissible resistance mechanisms *qnrA, qnrB, qnrS*, and *aac(6')Ib-cr,* in *Escherichia coli* and *Salmonella enterica* and determinations of wild-type distributions. *Journal of Clinical Microbiology*, 47:2751-2758
21. CDC. 2011. *National Salmonella Surveillance Overview*, http://www.cdc.gov/ncezid/dfwed ed. Atlanta, Georgia: USA Department of Health and Human Services: CDC
22. CDC. 2013. *Multistate Outbreak of Human Salmonella Typhimurium Infections linked to live poultry in backyard flocks (final update).*, http://www.cdc.gov/salmonella/typhimurium-live-poultry-04-13 ed. Atlanta, Georgia: USA Department of Health and Human Services,: Centers for Disease Control and Prevention
23. CHOPRA, I. & ROBERTS, M. 2001. Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance*. Microbiology and Molecular Biology Reviews*, 65:232-+
24. CLARK, L., PERRETT, C.A., MALT, L., HARWARD, C., HUMPHREY, S., JEPSON, K.A., MARTINEZ-ARGUDO, I., CARNEY, L.J., LA RAGIONE, R.M., HUMPHREY, T.J. & JEPSON, M.A. 2011. Differences in *Salmonella enterica* serovar Typhimurium strain invasiveness are associated with heterogeneity in SPI-1 gene expression*. Microbiology-Sgm*, 157:2072-2083
25. CODY, S.H., ABBOTT, S.L., MARFIN, A.A., SCHULZ, B., WAGNER, P., ROBBINS, K., MOHLE-BOETANI, J.C. & VUGIA, D.J. 1999. Two outbreaks of multidrug-resistant

Salmonella serotype Typhimurium DT104 infections linked to raw-milk cheese in Northern California. *The Journal of the American Medical Association*, 281:1805-1810

26. CROWTHER-GIBSON, P., GOVENDER, N., LEWIS, D.A., BAMFORD, C., BRINK, A., VON GOTTBERG, A., KLUGMAN, K., DU PLESSIS, M., FALI, A., HARRIS, B., KEDDY, K. & BOTHA, M. 2011. Part IV. Human infections and antibiotic resistance. *South African Medical Journal = Suid-Afrikaanse Tydskrif vir Geneeskunde*, 101:567-578
27. CRUM-CIANFLONE, N.F. 2008. Salmonellosis and the gastrointestinal tract: More than just peanut butter. *Current Gastroenterology Reports*, 10:424-431
28. DANTAS, G., SOMMER, M.O.A., OLUWASEGUN, R.D. & CHURCH, G.M. 2008. Bacteria subsisting on antibiotics. *Science (New York, N.Y.)*, 320:100-103
29. DAVIES, J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. *Science (New York, N.Y.)*, 264:375-382
30. DAVIES, J. & DAVIES, D. 2010. Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews*, 74:417-433
31. DEGROOTE, M.A., OCHSNER, U.A., SHILOH, M.U., NATHAN, C., MCCORD, J.M., DINAUER, M.C., LIBBY, S.J., VAZQUEZTORRES, A., XU, Y.S. & FANG, F.C. 1997. Periplasmic superoxide dismutase protects *Salmonella* from products of phagocyte NADPH-oxidase and nitric oxide synthase. *Proceedings of the National Academy of Sciences of the United States of America*, 94:13997-14001
32. DEMARCO, C.E., CUSHING, L.A., FREMPONG-MANSO, E., SEO, S.M., JARAVAZA, T.A.A. & KAATZ, G.W. 2007. Efflux-related resistance to norfloxacin, dyes, and biocides in bloodstream isolates of *Staphylococcus aureus. Antimicrobial Agents and Chemotherapy*, 51:3235-3239
33. DRECKTRAH, D., LEVINE-WILKINSON, S., DAM, T., WINFREE, S., KNODLER, L.A., SCHROER, T.A. & STEELE-MORTIMER, O. 2008. Dynamic behavior of *Salmonella*-induced membrane tubules in epithelial cells. *Traffic (Copenhagen, Denmark)*, 9:2117-2129
34. EAGAR H.A. 2008. A Survey of Antimicrobial Usage in Animals in South Africa with Specific Reference to Food Animals. Master of Veterinary Medicine (Pharmacology) Thesis or Dissertation, Pretoria/ South Africa
35. EFSA. 2010. Panel on Biological Hazards (BIOHAZ). Scientific Opinion on monitoring and assessment of the public health risk of *Salmonella* Typhimurium-like strains. *EFSA Journal*, 8 (10):1826-48
36. EHRBAR, K. & HARDT, W. 2005. Bacteriophage-encoded type III effectors in *Salmonella enterica* subspecies 1 serovar Typhimurium. *Infection, Genetics and Evolution : Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*, 5:1-9
37. EKDAHL, K., DE JONG, B., WOLLIN, R. & ANDERSSON, Y. 2005. Travel-associated non-typhoidal salmonellosis: geographical and seasonal differences and serotype distribution. *Clinical Microbiology and Infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, 11:138-144
38. EL-GEDAILY, A., PAESOLD, G. & KRAUSE, M. 1997. Expression profile and subcellular location of the plasmid-encoded virulence (Spv) proteins in wild-type *Salmonella* Dublin. *Infection and Immunity*, 65:3406-3411

39. EVANS, S. & DAVIES, R. 1996. Case control study of multiple-resistant *Salmonella* Typhimurium DT104 infection of cattle in Great Britain. *The Veterinary Record*, 139:557-558
40. EWING, W.H. 1972. The nomenclature of *Salmonella*, its usage, and definitions for the three species. *Canadian Journal of Microbiology*, 18:1629-1637
41. FÀBREGA, A. & VILA, J. 2013. *Salmonella enterica* serovar Typhimurium skills to succeed in the host: Virulence and regulation. *Clinical Microbiology Reviews*, 26:308-341
42. FARZAN, A., FRIENDSHIP, R.M., POPPE, C., MARTIN, L., DEWEY, C.E. & FUNK, J. 2008. Molecular epidemiology and antimicrobial resistance of *Salmonella* Typhimurium DTI04 on Ontario swine farms. *Canadian Journal of Veterinary Research = Revue Canadienne de Recherche Veterinaire*, 72:188-194
43. FEARNLEY, E., RAUPACH, J., LAGALA, F. & CAMERON, S. 2011. *Salmonella* in chicken meat, eggs and humans; Adelaide, South Australia, 2008. *International Journal of Food Microbiology*, 146:219-227
44. FEASEY, N.A., ARCHER, B.N., HEYDERMAN, R.S., SOOKA, A., DENNIS, B., GORDON, M.A. & KEDDY, K.H. 2010. Typhoid fever and invasive nontyphoid salmonellosis, Malawi and South Africa. *Emerging Infectious Diseases*, 16:1448-1451
45. FIERER, J., KRAUSE, M., TAUXE, R. & GUINEY, D. 1992. *Salmonella* Typhimurium Bacteremia - Association with the Virulence Plasmid. *Journal of Infectious Diseases*, 166:639-642
46. FIGUEROA-BOSSI, N. & BOSSI, L. 1999. Inducible prophages contribute to *Salmonella* virulence in mice. *Molecular Microbiology*, 33:167-76
47. FIGUEROA-BOSSI, N., UZZAU, S., MALORIOL, D. & BOSSI, L. 2001. Variable assortment of prophages provides a transferable repertoire of pathogenic determinants in *Salmonella*. *Molecular Microbiology*, 39:260-71
48. FRIEBEL, A., ILCHMANN, H., AELPFELBACHER, M., EHRBAR, K., MACHLEIDT, W. & HARDT, W.D. 2001. SopE and SopE2 from *Salmonella* Typhimurium activate different sets of RhoGTPases of the host cell. *Journal of Biological Chemistry*, 276:34035-34040
49. FUENTES, D.E., NAVARRO, C.A., TANTALEAN, J.C., ARAYA, M.A., SAAVEDRA, C.P., PEREZ, J.M., CALDERON, I.L., YOUDERIAN, P.A., MORA, G.C. & VASQUEZ, C.C. 2005. The product of the *qacC* gene of *Staphylococcus epidermidis* CH mediates resistance to beta-lactam antibiotics in Gram-positive and Gram-negative bacteria. *Research in microbiology*, 156:472-477
50. FURUKAWA, K., SUZUKI, T. & TONOMURA, K. 1969. Decomposition of Organic Mercurial Compounds by Mercury-Resistant Bacteria. *Agricultural and Biological Chemistry*, 33:128-&
51. GALAN, J.E. 2009. Common themes in the design and function of bacterial effectors. *Cell Host & Microbe*, 5:571-579
52. GERNER-SMIDT, P., HISE, K., KINCAID, J., HUNTER, S., ROLANDO, S., HYYTIA-TREES, E., RIBOT, E.M., SWAMINATHAN, B. & PULSENET TASKFORCE. 2006. PulseNet USA: a five-year update. *Foodborne Pathogens and Disease*, 3:9-19
53. GOUWS, P.A. & BROZEL, V.S. 2000. Antimicrobial resistance of *Salmonella* isolates associated with retail chicken and a poultry abattoir. *South African Journal of Science*, 96:254-256

54. GOVENDER, N., SMITH, A.M., KARSTAEDT, A.S., KEDDY, K.H. & GROUP FOR ENTERIC, RESPIRATORY AND MENINGEAL DISEASE SURVEILLANCE IN SOURTH AFRICA (GERMS-SA). 2009. Plasmid-mediated quinolone resistance in *Salmonella* from South Africa. *Journal of Medical Microbiology*, 58:1393-1394
55. GUINEY, D.G. & FIERER, J. 2011. The role of the *spv* genes in *Salmonella* pathogenesis. *Frontiers in Microbiology*, 2:129
56. GULIG, P.A., DANBARA, H., GUINEY, D.G., LAX, A.J., NOREL, F. & RHEN, M. 1993. Molecular Analysis of Spv Virulence Genes of the *Salmonella* Virulence Plasmids. *Molecular Microbiology*, 7:825-830
57. GYLES, C. & BOERLIN, P. 2014. Horizontally transferred genetic elements and their role in pathogenesis of bacterial disease. *Veterinary Pathology*, 51:328-340
58. HALL, B.G. & BARLOW, M. 2003. Structure-based phylogenies of the serine beta-lactamases. *Journal of Molecular Evolution*, 57:255-260
59. HALL, R.M. & COLLIS, C.M. 1995. Mobile gene cassettes and integrons: Capture and spread of genes by site-specific recombination. *Molecular Microbiology*, 15:593-600
60. HARAGA, A. & MILLER, S.I. 2003. A *Salmonella enterica* serovar Typhimurium translocated leucine-rich repeat effector protein inhibits NF-kappa B-dependent gene expression. *Infection and Immunity*, 71:4052-4058
61. HELMS, M., VASTRUP, P., GERNER-SMIDT, P. & MOLBAK, K. 2002. Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. *Emerging Infectious Diseases*, 8:490-495
62. HELMUTH, R., STEPHAN, R., BUNGE, C., HOOG, B., STEINBECK, A. & BULLING, E. 1985. Epidemiology of virulence-associated plasmids and outer membrane protein patterns within seven common *Salmonella* serotypes. *Infection and Immunity*, 48:175-182
63. HENDRIKSEN, R.S., VIEIRA, A.R., KARLSMOSE, S., LO FO WONG, D.M.A., JENSEN, A.B., WEGENER, H.C. & AARESTRUP, F.M. 2011. Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. *Foodborne Pathogens and Disease*, 8:887-900
64. HOLLINGSHEAD, S. & VAPNEK, D. 1985. Nucleotide-Sequence Analysis of a Gene Encoding a Streptomycin Spectinomycin Adenyltransferase. *Plasmid*, 13:17-30
65. HUOVINEN, P., SUNDSTROM, L., SWEDBERG, G. & SKOLD, O. 1995. Trimethoprim and Sulfonamide Resistance. *Antimicrobial Agents and Chemotherapy*, 39:279-289
66. HUOY, L., PORNRUANGWONG, S., PULSRIKARN, C. & CHATURONGAKUL, S. 2014. Molecular characterization of Thai *Salmonella enterica* serotype Typhimurium and serotype 4,5,12:i:- reveals distinct genetic deletion patterns. *Foodborne Pathogens and Disease*, 11:589-592
67. ISSENHUTH-JEANJEAN, S., ROGGENTIN, P., MIKOLEIT, M., GUIBOURDENCHE, M., DE PINNA, E., NAIR, S., FIELDS, P.I. & WEILL, F. 2014. Supplement 2008-2010 (no. 48) to the White-Kauffmann-Le Minor scheme. *Research in Microbiology*, 165:526-530
68. JAGLIC, Z. & CERVINKOVA, D. 2012. Genetic basis of resistance to quaternary ammonium compounds - the *qac* genes and their role: a review. *Veterinarni medicina*, 57:275-281

69. JENSEN, A.N., DALSGAARD, A., STOCKMARR, A., NIELSEN, E.M. & BAGGESEN, D.L. 2006. Survival and transmission of *Salmonella enterica* serovar Typhimurium in an outdoor organic pig farming environment. *Applied and Environmental Microbiology*, 72:1833-1842
70. JONES, B.A., GRACE, D., KOCK, R., ALONSO, S., RUSHTON, J., SAID, M.Y., MCKEEVER, D., MUTUA, F., YOUNG, J., MCDERMOTT, J. & PFEIFFER, D.U. 2013. Zoonosis emergence linked to agricultural intensification and environmental change. *Proceedings of the National Academy of Sciences of the United States of America*, 110:8399-8404
71. JONES, F., AXTELL, R.C., TARVER, F.R., RIVES, D.V., SCHEIDELER, S.E. & WINELAND, M.J. 1991. Environmental Factors Contributing to *Salmonella* Colonization of Chickens, in *Colonization Control of Human Bacterial Enteropathologens in Poultry*, edited by L.C. Blankenship, : Academic Press: 3-21
72. KAGAMBEGA, A., LIENEMANN, T., AULU, L., TRAORE, A.S., BARRO, N., SIITONEN, A. & HAUKKA, K. 2013. Prevalence and characterization of *Salmonella enterica* from the faeces of cattle, poultry, swine and hedgehogs in Burkina Faso and their comparison to human *Salmonella* isolates. *BMC Microbiology*, 13:(11 November 2013)-(11 November 2013)
73. KAISER G AND SUCHMAN E. 2013. *Transfer of conjugative plasmids and mobilizable plasmids in Gram-Negative Bacteria.*, www.microbelibrary.org/.../bacteria/3810 ed
74. KARIUKI, S., REVATHI, G., KARIUKI, N., KIIRU, J., MWITURIA, J., MUYODI, J., GITHINJI, J.W., KAGENDO, D., MUNYALO, A. & HART, C.A. 2006. Invasive multidrug-resistant non-typhoidal *Salmonella* infections in Africa: zoonotic or anthroponotic transmission? *Journal of Medical Microbiology*, 55:585-591
75. KEDDY, K.H., DWARIKA, S., CROWTHER, P., PEROVIC, O., WADULA, J., HOOSEN, A., SOOKA, A., CREWE-BROWN, H.H. & SMITH, A.M. 2009. Genotypic and demographic characterization of invasive isolates of *Salmonella* Typhimurium in HIV co-infected patients in South Africa. *Journal of infection in Developing Countries*, 3:585-592
76. KIDANEMARIAM, A., ENGELBRECHT, M. & PICARD, J. 2010. Retrospective study on the incidence of *Salmonella* isolations in animals in South Africa, 1996 to 2006. *Journal of the South African Veterinary Association*, 81:37-44
77. KNODLER, L.A., CELLI, J., HARDT, W., VALLANCE, B.A., YIP, C. & FINLAY, B.B. 2002. *Salmonella* effectors within a single pathogenicity island are differentially expressed and translocated by separate type III secretion systems. *Molecular Microbiology*, 43:1089-1103
78. KOHLER, A.C., SPANÒ, S., GALÁN, J.E. & STEBBINS, C.E. 2014. Structural and enzymatic characterization of a host-specificity determinant from *Salmonella. Acta Crystallographica Section D: Biological Crystallography*, 70:384-391
79. KONDO, I., ISHIKAWA, T. & NAKAHARA, H. 1974. Mercury and Cadmium Resistances Mediated by Penicillinase Plasmid in *Staphylococcus Aureus. Journal of Bacteriology*, 117:1-7
80. KRUGER, T., SZABO, D., KEDDY, K.H., DEELEY, K., MARSH, J.W., HUJER, A.M., BONOMO, R.A. & PATERSON, D.L. 2004. 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

81. KUCKEN, D., FEUCHT, H.H. & KAULFERS, P.M. 2000. Association of *qacE* and *qacE* Delta 1 with multiple resistance to antibiotics and antiseptics in clinical isolates of Gram-negative bacteria. *FEMS microbiology letters*, 183:95-98
82. LEEKITCHAROENPHON, P., FRIIS, C., ZANKARI, E., SVENDSEN, C.A., PRICE, L.B., RAHMANI, M., HERRERO-FRESNO, A., FASHAE, K., VANDENBERG, O., AARESTRUP, F.M. & HENDRIKSEN, R.S. 2013. Genomics of an emerging clone of *Salmonella* serovar Typhimurium ST313 from Nigeria and the Democratic Republic of Congo. *Journal of Infection in Developing Countries*, 7:696-706
83. LEVIN, B.R., LIPSITCH, M. & BONHOEFFER, S. 1999. Population biology, evolution, and infectious disease: convergence and synthesis. *Science (New York, N.Y.)*, 283:806-809
84. LINDSTEDT, B., HEIR, E., NYGARD, I. & KAPPERUD, G. 2003. Characterization of class I integrons in clinical strains of *Salmonella enterica* subsp. enterica serovars Typhimurium and Enteritidis from Norwegian hospitals. *Journal of Medical Microbiology*, 52:141-149
85. LIU, B. & POP, M. 2009. ARDB--Antibiotic Resistance Genes Database. *Nucleic Acids Research*, 37:D443-7
86. LIVERMORE, D.M. 1995. Beta-Lactamases in Laboratory and Clinical Resistance. *Clinical Microbiology Reviews*, 8:557-&
87. LOPEZ, F.E., DE LAS MERCEDES PESCARETTI, M., MORERO, R. & DELGADO, M.A. 2012. *Salmonella* Typhimurium general virulence factors: A battle of David against Goliath?. *Food Research International*, 45:842-851
88. LÓPEZ, F.E., DE LAS MERCEDES PESCARETTI, M., MORERO, R. & DELGADO, M.A. 2012. *Salmonella* Typhimurium general virulence factors: A battle of David against Goliath?. *Food Research International*, 45:842-851
89. LY, K.T. & CASANOVA, J.E. 2007. Mechanisms of *Salmonella* entry into host cells. *Cellular Microbiology*, 9:2103-2111
90. MAJOWICZ, S.E., MUSTO, J., SCALLAN, E., ANGULO, F.J., KIRK, M., O'BRIEN, S.J., JONES, T.F., FAZIL, A., HOEKSTRA, R.M. & INTERNATIONAL COLLABORATION ON ENTERIC DISEASE 'BURDEN OF ILLNESS' STUDIES. 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 50:882-889
91. MANIE, T., KHAN, S., BROZEL, V.S., VEITH, W.J. & GOUWS, P.A. 1998. Antimicrobial resistance of bacteria isolated from slaughtered and retail chickens in South Africa. *Letters in Applied Microbiology*, 26:253-258
92. MATTHEW, M. 1979. Plasmid-Mediated Beta-Lactamases of Gram-Negative Bacteria - Properties and Distribution. *Journal of Antimicrobial Chemotherapy*, 5:349-358
93. METTEE ZARECKI, S.L., BENNETT, S.D., HALL, J., YAEGER, J., LUJAN, K., ADAMS-CAMERON, M., WINPISINGER QUINN, K., BRENDEN, R., BIGGERSTAFF, G., HILL, V.R., SHOLTES, K., GARRETT, N.M., LAFON, P.C., BARTON BEHRAVESH, C., SODHA, S.V. & SALMONELLA TYPHIMURIUM OUTBREAK INVESTIGATION TEAM. 2013. US outbreak of human *Salmonella* infections associated with aquatic frogs, 2008-2011. *Pediatrics*, 131:724-731

94. MIAO, E.A., BRITTNACHER, M., HARAGA, A., JENG, R.L., WELCH, M.D. & MILLER, S.I. 2003. *Salmonella* effectors translocated across the vacuolar membrane interact with the actin cytoskeleton. *Molecular Microbiology*, 48:401-415
95. MIAO, E.A. & MILLER, S.I. 1999. Bacteriophages in the evolution of pathogen-host interactions. *Proceedings of the National Academy of Sciences of the United States of America*, 96:9452-4
96. MINDLIN, M.J., LANG, N., MAGUIRE, H., WALSH, B., VERLANDER, N.Q., LANE, C., TAYLOR, C., BISHOP, L.A. & CROOK, P.D. 2013. Outbreak investigation and case-control study: penta-resistant *Salmonella* Typhimurium DT104 associated with biltong in London in 2008. *Epidemiology and Infection*, 141:1920-1927
97. MOLLA, B., MIKO, A., PRIES, K., HILDEBRANDT, G., KLEER, J., SCHROETER, A. & HELMUTH, R. 2007. Class 1 integrons and resistance gene cassettes among multidrug resistant *Salmonella* serovars isolated from slaughter animals and foods of animal origin in Ethiopia. *Acta Tropica*, 103:142-149
98. MORAR, M. & WRIGHT, G.D. 2010. The genomic enzymology of antibiotic resistance. *Annual Review of Genetics*, 44:25-51
99. MORPETH, S.C., RAMADHANI, H.O. & CRUMP, J.A. 2009. Invasive non-Typhi *Salmonella* disease in Africa. *Clinical Infectious Diseases : an official publication of the Infectious Diseases Society of America*, 49:606-611
100. MOYANE, J.N., JIDEANI, A.I.O. & AIYEGORO, O.A. 2013. Antibiotics usage in food-producing animals in South Africa and impact on human: antibiotic resistance. *African Journal of Microbiology Research*, 7:2990-2997
101. NORMAN, A., HANSEN, L.H. & SORENSEN, S.J. 2009. Conjugative plasmids: vessels of the communal gene pool. *Philosophical Transactions of the Royal Society of London.Series B, Biological Sciences*, 364:2275-2289
102. OBI, C.L., POTGIETER, A., MUSIE, E.M., BESSONG P.O., SAMIE A. & VENTER S.N. 2007. *Human and environmental associated Salmonella isolates from different sources in the Venda region of South Africa.*, Cap Town / South Africa ed. Cap Town: Water SA
103. OLSEN, S.J., BISHOP, R., BRENNER, F.W., ROELS, T.H., BEAN, N., TAUXE, R.V. & SLUTSKER, L. 2001. The changing Epidemiology of *Salmonella*: trends in serotypes isolated from humans in the United States, 1987-1997. *The Journal of Infectious Diseases*, 183:753-761
104. OPITZ, H.M. 1992. Progress being made in *Salmonella* Enteritidis reduction on the farm. *Poultry Digest*, 51:16...22-16...22
105. OZFOODNET WORKING GROUP. 2010. Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: annual report of the OzFoodNet Network, 2009. *Communicable Diseases Intelligence Quarterly Report*, 34:396-426
106. PADUNGTOD, P. & KANEENE, J.B. 2006. *Salmonella* in food animals and humans in northern Thailand. *International Journal of Food Microbiology*, 108:346-354
107. PAGOTTO, F., CORNEAU, N., SCHERF, C., LEOPOLD, P., CLARK, C. & FARBER, J.M. 2005. Molecular typing and differentiation of foodborne bacterial pathogens., in ***Foodborne Pathogens: Microbiology and Molecular Biology***, edited by Fratamico, P. M. Bhunia, A. K. Smith,J.L., U K: Caister Academic Press: 75

108. POPOFF, M.Y., BOCKEMUHL, J. & GHEESLING, L.L. 2003. Supplement 2001 (no. 45) to the Kauffmann-White scheme. *Research in Microbiology*, 154:173-174
109. RABSCH, W., ANDREWS, H.L., KINGSLEY, R.A., PRAGER, R., TSCHAPE, H., ADAMS, L.G. & BAUMLER, A.J. 2002. *Salmonella* enterica serotype Typhimurium and its host-adapted variants. *Infection and Immunity*, 70:2249-2255
110. RILEY L.W. 2004. *Molecular Epidemiology of Infectious Diseases. Principles and Practices.* . Washington D.C: ASM Press
111. ROBERTS, M.C. 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS microbiology reviews*, 19:1-24
112. ROUCH, D.A., CRAM, D.S., DIBERARDINO, D., LITTLEJOHN, T.G. & SKURRAY, R.A. 1990. Efflux-Mediated Antiseptic Resistance Gene *Qaca* from *Staphylococcus Aureus* - Common Ancestry with Tetracycline-Transport and Sugar-Transport Proteins*. Molecular microbiology*, 4:2051-2062
113. RUIZ, J., HERRERA-LEON, S., MANDOMANDO, I., MACETE, E., PUYOL, L., ECHEITA, A. & ALONSO, P.L. 2008. Detection of *Salmonella enterica* serotype Typhimurium DT104 in Mozambique. *The American Journal of Tropical Medicine and Hygiene*, 79:918-920
114. RUSSELL, A.D. 2000. Do biocides select for antibiotic resistance?*. Journal of Pharmacy and Pharmacology*, 52:227-233
115. RYCHLIK, I., GREGOROVA, D. & HRADECKA, H. 2006. Distribution and function of plasmids in *Salmonella enterica. Veterinary Microbiology*, 112:1-10
116. SABBAGH, S.C., FOREST, C.G., LEPAGE, C., LECLERC, J. & DAIGLE, F. 2010. So similar, yet so different: uncovering distinctive features in the genomes of *Salmonella enterica* serovars Typhimurium and Typhi. *FEMS Microbiology Letters*, 305:1-13
117. SANDT, C.H., FEDORKA-CRAY, P.J., TEWARI, D., OSTROFF, S., JOYCE, K. & M'IKANATHA, N.M. 2013. A comparison of non-typhoidal *Salmonella* from humans and food animals using pulsed-field gel electrophoresis and antimicrobial susceptibility patterns. *PLoS ONE*, 8:e77836-e77836
118. SCHMIDT, H. & HENSEL, M. 2004. Pathogenicity islands in bacterial pathogenesis. *Clinical Microbiology Reviews*, 17:14-56
119. SCHMIEGER, H. & SCHICKLMAIER, P. 1999. Transduction of multiple drug resistance of *Salmonella enterica* serovar Typhimurium DT104. *FEMS Microbiology Letters*, 170:251-256
120. SCHOTTEL, J., MANDAL, A., CLARK, D. & SILVER, S. 1974. Volatilization of Mercury and Organomercurials Determined by Inducible R-Factor Systems in Enteric Bacteria*. Nature*, 251:335-337
121. SCHWARZ, S., KEHRENBERG, C. & WALSH, T.R. 2001. Use of antimicrobial agents in veterinary medicine and food animal production. *International journal of antimicrobial agents*, 17:431-437
122. SHAW, K.J., RATHER, P.N., HARE, R.S. & MILLER, G.H. 1993. Molecular-Genetics of Aminoglycoside Resistance Genes and Familial Relationships of the Aminoglycoside-Modifying Enzymes*. Microbiological Reviews*, 57:138-163
123. SHAW, W.V. 1983. Chloramphenicol Acetyltransferase - Enzymology and Molecular-Biology*. CRC critical reviews in biochemistry*, 14:1-46

124. SILVA C., WIESNER M., CALVA E. 2012. The Importance of Mobile Genetic Elements in the Evolution of *Salmonella*: Pathogenesis, Antibiotic Resistance and Host Adaptation., in *Salmonella - A Diversified Superbug*, edited by Kumar Y., available from http://www.intechopen.com/books: InTech: 231-254
125. SMITH, A.M., ISMAIL, H., HENTON, M.M., KEDDY, K.H. & GERMS-SA SURVEILLANCE NETWORK. 2014. Similarities between *Salmonella* Enteritidis isolated from humans and captive wild animals in South Africa. *Journal of infection in Developing Countries*, 8:1615-9
126. SPA. 2015a. *Broiler industry production report: May 2015*, http://www.sapoultry.co.za/pdf%20annualreports/EO%20REPORT.pdf ed: South African Poultry Association.
127. SPA. 2015b. *Egg industry report for July 2015.*, http://www.sapoultry.co.za/pdf%annual report ed: South African Poultry Association.
128. STANLEY, T.L., ELLERMEIER, C.D. & SLAUCH, J.M. 2000. Tissue-specific gene expression identifies a gene in the lysogenic phage Gifsy-1 that affects *Salmonella enterica* serovar Typhimurium survival in Peyer's patches. *Journal of Bacteriology*, 182:4406-13
129. STATISTICS SOUTH AFRICA. 2014. *Mid-year population estimates 2014.*, http://beta2.statssa.gov.za/publications/P0302/P03022013.pdf ed: Statistics South Africa
130. SWAMINATHAN, B., BARRETT, T.J., HUNTER, S.B., TAUXE, R.V. & CDC PULSENET TASK FORCE. 2001. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. *Emerging Infectious diseases*, 7:382-9
131. TEZUKA, T. & TONOMURA, K. 1978. Purification and Properties of a Second Enzyme Catalyzing Splitting of Carbon-Mercury Linkages from Mercury-Resistant Pseudomonas K-62. *Journal of Bacteriology*, 135:138-143
132. THORNTON, P.K. 2010. Livestock production: recent trends, future prospects. *Philosophical transactions of the Royal Society of London.Series B, Biological sciences*, 365:2853-2867
133. THRELFALL, E.J. 2000. Epidemic *Salmonella* Typhimurium DT 104--a truly international multiresistant clone. *The Journal of Antimicrobial Chemotherapy*, 46:7-10
134. THRELFALL, E., WARD, L. & ROWE, B. 1998. Multiresistant *Salmonella* Typhimurium DT 104 and *Salmonella* bacteraemia. *The Lancet*, 352:287-288
135. TINDALL, B.J., GRIMONT, P.A.D., GARRITY, G.M. & EUZEBY, J.P. 2005. Nomenclature and taxonomy of the genus *Salmonella*. *International Journal of Systematic and Evolutionary Microbiology*, 55:521-524
136. TRAN, J.H., JACOBY, G.A. & HOOPER, D.C. 2005. Interaction of the plasmid-encoded quinolone resistance protein Qnr with *Escherichia coli* DNA gyrase. *Antimicrobial Agents and Chemotherapy*, 49:118-125
137. VAN NIEROP, W., DUSE, A.G., MARAIS, E., AITHMA, N., THOTHOBOLO, N., KASSEL, M., STEWART, R., POTGIETER, A., FERNANDES, B., GALPIN, J.S. & BLOOMFIELD, S.F. 2005. Contamination of chicken carcasses in Gauteng, South Africa, by *Salmonella*, *Listeria monocytogenes* and *Campylobacter*. *International Journal of Food Microbiology*, 99:1-6

138. VONAESCH, P., SELLIN, M.E., CARDINI, S., SINGH, V., BARTHEL, M. & HARDT, W.-. 2014. The *Salmonella*Typhimurium effector protein SopE transiently localizes to the early SCV and contributes to intracellular replication*. Cellular Microbiology*, 16:1723-1735

139. WALL, P.G., MORGAN, D., LAMDEN, K., RYAN, M., GRIFFIN, M., THRELFALL, E.J., WARD, L.R. & ROWE, B. 1994. A case control study of infection with an epidemic strain of multi-resistant *Salmonella* Typhimurium DT104 in England and Wales. *Communicable Disease Report Review*, 4:R130-5

140. WANG, Y., ZHANG, L., PICKING, W.L., PICKING, W.D. & DE GUZMAN, R.N. 2008. Structural dissection of the extracellular moieties of the type III secretion apparatus. *Molecular bioSystems*, 4:1176-1180

141. WRIGHT, G.D. 2007. The antibiotic resistome: the nexus of chemical and genetic diversity. *Nature Reviews Microbiology*, 5:175-186

142. ZHANG, S., SANTOS, R.L., TSOLIS, R.M., STENDER, S., HARDT, W., BAUMLER, A.J. & ADAMS, L.G. 2002. The *Salmonella enterica* serotype Typhimurium effector proteins SipA, SopA, SopB, SopD, and SopE2 act in concert to induce diarrhoea in calves. *Infection and Immunity*, 70:3843-3855

143. ZHAO, W., CHEN, G., ITO, R., KIMURA, S. & HU, Z. 2012. Identification of a plasmid-borne *bla(IMP-11)* gene in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae. Journal of medical microbiology*, 61:246-251

CHAPTER 3. VIRULENCE CHARACTERIZATION AND MOLECULAR SUBTYPING OF *SALMONELLA* TYPHIMURIUM ISOLATED BETWEEN 1995 AND 2002 FROM ORGANS AND ENVIRONMENTS OF DISEASED POULTRY IN SOUTH AFRICA.

3.1. Introduction

Salmonella Typhimurium is a non - typhoidal *Salmonella* (NTS) serovar that has been associated with salmonellosis in humans and a wide range of animals' worldwide (Hendriksen *et al.* 2011). *Salmonella* Typhimurium infections are mostly characterized by self-limiting gastroenteritis (Crum-Cianflone 2008) and in some instances complications including bacteraemia (Threlfall, Ward & Rowe 1998), meningitis (Van Sorge *et al.* 2011), osteomyelitis (Schulze *et al.* 2009) and chronic reactive arthritis (Lee, Hall & Pile 2005) especially in immune deficient patients. *Salmonella* Typhimurium illnesses are accompanied with economic losses which are mostly associated with treatment and death of patients (Ailes *et al.* 2013).

Animals are considered reservoirs while humans are mostly infected by ingesting undercooked poultry products including meat, eggs (Fearnley *et al.* 2011) and contaminated water (Obi *et al.* 2007). Twenty six per cent (26.6%) of 182 *Salmonella* serotypes collected between 1996 and 2006 from animals in all provinces of South Africa were *S.* Typhimurium, and 70.5 % of these were from poultry and various birds (Kidanemariam, Engelbrecht & Picard 2010). Between 2006 and 2007, *S.* Typhimurium constituted 61% of *Salmonella* isolates which were recovered mainly from HIV-positive patients in the Gauteng province, South Africa (Keddy *et al.* 2009). Furthermore, 25% of the 317 human *Salmonella* isolates from Eastern Cape Province (South Africa) were also identified as *S.* Typhimurium (Bisi-Johnson *et al.* 2011).

Infections caused by *S.* Typhimurium vary depending on the pathogen's virulence factors and the host immune system (Foley *et al.* 2013). A number of virulence factors and markers that

have been shown to play a role in *Salmonella* pathogenesis are carried on *Salmonella* pathogenicity islands (SPIs) (Schmidt & Hensel 2004), bacteriophages (Brussow, Canchaya & Hardt 2004) and plasmids (Gulig *et al.* 1993). For example, SPI1-encoded genes: invasion gene (*invA*), and *Salmonella* outer protein (*sopB*) alter the host cell recognition mechanisms resulting in host actin cytoskeleton rearrangements and membrane ruffling which favours effector proteins to penetrate into host cells (Ly & Casanova 2007). SPI-2-encoded gene, *Salmonella*-induced filament (*sifA*), maintains the integrity of the phagosome (Stein *et al.* 1996). SifA is required for phagosome tubulation which promotes bacterial replication (Stein *et al.* 1996) and contributes to intestinal disease and inflammation (Waterman & Holden 2003). Other virulence genes encoding effector proteins are carried on temperate bacteriophages (Brussow *et al.* 2004). The Gisfy-2 bacteriophage harbours genes (*SodC-1, GtgE, GtgB*) encoding respectively effector proteins SodC-1, GtgE, GtgB (Ehrbar & Hardt 2005, Ho *et al.* 2002). The GtgB protein is responsible for the polymerization of the host actin cytoskeleton resulting in the introduction of effector proteins into the host cytoplasm (Miao *et al.* 2003). The SodC-1 protein prevents the formation of toxic peroxinitrite through hydrolysis of peroxide produced by macrophages subsequent to intra-macrophage survival (De Groote *et al.* 1997). The *gtgE* gene encodes a protein which inhibits the entry of the host antimicrobial factors such as cytokines and chemokine's into *Salmonella*-containing vacuoles (SCV) (Kohler *et al.* 2014). The *sopE* gene is carried by the SopE ø phage and contributes to host cell membrane ruffling and invasion of intestinal cells (Mirold *et al.* 1999). A Gifsy-3 bacteriophage-encoded effector, "*Salmonella* secreted protein" (SspH1), is also involved in invasion by down-modulating Interleukin (IL)-8 production (Haraga & Miller 2003). GipA is essential for bacterial growth and survival in Peyer's patches (Stanley, Ellermeier & Slauch 2000) while SspH2 modulates innate immunity (Bhavsar *et al.* 2013).

Various genes encoding *Salmonella* Typhimurium effector proteins including *spvC, pefA, rck, srgA* and *mig5* are encoded on plasmids (Gulig *et al.* 1993, Guiney & Fierer 2011). Most *S.*

Typhimurium (88%) harbour a serotype specific 90 kb virulence plasmid (Helmuth *et al.* 1985) which is mostly found in non-typhoid bacteraemia strains (Gulig *et al.* 1993, Guiney & Fierer 2011). An 8 kb operon on the *Salmonella* virulence plasmid (spv) encodes *spv* genes *spv ABCD* (Gulig *et al.* 1993). Most *spv* genes encode proteins which are responsible for intra-macrophage survival (Matsui *et al.* 2001). The *spvR* gene is essential for the regulation of transcription of the spv ABCD operon while the *spvB* gene inhibits actin polymerization and softens the macrophages cytoskeleton leading to cell death by apoptosis (Guiney & Fierer 2011). The *spvC* gene contributes to invasion, survival and multiplication via inhibition of the host cell mitogen - activated protein kinases (MAPK) (Li *et al.* 2007). The 90 kb spv plasmid carries other loci including *pef* (plasmid–encoded fimbriae), *srgA* (SdiA regulated gene which is a putative disulphide oxidoreductase), *rck* (resistance to complement killing) gene and *mig5 (*a macrophage inducible gene) involved in macrophage survival (Rychlik, Gregorova & Hradecka 2006). The *pef* locus harbours four important genes (*pef BACDI*) which are responsible for attachment, adhesion and invasion of *S.* Typhimurium to the small intestine (Baumler, Tsolis & Heffron 1996).

Molecular subtyping methods are used to track foodborne pathogens outbreaks (Swaminathan *et al.* 2001). Pulse field gel electrophoresis (PFGE) is the method of choice for subtyping enteric foodborne pathogens because of its discriminatory power, usefulness in *Salmonella* intra-serovars differentiation, reproducibility and concordance with epidemiologic investigations (Swaminathan *et al.* 2001). Currently, there is no information on the PFGE profiles of *S.* Typhimurium isolates from poultry in South Africa. Although virulotyping studies have been carried out to characterize *S.* Typhimurium isolates and other *Salmonella* serovars (Huehn *et al.* 2010), little is known about virulence factors and markers of *S.* Typhimurium isolates from South Africa. Therefore, the objective of this study was to characterize and subtype *S.* Typhimurium isolates that were recovered between 1995 and 2002 from organs and environments of diseased poultry in South Africa using virulotyping, PFGE, and plasmid profiling. In this study, *S.*

Typhimurium isolates which were recovered between 1995 and 2002 from organs and environments of diseased poultry in South Africa were characterized in terms of their virulence profiles and examined by PFGE to assess relatedness among isolates. Overall aim is to contribute to *Salmonella* surveillance through molecular subtyping and virulotyping of *S.* Typhimurium isolates from South Africa.

3.2. Materials and methods

3.2.1. Bacterial isolates

A total of 141 *S.* Typhimurium isolates were used for this study. These isolates were recovered over a period of 8 years (1995 - 2002) from more than 22 poultry farms (grower and hatcheries) in 5 provinces of South Africa (Gauteng, Limpopo, KwaZulu-Natal, North West, and Free State). One hundred and fifteen (115) of these isolates were recovered from poultry organs (lungs, liver, and intestines) and 26 from poultry environments (litter, dust) (Appendix 3.1). All environmental isolates were collected from F20 between February and May 2002. *Salmonella* Typhimurium isolates were identified and serotyped according to the White-Kauffman-Le Minor Scheme (Popoff *et al.* 2001) at the Onderstepoort Veterinary Research Institute, Onderstepoort, South Africa. Pure cultures were kept lyophilized in the bacteriology laboratory at Faculty of Veterinary Science, University of Pretoria. In this study, poultry farms were designated with letter F followed by an Arabic number. Zero (0) was used when the origin of isolates could not be traced (Appendix 3.1).

3.2.2. DNA extraction

Template DNA was extracted by the boiling method as described previously (De Medici *et al.* 2003). Briefly, one loop-full of an overnight bacterial culture (horse blood) was mixed in 1.5 ml eppendorf tube containing 1ml FA buffer (Becton Dickinson and Company Sparks, USA). The cell suspension was vortexed and centrifuged for 5 min. The supernatant was discarded and the

cell pellet was re-suspended in FA buffer. This procedure was repeated twice. A homogeneous cell suspension was boiled at 100˚C for 15 min then stored at -20˚C for further processing.

3.2.3. Confirmation of *Salmonella* Typhimurium by PCR

A total of 141 *S.* Typhimurium isolates were confirmed by PCR as *Salmonella* Typhimurium by screening for three specific antigenic genes (*rfbJ, fliC* and *fljB*) encoding somatic (O4) and flagellar antigens (H: i and H: 1, 2) respectively using specific primer pairs (Table 3.1) and a slightly modified PCR protocol (Lim *et al.* 2003). Briefly, the 25 µl PCR reaction mixture contained 1 x reaction buffer; 200 mM of each dNTP (New England Biolabs, USA); 0.3 µM (Lim *et al.* 2003) of each primer (Integrated DNA Technologies, USA) , 1.25 U of *Taq* DNA polymerase (New England Biolabs, USA) and 3 µl of DNA template. Cycling conditions were as follows: initial denaturation at 94˚C for 3 min, 30 cycles of denaturation at 94˚C for 30 seconds, annealing at 60˚C (Lim *et al.* 2003) for 1 min (Table 3.1), extension at 72˚C for 1 min, and 1 cycle of final extension at 72˚C for 8 min. PCR products were electrophoresed on 1.5% agarose containing 0.5 mg/ml ethidium bromide and photographed under UV light (Gel Doc system (Bio-Rad, USA).

Table 3.1. Primers used to confirm *Salmonella* Typhimurium status

Genes	Primers	Sequences	Tm (˚C)	Size (bp)	References
rfbJ	RfbJ-s	CCA GCA CCA GTT CCA ACT TGA TAC	60	663	(Lim *et al.* 2003)
	RfbJ-as	GGC TTC CGG CTT TAT TGG TAA GCA			
fljB	fljB-s	ATA GCC ATC TTT ACC AGT TCC CCC		183	
	fljB-as	GCT GCA ACT GTT ACA GGA TAT GCC			
fliC	fliC-s	ACG AAT GGT ACG GCT TCT GTA ACC		526	
	fliC-as	TAC CGT CGA TAG TAA CGA CTT CGG			

Tm = melting or annealing temperature

3.2.4. Virulotyping

Salmonella Typhimurium isolates (n=141) were screened for bacteriophages, plasmid and SPIs-encoded virulence genes (virulotyping) that are essential for invasion (*invA, sopB, gtgB, sspH1, sopE, spvC, and pefA*), survival (*sifA, gipA, sodC1, gtgE, mig5,* and *sspH2*), and serum resistance (*rck,* and *srgA*) in the host (Table 3.2). Virulence genes were detected by PCR using slightly modified cycling conditions as described previously (Capuano *et al.* 2013). Briefly, the 20 µl PCR reaction mixture contained 1X buffer; 200 mM of each dNTP (New England Biolabs, USA); 0.2 µM of each primer (Integrated DNA Technologies, USA), 1.25 U of *Taq* DNA polymerase (New England Biolabs, USA), 2.5 mM $MgSO_4$ and 3 µl of DNA template. Amplification was performed in a Veriti thermal cycler (Applied Biosystems, California, USA) as follows: initial denaturation at 94˚C for 3 min, 30 cycles of denaturation at 94˚C for 30 seconds, annealing temperature (Table 3.2) for 1 min, extension at 72˚C for 1 min, and final extension at 72˚C for 8 min. PCR products were electrophoresed on 1.5% agarose containing 0.5 mg/ml ethidium bromide and photographed under UV light Gel Doc system (Bio-Rad, USA). Virulotypes were designated by the letter V followed with an Arabic number starting from number 1. Number (1) indicates that isolates carried all 15 virulence genes. If there were more than one virulotype carrying the same number of genes but differing by gene combinations, letters in alphabetic order were added to the virulotype code after an Arabic number.

3.2.5. Pulsed field gel electrophoresis (PFGE)

PFGE was performed according to the PulseNet protocol (PulseNet 2013). The DNA-containing plug slices were digested with 50U/ plug of *XbaI* (New England Biolabs, USA) for 2 hrs. at 37˚C, then electrophoresed in 1% SeaKem Gold Agarose gel (Bio-Rad, USA) with initial switch time (2.2 sec) and final switch time (63.8 sec) using CHEF Mapper DRIII (Bio-Rad, USA) for 20 hrs. The gel was stained in 1% ethidium bromide and visualized under UV light using the Gel Doc system (Bio-Rad, USA). *Salmonella* serovar Braenderup H 9812 (restriction digests) was

used as a reference strain (DNA ladder). Pulsotypes were analysed by BioNumerics version 6.6 (Applied Maths, Belgium) software. Matching of band patterns was calculated using Dice – coefficients with a 3% optimization and 3% band tolerance. A dendrogram was constructed using the unweighted pair group method with averages (UPGMA) to determine relatedness among isolates. The Simpson's index of diversity was calculated as previously described (Hunter & Gaston 1988). A pulsotype was defined as a set of *S.* Typhimurium strains which were related at 100% of genetic similarity. Pulsotypes were labelled with Arabic numbers preceded by X.

3.2.6. Plasmid profiling

Plasmid DNA was extracted from 43 *S.* Typhimurium isolates that were randomly selected from different major pulsotypes. Plasmid DNA was extracted using the Qiagen plasmid midi kit. Plasmid DNA was detected by gel electrophoresis (1% agarose). A supercoiled DNA ladder (New England Biolabs, USA) and the plasmids of *E. coli* EDL 933 were used as DNA makers to measure the size of plasmids.

3.2.7. Statistical analysis

Descriptive statistics were carried out on all data. The linear relationship between virulence genes was calculated using pairwise correlation. Differences between proportions of virulence genes in environmental and organ isolates were evaluated using two sample t - test. The intercooled Stata 9 software (StataCorp, USA) was used for statistical analysis. Binary virulotyping data were analysed by BioNumerics version 6.6 (Applied Maths, Belgium) software using categorical data and UPGMA.

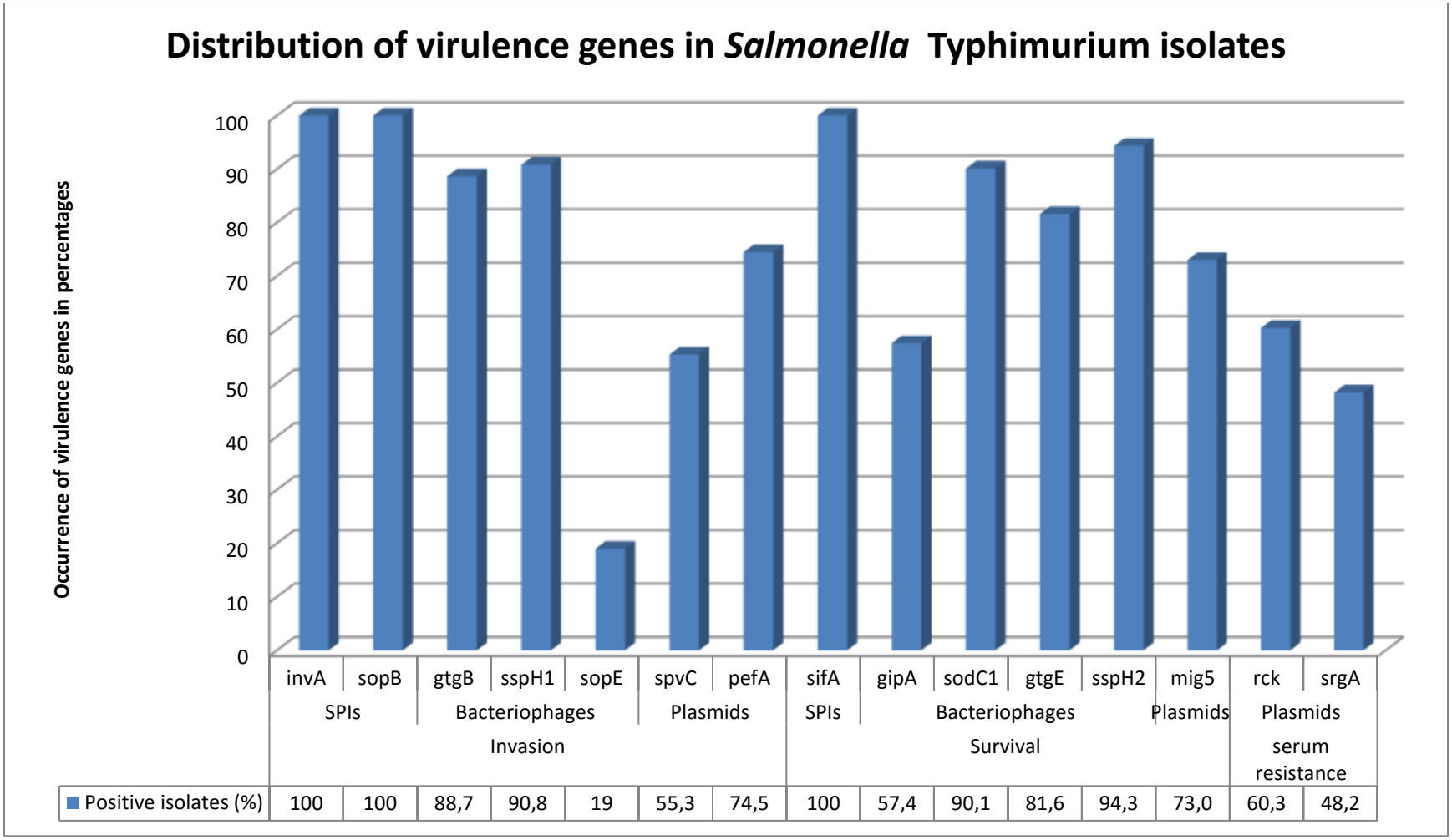
Table 3.2. List of *Salmonella* Typhimurium virulence genes, primers, primer concentrations and PCR conditions used for amplification

	Gene	Location	Function	Tm (˚C)	Size bp	Primer sequence (5' to 3')	References
1	*invA*	SPI -1	Invasion	57	244	F - ACAGTGCTCGTTTACGACCTGAAT R - AGACGACTGGTACTGATCGATAAT	(Chiu & Ou 1996)
2	*sopB*	SPI -1	Invasion of macrophages	64	220	F- CGGACCGGCCAGCAACAAAACAAAGAAGAAG R- TAGTGATGCCCGTTATGCGTGAGTGTATT	(Skyberg, Logue & Nolan 2006)
3	*gtgB*	Gifsy-2	Invasion	58	436	F - TGCACGGGGAAAACTACTTC R - TGATGGGCTGAAACATCAAA	(Mikasova *et al.* 2005)
4	*sspH1*	Gifsy-3	Invasion	60	246	F - TGCAGAAAAAGGGGAATACG R - GCAGCCTGAAGGTCTGAAAC	(Borriello *et al.* 2012)
5	*sopE*	sopEø	Invasion & intestinal inflammation	58	362	F - CGAGTAAAGACCCCGCATAC R - GAGTCGGCATAGCACACTCA	(Drahovska *et al.* 2007)
6	*spvC*	PSLT038	Invasion, survival and multiplication	57	570	F - ACTCCTTGCACAACCAAATGCGGA R - TGTCTTCTGCATTTCGCCACCATCA	(Chiu & Ou 1996)
7	*pefA*	PSLT018	adhesion and invasion	66	157	F - GCGCCGCTCAGCCGAACCAG R - GCAGCAGAAGCCCAGGAAACAGTG	(Skyberg *et al.* 2006)
8	*sifA*	SPI -2	Survival within macrophages	67	449	F- TTTGCCGAAGAACGCGCCCCCCACACG R- GTTGCCTTTTCTTGCGCTTTCCACCCATCT	
9	*gipA*	Gifsy-1	Growth and survival in Payer's Patches	58	212	F - GCAAGCTGTACATGGCAAAG R - GGTATCGGTGACGAACAAAT	(Mikasova *et al.* 2005)
10	*sodC1*	Gifsy-2	Survival within macrophages	50	467	F - TATTGTCGCTGGTAGCTG R - CAGGTTTATCCGAGTAAT	(Bacciu *et al.* 2004)
11	*gtgE*	Gifsy-2	Intracellular survival	50	1113	F - AGGAGGAGTGTAAAGGT R - GTAGAACTGGTTTATGAC	
12	*mig5*	PSLT	Intra-macrophage survival	58	248	F - AACCAACCAGACCAACCTTC R - GCAATACTGTTGCGCTTCTG	(Borriello *et al.* 2012)
13	*sspH2*	Phage Remnant	Modulation of innate immunity (survival)	58	203	F - GCACAACTGGCTGAAGATGA R - TTTCCCAGACGGAACATCTC	
14	*rcK*	PSLT	Resistance to complement killing	58	189	F - AACGGACGGAACACAGAGTC R - TGTCCTGACGAAAGTGCATC	
15	*srgA*	PSLT	serum resistance	58	344	F - TGTTCCGGTCATAATGCAGA R - TTTTGAGGCCATCGAATACC	

3.3. Results

3.3.1. Virulotyping

SPIs-encoded genes (*invA, sopB* and *sifA*) were found in all isolates. Bacteriophage-encoded genes occurred as follows: *sspH2* 94% (133/141), *sspH1* 90.8% (128/141), *sodC1* 90.1% (127/141), *gtgB* 88.7% (125/141), *gtgE* 81.6% (115/141), *gipA* 57.4% (81/141), and *sopE* 19% (27/141). Apart from *gipA* and *sopE*, all the other bacteriophage-encoded virulence genes (*sspH2*, *sspH1*, *sodC1*, *gtgB*, *gtgE*) were observed in 74.5% (105/141) of the isolates. The frequency of plasmid-encoded genes was: *pefA* 74.5% (105/141), *mig5* 73.0% (103/141), *rck* 60.3% (85/141), *spvC* 55.3% (79/141), and *srgA* 48.2% (68/141) (Figure 3.1). Five plasmid-encoded virulence genes (*pefA, mig5, rck, spvC, srgA*) were observed in 43.9% (62/141) of the isolates while *spvC* and *pefA* were observed in 49.6% (70/141) of the isolates. Pairwise correlation revealed that there was a linear relationship between virulence genes encoded on the same location such as bacteriophages or plasmids. For example, the proportion of linear relationship between plasmid-encoded virulence genes: *srgA* and *spvC* was 72.5% while association between one gene encoded on a phage and another one located on a plasmid was very low (Appendix 3.2). The following virulence genes *gtgB, spvC, gipA, gtgE, mig5, rck* and *srgA* were more frequent ($p \leq 0.05$) in *S.* Typhimurium isolates from environments (Table 3.3; Appendix 3.4).



SPIs: *Salmonella* pathogenicity islands (*invA, sopB,* and *sifA*)

Figure 3.1. Frequencies of virulence genes of *Salmonella* Typhimurium isolates identified from diseased poultry between 1995 and 2002 in South Africa.

Table 3.3. Frequencies of virulence genes among *Salmonella* Typhimurium according to the sources of isolates

Genes	Total n=141	Environment	Organs	*p* value
		Positive isolates n=26 (%)	Positive isolates n=115 (%)	
invA	141 (100.0)	26 (100.0)	115 (100.0)	-
sopB	141 (100.0)	26 (100.0)	115 (100.0)	-
gtgB	125 (88.7)	26 (100.0)	99 (86.1)	0.045*
sspH1	128 (90.8)	26 (100.0)	102 (88.7)	0.074
sopE	27 (19.0)	5 (19.2)	22 (19.1)	0.99
spvC	79 (55.3)	24 (92.3)	55 (47.8)	0.001*
pefA	105 (74.5)	23 (88.5)	82 (71.3)	0.071
sifA	141 (100.0)	26 (100.0)	115 (100.0)	-
gipA	81 (57.4)	24 (92.3)	57 (49.6)	0.001*
mig5	103 (74.5)	24 (92.3)	79 (68.7)	0.016*
sodC1	127 (90.1)	26 (100.0)	101 (87.8)	0.063
gtgE	115 (81.6)	25 (96.2)	90 (78.3)	0.035*
sspH2	133 (94.3)	26 (100.0)	107 (93.0)	0.167
rck	85 (60.3)	22 (84.6)	63 (54.8)	0.005*
srgA	68 (48.2)	23 (88.5)	45 (39.1)	0.001*

*: significant: *gtgB, spvC, gipA, gtgE, mig5, rck* and *srgA*

Virulotyping clustered 141 isolates into 59 distinct virulence profiles (virulotypes). Each virulotype clustered virulence genes involved in invasion, and survival. Virulence genes (*rck* and *srgA*) which are responsible for resistance to serum killing were not found in 36.8% (52/141) of the isolates of which 34.7% (49/141) were from poultry organs. Among the 59 virulotypes, five (V2, V3a, V5a, V4a, and V1) were considered dominant virulotypes. In this study, a dominant virulotype is a virulotype which clustered 5 or more isolates carrying an identical combination of genes (Table 3.4, Appendix 3.4). Virulotype V2 contained 14 virulence genes (all virulence genes tested except *sopE*) and clustered 17.7% (25/141) of the isolates including 20 that were isolated from poultry environments and 5 isolates identified from poultry organs. Virulotype V3a contained 13 virulence genes (all virulence genes tested except *gipA* and *sopE*) each and clustered 17.7% (25/141) of the isolates recovered from organs. Virulotype V5a contained 10 virulence genes and clustered 5.7% (8/141) of *S.* Typhimurium isolated from organs. Virulotype V4a clustered 3.5%

(5/141) of the isolates recovered from organs and carrying 11 virulence genes. Virulotype V1 clustered 4.3% (6/141) of the isolates carrying all 15 virulence genes tested in this study. Among the 6 isolates, 4 were recovered from poultry environments while 2 isolates were identified from organs (Table 3.4, Appendix 3.4). Ninety two per cent (92.3%, 24/26) of poultry environmental isolates carried at least 14 virulence genes. Simpson's diversity index for virulotyping was 0.93.

Table 3.4. Dominant virulotypes of *Salmonella* Typhimurium isolated between 1995 and 2002 from diseased poultry in South Africa.

Dominant virulotypes detected	Identity	No. of genes	No. of positive isolates
invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-sopE-spvC-rck-pefA-mig5-srgA	V1	15	6 (4.3%)
invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA	V2	14	25 (17.7%)
invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA	V3a	13	25 (17.7%)
invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-rck-pefA	V4a	11	5 (3.5%)
invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-pefA	V5a	10	7 (4.9%)

V: virulotype; letter "a" indicates that there is another different virulotype containing the same number of genes.

3.3.2. Pulsed Field Gel Electrophoresis (PFGE)

PFGE analysis of normalized gels (Figure 3.2) generated a dendrogram (Figure 3.3) of 140 *S.* Typhimurium isolates which were clustered into 55 pulsotypes showing a genetic similarity ≥ 79%. Of the 55 pulsotypes, five (X25, X5, X13, X45 and X3) were considered major pulsotypes because they clustered 5 or more isolates. A total of 47.1% (66/140) of the isolates belonged to the 5 major pulsotypes. Each pulsotype individually clustered X25 - 22.8% (32/141), X5 - 9.3%

(13/141), X13 - 7.1% (10/141), X45 - 4.3% (6/141) and X3 - 3.6% (5/141) of the isolates. There were 7 and 10 pulsotypes which contained respectively 3 and 2 isolates while 33 isolates represented single isolate pulsotypes (Figure 3.3). Pulsotypes: X25, X5, X45 and X3 clustered respectively 31/32, 12/13, 6/6 and 5/5 isolates that were all recovered from poultry organs while pulsotype X13 clustered only 10/10 environmental isolates. Simpson's diversity index for PFGE profiles was 0.93. Pulsotypes corresponded mostly to virulotypes by clustering isolates that shared the same gene makeup (Figure 3.3).

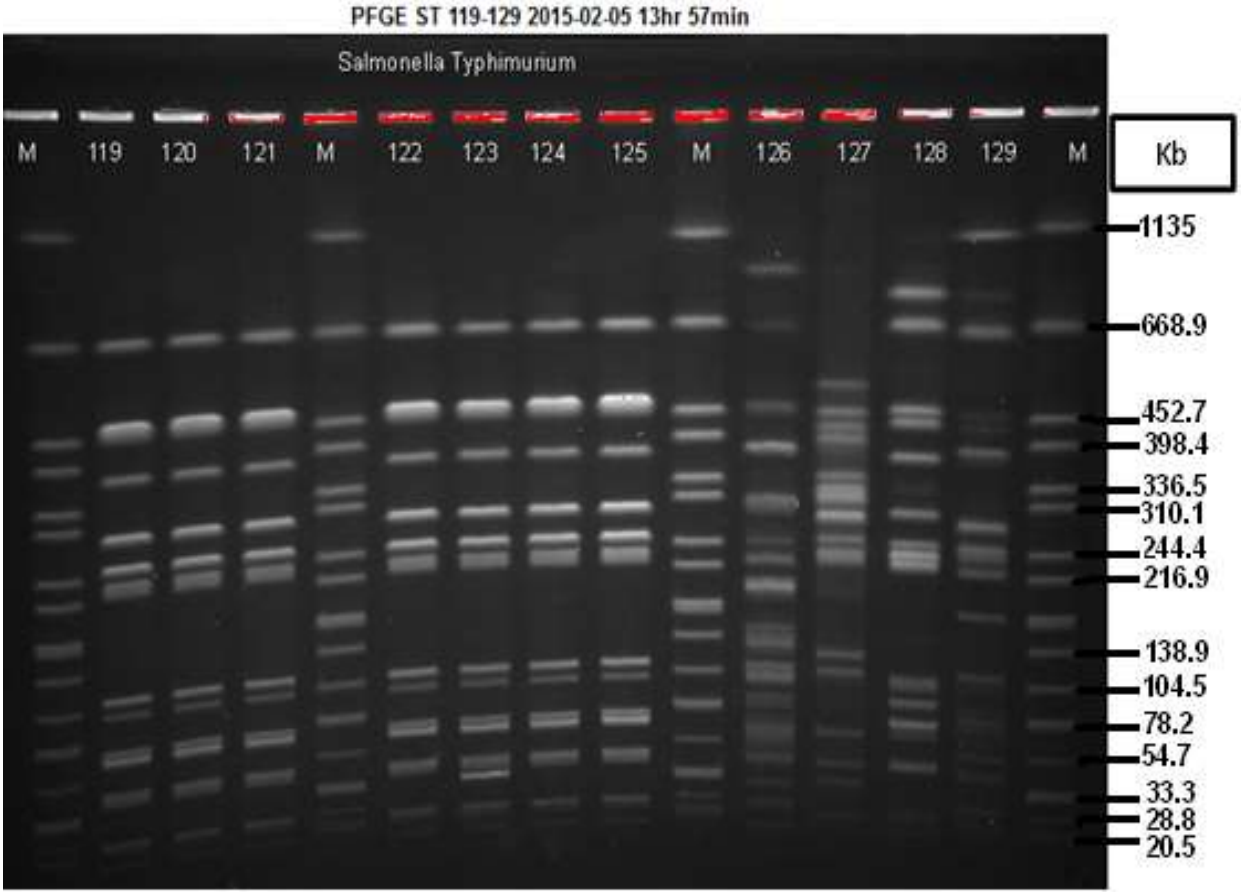
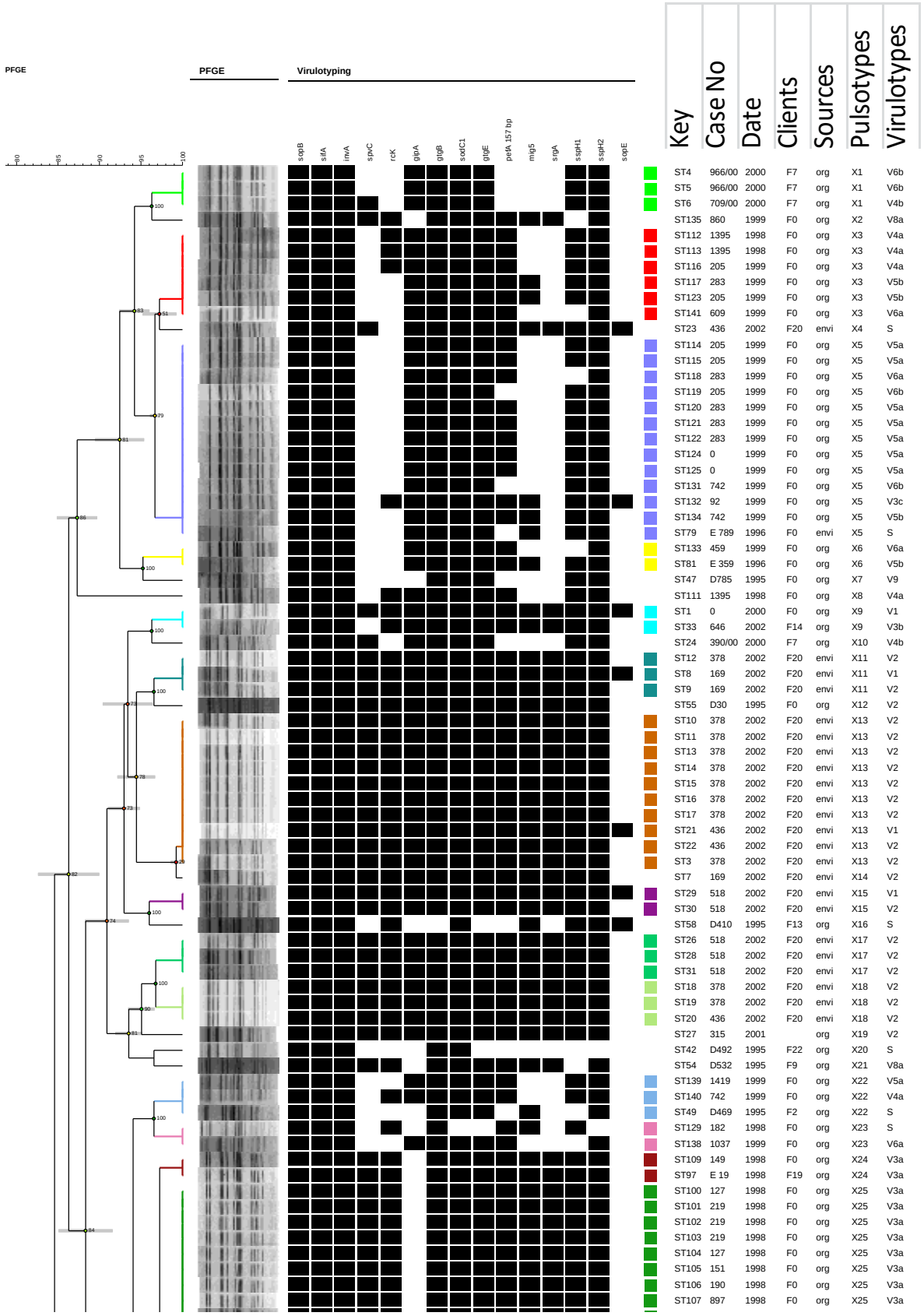


Figure 3.2. Pulsed field gel electrophoresis image showing *Salmonella* Typhimurium *XbaI* patterns. Lanes 1, 5, 10 and 15 contained the *Salmonella* Braenderup (Marker); lanes 119 -121; 122-125; 126 – 129 contained *Salmonella* Typhimurium isolates.



Key	Case No	Date	Clients	Sources	Pulsotypes	Virulotypes
ST4	966/00	2000	F7	org	X1	V6b
ST5	966/00	2000	F7	org	X1	V6b
ST6	709/00	2000	F7	org	X1	V4b
ST135	860	1999	F0	org	X2	V8a
ST112	1395	1998	F0	org	X3	V4a
ST113	1395	1998	F0	org	X3	V4a
ST116	205	1999	F0	org	X3	V4a
ST117	283	1999	F0	org	X3	V5b
ST123	205	1999	F0	org	X3	V5b
ST141	609	1999	F0	org	X3	V6a
ST23	436	2002	F20	envi	X4	S
ST114	205	1999	F0	org	X5	V5a
ST115	205	1999	F0	org	X5	V5a
ST118	283	1999	F0	org	X5	V6a
ST119	205	1999	F0	org	X5	V6b
ST120	283	1999	F0	org	X5	V5a
ST121	283	1999	F0	org	X5	V5a
ST122	283	1999	F0	org	X5	V5a
ST124	0	1999	F0	org	X5	V5a
ST125	0	1999	F0	org	X5	V5a
ST131	742	1999	F0	org	X5	V6b
ST132	92	1999	F0	org	X5	V3c
ST134	742	1999	F0	org	X5	V5b
ST79	E 789	1996	F0	envi	X5	S
ST133	459	1999	F0	org	X6	V6a
ST81	E 359	1996	F0	org	X6	V5b
ST47	D785	1995	F0	org	X7	V9
ST111	1395	1998	F0	org	X8	V4a
ST1	0	2000	F0	org	X9	V1
ST33	646	2002	F14	org	X9	V3b
ST24	390/00	2000	F7	org	X10	V4b
ST12	378	2002	F20	envi	X11	V2
ST8	169	2002	F20	envi	X11	V1
ST9	169	2002	F20	envi	X11	V2
ST55	D30	1995	F0	org	X12	V2
ST10	378	2002	F20	envi	X13	V2
ST11	378	2002	F20	envi	X13	V2
ST13	378	2002	F20	envi	X13	V2
ST14	378	2002	F20	envi	X13	V2
ST15	378	2002	F20	envi	X13	V2
ST16	378	2002	F20	envi	X13	V2
ST17	378	2002	F20	envi	X13	V2
ST21	436	2002	F20	envi	X13	V1
ST22	436	2002	F20	envi	X13	V2
ST3	378	2002	F20	envi	X13	V2
ST7	169	2002	F20	envi	X14	V2
ST29	518	2002	F20	envi	X15	V1
ST30	518	2002	F20	envi	X15	V2
ST58	D410	1995	F13	org	X16	S
ST26	518	2002	F20	envi	X17	V2
ST28	518	2002	F20	envi	X17	V2
ST31	518	2002	F20	envi	X17	V2
ST18	378	2002	F20	envi	X18	V2
ST19	378	2002	F20	envi	X18	V2
ST20	436	2002	F20	envi	X18	V2
ST27	315	2001		org	X19	V2
ST42	D492	1995	F22	org	X20	S
ST54	D532	1995	F9	org	X21	V8a
ST139	1419	1999	F0	org	X22	V5a
ST140	742	1999	F0	org	X22	V4a
ST49	D469	1995	F2	org	X22	S
ST129	182	1998	F0	org	X23	S
ST138	1037	1999	F0	org	X23	V6a
ST109	149	1998	F0	org	X24	V3a
ST97	E 19	1998	F19	org	X24	V3a
ST100	127	1998	F0	org	X25	V3a
ST101	219	1998	F0	org	X25	V3a
ST102	219	1998	F0	org	X25	V3a
ST103	219	1998	F0	org	X25	V3a
ST104	127	1998	F0	org	X25	V3a
ST105	151	1998	F0	org	X25	V3a
ST106	190	1998	F0	org	X25	V3a
ST107	897	1998	F0	org	X25	V3a

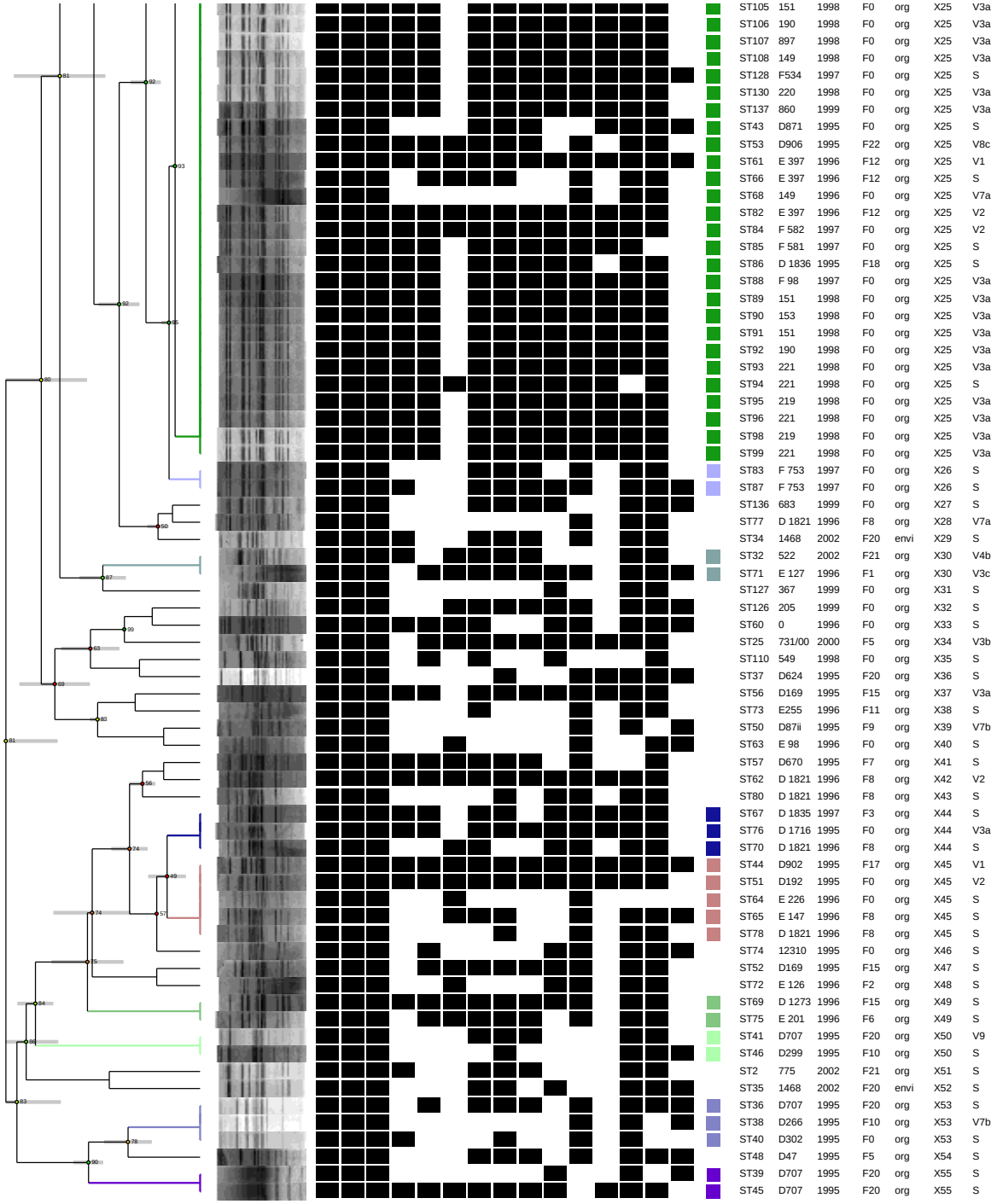


Figure 3.3. Unweighted pair group method with averages dendrogram showing pulsed field gel electrophoresis-*Xba*I patterns and character values from virulotyping of *Salmonella* Typhimurium collected from poultry farms in South Africa. Black boxes indicate the presence of a virulence

gene and white spaces indicate its absence. V3a and V3b were two virulotypes with the same number and similar gene makeup except for two genes (*gipA* absent for V3a, and *spvC* absent for V3b); ST: *Salmonella* Typhimurium; F0: unknown source. S: single virulotype.

3.3.3. Plasmid profiling

Plasmid extraction from 43 *S.* Typhimurium isolates revealed ten plasmid profiles (Table 3.5; Appendix 3.5). The size of plasmids ranged from 2 Kb to 90 Kb (Table 3.5). Two groups of plasmids were identified: large (75 kb and 90 kb), and small plasmids (≤ 10 kb). Out of 43 isolates, 77.5 % (33/43) possessed at least 1 plasmid while the remaining 23.3% (10/43) of the isolates did not reveal any plasmid (Table 3.5). Four out of 43 (9.3%) isolates carried only small plasmids (2-8Kb) while the remaining 29/43 (67.4%) possessed both small and large plasmids (2-90Kb). In terms of plasmid profiles identified, 8 distinct profiles clustered only isolates that were recovered from poultry organs. Profile A was represented by one isolate (ST1) which was recovered from a poultry organ. This isolate possessed a high number of plasmids (4) with the following sizes: 90kb, 75 kb, 3 kb and 2 kb. The major plasmid profiles (observed in 5 or more than 5 isolates) included plasmid profile C, D, and E. Plasmid profile C clustered 5 isolates (ST93, ST97, ST99, ST102, and ST107) from organs and possessed 2 plasmids with 90 kb and 3.5 kb. Plasmid profile D clustered 6 isolates (ST112, ST114, ST116, ST122, ST123, and ST126) from poultry organs and possessed two plasmids (90 kb and 2 kb). Plasmid profile E that carried one large plasmid (90 kb) clustered 12 isolates of which 5 (ST7, ST8, ST13, ST16, and ST30) were from poultry environments and other 7 (ST42, ST54, ST55, ST57, ST119, ST133, and ST137) from poultry organs (Table 3.5; Appendix 3.5).

Table 3.5. Plasmid profiles of 43 selected *Salmonella* Typhimurium isolated between 1995 and 2002 from diseased poultry in South Africa.

N_o profiles	Estimation of plasmid size in Kilobase (Kb)								N_o of plasmids	N_o of isolates
	90	75	10	8	4	3.5	3	2		
A	+	+	-	-	-	-	+	+	4	1
B	+	-	+	-	-	-	-	+	3	2
C	+	-	-	-	-	+	-	-	2	5
D	+	-	-	-	-	-	-	+	2	6
E	+	-	-	-	-	-	-	-	1	12
F	-	+	-	-	-	-	-	+	2	1
G	-	-	-	+	+	-	-	-	2	2
H	-	-	-	-	+	-	-	-	1	1
I	-	-	-	-	-	+	-	-	1	2
J	-	-	-	-	-	-	-	+	1	1
Total										**33**

3.4. Discussion

Salmonella Typhimurium isolates are among important zoonotic *Salmonella* serovars which are estimated to account for approximately 80% of human gastroenteritis cases (EFSA 2008, Kariuki *et al.* 2005). Occurrence and widespread of human *S.* Typhimurium infections have been associated with ingestion of poultry products (Fearnley *et al.* 2011). The severity of *S.* Typhimurium infections depends on both virulence makeup of the pathogen and immunity status of the host (Foley *et al.* 2013). In this study, *S.* Typhimurium isolates (n=141) were characterized with regards to SPIs, bacteriophages and plasmids- encoded virulence genes, and plasmids profiles. Relatedness among isolates was also evaluated using molecular DNA fingerprinting (PFGE). Most of *S.* Typhimurium isolates (85%) carried at least 5 different virulence genes supporting the statement that *S.* Typhimurium isolates carry virulence genes that have been incriminated in *Salmonella* infections (Huehn *et al.* 2010, Capuano *et al.* 2013, Borriello *et al.* 2012). While SPI-encoded virulence genes were present in *S.* Typhimurium isolates under study, a high level of genetic diversity was observed from both virulotyping and PFGE. Diversity was mainly observed among bacteriophages (*gip A* and *sopE*) and plasmid- encoded (*rck*) virulence genes.

The presence of SPIs-encoded genes in 100% of *S.* Typhimurium isolates is congruent with findings obtained previously in *S.* Typhimurium isolates elsewhere (Hughes *et al.* 2008, Skyberg *et al.* 2006,Huehn *et al.* 2010). Therefore, SPIs-encoded *invA, sopB,* and *sifA* genes produced respective proteins which contributed in the invasion process and survival (Table 3.2) of the isolates with disease manifestations in chickens as a result. Taken together the findings of the present study and data from different studies (Skyberg *et al.* 2006, Hughes *et al.* 2008), confirm that SPIs-encoded *invA, sopB* and *sifA* genes are virulence markers conserved within *S.* Typhimurium chromosome (Anjum *et al.* 2005, Amavisit *et al.* 2003). The PCR method screening for the presence of SPIs-encoded genes (*invA, sopB*, and *sifA*) could be a rapid alternative to the conventional culture and serotyping method for identification of *Salmonella* species from various host species.

Among bacteriophage-encoded genes, *sspH2, sspH1, sodC1, gtgB,* and *gtgE* were present in the majority (74.5%) of isolates while *sopE* was less frequent (19%). This is concordant with findings that have been reported in previous studies (Huehn *et al.* 2010, Capuano *et al.* 2013, Mikasova *et al.* 2005). Gifsy-2-encoded genes: *sodC1, gtgB* and *gtgE* were simultaneously present in 81% of *S.* Typhimurium isolates. This confirms the opinion that all *S.* Typhimurium harbour the Gisfy-2 (Figueroa-Bossi *et al.* 2001) and suggests that genetic exchanges via lysogenic bacteriophages are very limited within the Gisf-2 sequence. This was also supported by the pairwise correlation which showed a significant linear correlation among Gisfy2-encoded virulence genes (Appendix 3.2). The findings of this study also confirm that the *gtgB* gene is frequent in *S.* Typhimurium isolates whereas *sopE* is less frequent (Ehrbar & Hardt 2005). Interestingly, the frequency of Gisfy-3 encoded *sspH1* gene in this study (90.8%) was higher compared to the results obtained by Capuano *et al.* (2013), Borriello *et al.* (2012) and is in disagreement with the study by (Tsolis *et al.* 1999) that stated that *sspH1* is present only in few *S.* Typhimurium isolates. The high frequency of *sspH1* in this study may be probably associated

either with high level of virulence and resultant death or host adaptation. The presence of bacteriophages-encoded genes highlights the pathogenic ability of *S.* Typhimurium since these genes are involved in different steps of invasion and survival of the pathogen in the host (Table 3.2). Bacteriophage-encoded genes also represent a high risk because they are prone to their dissemination in non- pathogenic salmonellae or other related bacteria through bacteriophage lysogenesis.

There was a certain association between plasmid - encoded virulence genes since all plasmid-encoded virulence genes tested in this study were simultaneously observed in 43.9% of the isolates. While *spvC* and *pefA* were simultaneously observed in 49.6% of the isolates, 51.7% (73/141) of isolates carried simultaneously *spvC* and *mig5*. Furthermore, 58.1% (85/141) of *pefA* positive isolates carried also *mig5* while 56.0% (79/141) of isolates carried simultaneously *pefA* and *rck*. This was also in agreement with the results from pairwise correlation which showed a significant linear relationship among plasmid-encoded virulence genes (Appendix 3.2). The concurrent presence of more than two plasmid-encoded virulence genes in 50% of isolates indicates that the presence of plasmid-encoded virulence genes is associated with the presence of the *Salmonella* virulence plasmid (*spv*). The frequency of *pefA*, *mig5, rck*, *spvC*, and *srgA* were comparable to the results that have been found elsewhere (54%) for *spvC* (Borriello *et al.* 2012); and (51.4%) for *srgA* (Capuano *et al.* 2013). Lower rates for *spvC*, *pefA, rck* and *mig5* were reported elsewhere (Capuano *et al.* 2013, Mohamed *et al.* 2014). The rates of *spvC* were lower compared to those reported for poultry *S.* Typhimurium strains in other studies (Huehn *et al.* 2010, Diarra *et al.* 2014). These results indicate that the presence of *spvC* varies from one study to another (Huehn *et al.* 2010, Capuano *et al.* 2013, Diarra *et al.* 2014, Mohamed *et al.* 2014). *SpvC* is generally present in a broad host range *Salmonellae* isolates such as *S.* Typhimurium, *S.* Heidelberg and *S.* Enteritis (Libby *et al.* 1997). The presence of *pefA* (plasmid-encoded fimbria) gene in 74.5% of isolates observed in this study was comparable to 88.7% reported in Japan

(Futagawa-Saito *et al.* 2010) but higher compared to the results reported in other studies in which lower rates were found (Capuano *et al.* 2013, Skyberg *et al.* 2006, Borriello *et al.* 2012). The high rate of *pefA* among *S.* Typhimurium explains the contribution of this gene in the attachment and invasion of isolates in chickens.

The virulence genes (*gtgB, spvC, gipA, gtgE, mig5, rck* and *srgA*) were more frequent ($p \leq 0.05$) in poultry environmental isolates compared to isolates from poultry organs (Table 3.3). The function of genes (*gtgB, spvC, gipA, gtgE, mig5, rck* and *srgA*) may explain why they were significantly more frequent in the litter. GtgB and spvC were produced in response of *S.* Typhimurium to invade intestinal epithelial cells (Miao *et al.* 2003). GtgE, gipA, mig5 were produced by *S.* Typhimurium isolates to survive within intestinal payer's patches (De Groote *et al.* 1997, Kohler *et al.* 2014, Stanley *et al.* 2000) while rck and srgA were produced to resist the serum killing within intestinal blood vessels. This is not surprising since the invasion gene (*invA*) has been used for identification of *Salmonella* serovars from faeces (Chiu & Ou 1996) indicating that some *Salmonella* isolates harbouring genes which are involved in invasion and survival might cause only self-limiting gastroenteritis. Therefore, *S.* Typhimurium isolates recovered from environments produced these genes which were responsible for the colonization of intestinal epitheliums and were shed within faeces after causing gastroenteritis in chickens (Chiu & Ou 1996, Barrow *et al.* 2004). All environmental isolates were collected from farm F20 at different dates between February and May 2002 and only litter was sampled for diagnostic purpose. The fact that isolates from litter carried at least 14 out of 15 virulence genes tested indicates that the litter was contaminated by diarrheal faeces suggesting that there was an endemic *S.* Typhimurium infection that was characterized by gastroenteritis in F20. However, a well-structured study (with representative sample size and identical husbandry conditions) on frequencies of virulence genes of *S.* Typhimurium isolates from organs and environments of diseased poultry will support our findings since we used the isolates that were available.

While most of the virulotypes observed in this study were single strain profiles, 69% of the isolates belonged to the major clusters. Virulotyping clustered 141 isolates into 59 distinct virulotypes of which only 5 were considered dominant (Table 3. 4) while 44 were single isolate distinct virulotypes (S). Major clusters included isolates that were mostly recovered from same sources during the same period (Appendix 3. 4). The high variation observed among virulotypes was related to the presence or absence of virulence genes encoded on bacteriophages and plasmids. This was expected since these mobile genetic elements are characterized by high level of gain and loss of genes as a rapid mode of evolution in *S.* Typhimurium strains (Brussow *et al.* 2004, Heuer, Abdo & Smalla 2008). The presence of different combinations of virulence genes is a reflection of a diverse genetic makeup of *S.* Typhimurium strains and their capacity to survive hostile environments in the host (macrophages, and serum killing) and even cause fatal invasive salmonellosis in susceptible hosts. The existence of 59 distinct virulotypes of which 44 were represented by a single isolate while 97 isolates were grouped in five major profiles indicates that while there might be clones of *S.* Typhimurium circulating among investigated diseased poultry farms, there are also unique isolates of *S.* Typhimurium with particular virulence features.

PFGE revealed a considerable level of genetic diversity among poultry *S.* Typhimurium isolates (D = 0.93). This was expected as isolates of this study were sourced from various poultry farms and for a long period spanning 8 years. The considerable diversity among *S.* Typhimurium isolates have been observed elsewhere (Benacer *et al.* 2010, Tamamura *et al.* 2011). Although, a high genetic diversity of poultry *S.* Typhimurium isolates was observed in this study, 32 (22.8%) *S.* Typhimurium strains that were collected from multiple sources between 1995 and 1999 clustered into a major pulsotype X25 (Figure 3.3). This cluster of isolates that were collected at different times from various sources indicates that there exists a common *S.* Typhimurium clone which is persistent and widespread on the farms from where the isolates belonging to this cluster were recovered. It would be also interesting to investigate to what extent such a clone may be

also prevalent among *S.* Typhimurium recovered from human populations through comparative genomic studies.

Virulotyping profiles corresponded to a large extent with PFGE profiles. Furthermore virulotyping and PFGE showed the same discriminatory power (D = 0.93). A consistent relationship between virulotypes and pulsotypes was also found in a study by (Rychlík, Hradecka & Malcova 2008) that characterized *S.* Typhimurium by multiplex PCR and PFGE. Rychlík *et al.* (2008) also showed that the discriminatory power of virulotyping was the same as that of PFGE. Another study which compared virulotyping and microarray showed also a strong correlation between these two methods (Huehn *et al.* 2010). Data obtained in the current study indicate that virulotyping may be an acceptable subtyping method for *S.* Typhimurium in laboratories where PFGE is not available.

Ten plasmid profiles varying from 2 kb to 90 kb were observed among 43 *S.* Typhimurium isolates. However, (Benacer *et al.* 2010) found among 47 *S.* Typhimurium isolates 22 different plasmid profiles ranging (2 kb – 95 kb). In the present study only two isolates (ST19 and ST 28) from F20 had an 8 kb plasmid. However, this plasmid was not found in other studies (Benacer *et al.* 2010, Baggesen, Olsen & Bisgaard 1992). The absence of plasmids in 23.3% of the isolates in this study is congruent with the results obtained by (Benacer *et al.* 2010) and (Helmuth *et al.* 1985) who reported similar results. This absence could be attributed to the shearing of large plasmids or precipitation with chromosomal DNA during plasmid extraction process or due to plasmid instability. The insertion and deletion of mobile genetic elements including plasmids are common in bacterial species including *S.* Typhimurium (Gyles & Boerlin 2014, Silva, Wiesner & Calva 2012). Although, plasmid profiles of only 30% of *S.* Typhimurium isolates were investigated in this study, plasmid profiles corresponded approximately to pulsotypes and virulotypes. Most of isolates that belonged to the same plasmid profile shared the same pulsotype and same virulotype (Appendix 3.6). The presence of plasmids and corresponding plasmid-encoded virulence genes

indicate the potential ability of these *S.* Typhimurium isolates to disseminate plasmid-encoded virulence genes to other non-pathogenic bacteria (microflora) infecting birds with invasive infections within poultry industries.

3.5. Conclusion

In conclusion, the present study is the first one to carry out virulence genes characterization and DNA fingerprinting in poultry *S.* Typhimurium isolates in South Africa. Virulotyping showed that 85.8% of the *S.* Typhimurium isolates carried at least 9 to 15 virulence genes that are usually incriminated in severe human outbreaks. These genes were encoded on genetic mobile elements (SPIs, bacteriophages and plasmids) which disseminate virulence genes among *Salmonella* and have been associated with the emergence and dissemination of highly virulent *Salmonella* pathovars. Virulotyping and PFGE showed the same discriminatory power (D = 0.93) and there was a very strong agreement between the two molecular methods. Thus, virulotyping can be recommended for subtyping of *Salmonella* serovars in laboratories where PFGE is not available. Poultry *S.* Typhimurium isolates were also found to be genetically diverse. The genetic variation that was shown by both virulotyping and PFGE among *S.* Typhimurium isolates (D = 0.93) under study indicates that *S.* Typhimurium isolates which were from multiple sources have acquired diverse genetic makeup to adapt to different environmental changes and have the capacity to cause invasive Salmonellosis in poultry. More comparative studies on poultry and human *S.* Typhimurium isolates are needed to determine to what extent the isolates investigated in this study may be a cause of human *S.* Typhimurium infections. *Salmonella* surveillance programs aiming at reducing contamination at the poultry farm level and along the food chain should be enhanced to ensure food safety.

3.6. Acknowledgements

Our gratitude goes to the University of Pretoria and the National Research Foundation (NRF) for funding this project. We thank also the Laboratory of Bacteriology, Faculty of Veterinary Science, University of Pretoria for providing the isolates.

3.7. References

1. AILES, E., BUDGE, P., SHANKAR, M., COLLIER, S., BRINTON, W., CRONQUIST, A., CHEN, M., THORNTON, A., BEACH, M.J. & BRUNKARD, J.M. 2013. Economic and health impacts associated with a *Salmonella* Typhimurium drinking water outbreak-Alamosa, CO, 2008. *PloS one*, 8:e57439
2. AMAVISIT, P., LIGHTFOOT, D., BROWNING, G.F. & MARKHAM, P.F. 2003. Variation between pathogenic serovars within *Salmonella* pathogenicity islands. *Journal of Bacteriology*, 185:3624-3635
3. ANJUM, M.F., MAROONEY, C., FOOKES, M., BAKER, S., DOUGAN, G., IVENS, A. & WOODWARD, M.J. 2005. Identification of core and variable components of the *Salmonella enterica* subspecies I genome by microarray. *Infection and Immunity*, 73:7894-7905
4. BACCIU, D., FALCHI, G., SPAZZIANI, A., BOSSI, L., MAROGNA, G., LEORI, G.S., RUBINO, S. & UZZAU, S. 2004. Transposition of the heat-stable toxin astA gene into a gifsy-2-related prophage of *Salmonella enterica* serovar Abortusovis. *Journal of Bacteriology*, 186:4568-4574
5. BAGGESEN, D.L., OLSEN, J.E. & BISGAARD, M. 1992. Plasmid Profiles and Phage Types of *Salmonella* Typhimurium Isolated from Successive Flocks of Chickens on 3 Parent Stock Farms. *Avian Pathology*, 21:569-579
6. BARROW, P.A., BUMSTEAD, N., MARSTON, K., LOVELL, M.A. & WIGLEY, P. 2004. Faecal shedding and intestinal colonization of *Salmonella enterica* in in-bred chickens: the effect of host-genetic background. *Epidemiology and Infection*, 132:117-126
7. BAUMLER, A.J., TSOLIS, R.M. & HEFFRON, F. 1996. Contribution of fimbrial operons to attachment to and invasion of epithelial cell lines by *Salmonella* Typhimurium. *Infection and Immunity*, 64:1862-1865
8. BENACER, D., THONG, K.L., WATANABE, H. & PUTHUCHEARY, S.D. 2010. Characterization of Drug-Resistant *Salmonella enterica* Serotype Typhimurium by Antibiograms, Plasmids, Integrons, Resistance Genes, and PFGE. *Journal of Microbiology and Biotechnology*, 20:1042-1052
9. BHAVSAR, A.P., BROWN, N.F., STOEPEL, J., WIERMER, M., MARTIN, D.D.O., HSU, K.J., IMAMI, K., ROSS, C.J., HAYDEN, M.R., FOSTER, L.J., LI, X., HIETER, P. & IMAMI, K. 2013. The *Salmonella* Type III Effector SspH2 Specifically Exploits the NLR Co-chaperone Activity of SGT1 to Subvert Immunity. *Plos Pathogens*, 9:e1003518
10. BISI-JOHNSON, M.A., OBI, C.L., VASAIKAR, S.D., BABA, K.A. & HATTORI, T. 2011. Molecular basis of virulence in clinical isolates of *Escherichia coli* and *Salmonella* species from a tertiary hospital in the Eastern Cape, South Africa. *Gut Pathogens*, 3:9
11. BORRIELLO, G., LUCIBELLI, M.G., PESCIAROLI, M., CARULLO, M.R., GRAZIANI, C., AMMENDOLA, S., BATTISTONI, A., ERCOLINI, D., PASQUALI, P. & GALIERO, G. 2012. Diversity of *Salmonella* spp. serovars isolated from the intestines of water buffalo calves with gastroenteritis. *BMC Veterinary Research*, 8:201

12. BRUSSOW, H., CANCHAYA, C. & HARDT, W. 2004. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiology and Molecular Biology Reviews*, 68:560-602tabeofontents
13. CAPUANO, F., MANCUSI, A., CAPPARELLI, R., ESPOSITO, S. & PROROGA, Y.T.R. 2013. Characterization of drug resistance and virulotypes of *Salmonella* strains isolated from food and humans. *Foodborne Pathogens and Disease*, 10:963-968
14. CHIU, C.H. & OU, J.T. 1996. Rapid identification of *Salmonella* serovars in faeces 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
15. CRUM-CIANFLONE, N.F. 2008. Salmonellosis and the gastrointestinal tract: More than just peanut butter. *Current Gastroenterology Reports*, 10:424-431
16. DE GROOTE, M.A., OCHSNER, U.A., SHILOH, M.U., NATHAN, C., MCCORD, J.M., DINAUER, M.C., LIBBY, S.J., VAZQUEZ-TORRES, A., XU, Y. & FANG, F.C. 1997. Periplasmic superoxide dismutase protects *Salmonella* from products of phagocyte NADPH-oxidase and nitric oxide synthase. *Proceedings of the National Academy of Sciences of the United States of America*, 94:13997-4001
17. DE MEDICI, D., CROCI, L., DELIBATO, E., DI PASQUALE, S., FILETICI, E. & TOTI, L. 2003. Evaluation of DNA extraction methods for use in combination with SYBR green I real-time PCR to detect *Salmonella enterica* serotype Enteritidis in poultry. *Applied and Environmental Microbiology*, 69:3456-3461
18. DIARRA, M.S., DELAQUIS, P., REMPEL, H., BACH, S., HARLTON, C., ASLAM, M., PRITCHARD, J. & TOPP, E. 2014. Antibiotic resistance and diversity of *Salmonella enterica* serovars associated with broiler chickens. *Journal of Food Protection*, 77:40-49
19. DRAHOVSKA, H., MIKASOVA, E., SZEMES, T., FICEK, A., SASIK, M., MAJTAN, V. & TURNA, J. 2007. Variability in occurrence of multiple prophage genes in *Salmonella* Typhimurium strains isolated in Slovak Republic. *FEMS Microbiology Letters*, 270:237-244
20. EFSA. 2008. Scientific Opinion of the Panel on Biological Hazards on a request from the European Commission on a quantitative microbiological risk assessment on *Salmonella* in meat: Source attribution for human salmonellosis from meat. *The EFSA Journal*, 625:1-32
21. EHRBAR, K. & HARDT, W. 2005. Bacteriophage-encoded type III effectors in *Salmonella enterica* subspecies 1 serovar Typhimurium. *Infection, Genetics and Evolution : Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*, 5:1-9
22. FEARNLEY, E., RAUPACH, J., LAGALA, F. & CAMERON, S. 2011. *Salmonella* in chicken meat, eggs and humans; Adelaide, South Australia, 2008. *International Journal of Food Microbiology*, 146:219-227
23. FIGUEROA-BOSSI, N., UZZAU, S., MALORIOL, D. & BOSSI, L. 2001. Variable assortment of prophages provides a transferable repertoire of pathogenic determinants in *Salmonella*. *Molecular Microbiology*, 39:260-71
24. FOLEY, S.L., JOHNSON, T.J., RICKE, S.C., NAYAK, R. & DANZEISEN, J. 2013. *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. *Microbiology and Molecular Biology Reviews*, 77:582-607
25. FUTAGAWA-SAITO, K., OKATANI, A.T., SAKURAI-KOMADA, N., BA-THEIN, W. & FUKUYASU, T. 2010. Epidemiological Characteristics of *Salmonella enterica* Serovar Typhimurium from Healthy Pigs in Japan. *Journal of Veterinary Medical Science*, 72:61-66
26. GUINEY, D.G. & FIERER, J. 2011. The role of the *spv* genes in *Salmonella* pathogenesis. *Frontiers in Microbiology*, 2:129

27. GULIG, P.A., DANBARA, H., GUINEY, D.G., LAX, A.J., NOREL, F. & RHEN, M. 1993. Molecular Analysis of Spv Virulence Genes of the *Salmonella* Virulence Plasmids. *Molecular Microbiology*, 7:825-830
28. GYLES, C. & BOERLIN, P. 2014. Horizontally transferred genetic elements and their role in pathogenesis of bacterial disease. *Veterinary Pathology*, 51:328-340
29. HARAGA, A. & MILLER, S.I. 2003. A *Salmonella enterica* serovar Typhimurium translocated leucine-rich repeat effector protein inhibits NF-kappa B-dependent gene expression. *Infection and Immunity*, 71:4052-4058
30. HELMUTH, R., STEPHAN, R., BUNGE, C., HOOG, B., STEINBECK, A. & BULLING, E. 1985. Epidemiology of virulence-associated plasmids and outer membrane protein patterns within seven common *Salmonella* serotypes. *Infection and Immunity*, 48:175-182
31. HENDRIKSEN, R.S., VIEIRA, A.R., KARLSMOSE, S., LO FO WONG, D.M.A., JENSEN, A.B., WEGENER, H.C. & AARESTRUP, F.M. 2011. Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. *Foodborne Pathogens and Disease*, 8:887-900
32. HEUER, H., ABDO, Z. & SMALLA, K. 2008. Patchy distribution of flexible genetic elements in bacterial populations mediates robustness to environmental uncertainty. *FEMS Microbiology Ecology*, 65:361-371
33. HO, T.D., FIGUEROA-BOSSI, N., WANG, M.H., UZZAU, S., BOSSI, L. & SLAUCH, J.M. 2002. Identification of GtgE, a novel virulence factor encoded on the Gifsy-2 bacteriophage of Salmonella enterica serovar Typhimurium. *Journal of Bacteriology*, 184:5234-5239
34. HUEHN, S., LA RAGIONE, R.M., ANJUM, M., SAUNDERS, M., WOODWARD, M.J., BUNGE, C., HELMUTH, R., HAUSER, E., GUERRA, B., BEUTLICH, J., BRISABOIS, A., PETERS, T., SVENSSON, L., MADAJCZAK, G., LITRUP, E., IMRE, A., HERRERA-LEON, S., MEVIUS, D., NEWELL, D.G. & MALORNY, B. 2010. Virulotyping and antimicrobial resistance typing of *Salmonella enterica* serovars relevant to human health in Europe. *Foodborne Pathogens and Disease*, 7:523-535
35. HUGHES, L.A., SHOPLAND, S., WIGLEY, P., BRADON, H., LEATHERBARROW, A.H., WILLIAMS, N.J., BENNETT, M., DE PINNA, E., LAWSON, B., CUNNINGHAM, A.A. & CHANTREY, J. 2008. Characterisation of *Salmonella enterica* serotype Typhimurium isolates from wild birds in northern England from 2005-2006. *BMC Veterinary Research*, 4
36. HUNTER, P.R. & GASTON, M.A. 1988. Numerical Index of the Discriminatory Ability of Typing Systems - an Application of Simpsons Index of Diversity. *Journal of Clinical Microbiology*, 26:2465-2466
37. KARIUKI, S., REVATHI, G., KARIUKI, N., MUYODI, J., MWITURIA, J., MUNYALO, A., KAGENDO, D., MURUNGI, L. & HART, C.A. 2005. Increasing prevalence of multidrug-resistant non-typhoidal salmonellae, Kenya, 1994-2003. *International Journal of Antimicrobial Agents*, 25:38-43
38. KEDDY, K.H., DWARIKA, S., CROWTHER, P., PEROVIC, O., WADULA, J., HOOSEN, A., SOOKA, A., CREWE-BROWN, H.H. & SMITH, A.M. 2009. Genotypic and demographic characterization of invasive isolates of *Salmonella* Typhimurium in HIV co-infected patients in South Africa. *Journal of infection in Developing Countries*, 3:585-592
39. KIDANEMARIAM, A., ENGELBRECHT, M. & PICARD, J. 2010. Retrospective study on the incidence of *Salmonella* isolations in animals in South Africa, 1996 to 2006. *Journal of the South African Veterinary Association*, 81:37-44
40. KOHLER, A.C., SPANÒ, S., GALÁN, J.E. & STEBBINS, C.E. 2014. Structural and enzymatic characterization of a host-specificity determinant from *Salmonella. Acta Crystallographica Section D: Biological Crystallography*, 70:384-391

41. LEE, A.T.Y., HALL, R.G. & PILE, K.D. 2005. Reactive joint symptoms following an outbreak of *Salmonella* Typhimurium phage type 135a*. Journal of Rheumatology*, 32:524-527
42. LI, H., XU, H., ZHOU, Y., ZHANG, J., LONG, C., LI, S., CHEN, S., ZHOU, J. & SHAO, F. 2007. The phosphothreonine lyase activity of a bacterial type III effector family*. Science*, 315:1000-1003
43. LIBBY, S.J., ADAMS, L.G., FICHT, T.A., ALLEN, C., WHITFORD, H.A., BUCHMEIER, N.A., BOSSIE, S. & GUINEY, D.G. 1997. The *spv* genes on the *Salmonella* Dublin virulence plasmid are required for severe enteritis and systemic infection in the natural host*. Infection and Immunity*, 65:1786-1792
44. LIM, Y., HIROSE, K., IZUMIYA, H., ARAKAWA, E., TAKAHASHI, H., TERAJIMA, J., ITOH, K., TAMURA, K., KIM, S. & WATANABE, H. 2003. Multiplex polymerase chain reaction assay for selective detection of *Salmonella enterica* serovar Typhimurium. *Japanese Journal of Infectious Diseases*, 56:151-155
45. LY, K.T. & CASANOVA, J.E. 2007. Mechanisms of *Salmonella* entry into host cells. *Cellular Microbiology*, 9:2103-2111
46. MATSUI, H., BACOT, C.M., GARLINGTON, W.A., DOYLE, T.J., ROBERTS, S. & GULIG, P.A. 2001. Virulence plasmid-borne spvB and spvC genes can replace the 90-kilobase plasmid in conferring virulence to *Salmonella enterica* serovar Typhimurium in subcutaneously inoculated mice. *Journal of Bacteriology*, 183:4652-4658
47. MIAO, E.A., BRITTNACHER, M., HARAGA, A., JENG, R.L., WELCH, M.D. & MILLER, S.I. 2003. *Salmonella* effectors translocated across the vacuolar membrane interact with the actin cytoskeleton*. Molecular Microbiology*, 48:401-415
48. MIKASOVA, E., DRAHOVSKA, H., SZEMES, T., KUCHTA, T., KARPISKOVA, R., SASIK, M. & TURNA, J. 2005. Characterization of *Salmonella enterica* serovar Typhimurium strains of veterinary origin by molecular typing methods. *Veterinary Microbiology*, 109:113-120
49. MIROLD, S., RABSCH, W., ROHDE, M., STENDER, S., TSCHAPE, H., RUSSMANN, H., IGWE, E. & HARDT, W.D. 1999. Isolation of a temperate bacteriophage encoding the type III effector protein SopE from an epidemic *Salmonella* Typhimurium strain*. Proceedings of the National Academy of Sciences of the United States of America*, 96:9845-9850
50. MOHAMED, T., ZHAO, S., WHITE, D.G. & PARVEEN, S. 2014. Molecular characterization of antibiotic resistant *Salmonella* Typhimurium and *Salmonella* Kentucky isolated from pre- and post-chill whole broilers carcasses*. Food Microbiology*, 38:6-15
51. OBI, C.L., POTGIETER, A., MUSIE, E.M., BESSONG P.O., SAMIE A. & VENTER S.N. 2007. *Human and environmental associated Salmonella isolates from different sources in the Venda region of South Africa.*, Cap Town / South Africa ed. Cap Town: Water SA
52. POPOFF, M.Y., BOCKEMUHL, J., BRENNER, F.W. & GHEESLING, L.L. 2001. Supplement 2000 (no. 44) to the Kauffmann-White scheme*. Research in Microbiology*, 152:907-909
53. PULSENET. 2013. *Standard operating procedure for Pulsenet PFGE of Escherichia coli O157:H7, Escherichia coli non-O157 (STEC), Salmonella serotypes, Shigella sonnei and Shigella flexneri*, http://www.pulsenetinternational.org/assets/PulseNet/uploads/pfge/PNLO5 Ec-Sal-ShigPFGEprotol.pdf; ed
54. RYCHLIK, I., GREGOROVA, D. & HRADECKA, H. 2006. Distribution and function of plasmids in *Salmonella enterica. Veterinary Microbiology*, 112:1-10
55. RYCHLÍK, I., HRADECKA, H. & MALCOVA, M. 2008. *Salmonella enterica* serovar Typhimurium typing by prophage-specific PCR*. Microbiology*, 154:1384-1389
56. SCHMIDT, H. & HENSEL, M. 2004. Pathogenicity islands in bacterial pathogenesis. *Clinical Microbiology Reviews*, 17:14-56

57. SCHULZE, T., LÜDTKE, A., RAHLFF, I., TUNN, P.-. & HOHENBERGER, P. 2009. *Salmonella* osteomyelitis in an immunocompromized patient presenting as a primary lymphoma of the bone. *International Journal of Infectious Diseases*, 13:e67-e70
58. SILVA C., WIESNER M. & CALVA E. 2012. The Importance of Mobile Genetic Elements in the Evolution of *Salmonella*: Pathogenesis, Antibiotic Resistance and Host Adaptation., in *Salmonella - A Diversified Superbug*, edited by Kumar Y., available from http://www.intechopen.com/books: InTech: 231-254
59. SKYBERG, J.A., LOGUE, C.M. & NOLAN, L.K. 2006. Virulence genotyping of *Salmonella* spp. with multiplex PCR. *Avian Diseases*, 50:77-81
60. STANLEY, T.L., ELLERMEIER, C.D. & SLAUCH, J.M. 2000. Tissue-specific gene expression identifies a gene in the lysogenic phage Gifsy-1 that affects *Salmonella enterica* serovar Typhimurium survival in Peyer's patches. *Journal of Bacteriology*, 182:4406-13
61. STEIN, M.A., LEUNG, K.Y., ZWICK, M., GARCIADELPORTILLO, F. & FINLAY, B.B. 1996. Identification of a *Salmonella* virulence gene required for formation of filamentous structures containing lysosomal membrane glycoproteins within epithelial cells. *Molecular Microbiology*, 20:151-164
62. SWAMINATHAN, B., BARRETT, T.J., HUNTER, S.B., TAUXE, R.V. & CDC PULSENET TASK FORCE. 2001. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. *Emerging Infectious diseases*, 7:382-9
63. TAMAMURA, Y., UCHIDA, I., TANAKA, K., OKAZAKI, H., TEZUKA, S., HANYU, H., KATAOKA, N., MAKINO, S., KISHIMA, M., KUBOTA, T., KANNO, T., HATAMA, S., ISHIHARA, R., HATA, E., YAMADA, H., NAKAOKA, Y. & AKIBA, M. 2011. Molecular Epidemiology of *Salmonella enterica* Serovar Typhimurium Isolates from Cattle in Hokkaido, Japan: Evidence of Clonal Replacement and Characterization of the Disseminated Clone. *Applied and Environmental Microbiology*, 77:1739-1750
64. THRELFALL, E., WARD, L. & ROWE, B. 1998. Multiresistant *Salmonella* Typhimurium DT 104 and *Salmonella* bacteraemia. *The Lancet*, 352:287-288
65. TSOLIS, R.M., TOWNSEND, S.M., MIAO, E.A., MILLER, S.I., FICHT, T.A., ADAMS, L.G. & BAUMLER, A.J. 1999. Identification of a putative *Salmonella enterica* serotype Typhimurium host range factor with homology to IpaH and YopM by signature-tagged mutagenesis. *Infection and Immunity*, 67:6385-6393
66. VAN SORGE, N.M., ZIALCITA, P.A., BROWNE, S.H., QUACH, D., GUINEY, D.G. & DORAN, K.S. 2011. Penetration and activation of brain endothelium by *Salmonella enterica* serovar Typhimurium. *Journal of Infectious Diseases*, 203:401-405
67. WATERMAN, S.R. & HOLDEN, D.W. 2003. Functions and effectors of the *Salmonella* pathogenicity island 2 type III secretion system. *Cellular Microbiology*, 5:501-511

CHAPTER 4: ANTIMICROBIAL RESISTANCE PROFILES, PREVALENCE OF INTEGRONS AND *SALMONELLA* TYPHIMURIUM DEFINITIVE PHAGE TYPE (DT) 104 AMONG *SALMONELLA* TYPHIMURIUM ISOLATED BETWEEN 1995 AND 2002 FROM ORGANS AND ENVIRONMENTS OF DISEASED POULTRY IN SOUTH AFRICA.

4.1. Introduction

Non-typhoidal *Salmonella* (NTS) *enterica* serovars are major foodborne pathogens which cause annually 93.8 million of human gastroenteritis and 150,000 deaths worldwide (Majowicz *et al.* 2010). At least 2.5 million illnesses and 4 100 deaths have been ascribed to non-typhoidal salmonellae in Africa (Majowicz *et al.* 2010). In South Africa, 16,211 cases of human NTS salmonellosis were reported between 2003 and 2010 (Crowther-Gibson *et al.* 2011). *Salmonella* Typhimurium is the second serovar which causes non-typhoidal salmonellosis characterised by gastroenteritis in humans (Hendriksen *et al.* 2011). Human *S.* Typhimurium outbreaks are mainly caused by ingestion of contaminated poultry products (Fearnley *et al.* 2011). Although, *S.* Typhimurium infections are mainly associated with gastroenteritis which often resolves without treatment, in some cases, *S.* Typhimurium strains can be invasive and cause systemic infections and various complications that require antimicrobial therapy (Feasey *et al.* 2015). Complications include bacteraemia (Threlfall, Ward & Rowe 1998), meningitis (Van Sorge *et al.* 2011), osteomyelitis (Schulze *et al.* 2009), and arthritis (Lee, Hall & Pile 2005). In South Africa, systemic infections caused by *S.* Typhimurium have been mainly associated with bacteraemia in immune-compromised patients (Keddy *et al.* 2009).

In most instances, severe and persistent *S.* Typhimurium infections have been primarily attributed to multidrug resistant *S.* Typhimurium strains (Threlfall 2000). Most *S.* Typhimurium isolates associated with persistent and systemic infections are invasive and commonly resistant to ampicillin, chloramphenicol, nalidixic acid, trimethoprim, trimethoprim - sulfamethoxazole, and

ceftriaxone (Kariuki *et al.* 2015). Invasive *S.* Typhimurium isolates that are multi-drug resistant have also been reported in Malawi (Feasey *et al.* 2015), and South Africa (Keddy *et al.* 2009). Infections caused by multi-drug resistant *S.* Typhimurium in humans are characterised by longer hospitalisation and high risk of death (Helms *et al.* 2002). The mechanism of antimicrobial resistance consists of enzymatic reactions resulting in changes that affect bacterial cell permeability, drug activity site modification, destruction or inhibition and energetic rejection of antimicrobials by efflux pumps located on bacterial membrane (Morar & Wright 2010, Schwarz, Kehrenberg & Walsh 2001). These enzymes are produced via expression of resistance genes such as *blaTEM-1, blaSHV-1, blaCMY-1* and *blaPSE-1* for β-lactams (Livermore 1995); chloramphenicol acetyltransferases (*cat1* and *cmlA*), and florfenicol and chloramphenicol resistance genes (*flo / floR*) (Bissonnette *et al.* 1991). Other genes which produce enzymes are: *tetA, tetB, tetC,* and *tetG* for tetracycline (Roberts 1996); aminoglycosides acetyltransferases (*aac*), aminoglycosides adenylytransferases (*ant* or *aad*), and aminoglycosides phosphotransferases *(aph)* (Shaw *et al.* 1993). Others include plasmid mediated – quinolones resistance (QMQR) genes such as *qnrA, qnrB,* and *qnrS* (Tran, Jacoby & Hooper 2005)*;* dihydrofolate reductase (*dhfr*) for trimethoprim (Huovinen *et al.* 1995); *sul1, sul2* and *sul3* for sulphonamides (Schwarz *et al.* 2001); *mer A, mer B, mer C, mer D, mer R,* and *mer T* for mercury and organomercury (Kondo, Ishikawa & Nakahara 1974); and *qacE,* and *qacEΔ1* for quaternary ammonium (Kucken 2000). These resistance genes are mainly located on mobile genetic elements such as plasmids, transposons, and integrons (Schwarz *et al.* 2001).

Integrons are genetic elements composed of integrase gene (*int*), a specific recombination site (*attI*), and a promoter (Hall & Collis 1995). The integrase catalyses the integration of mobile gene cassettes at a specific recombination site (*attI*) while the promoter is responsible for the expression of gene cassettes which harbour antibiotic resistance genes. This process contributes to the occurrence and dissemination of resistance genes in bacterial species (Hall & Collis 1995,

Cambray, Guerout & Mazel 2010). There are three well-documented classes (class1, 2, and 3) of integrons which are associated with multi-drug resistance but only class 1 integron was frequently observed in *S.* Typhimurium definitive phage 104 (DT 104) strains (Boyd *et al.* 2001). Although resistant integrons have been reported in several studies (Lindstedt *et al.* 2003, Maka *et al.* 2015, Ahmed & Shimamoto 2012, Yu *et al.* 2011), there are no reports on the prevalence of integrons in *S.* Typhimurium isolates from poultry in South Africa.

DT 104 is the prototype of multi-resistant *Salmonella* strains that was first identified in United Kingdom from cattle in 1984 (Schmieger & Schicklmaier 1999) before becoming a common pathogen of many food-producing animals such as poultry, pigs, sheep and humans (Threlfall 2000). The sources of human infections with multi-drug resistant DT104 strain include the ingestion of chicken (Wall *et al.* 1994). DT104 harbours five resistance genes in its DNA segment referred to as *Salmonella* genomic island (SGI) (Boyd *et al.* 2001, Briggs & Fratamico 1999). Those genes include *pse-1, flo, aadA2, sul1,* and *tetG* encoding respectively resistance to ampicillin, chloramphenicol / florfenicol, streptomycin / spectinomycin, sulphonamides and tetracycline. This resistance type is also termed ACSSuT (Boyd *et al.* 2001, Briggs & Fratamico 1999). Since its discovery, DT104 has become a public health concern due to its worldwide spread, and its ability to acquire additional resistance patterns to other clinically important antimicrobials including amoxicillin - clavulanic acid, nalidixic acid and ciprofloxacin (Helms, Simonsen & Molbak 2004, Mindlin *et al.* 2013, Ruiz *et al.* 2008). DT104 is also known to be more virulent and widespread than other non-DT104 *Salmonella* (Helms 2005). Between 2006 and 2007, ninety three per cent (93%) of 857 *S.* Typhimurium were isolated from HIV-positive patients in Gauteng province, South Africa, and 13% exhibited resistance to ampicillin; chloramphenicol, streptomycin, sulphonamide, tetracycline and nalidixic acid (ACSSuTN) (Keddy *et al.* 2009). This resistance type is characteristic of the DT104 strain (Boyd *et al.* 2001). This is the only report on the prevalence of ACSSuT isolates in South Africa within the limits of our literature search.

In South Africa, the poultry industry is among the largest consumers of antimicrobial agents used for metaphylaxis, growth promotion and therapy (Eagar 2008). *Salmonella* isolates that were mainly resistant to streptomycin, sulphonamides, and tetracycline have been isolated from poultry carcasses in South Africa (Manie *et al.* 1998, Gouws & Brozel 2000). Although foodborne outbreaks are under-reported in South Africa, chickens are important reservoirs of human *Salmonella* outbreaks and play an important role in the transmission of antimicrobial resistance to humans (Fearnley *et al.* 2011). Data from South Africa indicate that *S.* Typhimurium is the most NTS commonly isolated in poultry (Kidanemariam, Engelbrecht & Picard 2010). However, there is a paucity of information on antimicrobial resistance profiles and genetic determinants of antimicrobial resistance in *S.* Typhimurium isolates of poultry origin from South Africa. The objectives of this study therefore were: (1) to determine antimicrobial resistance profiles of *S.* Typhimurium from poultry organs and production environments by disk diffusion method, (2) to identify the genetic determinants of resistance through genotyping, and (3) to determine the prevalence of DT104 strains using disk diffusion and genotyping. The isolates under study were recovered from poultry organs and production environments during a period spanning from 1995 to 2002 from various poultry farms in South Africa.

4.2. Materials and methods

4.2.1. Samples

A total of 141 *S.* Typhimurium isolates from poultry organs and poultry production environments that were isolated from 1995 - 2002 were used in this study. One hundred and fifteen (115) isolates were from poultry organs (liver, lungs, intestines, and peritoneum) and 26 from poultry house environments (litter and dust). Sixty nine (69) isolates were recovered from samples that were collected from more than 22 poultry farms (F1-F22) located in 5 provinces of South Africa (Gauteng, Limpopo, KwaZulu-Natal, North West, and Free State) while 72 isolates were of unknown origin (data on origin of isolates was unavailable). The isolates were serotyped according to the White-Kauffman-Le Minor Scheme by agglutination with O and H antigen

(Onderstepoort *Salmonella* Reference Laboratory) against specific antisera. The isolates were diagnostic and surveillance samples belonging to the Bacteriology laboratory at the Faculty of Veterinary Science, University of Pretoria.

4.2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility test was performed by the disk diffusion method (Bauer *et al.* 1966) against a panel of 16 antimicrobial agents including ampicillin (AM: 10 µg), amoxicillin-clavulanic acid (AMC: 30 µg), cefotaxime (CTX: 30 µg), kanamycin (K: 30 µg), gentamicin (GM: 10 µg), streptomycin (S10: 10 µg), colistin sulphate (CT: 10 µg), tetracycline (TE: 30 µg), sulfamethoxazole - trimethoprim (SXT: 25 µg), enrofloxacin (ENR: 5 µg), sulphonamides (S3: 30 µg) (Oxoid, UK); cephalothin (CF: 30 µg), ceftazidime (CAZ: 30 µg), chloramphenicol (C: 30 µg), nalidixic acid (NA: 30 µg), and ciprofloxacin (CIP: 5 µg) (Becton Dickinson, Australia). Bacteria were adjusted to a McFarland, 0.5 turbidity standard. Swabs soaked in adjusted bacterial suspension were spread on Mueller-Hinton agar (MHA) plates. Disks impregnated with antimicrobial agents were applied with a disk dispenser on MHA plates that were incubated at 35°C for 18 hrs. *Escherichia coli* 25922 was used as a reference strain. Antimicrobial sensitivity was evaluated using zone diameter interpretive criteria according to the Clinical and Laboratory Standards Institute (CLSI. 2013). Isolates were first classified as susceptible, intermediate or resistant to each antimicrobial agent, then the intermediate readings were assigned to the resistant category. In this study, an antimicrobial resistance profile (phenotype, and genotype) was considered as a predominant profile if it was observed in 2 or more isolates.

4.2.3. DNA extraction

DNA was extracted from cultures of *S.* Typhimurium isolates by the boiling method as described previously (De Medici *et al.* 2003). Briefly, one loop-full of bacterial culture from overnight incubation was mixed with 1 ml FA buffer (Becton Dickinson and Company Sparks, USA) in 1.5 ml eppendorf tube. The cell suspension was vortexed and centrifuged for 5 min. The

supernatant was discarded and the cell pellet re-suspended in FA buffer. This procedure was repeated 3 times. The homogeneous cell suspension was boiled at 100˚C for 30 min and, stored at -20˚C until use.

4.2.4. Antimicrobial resistance genes and integrons detection

PCR was performed to screen for the presence of 27 resistance genes including *blaTEM, blaCMY-2,* for beta lactams, *sul3* for sulphonamides (Kozak *et al.* 2009); *blaSHV,* and *blaPSE* for beta lactams*, cat1, flo,* and *cmlA* for chloramphenicol, *ant(3')-Ia* for streptomycin*, aac(3)-Iva* for gentamycin*, aphA(3')-IIa* for kanamycin*, dfrI, dfrXII,* and *dfrXIII* for sulfamethoxazole – trimethoprim (Chen *et al.* 2004); *tetA,* and *tetC* for tetracycline (Lanz, Kuhnert & Boerlin 2003); *tetB* for tetracycline (Goswami *et al.* 2008); *tetG* for tetracycline, and *str* for streptomycin (Chiu *et al.* 2006); *aadB* for gentamycin*, aphA2* for kanamycin (Travis *et al.* 2006); *aphA1* for kanamycin (Maynard *et al.* 2003); *qnrA, qnrB, qnrS* for ciprofloxacin, enrofloxacin and nalidixic acid (Gay *et al.* 2006); *sul1* for sulphonamides (Kerrn *et al.* 2002); *sul2* for sulphonamides (Aarestrup *et al.* 2003). Briefly, PCR reaction (20 µl) contained 1x Buffer, 200 mM of each dNTP, and 1.25 U of *Taq* DNA polymerase; (New England Biolabs, USA), 2.5 mM $MgCl_2$, 2.5 µl of template DNA, and primers (Integrated DNA Technologies , USA) (Appendix 4.1). PCR was performed in either a Veriti Thermal cycler (Applied BioSystems, USA) or a CT1000 Touch Thermal Cycler (Bio-Rad, USA). PCR conditions for multiplex and single PCRs for detection of resistance genes are described in Appendix 4.1. Briefly, cycling conditions consisted of one cycle of initial denaturation at 94˚C for 3 min, 30 cycles of denaturation at 94˚C for 30 sec, various annealing respective temperatures for respective genes (Appendix 4.1), extension at 72˚C for 1 min followed by a final extension at 72˚C for 10 min. PCR products were electrophoresed on 1.5% agarose containing 0.5 mg/ml ethidium bromide and visualized under UV light in a Gel Doc system (Bio-Rad, USA).

Isolates were also screened by PCR for the presence of *int1, int2* and *int3* genes that are carried on class 1, 2 and 3 integrons respectively. Primer and PCR conditions were according to

previously described protocols (Lindstedt *et al.* 2003, White, McIver & Rawlinson 2001) (Appendix 4.1). Briefly, PCR reactions (20 µl) contained 1x buffer, 2.5 mM $MgCl_2$, 200 mM of each dNTP (New England BioLabs USA); primer (Integrated DNA Technology, USA) (Appendix 4.1), 1.25 U of *Taq* DNA polymerase (New England BioLabs, USA) and 2.5 µl of DNA template. PCR cycling conditions consisted of an initial denaturation at 94˚C for 3 min, 30 cycles of denaturation at 94˚C for 30 sec, various annealing temperatures for respective genes 30 sec (White *et al.* 2001) (Appendix 4.1), extension at 72˚C for 30 sec, and 1 cycle of final extension at 72˚C for 8 min (Appendix 4.1). PCR products were electrophoresed on 1.5% agarose containing 0.5 mg/ml ethidium bromide and photographed under UV light in Gel Doc system (Bio-Rad, USA).

4.2.5. Detection of DT104

Salmonella Typhimurium definitive type 104 isolates were identified phenotypically using the disk diffusion method by screening for 5 antimicrobial agents (ACSSuT resistance – type) including: ampicillin (AM: 10 µg), streptomycin (S10: 10 µg), tetracycline (TE: 30 µg), sulphonamides (S3: 30 µg) (Oxoid, UK); and chloramphenicol (C: 30 µg), (Becton Dickinson, Australia). The isolates were also confirmed by multiplex PCR as previously described by (Khan *et al.* 2000). The PCR protocol targeted gene sequences encoding florofenicol - chloramphenicol (flo_{st}) resistance, class 1 integrons (*int1*), invasion gene (*invA*), and plasmid- encoded virulence gene (*spvC*). Briefly, PCR mixture (20 µl) contained 1x buffer, 200 mM of each dNTP, 1.25 U of *Taq* DNA polymerase (New England Biolabs, USA), 0.3 µM of each primer (Integrated DNA Technologies, USA), 2.5 mM of $MgCl_2$, 3 µl of template DNA. Amplification was performed in either a Veriti thermal cycler (Applied Biosystems, USA) or a CT1000 Touch thermal cycler (Bio-Rad, USA) using the following PCR conditions: one cycle of initial denaturation at 94˚C for 3 min, 30 cycles of denaturation at 94˚C for 45 sec, annealing at 60˚C for 45 sec, extension at 72˚C for 90 sec, and the final extension at 72˚C for 5 min. PCR products were electrophoresed on 1.5%

agarose containing 0.5 mg/ml ethidium bromide and visualized under UV light in a Gel Doc system (Bio-Rad, USA).

4.2.6. Statistical analysis

Descriptive statistics were conducted on all data. Significant differences of antimicrobial resistance (phenotype and genotype) between isolates from organs and those from environments were evaluated using the two sample t-test. The Kappa value (coefficient of agreement) was calculated to establish the agreement between values of antimicrobial resistance phenotype and those of corresponding genotypes. Statistical analysis was performed using intercooled Stata 9 software (Stata Corporation, College Station, TX, USA).

4.3. Results

4.3.1. Antimicrobial susceptibility testing

Only 2.1% (3/141) of the isolates (ST 41, ST50 and ST73) were susceptible to all 16 antimicrobial agents while 1.4% (2/141) of the isolates (ST100 and ST103) were resistant to all 16 antimicrobial agents. Out of 141 isolates, multi-drug resistance (resistance to 2 or more antimicrobials) was observed in 97.2% (137/141) of isolates and 79.4% (112/141) of the isolates were from organs while 17.7% (25/141) were from poultry environments. Over seventy five per cent (75.8%, 107/141) of the isolates were resistant to at least 5 different combinations of antimicrobial agents. Fourteen per cent (14.2%, 20/141) of the isolates were resistant to at least 8 different combinations of antimicrobial agents (Table 4.1; Appendix 4.2).

Table 4.1. Multi-drug resistance of *Salmonella* Typhimurium isolated from diseased poultry

No. of antimicrobials	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
N_o of resistant isolates	3	1	6	10	14	19	13	14	20	13	11	8	3	2	0	2	2	141

The frequencies of resistance in descending order were as follows: streptomycin 94.3% (133/141), sulphonamides 86.5% (123/141), ciprofloxacin 79.4% (112/141), tetracycline 61% (86/141), cefotaxime 55.3% (78/141), ampicillin 46.1% (65/141), chloramphenicol 44% (62/141), and kanamycin 42.6% (60/141) (Figure 4.1). The 18.4% (26/141) of the isolates resistant to amoxicillin - clavulanic acid were also resistant to ampicillin. Simultaneous resistance to cephalothin, cefotaxime, and ceftazidime (extended-spectrum cephalosporins) was observed in 20.6% (29/141) of the isolates. Simultaneous resistance to ampicillin, cephalothin, cefotaxime and ceftazidime (broad-spectrum β-lactams) was observed in 14.1% (20/141) of the isolates. Eleven per cent: 11% (16/141) of the isolates were resistant to streptomycin, gentamicin and kanamycin. Five per cent 5.7% (8/141) of isolates showed concurrent resistance to ciprofloxacin, enrofloxacin and nalidixic acid (Appendix 4.2). All 19.1% (27/141) of the isolates from organs that were resistant to sulfamethoxazole – trimethoprim were also resistant to sulphonamides (Appendix 4.2).

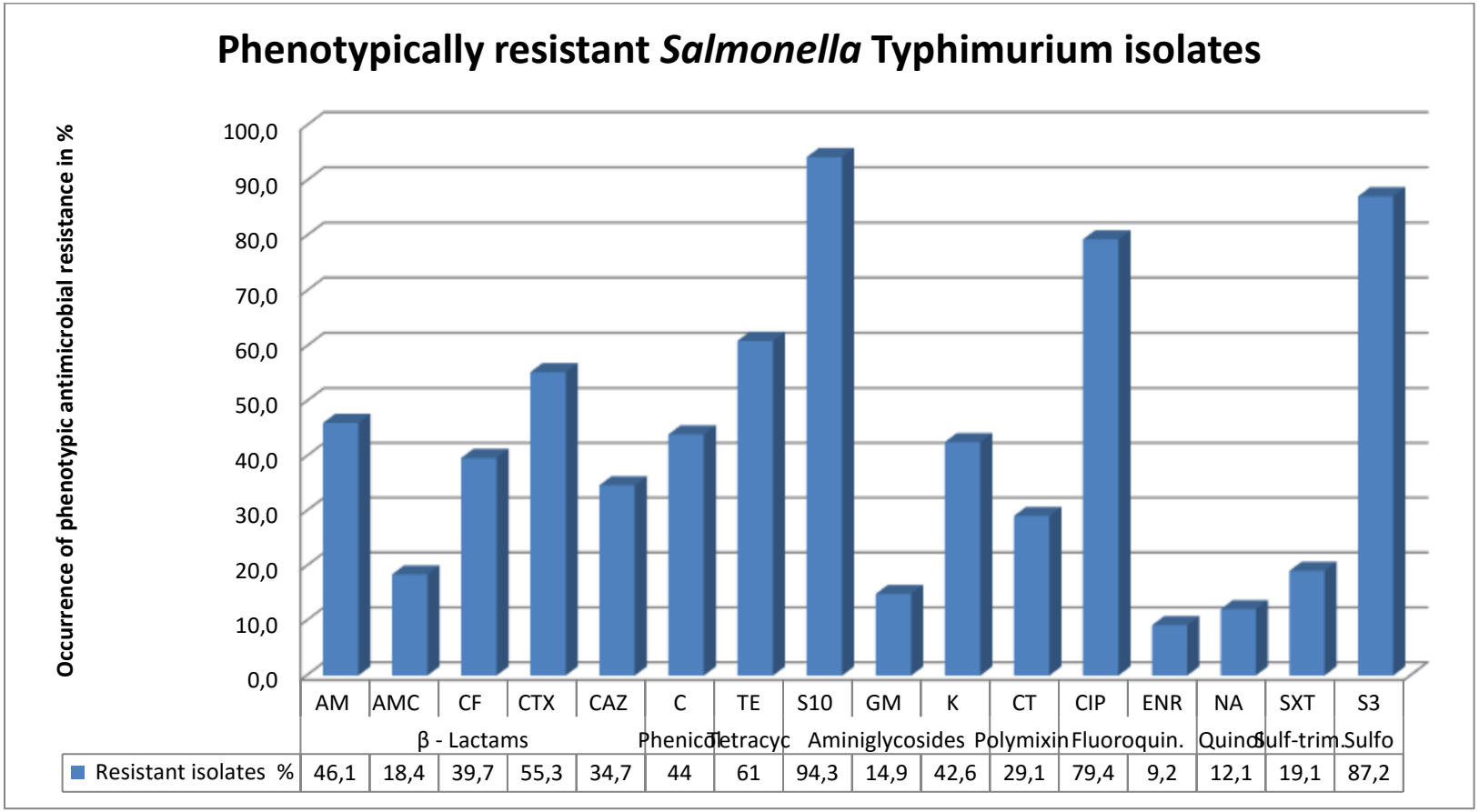


Figure 4.1. Prevalence of resistance to different antimicrobial agents among *S.* Typhimurium isolated from diseased poultry

AM: ampicillin; AMC: amoxicillin - clavulanic acid; CF: cephalotin; CTX: cefotaxime; CAZ: ceftazidime; C: chloramphenicol; TE: tetracycline; S10: streptomycin; GM: gentamycin; K: kanamycin; CT: colistin; CIP: ciprofloxacin; ENR: enrofloxacin; NA: nalidixic acid; SXT: sulfamethoxazole - trimethoprim; S3: sulphonamides.

The levels of resistance that were recorded in isolates from poultry organs in descending order were as follows: streptomycin 93% (107/115), sulphonamides 85.2% (98/115), ciprofloxacin 77.4% (89/115), tetracycline 74.8% (86/115), chloramphenicol 53.9% (62/115), ampicillin 52.2% (60/115) (Table 4.2). The frequencies of resistance that were recovered in isolates from poultry environments in descending order were as follows: streptomycin 100% (26/26), sulphonamides 96.2% (25/26), ciprofloxacin 88.5% (23/26), cefotaxime 84.6% (22/26), cephalothin 65.4% (17/26) (Table 4.2). Isolates from poultry organs were more resistant ($p \leq 0.05$) to ampicillin, amoxicillin - clavulanic acid, chloramphenicol, tetracycline and sulfamethoxazole - trimethoprim while isolates collected from poultry environments were more resistant ($p \leq 0.05$) to cephalothin, cefotaxime, cefotazidime, colistin sulphate and nalidixic acid (Table 4.2).

Table 4.2. Prevalence of poultry *Salmonella* Typhimurium resistant isolates to antimicrobials per origin of isolation.

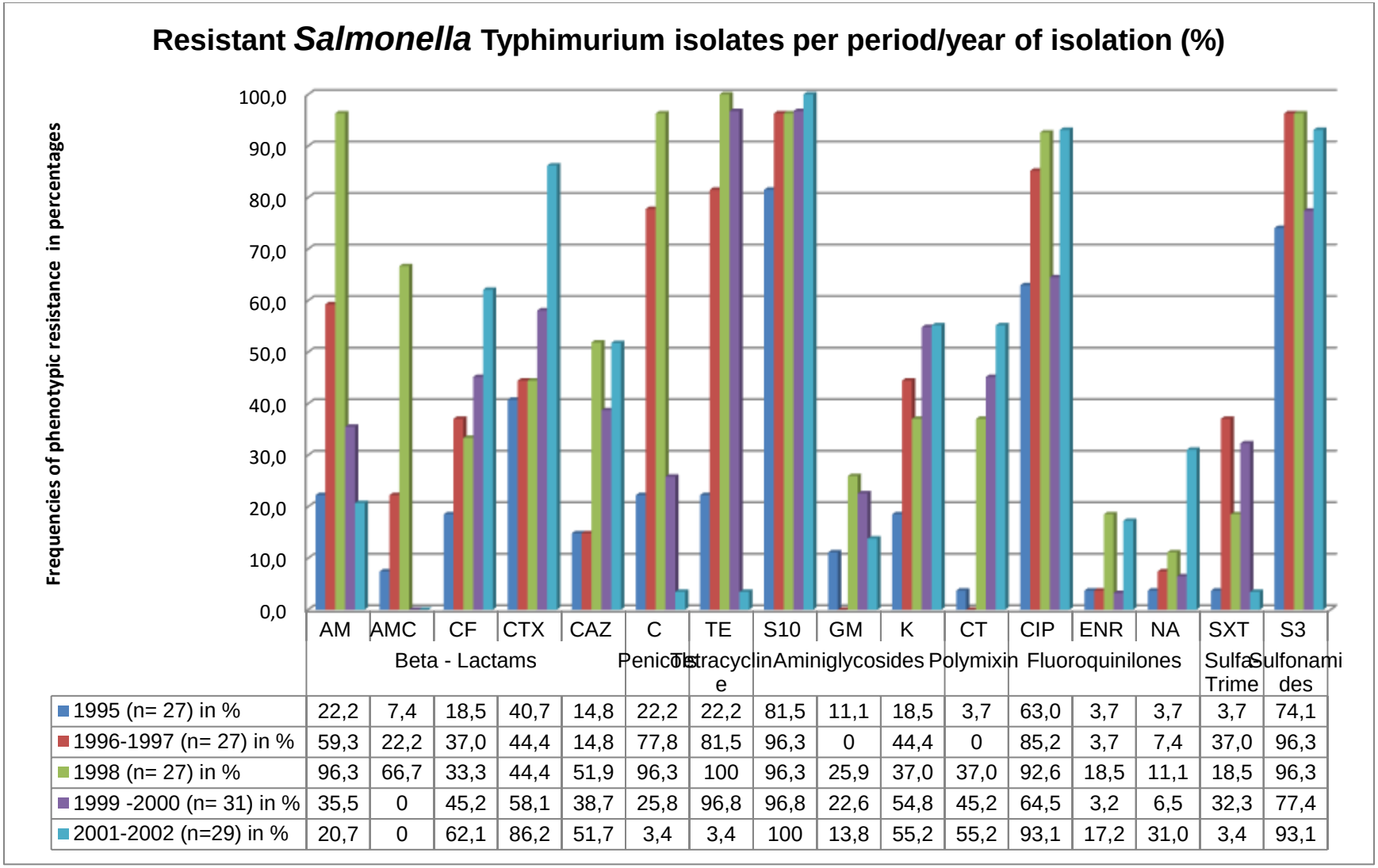
Classes	**Antibiotics**	**Resistant isolates n=141 (%)**	**Resistant isolates from organs n=115 (%)**	**Resistant isolates from envir. n= 26 (%)**	***p value***
Beta - Lactams	AM	65 (46.1)	60 (52.2)	5 (19.2)	0.002*
	AMC	26 (18.4)	26 (22.6)	0 (0.0)	0.008*
	CF	56 (39.7)	39 (33.9)	17 (65.4)	0.004*
	CTX	78 (44.9)	56 (48.7)	22 (84.6)	0.001*
	CAZ	49 (40.4)	35 (30.4)	14 (53.8)	0.025*
Phenicol	C	62 (44)	62 (53.9)	0 (0.0)	0.000*
Tetracycline	TE	86 (61)	86 (74.8)	0 (0.0)	0.000*
Aminiglyco-sides	S10	133 (94.3)	107(93.0)	26(100.0)	0.167
	GM	21 (14.9)	17 (14.8)	4 (15.4)	0.938
	K	60 (42.6)	46 (40.0)	14 (53.8)	0.201
Polymixin	CT	41 (29.1)	26 (22.6)	15 (57.7)	0.001*
Fluoroquinolones & quinolones	CIP	112 (79.4)	89 (77.4)	23 (88.5)	0.208
	ENR	13 (9.2)	9 (7.8)	4 (15.4)	0.228
	SXT	27 (19.1)	27 (23.5)	0 (0.0)	0.006*
	NA	17 (12.1)	9 (7.8)	8 (30.8)	0.001*
Sulphonamide	S3	133 (87.2)	98 (85.2)	25 (96.2)	0.131

*: significant

Salmonella Typhimurium isolated in 1998 were resistant to tetracycline 100% (27/27), ampicillin 96.3% (26/27), chloramphenicol 96.3% (26/27), sulphonamides 96.3% (26/27), amoxicillin - clavulanic acid 66.7% (18/27), ceftazidime 51.9% (14/27), gentamycin 25.8% (7/27), and enrofloxacin 18.5% (5/27). Isolates that were recovered between 2001 and 2002 were resistant to streptomycin 100% (29/29), ciprofloxacin 93.1% (25/29), cefotaxime 86.1% (25/29), cephalothin 62.1% (18/29), kanamycin 55.2% (16/29), colistin 55.2% (16/29), and nalidixic acid 31% (9/29) (Figure 4. 2).

4.3.2. Distribution of resistance genes and integrons in *Salmonella* Typhimurium isolates from diseased poultry

The highest frequencies of resistance genes in descending order were: *qnrA* which was recovered from 79.4% (112/141) of the isolates resistant to fluoroquinolones (ciprofloxacin, enrofloxacin and nalidixic acid) followed by *sul1* 66% (92/141) for sulphonamides, ***ant3'la*** 60.3% (85/141) for streptomycin, and *blaSHV* 57.4% (81/141) for beta lactams. The other resistance genes were: *str* 50.4% (71/141) for streptomycin, *aphA1* 47.5% (67/141) for kanamycin, *blaTEM* 42.5% (60/141) for beta lactams (Figure 4.3). Only two isolates (ST90 and ST94) that were isolated from organs in 1998 carried both *flo* and *cmlA* concurrently. Eight per cent 8.5% (12/141) of the isolates carried concurrently *tetA* and *tetB*; other 8.5% (12/141) of the isolates were positive to both *tetB* and *tetG*. Twenty two per cent 22.7% (32/141) of the isolates carried simultaneously *sul1* and *sul3* (Appendix 4.2).



	AM	AMC	CF	CTX	CAZ	C	TE	S10	GM	K	CT	CIP	ENR	NA	SXT	S3
1995 (n= 27) in %	22,2	7,4	18,5	40,7	14,8	22,2	22,2	81,5	11,1	18,5	3,7	63,0	3,7	3,7	3,7	74,1
1996-1997 (n= 27) in %	59,3	22,2	37,0	44,4	14,8	77,8	81,5	96,3	0	44,4	0	85,2	3,7	7,4	37,0	96,3
1998 (n= 27) in %	96,3	66,7	33,3	44,4	51,9	96,3	100	96,3	25,9	37,0	37,0	92,6	18,5	11,1	18,5	96,3
1999 -2000 (n= 31) in %	35,5	0	45,2	58,1	38,7	25,8	96,8	96,8	22,6	54,8	45,2	64,5	3,2	6,5	32,3	77,4
2001-2002 (n=29) in %	20,7	0	62,1	86,2	51,7	3,4	3,4	100	13,8	55,2	55,2	93,1	17,2	31,0	3,4	93,1

Figure 4.2. Frequencies of resistant *Salmonella* Typhimurium isolates per years of isolation

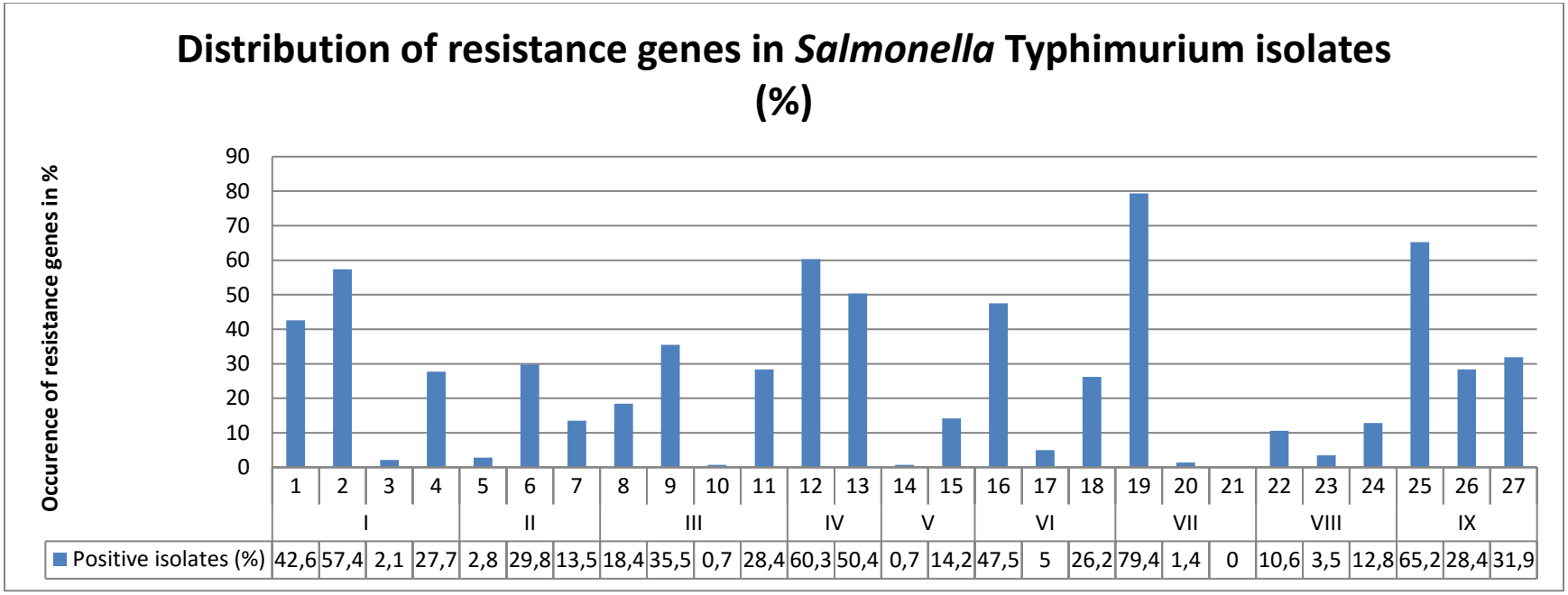


Figure 4.3. Distribution of resistance genes in *Salmonella* Typhimurium isolated from diseased poultry in South Africa

1: *blaTEM-1*, **2:** *blaSHV-1*, **3:** *blaCMY-1*, **4:** *blaPSE-1*, **5:** *cat1*, **6:** *flo*, **7:** *cmlA*, **8:** *tetA*, **9:** *tetB*, **10:** *tetC*, **11:** *tetG*, **12:** *ant3'-Ia*, **13:** *str*, **14:** *aadB*, **15:** *aac-Iva*, **16:** *aphA1*, **17:** *aphA2*, **18:** *aph3'-IIa*, **19:** *qnrA*, **20:** *qnrB*, **21**: *qnrS,* **22:** *dhfr I*, **23:** *dhfrXII*, **24:** *dhfrXIII*, **25:** *sul1*, **26:** *sul2*, **27:** *sul3*

I: β-lactams, **II:** chloramphenicol, **III:** tetracycline, **IV:** streptomycin, **V:** gentamycin, **VI:** kanamycin, **VII:** ciprofloxacin – enrofloxacin – nalidixic acid, **VIII:** sulfamethoxazole - trimethoprim, **IX:** sulphonamides

The frequencies of resistance genes in isolates from poultry organs occurred at the following rates: for β-lactams: *blaSHV* 53.9% (62/115), *blaTEM* 32.2% (37/115), and *blaPSE* 33.9% (39/115); for chloramphenicol: *flo* 36.5% (39/115), *cmlA* 16.5% (19/115); for tetracycline: *tetB* 43.5% (50/115), *tetG* 34.8% (40/115), and *tetA* 22.6% (26/115); for streptomycin: *str* 56.5% (65/115), and *ant3'-Ia* 53.0% (61/115); for kanamycin: *aphA1* 44.3% (51/115); for ciprofloxacin: *qnrA* 78.3% (90/115); for sulphonamides: *sul1* 72.2% (83/115), *sul3* 32.2% (37/115), and *sul2* 21.7% (25/115) (Table 4.3). The frequencies of resistance genes in isolates from poultry environments were for β-lactamases: *blaTEM* 88.5% (23/26), *blaSHV* 69.2% (18/26), for streptomycin: *ant3'-Ia* 96.2% (25/26) and *str* 23.1% (6/26); for kanamycin: *aphA1* 61.5% (16/26); for ciprofloxacin: *qnrA* 88.5% (23/26); for sulphonamides: *sul1* 34.6% (9/26), *sul3* 30.8% (8/141), and *sul2* 53.8% (14/26) (Table 4.3). Resistance genes that were more frequent ($p \leq 0.05$) in poultry organs included *blaTEM* and *blaPSE* for β-lactams, *cmlA* and *flo* for chloramphenicol, *tetA, tetB* and *tetG* for tetracycline *ant3'-Ia*, and *str* for streptomycin, *aac3-Iva* for gentamycin, *aph3'-IIa* for kanamycin, *dfrXIII for* sulfamethoxazole-trimethoprim, *sul1* for sulphonamides. Only *sul2* was more frequent in isolates from poultry environments *($p \leq 0.05$)* (Table 4.3).

Table 4.3. Distribution of resistance genes of *Salmonella* Typhimurium isolates per origin of isolation.

Class/Antibiotics	Genes	All positive isolates n=141(%)	Organs	Environments	*p value*
			No. of positive isolates N=115 (%)	No. of positive isolates N=26 (%)	
β-lactams	*blaTEM*	60 (42.2)	37 (32.2)	23 (88.5)	0.000*
	blaSHV	80 (56.7)	62 (53.9)	18 (69.2)	0.157
	blaCMY	3 (2.1)	3 (2.6)	0 (0.0)	0.407
	blaPSE	39 (27.7)	39 (33.9)	0 (0.0)	0.001*
Chloramph-enicol	*cat1*	4 (2.8)	4 (3.5)	0 (0.0)	0.335
	cmlA	19 (13.5)	19 (16.5)	0 (0.0)	0.027*
	flo	42 (29.8)	42 (36.5)	0 (0.0)	0.001*

tetracycline	*tetA*	26 (18.4)	26 (22.6)	0 (0.0)	0.008*
	tetB	50 (35.5)	50 (43.5)	0 (0.0)	0.000*
	tetC	1 (0.7)	1 (0.9)	0 (0.0)	0.628
	tetG	40 (28.4)	40 (34.8)	0 (0.0)	0.001*
streptomycin	*ant3'Ia*	86 (61.0)	61 (53.0)	25 (96.2)	0.001*
	str	71 (50.4)	65 (56.5)	6 (23.1)	0.003*
gentamycin	*aadB*	3 (2.1)	3 (2.6)	0 (0.0)	0.407
	aac3-Iva	23 (16.3)	19 (16.5)	4 (15.4)	0.891
kanamycin	*aphA1*	67 (47.5)	51 (44.3)	16 (61.5)	0.115
	aphA2	7 (5.0)	7 (6.1)	0 (0.0)	0.198
	aph3'-Iia	37 (26.2)	25 (21.7)	12 (46.2)	0,011*
cipro-enro-floxacin, nalidixic acid	*qnrA*	113 (80.1)	90 (78.3)	23 (88.5)	0.241
	qnrB	2 (1.4)	2 (1.7)	0 (0.0)	0.504
	qnrS	0 (0.0)	0 (0.0)	0 (0.0)	-
sulfamethoxazole-trimethoprim	*dfrI*	15 (10.6)	15 (13.0)	0 (0.0)	0.054
	dfrXII	5 (3.5)	5 (4.3)	0 (0.0)	0.284
	dfrXIII	18 (12.8)	18 (15.7)	0 (0.0)	0.032*
sulphonamides	*sul1*	92 (65.2)	83 (72.2)	9 (34.6)	0.001*
	sul2	39 (27.7)	25 (21.7)	14 (53.8)	0.001*
	sul3	45 (31.9)	37 (32.2)	8 (30.8)	0.890

*: significant

Antimicrobial resistance profiles (phenotype and genotype) of all isolates are shown in Appendix 4.2. The total number of phenotypic antimicrobial resistance profiles was 108 including 21 predominant resistance profiles and 87 single isolate profiles. Among the 21 predominant phenotypic profiles, only two profiles (ampicillin - amoxicillin clavulanic acid – chloramphenicol - tetracycline – streptomycin - ciprofloxacin - sulphonamides; and ampicillin – chloramphenicol – tetracycline – streptomycin – ciprofloxacin - sulphonamides) were the most predominant because they were observed in 6 and 5 isolates respectively (Table 4.4). Seven (7) genotypic antimicrobial resistance profiles were considered predominant profiles of which 2 profiles (G1, and G4) were observed in 5 and 3 isolates respectively while the remaining 5 profiles were each observed in 2

isolates (Table 4.5). Predominant phenotypic resistance profiles did not correspond with predominant genotypic resistance profiles (Appendix 4.2).

Table 4.4. Predominant phenotypic antimicrobial resistance profiles of *Salmonella* Typhimurium isolated from diseased poultry.

Profiles	lab code	Date	Clients /Farms	Source	Case number	Predominant phenotypic antimicrobial resistance profiles
	ST 82	1996	F12	organ	E 395	
	ST 86	1995	F18	organ	D 1836	
	ST 87	1997	F0	organ	F 753	
	ST 89	1998	F0	organ	151	
	ST 90	1998	F0	organ	153	
P1	ST 111	1998	F0	organ	1395	AM,C,TE,S10,CIP,S3
	ST 98	1998	F0	organ	219	
P2	ST 99	1998	F0	organ	221	AM,CAZ,C,TE,S10,CIP,S3
	ST 43	1995	F0	organ	D 871	
	ST 88	1997	F0	organ	F 98	
	ST 91	1998	F0	organ	151	
	ST 94	1998	F0	organ	221	
	ST 95	1998	F0	organ	219	
P3	ST 96	1998	F0	organ	221	AM,AMC,C,TE,S10,CIP,S3
	ST68	1996	F0	organ	149	
P4	ST93	1998	F0	organ	221	AM,AMC,C,TE,S10,K,CIP,S3
	ST60	1996	F0	organ	0	
	ST61	1996	F12	organ	E397	
P5	ST97	1998	F19	organ	E 19	AM,AMC,CF,C,TE,S10,CIP,S3
	ST110	1998	F0	organ	1395	
P6	ST112	1998	F0	organ	149	AM,CTX,C,TE,S10,CT,CIP,S3
	ST53	1995	F22	organ	F 581	
P7	ST85	1997	F0	organ	190	AM,CF,CTX,CAZ,C,TE,S10,CIP,S3
	ST 65	1996	F8	organ	E 127	
	ST 71	1996	F1	organ	D 1821	AM,CF,CTX,C,TE,S10,K,CIP,SXT,
P8	ST 78	1996	F8	organ	D 410	S3
	ST 131	1999	F0	organ	742	
P9	ST 140	1999	F0	organ	742	AM,CF,C,TE,S10,K,STX,S3
	ST 32	2002	F 21	organ	522	
P10	ST 77	1996	F 8	organ	D1821	CTX,C,TE,S10,K,CIP,SXT,S3
	ST 51	1995	F0	organ	D47	
P11	ST 74	1995	F0	organ	12310	S10,S3

	ST141	1999	F0	organ	609	
P12	ST63	1996	F0	organ	E 98	TE,S10,S3
	ST 52	1995	F15	organ	D 169	
	ST 54	1995	F9	organ	D 532	
	ST 76	1995	F0	organ	D 1716	
P13	ST 81	1996	F0	organ	E 359	CTX,S10,CIP,S3
	ST 33	2002	F14	organ	646	
P14	ST 38	1995	F10	organ	D 266	CTX,S10,CIP
	ST28	2002	F20	Env	518	
P15	ST40	1995	F0	organ	D 302	CTX,CAZ,S10,CIP,S3
	ST 27	2001	F0	organ	315	
P16	ST 75	1996	F6	organ	E 201	CTX,S10,K,CIP,S3
	ST29	2002	F20	Env	518	
P17	ST30	2002	F20	Env	518	CF,CTX,CAZ.S10,K,CIP,S3
	ST16	2002	F20	Env	378	
P18	ST17	2002	F20	Env	378	CTX,S10,CT,CIP,S3
	ST22	2002	F20	Env	169	AM,CF,CTX,CAZ,S10,K,CT,CIP,N
P19	ST9	2002	F20	Env	378	A,S3
	ST 101	1998	F0	organ	219	AM,AMC,CF,CTX,CAZ,C,TE,S10,
P20	ST 103	1998	F0	organ	219	GM,K,CT,CIP,ENR,SXT,S3
	ST 100	1998	F0	organ	219	AM,AMC,CF,CTX,CAZ,C,TE,S10,
P21	ST 103	1998	F0	organ	149	GM,K,CT,CIP,ENR, NA,SXT,S3

Table 4.5. Predominant genotypic antimicrobial resistance profiles of *Salmonella* Typhimurium isolated from diseased poultry

Profiles	Lab code	Date	Clients / Farms	Source	Case number	Predominant antimicrobial resistance genes profiles
	ST21	2002	F20	Env	436	
	ST22	2002	F20	Env	436	
G1	ST27	2001	F0	Organ	315	*blaTEM, blaSHV,ant3'-Ia,aphA1,aph3-Iia,qnrA,sul2*
	ST30	2002	F20	Env	518	
	ST31	2002	F20	Env	518	
G2	ST28	2002	F20	Env	518	*blaTEM,blaSHV,ant3'-Ia,qnrA,sul1*
	ST52	1995	F15	Organ	D169	
G3	ST40	1995	F0	Organ	D302	*blaSHV,ant3'-Ia,qnrA,sul1*
	ST54	1995	F9	Organ	D532	
	ST89	1998	F0	Organ	151	
G4	ST96	1998	F0	Organ	221	*blaPSE,flo,tetG,ant3'-Ia,qnrA,sul1*
	ST98	1998	F0	Organ	219	

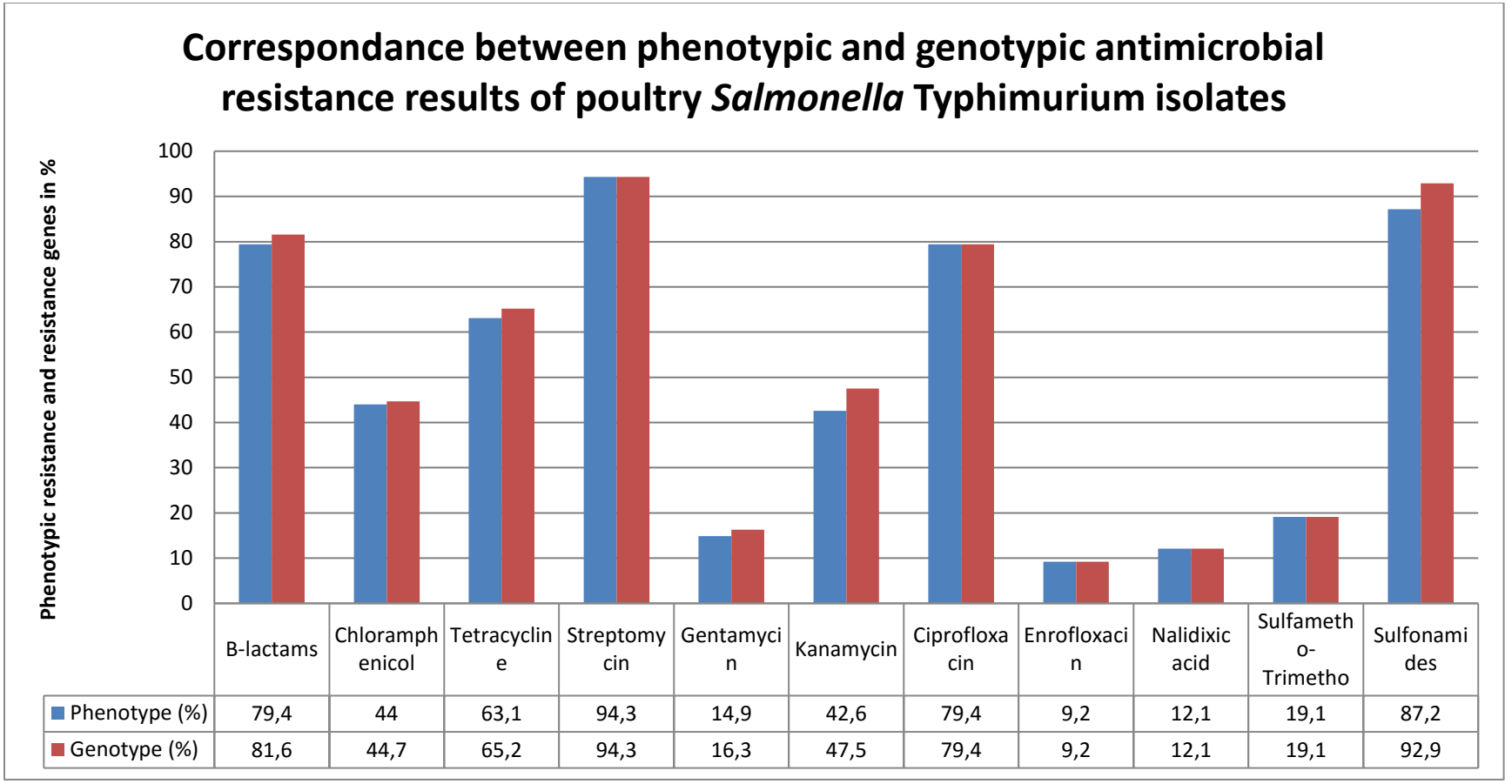
G5	ST 99	1998	F0	Organ	221	*blaPSE,blaSHV,flo,tetG,ant3'-Ia,qnrA,sul1*
	ST 105	1998	F0	Organ	151	
G6	ST 115	1999	F0	Organ	205	*blaSHV,tetB,str,qnrA*
	ST 119	1999	F0	Organ	205	
G7	ST122	1999	F0	Organ	283	*blaSHV,tetB,str,aphA1,aph3'-Iia,qnrA,sul2*
	ST125	1999	F0	Organ	0	

Antimicrobial resistance phenotypes corresponded with PCR results. For each phenotypic resistance recorded, corresponding gene(s) were detected with an agreement varying from good to perfect (kappa = 0.607 – 1). There was a perfect agreement for streptomycin, ciprofloxacin, enrofloxacin, nalidixic acid and sulfamethoxazole - trimethoprim. There was a very good agreement for beta lactams, chloramphenicol, tetracycline, gentamycin, and kanamycin while a good agreement was observed for sulphonamides (Table 4.6; Figure 4.4; Appendix 4. 4). For these antimicrobial agents for whom a perfect agreement was not observed, resistance genes were found in few susceptible isolates. Resistance genes were found in 3, 1, 3,1,7,9 susceptible isolates for beta – lactams, chloramphenicol, tetracycline, gentamycin, kanamycin and sulphonamides respectively (Appendix 4. 4).

Table 4.6. Statistical concordance (Kappa agreement) between phenotypic and genotypic results of antimicrobial resistance in *S.* Typhimurium isolates.

Class and / or antimicrobials	Agreement	Expected agreement	Kappa	Standard error	Z	P *value*
Beta lactams	97.16%	69.00%	0.9085	0.084	10.83	0.000
Chloramphenicol	99.29%	50.64%	0.9856	0.0842	11.7	0.000
Tetracycline	97.87%	52.88%	0.9548	0.0841	11.35	0.000
Streptomycin	100.00%	89.30%	1	0.0842	11.87	0.000
Gentamycin	98.58%	73.65%	0.9462	0.0841	11.25	0.000
Kanamycin	95.04%	50.37%	0.9	0.0838	10.74	0.000
Aminoglycoside	99.29%	91.20%	0.9194	0.0839	10.95	0.000
Ciprofloxacin	100.00%	67.33%	1	0.0842	11.87	0.000
Enrofloxacin	100.00%	83.26%	1	0.0842	11.87	0.000
Nalidixic acid	100.00%	78.79%	1	0.0842	11.87	0.000
Fluoroquinolones	100.00%	67.33%	1	0.0842	11.87	0.000
Sulfamethoxazole - Trimethoprim	100.00%	69.04%	1	0.0842	11.87	0.000
Sulphonamides	92.91%	81.95%	0.607	0.0799	7.59	0.000
DT104	91.49%	53.79%	0.8158	0.0841	9.71	0.000

If kappa = 0 indicates that two tests agree as well as would be expected by chance and a kappa of 1 indicates a perfect agreement. If kappa <0 = no agreement, 0.0 – 0.20 = poor agreement, 0.21 – 0.40 = fair, 0.41 – 0.60 = moderate, 0.61 – 0.80 = substantial or good and 0.81 – 1.00 = very good agreement.



	B-lactams	Chloramphenicol	Tetracycline	Streptomycin	Gentamycin	Kanamycin	Ciprofloxacin	Enrofloxacin	Nalidixic acid	Sulfameth o-Trimetho	Sulfonamides
Phenotype (%)	79,4	44	63,1	94,3	14,9	42,6	79,4	9,2	12,1	19,1	87,2
Genotype (%)	81,6	44,7	65,2	94,3	16,3	47,5	79,4	9,2	12,1	19,1	92,9

Figure 4.4. Correspondence between results of phenotypic and genotypic antimicrobial resistance among *Salmonella* Typhimurium isolates

Class 1 and class 2 integrons were detected in 82.9% (117/141) and 0.7% (1/141) of the isolates respectively while class 3 integrons were not observed. The *int1* gene was observed in 82.6% (95/115) of the *S.* Typhimurium isolated from poultry organs and in 84.6% (22/26) of the isolates from poultry environments. One isolate (ST 60) carried simultaneously *int1* and *int2*.

4.3.3. Characterization of DT104

Antimicrobial sensitivity testing showed that 37.6% (53/141) of the isolates displayed (ACSSuT) resistance phenotype which is characteristic of *S.* Typhimurium DT104 strains. However, only 84.9% (45/53) of the ACSSuT isolates were confirmed by PCR as *S.* Typhimurium DT104 and the remaining 15.1% (8/53) of the ACSSuT isolates did not yield all the 4 amplicons searched for. Conversely, 1 and 3 isolate (s) which exhibited ACSSuT closely related phenotypes including 1 SSuT, and 3 CSSuT were also confirmed by PCR as *S.* Typhimurium DT 104. Therefore, a total of 34.7% (49/141) of the isolates were confirmed phenotypically and genetically as *S.* Typhimurium DT104. In this study, DT104 isolates were from poultry organs isolated between 1995 and 1999 from eight poultry farms: F0, F1, F3, F8, F12, F15, F18, and F22 (Table 4.6). There was also a very good agreement (Kappa = 0.82) between DT104 phenotype (ACSSuT) and PCR results (Table 4.6, Table 4.7).

Table 4.7. List of *Salmonella* Typhimurium DT104 isolated between 1995 and 2002 from diseased poultry in South Africa

Lab Code	Date	Clients	Sources	Case number	DT104 Phenotype	DT104 Genotype			
						spvC	*invA*	*int1*	flo_{st}
ST43	1995	F0	organ	D871	ACSSuT	+	+	+	+
ST53	1995	F22	organ	D906	ACSSuT	+	+	+	+
ST86	1995	F18	organ	D1836	ACSSuT	+	+	+	+
ST60	1996	F0	organ	-	ACSSuT	+	+	+	+
ST62	1996	F8	organ	D1821	ACSSuT	+	+	+	+
ST65	1996	F8	organ	E 147	ACSSuT	+	+	+	+
ST70	1996	F8	organ	D1821	CSSuT	+	+	+	+

ST78	1996	F8	organ	D1821	ACSSuT	+	+	+	+
ST80	1996	F8	organ	D1821	CSSuT	+	+	+	+
ST64	1996	F0	organ	E 226	SSuT	+	+	+	+
ST61	1996	F12	organ	E 397	ACSSuT	+	+	+	+
ST66	1996	F12	organ	E 397	ACSSuT	+	+	+	+
ST82	1996	F12	organ	E 397	ACSSuT	+	+	+	+
ST68	1996	F0	organ	149	ACSSuT	+	+	+	+
ST69	1996	F15	organ	D1273	ACSSuT	+	+	+	+
ST71	1996	F1	organ	E 127	ACSSuT	+	+	+	+
ST67	1997	F3	organ	D1835	CSSuT	+	+	+	+
ST84	1997	F0	organ	F 582	ACSSuT	+	+	+	+
ST85	1997	F0	organ	F 581	ACSSuT	+	+	+	+
ST87	1997	F0	organ	F 753	ACSSuT	+	+	+	+
ST88	1997	F0	organ	F 98	ACSSuT	+	+	+	+
ST89	1998	F0	organ	151	ACSSuT	+	+	+	+
ST90	1998	F0	organ	153	ACSSuT	+	+	+	+
ST91	1998	F0	organ	151	ACSSuT	+	+	+	+
ST92	1998	F0	organ	190	ACSSuT	+	+	+	+
ST93	1998	F0	organ	221	ACSSuT	+	+	+	+
ST94	1998	F0	organ	221	ACSSuT	+	+	+	+
ST95	1998	F0	organ	219	ACSSuT	+	+	+	+
ST96	1998	F0	organ	221	ACSSuT	+	+	+	+
ST97	1998	F19	organ	E 19	ACSSuT	+	+	+	+
ST98	1998	F0	organ	219	ACSSuT	+	+	+	+
ST99	1998	F0	organ	221	ACSSuT	+	+	+	+
ST100	1998	F0	organ	127	ACSSuT	+	+	+	+
ST101	1998	F0	organ	219	ACSSuT	+	+	+	+
ST102	1998	F0	organ	219	ACSSuT	+	+	+	+
ST103	1998	F0	organ	219	ACSSuT	+	+	+	+
ST104	1998	F0	organ	127	ACSSuT	+	+	+	+
ST105	1998	F0	organ	151	ACSSuT	+	+	+	+
ST106	1998	F0	organ	190	ACSSuT	+	+	+	+
ST107	1998	F0	organ	897	ACSSuT	+	+	+	+
ST108	1998	F0	organ	149	ACSSuT	+	+	+	+
ST109	1998	F0	organ	149	ACSSuT	+	+	+	+
ST110	1998	F0	organ	549	ACSSuT	+	+	+	+
ST111	1998	F0	organ	1395	ACSSuT	+	+	+	+
ST112	1998	F0	organ	1395	ACSSuT	+	+	+	+
ST123	1999	F0	organ	205	ACSSuT	+	+	+	+
ST128	1997	F0	organ	-	ACSSuT	+	+	+	+
ST129	1998	F0	organ	-	ACSSuT	+	+	+	+

ST130	1998	F0	organ	-	ACSSuT	+	+	+	+
Total						49			

4.4. Discussion

Salmonella Typhimurium is an important foodborne pathogen that causes disease outbreaks in humans, and affects different animal hosts including poultry (CDC. 2013). Poultry products are the main sources of *Salmonella* infections in humans (Fearnley *et al.* 2011). Antimicrobial resistance has become a serious public health concern among *S.* Typhimurium due to the limitation of therapeutic options in humans (Feasey *et al.* 2015, Helms *et al.* 2002). In this study, *S.* Typhimurium isolated from organs and environments of diseased poultry in South Africa were screened for antimicrobial resistance phenotypes and antimicrobial resistance determinants. The findings in this study indicate that antimicrobial resistance is widespread among *S.* Typhimurium isolates that were recovered in South Africa between 1995 and 2002 from organs and environments of diseased poultry. Seventy five per cent: 75.8% of isolates were resistant to at least five different antimicrobial agents mainly streptomycin, sulphonamides, ciprofloxacin and tetracycline. In addition, multi-drug resistance was observed in 97.2% of isolates (Table 4.1).

Resistance to two or multiple antimicrobial agents especially streptomycin, sulphonamides, ciprofloxacin, tetracycline, and cefotaxime was also widespread among *S.* Typhimurium under study. Treatment failure and resultant death in diseased poultry from which the *S.* Typhimurium were recovered from may have been most probably due to the high level of multi-drug resistance that was widespread among isolates. The findings of this study are consistent with similar studies which have been carried out elsewhere in which *S.* Typhimurium that were isolated from organs of diseased poultry showed also a high frequency of multi-resistance (Ahmed & Shimamoto 2012, Zhao *et al.* 2007). Altogether, these findings indicate that multi-drug resistance is common in isolates recovered from organs of diseased poultry.

Occurrence of resistance to most of these antimicrobials limits their effectiveness in both human and animal salmonellosis therapy and also warrants sustained surveillance to determine sources and transmission patterns of emerging ciprofloxacin and cephalosporin resistant isolates.

High levels of resistance were recorded for antimicrobials usually used in poultry operations: streptomycin, sulphonamides, and tetracycline (Hofacre, Fricke & Inglis 2013). Tetracycline and sulphonamides are approved antimicrobials for growth promotion and treatment in poultry operations in South Africa (Eagar 2008). Therefore, resistance to tetracycline and sulphonamides may be due to selective pressure exerted by the use of these antimicrobials in poultry operations. Resistance to tetracycline and sulphonamides has also been observed in studies conducted in South Africa (Manie *et al.* 1998, Gouws & Brozel 2000) and in other countries (Ahmed & Shimamoto 2012, Louden *et al.* 2012, Ke *et al.* 2014, Farzan *et al.* 2008). Although streptomycin is approved for treatment and growth promotion in poultry production in other countries (Hofacre *et al.* 2013), it is not approved in South Africa (Eagar 2008). However, streptomycin was the most inefficient (94.3%) amongst antimicrobials tested in this study. Furthermore, the high level of resistance to streptomycin observed in this study was consistent with reports from previous studies in South Africa: 97% (Gouws & Brozel 2000) and 86.4% (Manie *et al.* 1998) and in other countries such as the USA (89.2%) (Lindsey *et al.* 2009), Egypt (94%) (Ahmed & Shimamoto 2012) and Spain (91%) (Glenn *et al.* 2011). These results support the observation that resistance to streptomycin is common in *Salmonella* isolates (Chen *et al.* 2004). The high resistance observed in this study may be associated with cross-resistance to spectinomycin or streptogramins, which are approved for use in poultry production in South Africa respectively for the treatment of *Mycoplasma* and coliform infections and for growth promotion (Eagar 2008). Furthermore, the *ant3'-Ia* gene which encodes resistance to both streptomycin and spectinomycin was also observed in the majority (60.5%) of streptomycin resistant isolates under study.

Significant levels (more than 40%) of resistance to antimicrobials which are not used in poultry production but used to treat human salmonellosis were also observed in this study. These antimicrobials include chloramphenicol, ampicillin, cephalothin, cefotaxime, ceftazidime, colistin sulphate, and ciprofloxacin (Figure 4.1). More than 40% of *S.* Typhimurium isolates were resistant to ampicillin, or chloramphenicol which are considered primary drugs (empiric therapy) for salmonellosis in humans (H. Chen *et al.* 2013). In this study, resistance to extended spectrum cephalosporins (cephalothin, cefotaxime and ceftazidime) was also observed in 20.6% of the isolates. Third generation cephalosporins such as ceftazidime, cefotaxime, ceftriaxone and a fluoroquinolone are recommended to treat human salmonellosis in areas where resistance to the empiric therapy has been declared or in case of complications such as bacteraemia, osteomyelitis, and meningitis (H. Chen *et al.* 2013). The high level of resistance that was observed in this study among cephalosporins represents an important public health concern because the third generation cephalosporins are commonly used to treat salmonellosis in humans. A higher resistance level (74.5%) was observed for ciprofloxacin compared to the one reported 47% by (Ahmed & Shimamoto 2012). Occurrence of resistance to ciprofloxacin is of concern since it is one of the last resort antimicrobial for salmonellosis therapy in humans. Ciprofloxacin is also considered an alternative antimicrobial in the treatment of invasive *Salmonella* infections (H. Chen *et al.* 2013). To the best of our knowledge, this is first time such high level of resistance to ciprofloxacin in *Salmonella* isolates is reported in poultry production in South Africa. This high resistance may be associated with cross-resistance or co-selection between ciprofloxacin and norfloxacin or olaquindox due to their respective use for treatment and growth promotion in poultry production in South Africa (Eagar 2008). Furthermore ciprofloxacin and norfloxacin have similarities in their chemical structure (Robicsek, Jacoby & Hooper 2006). The level of resistance observed to colistin sulphate (29%) is also worrying as colistin remains among the last alternative drug to treat multi-drug resistant bacteria even those that are resistant to carbapenems (Arcilla *et al.* 2016). Resistance to colistin associated with the plasmid mediate gene (*mcr-1*) which can be

transmitted to other bacteria has been recently reported in 75% of *E. coli* isolated from poultry and humans in South Africa (Arcilla *et al.* 2016). This suggests that there may have been a transmission of colistin resistance inter-bacteria in South Africa since the results of the present study indicate that resistance to colistin that was already present in poultry *S.* Typhimurium between 1995 and 2002 has increased from 29% to 75% in 2015 in poultry *E. coli* isolates. A significant proportion of *S.* Typhimurium isolates (44%) was also resistant to chloramphenicol which has been banned from use in poultry operations in South Africa since 1980. Several studies have also reported on the occurrence of chloramphenicol resistance in *Salmonella* isolates from poultry in the absence of any previous selective pressure occurring due to the previous use of chloramphenicol or other phenicols in poultry operations (Manie *et al.* 1998, Gouws & Brozel 2000, SVARM 2001, CDC. 2003). In South African poultry production, there is no selective pressure for the above-mentioned antimicrobials because they are not authorised for use in poultry production. Therefore, the relatively high proportion of poultry *S.* Typhimurium isolates resistant to the antimicrobials may be attributed to the acquisition of resistance genes via horizontal gene transfer by mobile genetic elements, and co-selection or cross-resistance to other antimicrobials agents, heavy metals, and disinfectants. These phenomena could have been occurred before the introduction of *S.* Typhimurium isolates into poultry production. For example cross – resistance between chloramphenicol and florfenicol has been previously reported.

The findings from this study showed that *S.* Typhimurium isolates from poultry organs were more resistant ($p \leq 0.05$) to ampicillin, amoxicillin - clavulanic acid, chloramphenicol, tetracycline, and sulfamethoxazole - trimethoprim while isolates that were recovered from poultry environments were more resistant to cephalothin, cefotaxime, ceftazidime, colistin sulphate and nalidixic acid. Ampicillin, amoxicillin - clavulanic acid, chloramphenicol; cephalothin, cefotaxime, ceftazidime, colistin sulphate and nalidixic acid are not approved for use in South African poultry production (Eagar. 2008). Therefore, resistance to these antimicrobials was acquired before their

introduction in poultry production. They probably gained into poultry production through different routes such as contaminated litter, air, feeds, workers, unclean facilities, insects, rodents, wind (Jones *et al.* 1991) thereby justifying their dominance in poultry environments (litter and dust). The presence and dominance of antimicrobial resistance in all isolates recovered from poultry organs may be associated with invasiveness ability of the isolates. These conclusions will have to be supported with a well – structured study since the isolates used in this study were the only ones available in the laboratory. Although, *S.* Typhimurium isolated in 1998 and those isolated from 2001 to 2002 showed high levels of resistance to some antimicrobials in general, there was no increase or decline of resistance to antimicrobials among *S.* Typhimurium isolated between 1995 and 2002 indicating that the resistance to all antimicrobials was spread from 1995 to 2002.

Resistance to beta lactams is mainly mediated by beta lactamases which hydrolyse the beta lactam ring (Livermore 1995). Although, there are more than 75 beta lactamases, the most common beta lactamases observed in Gram negative bacteria are mostly produced by *blaTEM, blaSHV, blaPSE,* and *blaCMY-2* (Livermore 1995). In this study, *blaSHV-1, blaTEM-1, blaPSE-1* were associated with resistance to beta-lactams (Table 4.2, Figure 4.3). However*, blaPSE* was mostly recovered from isolates resistant to ampicillin and amoxicillin - clavulanic acid, consistent with what is known about the association of *blaPSE* with ampicillin resistance in DT104 isolates (Boyd *et al.* 2001). In South Africa, *blaTEM, blaSHV,* and *blaPSE* have been previously recovered in *Salmonella* isolates from humans and cattle at low levels(Kruger *et al.* 2004, Igbinosa 2015). However, the findings of this study for *blaTEM* and *blaPSE* are comparable to those previously reported elsewhere (Glenn *et al.* 2011, Anjum *et al.* 2011). The high rates of *blaSHV*, *blaTEM*, and *blaPSE* are the first findings reported in *S.* Typhimurium isolates from poultry in South Africa. These findings are of considerable concern as these genes are prone to wide dissemination to various bacterial populations due to their location on mobile genetic elements. Constant

monitoring of resistant *S.* Typhimurium isolates requires more effort within poultry breeder flocks and hatcheries since they supply multiple farms.

In this study, florfenicol resistance gene (*flo*) was mostly associated with chloramphenicol resistance (26.2%) followed by *cmlA* (13.5%) and *cat1* (2.8%). The occurrence of these genes in the same descending order of prevalence: 48.8%, 20% and 3.3% has been previously reported among *S.* Typhimurium isolates from humans (de Toro *et al.* 2011), and food animals including poultry (Glenn *et al.* 2011). The findings of this study on prevalence of *tetB* and *tetG* (Figure 4.3) were consistent with the rates reported elsewhere among *S.* Typhimurium isolates from humans (de Toro *et al.* 2011, De Vito *et al.* 2015) and animals (Glenn *et al.* 2011, Anjum *et al.* 2011). The *flo, cmlA, tetB,* and *tetG* genes are mostly located on mobile DNA elements (SGI and integrons), which are associated with multi-drug resistance in Gram - negative bacteria (Schwarz *et al.* 2001, Boyd *et al.* 2001). These genes which activate efflux pumps may be circulating within different animal species including humans and therefore represent a hazard to public health.

Resistance genes that were associated with streptomycin resistance included *ant (3')-Ia* 60.3% of the isolates and *str* 50.4%. A similar prevalence (57.6%) for *str* and a high prevalence (81.8%) for *ant3'-Ia* were observed in *S.* Typhimurium isolates from diseased animals including poultry (Glenn *et al.* 2011). These findings indicate that resistance to streptomycin in *Salmonella* is mainly associated with *ant3'-Ia* and *str* genes. The *ant3'-Ia* gene has been found on SGI which encode a multi-drug resistance cluster of widespread DT104 isolates (Boyd *et al.* 2001).

The plasmid-mediated quinolone resistance gene (*qnrA*) was identified in 79.4% of *S.* Typhimurium isolates that were phenotypically resistant to ciprofloxacin (Table 4.2; Figure 4.3). The low level of resistance to enrofloxacin and nalidixic acid observed in this study is not surprising since disk diffusion can only detect resistance to enrofloxacin and nalidixic acid in isolates lacking plasmid-mediated quinolone resistance genes (Cavaco & Aarestrup 2009). All isolates that were resistant to enrofloxacin and nalidixic acid were also resistant to ciprofloxacin.

Therefore, the *qnrA* gene that was observed in isolates that were resistant to enrofloxacin and nalidixic acid may be associated with resistance to ciprofloxacin. To the best of our knowledge, this is the first report on high resistance to ciprofloxacin which is by mediated by *qnrA* in South Africa. The low rates of *qnrA* gene were previously reported in other studies (Dierikx *et al.* 2012, Veldman *et al.* 2011, Agga *et al.* 2015). The increasing incidence of plasmid-mediated quinolone gene "*qnrA*" is worrying due to its capacity of transferability to other micro-organisms affecting humans. Fluoroquinolones are the last alternative drugs for the treatment of human salmonellosis.

Resistance to sulphonamides is mostly mediated with sulphonamides resistance genes. In this study, *sul1* was recovered mostly in sulphonamides resistant isolates (65.2%), followed by *sul3*: (31.9%), and *sul2:* 27.7%. The *sul1* gene has also been recovered in 60% of the *S.* Typhimurium isolates from humans in Spain (de Toro *et al.* 2011); and in 39% of the *S.* Typhimurium from animals (Anjum *et al.* 2011). The gene *sul2* was also recovered in 46.4% of the *Salmonella* isolates from the majority of retail poultry meat in Poland (Maka *et al.* 2015). In Portugal *sul1, sul2* and *sul3* occurred in 76%, 37% and 7% of the *Salmonella* spp including *S.* Typhimurium isolates (Antunes *et al.* 2005). In the USA, *sul1* and *sul2* were respectively observed in 90.9% and 51.5% of the *S.* Typhimurium isolated from animals including poultry (Glenn *et al.* 2011). *Sul* genes were detected in 70% of the *Salmonella* isolates from poultry meat in Tunisia (Soufi *et al.* 2012). These findings indicate that *sul1* is the most frequently observed in *S.* Typhimurium isolates resistant to sulphonamides. *Sul1* is a constituent of SGI, and is also located at 3' conserved segment of class 1 integron (Hall & Collis 1995, Boyd *et al.* 2001). SGIs and of class 1 integron are associated with multi-resistance in *S.* Typhimurium isolates (Hall & Collis 1995, Boyd *et al.* 2001)

For all resistance phenotypes recorded in this study, one or more corresponding resistance gene(s) was detected with an agreement varying from good to perfect (Table 4.5; Figure 4.4; Appendix 4.2). However, resistance genes were present in few susceptible isolates

(beta lactams: 3, tetracycline: 3, kanamycin: 7, and sulphonamides: 9) and this may be ascribed to the lack of expression of resistance genes carried by these susceptible isolates. The other explanation may be that mutations in these genes may have led to inactivation of produced enzymes. Data on antimicrobial resistance patterns (Appendix 4.2) demonstrate extensive heterogeneity of *S.* Typhimurium isolates and existence of various mechanisms of resistance due to the presence of more than one gene associated with resistance to one antimicrobial agent (Figure 4.3). This was expected since isolates were collected from different farms and for a long period (8 years).

Integrons are mobile genetic elements which are associated with multi-drug resistance via the propagation of resistance genes to different bacteria (Hall & Collis 1995). The high rate of class 1 integrons that was identified in 83% of the *S.* Typhimurium isolated from diseased poultry in this study is similar to the levels previously reported elsewhere in clinical *S.* Typhimurium isolates (Lindstedt *et al.* 2003, Antunes *et al.* 2005). These findings confirm what is known about class 1 integrons as being prevalent in *S.* Typhimurium clinical isolates (Recchia & Hall 1995). However, lower findings were reported among *Salmonella spp* in Egypt (42%) (Ahmed & Shimamoto 2012), and in Uganda and North Dakota (20.7%) (Mahero *et al.* 2013). In other studies, *int1* was present in 30% of the *Salmonella* spp. in Poland (Maka *et al.* 2015) and 61.2% of the *Salmonella* spp. in United States (Frye *et al.* 2011). The comparison of these different findings confirm that class 1 integrons are frequently found in *S.* Typhimurium isolates compared to other *Salmonella* serovars. Class 1 integrons were also frequently discovered in *S.* Typhimurium DT104 strains (Boyd *et al.* 2001). In this study, all DT104 isolates carried a specific sequence of *int1,* consistent with previous studies (Khan *et al.* 2000, Ebner & Mathew 2001) that showed the presence of *int1* in all DT104 isolates. A class 2 integrons was found only in one (0.7%) isolate while no isolate carried class 3 integron. This is not surprising as class 2 and class 3 integrons are not common in *Salmonella* (Lindstedt *et al.* 2003, Ahmed & Shimamoto 2012).

Class 1 and class 2 integrons have been identified worldwide in *S.* Typhimurium isolates (Lindstedt *et al.* 2003, Ahmed, Shimamoto & Shimamoto 2014) but interestingly, to the best of our knowledge, this is the first study that reports the simultaneous presence of a class 1 and class 2 integrons genes in a single poultry *S.* Typhimurium isolate. The high prevalence of integrons in poultry *S.* Typhimurium isolates represents a high risk as they are associated with the emergence and the spread of antimicrobial resistance in *Salmonella* and other bacteria affecting animals and humans.

Salmonella Typhimurium DT104 is a multi-drug resistant strain which exhibits ACSSuT resistance type (Boyd *et al.* 2001). DT104 strains have also been associated with increased virulence with high rates of illnesses and deaths (Helms *et al.* 2002). In this study, 37.6% of the *S.* Typhimurium isolates displayed ACSSuT resistance type, which is consistent with 34.3% of the ACSSuT resistance type reported by Capuano *et al.* (2013) but lower than the 49% reported by Chiu *et al.* (2006). The prevalence of the ACSSuT phenotype (37.6%) obtained in this study is consistent with that reported in the USA (CDC. 2012) but higher than that found in two studies carried out on human isolates: in South Africa (Keddy *et al.* 2009) and Mozambique (Ruiz *et al.* 2008). Among the 37.6% of the ACSSuT isolates of this study, PCR confirmed only 34.7% as *S.* Typhimurium DT104. The remaining ACSSuT isolates (5.7%) may be other phage types such as U302, DT102 (Chiu *et al.* 2006). Four closely - related ACSSuT isolates (SSuT: 1; CSSuT: 3) were also confirmed as DT 104 by PCR suggesting that resistance genes to ampicillin and chloramphenicol were not expressed in these isolates. The prevalence of DT104 strains observed in this study indicates that DT104 strains are widespread as has been reported worldwide (Threlfall 2000, Khan *et al.* 2000, Ebner & Mathew 2001). DT104 strains identified in this study also showed the same phenotypic and genetic characteristics as the ones observed in different countries: in USA (Mather *et al.* 2013), China (Ke *et al.* 2014), and Europe (Alessiani *et al.* 2014). This confirms that the multi-drug resistance cluster of DT104 strains is chromosomally integrated

(Boyd *et al.* 2001), and thus it may spread without any exposure to previous selection pressure. In this study, DT104 was found in isolates that were identified from poultry organs between 1995 and 1999 in different poultry farms (Table 4.4). These data indicate that DT104 isolates may have been persistent and widespread in South Africa. This high prevalence of *S.* Typhimurium DT104 is the first one to be reported in poultry *S.* Typhimurium isolates in South Africa. The role played by poultry DT104 in the occurrence of human disease will be worthy investigating since 13% of ACSSuT resistance type was previously reported among human *S.* Typhimurium in South Africa (Keddy *et al.* 2009). The increasing incidence of DT104 isolates poses a serious concern as DT104 has been incriminated in severe epidemics which were characterized by long period of hospitalizations and deaths. Therefore, the dangerous DT104 will require stringent control strategies aiming at stopping its widespread in order to protect human and animal health.

4.5. Conclusion

The findings of this study showed that 97.2% (137/141) of the poultry *S.* Typhimurium isolates were resistant to multiple antimicrobials especially those that are commonly used in poultry operations for prophylaxis and growth promotion (streptomycin, tetracycline and sulphonamide) as well as antimicrobials used for therapy in human salmonellosis including: ciprofloxacin, cefotaxime, ceftazidime, and ampicillin. The phenotypic and genotypic results on antimicrobial resistance of *S.* Typhimurium isolates that were observed in this study indicate that there is an increasing incidence and propagation of antimicrobial resistance in poultry *S.* Typhimurium isolates. The existence of high number of phenotypic resistance profiles (108) which did not correspond with genotypic resistance profiles indicate the diversity of poultry *S.* Typhimurium isolates and their complexity in resistance mechanisms as a strategy of *S.* Typhimurium isolates to adapt to environmental changes. The high rate (83%) of class 1 integron that was observed in this study is also worrying since integrons are associated with occurrence and dissemination of multi-drug resistance in various populations of bacteria. A high occurrence

of multi-resistant poultry *S.* Typhimurium isolates including DT104 (34.7%) isolates represents a public health concern since DT104 strain is associated with higher hospitalizations rates and deaths in humans. Therefore, the role played by poultry DT104 strains in the occurrence of human disease will be worth investigating further since DT104 resistance phenotype "ACSSuT" have been reported in human *S.* Typhimurium in South Africa. Therefore, stringent policies aiming at reducing contamination from poultry farms to the consumer are of paramount importance. Prudent use of antimicrobials and the establishment of solid *Salmonella* monitoring and surveillance systems remain the best way to reduce the misuse and abuse of antimicrobials and ensuring food safety for consumers.

4.6. Acknowledgements

We thank the University of Pretoria and National Research Foundation (South Africa) for founding this project. We thank the Bacteriology laboratory / Faculty of Veterinary Science/ University of Pretoria for providing the isolates used in this study.

4.7. References

1. AARESTRUP, F.M., LERTWORAPREECHA, M., EVANS, M.C., BANGTRAKULNONTH, A., CHALERMCHAIKIT, T., HENDRIKSEN, R.S. & WEGENER, H.C. 2003. Antimicrobial susceptibility and occurrence of resistance genes among *Salmonella* enterica serovar Weltevreden from different countries*. Journal of Antimicrobial Chemotherapy*, 52:715-718
2. AGGA, G.E., ARTHUR, T.M., DURSO, L.M., HARHAY, D.M. & SCHMIDT, J.W. 2015. Antimicrobial-Resistant Bacterial Populations and Antimicrobial Resistance Genes Obtained from Environments Impacted by Livestock and Municipal Waste. *PloS one*, 10:e0132586
3. AHMED, A.M. & SHIMAMOTO, T. 2012. Genetic analysis of multiple antimicrobial resistance in *Salmonella* isolated from diseased broilers in Egypt*. Microbiology and Immunology*, 56:254-261
4. AHMED, A.M., SHIMAMOTO, T. & SHIMAMOTO, T. 2014. Characterization of integrons and resistance genes in multidrug-resistant *Salmonella* enterica isolated from meat and dairy products in Egypt. *International Journal of Food Microbiology*, 189:39-44
5. ALESSIANI, A., SACCHINI, L., PONTIERI, E., GAVINI, J. & DI GIANNATALE, E. 2014. Molecular typing of *Salmonella enterica* subspecies enterica serovar Typhimurium isolated in Abruzzo region (Italy) from 2008 to 2010*. Veterinaria Italiana*, 50:31-39
6. ANJUM, M.F., CHOUDHARY, S., MORRISON, V., SNOW, L.C., MAFURA, M., SLICKERS, P., EHRICHT, R. & WOODWARD, M.J. 2011. Identifying antimicrobial

resistance genes of human clinical relevance within *Salmonella* isolated from food animals in Great Britain. *Journal of Antimicrobial Chemotherapy*, 66:550-559
7. ANTUNES, P., MACHADO, J., SOUSA, J.C. & PEIXE, L. 2005. Dissemination of sulphonamide resistance genes (*sul1, sul2*, and *sul3*) in Portuguese *Salmonella enterica* strains and relation with integrons. *Antimicrobial Agents and Chemotherapy*, 49:836-839
8. ARCILLA, M.S., VAN HATTEM, J.M., MATAMOROS, S., MEILES, D.C., PENDERS, J., DE JONG, M.D., SCHULTSZ, C. & COMBAT CONSORTIUM. 2016. Dissemination of the mcr-1 colistin resistance gene. *Lancet Infectious Diseases*, 16:147-149
9. BAUER, A.W., KIRBY, W.M., SHERRIS, J.C. & TURCK, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Technical bulletin of the Registry of Medical Technologists*, 36:49-52
10. BISSONNETTE, L., CHAMPETIER, S., BUISSON, J.P. & ROY, P.H. 1991. Characterization of the Nonenzymatic Chloramphenicol Resistance (*Cmla*) Gene of the In4 Integron of Tn1696 - Similarity of the Product to Transmembrane Transport Proteins. *Journal of Bacteriology*, 173:4493-4502
11. BOYD, D., PETERS, G.A., CLOECKAERT, A., BOUMEDINE, K.S., CHASLUS-DANCLA, E., IMBERECHTS, E. & MULVEY, M.R. 2001. Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar Typhimurium DT104 and its identification in phage type DT120 and serovar Agona. *Journal of Bacteriology*, 183:5725-5732
12. BRIGGS, C.E. & FRATAMICO, P.M. 1999. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella* Typhimurium DT104. *Antimicrobial Agents and Chemotherapy*, 43:846-849
13. CAMBRAY, G., GUEROUT, A.M. & MAZEL, D. 2010. Integrons. *Annual Review of Genetics*, 44:141-166
14. CAPUANO, F., MANCUSI, A., CAPPARELLI, R., ESPOSITO, S. & PROROGA, Y.T.R. 2013. Characterization of drug resistance and virulotypes of *Salmonella* strains isolated from food and humans. *Foodborne Pathogens and Disease*, 10:963-968
15. CAVACO, L.M. & AARESTRUP, F.M. 2009. Evaluation of quinolones for use in detection of determinants of acquired quinolone resistance, including the new transmissible resistance mechanisms *qnrA, qnrB, qnrS*, and *aac(6')Ib-cr,* in *Escherichia coli* and *Salmonella enterica* and determinations of wild-type distributions. *Journal of Clinical Microbiology*, 47:2751-2758
16. CDC. 2003. *National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): 2001 Annual Report.* Atlanta, Georgia: U.S. Department of Health and Human Services,: CDC, 2003
17. CDC. 2012. *National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): Human Isolates Final Report, 2010* . Atlanta, Georgia: U.S. Department of Health and Human Services
18. CDC. 2013. *Multistate Outbreak of Human Salmonella Typhimurium Infections linked to live poultry in backyard flocks (final update).*, http://www.cdc.gov/salmonella/typhimurium-live-poultry-04-13 ed. Atlanta, Georgia: USA Department of Health and Human Services,: Centers for Disease Control and Prevention
19. CHEN, H., WANG, Y., SU, L. & CHIU, C. 2013. Nontyphoid *Salmonella* Infection: Microbiology, Clinical Features, and Antimicrobial Therapy. *Pediatrics and Neonatology*, 54:147-152
20. CHEN, S., ZHAO, S., WHITE, D.G., SCHROEDER, C.M., LU, R., YANG, H., MCDERMOTT, P.F., AYERS, S. & MENG, J. 2004. Characterization of multiple-antimicrobial-resistant *Salmonella* serovars isolated from retail meats. *Applied and Environmental Microbiology*, 70:1-7

21. CHIU, CHENG-HSUN, SU, L., CHU, C., WANG, M., YEH, C., WEILL, F. & CHU, C. 2006. Detection of multidrug-resistant *Salmonella enterica* serovar Typhimurium phage types DT102, DT104, and U302 by multiplex PCR. *Journal of Clinical Microbiology*, 44:2354-2358
22. CLSI. 2013. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Test; Twenty-third Informational Supplement. CLSI document M100-S23.*, Vol. 33 No. 1 ed. Wayne PA/ USA: Clinical laboratory Standards Institute; 2013
23. CROWTHER-GIBSON, P., GOVENDER, N., LEWIS, D.A., BAMFORD, C., BRINK, A., VON GOTTBERG, A., KLUGMAN, K., DU PLESSIS, M., FALI, A., HARRIS, B., KEDDY, K. & BOTHA, M. 2011. Part IV. Human infections and antibiotic resistance. *South African Medical Journal = Suid-Afrikaanse Tydskrif vir Geneeskunde*, 101:567-578
24. DE MEDICI, D., CROCI, L., DELIBATO, E., DI PASQUALE, S., FILETICI, E. & TOTI, L. 2003. Evaluation of DNA extraction methods for use in combination with SYBR green I real-time PCR to detect *Salmonella enterica* serotype Enteritidis in poultry. *Applied and Environmental Microbiology*, 69:3456-3461
25. DE TORO, M., SAENZ, Y., CERCENADO, E., ROJO-BEZARES, B., GARCIA-CAMPELLO, M., UNDABEITIA, E. & TORRES, C. 2011. Genetic characterization of the mechanisms of resistance to amoxicillin/clavulanate and third-generation cephalosporins in *Salmonella enterica* from three Spanish hospitals. *International Microbiology*, 14:173-181
26. DE VITO, D., MONNO, R., NUCCIO, F., LEGRETTO, M., OLIVA, M., COSCIA, M.F., DIONISI, A.M., CALIA, C., CAPOLONGO, C. & PAZZANI, C. 2015. Diffusion and persistence of multidrug resistant *Salmonella* Typhimurium strains phage type DT120 in southern Italy. *BioMed Research International*, 2015:265042-265042
27. DIERIKX, C.M., VAN DUIJKEREN, E., SCHOORMANS, A.H.W., VAN ESSEN-ZANDBERGEN, A., VELDMAN, K., KANT, A., HUIJSDENS, X.W., VAN DER ZWALUW, K., WAGENAAR, J.A. & MEVIUS, D.J. 2012. Occurrence and characteristics of extended-spectrum--lactamase- and AmpC-producing clinical isolates derived from companion animals and horses. *Journal of Antimicrobial Chemotherapy*, 67:1368-1374
28. EAGAR H.A. 2008. A Survey of Antimicrobial Usage in Animals in South Africa with Specific Reference to Food Animals. Master of Veterinary Medicine (Pharmacology) Thesis or Dissertation, Pretoria/ South Africa
29. EBNER, P.D. & MATHEW, A.G. 2001. Three molecular methods to identify *Salmonella enterica* serotype Typhimurium DT104: PCR fingerprinting, multiplex PCR and rapid PFGE. *FEMS Microbiology Letters*, 205:25-29
30. FARZAN, A., FRIENDSHIP, R.M., POPPE, C., MARTIN, L., DEWEY, C.E. & FUNK, J. 2008. Molecular epidemiology and antimicrobial resistance of *Salmonella* Typhimurium DTI04 on Ontario swine farms. *Canadian Journal of Veterinary Research = Revue Canadienne de Recherche Veterinaire*, 72:188-194
31. FEARNLEY, E., RAUPACH, J., LAGALA, F. & CAMERON, S. 2011. *Salmonella* in chicken meat, eggs and humans; Adelaide, South Australia, 2008. *International Journal of Food Microbiology*, 146:219-227
32. FEASEY, N.A., MASESA, C., JASSI, C., FARAGHER, E.B., MALLEWA, J., MALLEWA, M., MACLENNAN, C.A., MSEFULA, C., HEYDERMAN, R.S. & GORDON, M.A. 2015. Three Epidemics of Invasive Multidrug-Resistant *Salmonella* Bloodstream Infection in Blantyre, Malawi, 1998-2014. *Clinical Infectious Diseases : an official publication of the Infectious Diseases Society of America*, 61 Suppl 4:S363-71
33. FRYE, J.G., LINDSEY, R.L., MEINERSMANN, R.J., BERRANG, M.E., JACKSON, C.R., ENGLEN, M.D., TURPIN, J.B. & FEDORKA-CRAY, P.J. 2011. Related Antimicrobial Resistance Genes Detected in Different Bacterial Species Co-isolated from Swine Fecal Samples. *Foodborne Pathogens and Disease*, 8:663-679

34. GAY, K., ROBICSEK, A., STRAHILEVITZ, J., PARK, C.H., JACOBY, G., BARRETT, T.J., MEDALLA, F., CHILLER, T.M. & HOOPER, D.C. 2006. Plasmid-mediated quinolone resistance in non-Typhi serotypes of *Salmonella enterica. Clinical Infectious Diseases*, 43:297-304
35. GLENN, L.M., LINDSEY, R.L., FRANK, J.F., MEINERSMANN, R.J., ENGLEN, M.D., FEDORKA-CRAY, P.J. & FRYE, J.G. 2011. Analysis of Antimicrobial Resistance Genes Detected in Multidrug-Resistant *Salmonella enterica* Serovar Typhimurium Isolated from Food Animals*. Microbial Drug Resistance*, 17:407-418
36. GOSWAMI, P.S., GYLES, C.L., FRIENDSHIP, R.M., POPPE, C., KOZAK, G.K. & BOERLIN, P. 2008. Effect of plasmid pTENT2 on severity of porcine post-weaning diarrhoea induced by an O149 enterotoxigenic *Escherichia coli. Veterinary Microbiology*, 131:400-405
37. GOUWS, P.A. & BROZEL, V.S. 2000. Antimicrobial resistance of *Salmonella* isolates associated with retail chicken and a poultry abattoir*. South African Journal of Science*, 96:254-256
38. HALL, R.M. & COLLIS, C.M. 1995. Mobile gene cassettes and integrons: Capture and spread of genes by site-specific recombination*. Molecular Microbiology*, 15:593-600
39. HELMS, M., SIMONSEN, J. & MOLBAK, K. 2004. Quinolone resistance is associated with increased risk of invasive illness or death during infection with *Salmonella* serotype Typhimurium*. Journal of Infectious Diseases*, 190:1652-1654
40. HELMS, M., VASTRUP, P., GERNER-SMIDT, P. & MOLBAK, K. 2002. Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium*. Emerging Infectious Diseases*, 8:490-495
41. HENDRIKSEN, R.S., VIEIRA, A.R., KARLSMOSE, S., LO FO WONG, D.M.A., JENSEN, A.B., WEGENER, H.C. & AARESTRUP, F.M. 2011. Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. *Foodborne Pathogens and Disease*, 8:887-900
42. HOFACRE, C.L., FRICKE, J.A. & INGLIS, T. 2013. Antimicrobial Drug Use in Poultry, in *Antimicrobial Therapy in Veterinary Medicine*: John Wiley & Sons, Inc: 569-587
43. HUOVINEN, P., SUNDSTROM, L., SWEDBERG, G. & SKOLD, O. 1995. Trimethoprim and Sulphonamide Resistance*. Antimicrobial Agents and Chemotherapy*, 39:279-289
44. IGBINOSA, I.H. 2015. Prevalence and detection of antibiotic-resistant determinant in *Salmonella* isolated from food-producing animals*. Tropical Animal Health and Production*, 47:37-43
45. JONES, F., AXTELL, R.C., TARVER, F.R., RIVES, D.V., SCHEIDELER, S.E. & WINELAND, M.J. 1991. Environmental Factors Contributing to *Salmonella* Colonization of Chickens, in *Colonization Control of Human Bacterial Enteropathologens in Poultry*, edited by L.C. Blankenship, : Academic Press: 3-21
46. KARIUKI, S., OKORO, C., KIIRU, J., NJOROGE, S., OMUSE, G., LANGRIDGE, G., KINGSLEY, R.A., DOUGAN, G. & REYATHI, G. 2015. Ceftriaxone-Resistant *Salmonella enterica* Serotype Typhimurium Sequence Type 313 from Kenyan Patients Is Associated with the bla(CTX-M-15) Gene on a Novel IncHI2 Plasmid*. Antimicrobial Agents and Chemotherapy*, 59:3133-3139
47. KE, B., SUN, J., HE, D., LI, X., LIANG, Z. & KE, C. 2014. Serovar distribution, antimicrobial resistance profiles, and PFGE typing of *Salmonella enterica* strains isolated from 2007-2012 in Guangdong, China*. Bmc Infectious Diseases*, 14:338
48. KEDDY, K.H., DWARIKA, S., CROWTHER, P., PEROVIC, O., WADULA, J., HOOSEN, A., SOOKA, A., CREWE-BROWN, H.H. & SMITH, A.M. 2009. Genotypic and demographic characterization of invasive isolates of *Salmonella* Typhimurium in HIV co-infected patients in South Africa. *Journal of infection in Developing Countries*, 3:585-592

49. KERRN, M.B., KLEMMENSEN, T., FRIMODT-MOLLER, N. & ESPERSEN, F. 2002. Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of *sul* genes conferring sulphonamide resistance. *Journal of Antimicrobial Chemotherapy*, 50:513-516
50. KHAN, A.A., NAWAZ, M.S., KHAN, S.A. & CERNIGLIA, C.E. 2000. Detection of multidrug-resistant *Salmonella* Typhimurium DT104 by multiplex polymerase chain reaction. *FEMS Microbiology Letters*, 182:355-360
51. KIDANEMARIAM, A., ENGELBRECHT, M. & PICARD, J. 2010. Retrospective study on the incidence of *Salmonella* isolations in animals in South Africa, 1996 to 2006. *Journal of the South African Veterinary Association*, 81:37-44
52. KONDO, I., ISHIKAWA, T. & NAKAHARA, H. 1974. Mercury and Cadmium Resistances Mediated by Penicillinase Plasmid in *Staphylococcus Aureus. Journal of Bacteriology*, 117:1-7
53. KOZAK, G.K., BOERLIN, P., JANECKO, N., REID-SMITH, R.J. & JARDINE, C. 2009. Antimicrobial resistance in *Escherichia coli* isolates from swine and wild small mammals in the proximity of swine farms and in natural environments in Ontario, Canada. *Applied and Environmental Microbiology*, 75:559-566
54. KRUGER, T., SZABO, D., KEDDY, K.H., DEELEY, K., MARSH, J.W., HUJER, A.M., BONOMO, R.A. & PATERSON, D.L. 2004. 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
55. LANZ, R., KUHNERT, P. & BOERLIN, P. 2003. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. *Veterinary Microbiology*, 91:73-84
56. LEE, A.T.Y., HALL, R.G. & PILE, K.D. 2005. Reactive joint symptoms following an outbreak of *Salmonella* Typhimurium phage type 135a. *Journal of Rheumatology*, 32:524-527
57. LEVESQUE, C., PICHE, L., LAROSE, C. & ROY, P.H. 1995. PCR Mapping of Integrons Reveals several Novel Combinations of Resistance Genes. *Antimicrobial Agents and Chemotherapy*, 39:185-191
58. LINDSEY, R.L., FEDORKA-CRAY, P.J., FRYE, J.G. & MEINERSMANN, R.J. 2009. Inc A/C Plasmids Are Prevalent in Multidrug-Resistant *Salmonella enterica* Isolates. *Applied and Environmental Microbiology*, 75:1908-1915
59. LINDSTEDT, B., HEIR, E., NYGARD, I. & KAPPERUD, G. 2003. Characterization of class I integrons in clinical strains of *Salmonella enterica* subsp. enterica serovars Typhimurium and Enteritidis from Norwegian hospitals. *Journal of Medical Microbiology*, 52:141-149
60. LIVERMORE, D.M. 1995. Beta-Lactamases in Laboratory and Clinical Resistance. *Clinical Microbiology Reviews*, 8:557-&
61. LOUDEN, B.C., HAARMANN, D., HAN, J., FOLEY, S.L. & LYNNE, A.M. 2012. Characterization of antimicrobial resistance in *Salmonella enterica* serovar Typhimurium isolates from food animals in the U.S. *Food Research International*, 45:968-972
62. MAHERO, M., BYARUGABA, D.K., DOETKOTT, D.K., OLET, S. & KHAITSA, M.L. 2013. Antimicrobial Resistance and Presence of Class 1 Integrons in *Salmonella* Serovars Isolated from Clinical Cases of Animals and Humans in North Dakota and Uganda. *Clinical Microbiology*, 2:128
63. MAJOWICZ, S.E., MUSTO, J., SCALLAN, E., ANGULO, F.J., KIRK, M., O'BRIEN, S.J., JONES, T.F., FAZIL, A., HOEKSTRA, R.M. & INTERNATIONAL COLLABORATION ON ENTERIC DISEASE 'BURDEN OF ILLNESS' STUDIES. 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 50:882-889

64. MAKA, L., MACKIW, E., SCIEZYNSKA, H., MODZELEWSKA, M. & POPOWSKA, M. 2015. Resistance to Sulphonamides and Dissemination of *sul* Genes Among *Salmonella* spp. Isolated from Food in Poland. *Foodborne Pathogens and Disease*, 12:383-389
65. MANIE, T., KHAN, S., BROZEL, V.S., VEITH, W.J. & GOUWS, P.A. 1998. Antimicrobial resistance of bacteria isolated from slaughtered and retail chickens in South Africa. *Letters in Applied Microbiology*, 26:253-258
66. MATHER, A.E., REID, S.W.J., MASKELL, D.J., PARKHILL, J., FOOKES, M.C., HARRIS, S.R., BROWN, D.J., COIA, J.E., MULVEY, M.R., GILMOUR, M.W., PETROVSKA, L., DE PINNA, E., KURODA, M., AKIBA, M., IZUMIYA, H., CONNOR, T.R., SUCHARD, M.A., LEMEY, P., MELLOR, D.J., HAYDON, D.T. & THOMSON, N.R. 2013. Distinguishable Epidemics of Multidrug-Resistant *Salmonella* Typhimurium DT104 in Different Hosts. *Science*, 341:1514-1517
67. MAYNARD, C., FAIRBROTHER, J.M., BEKAL, S., SANSCHAGRIN, F., LEVESQUE, R.C., BROUSSEAU, R., MASSON, L., LARIVIERE, S. & HAREL, J. 2003. Antimicrobial resistance genes in enterotoxigenic *Escherichia coli* O149: K91 isolates obtained over a 23-year period from pigs. *Antimicrobial Agents and Chemotherapy*, 47:3214-3221
68. MAZEL, D., DYCHINCO, B., WEBB, V.A. & DAVIES, J. 2000. Antibiotic resistance in the ECOR collection: Integrons and identification of a novel *aad* gene. *Antimicrobial Agents and Chemotherapy*, 44:1568-1574
69. MINDLIN, M.J., LANG, N., MAGUIRE, H., WALSH, B., VERLANDER, N.Q., LANE, C., TAYLOR, C., BISHOP, L.A. & CROOK, P.D. 2013. Outbreak investigation and case-control study: penta - resistant *Salmonella* Typhimurium DT104 associated with biltong in London in 2008. *Epidemiology and Infection*, 141:1920-1927
70. MORAR, M. & WRIGHT, G.D. 2010. The genomic enzymology of antibiotic resistance. *Annual Review of Genetics*, 44:25-51
71. RECCHIA, G.D. & HALL, R.M. 1995. Gene cassettes: A new class of mobile element. *Microbiology-UK*, 141:3015-3027
72. ROBERTS, M.C. 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS microbiology reviews*, 19:1-24
73. ROBICSEK, A., JACOBY, G.A. & HOOPER, D.C. 2006. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infectious Diseases*, 6:629-640
74. RUIZ, J., HERRERA-LEON, S., MANDOMANDO, I., MACETE, E., PUYOL, L., ECHEITA, A. & ALONSO, P.L. 2008. Detection of *Salmonella enterica* serotype Typhimurium DT104 in Mozambique. *The American Journal of Tropical Medicine and Hygiene*, 79:918-920
75. SCHMIEGER, H. & SCHICKLMAIER, P. 1999. Transduction of multiple drug resistance of *Salmonella enterica* serovar Typhimurium DT104. *FEMS Microbiology Letters*, 170:251-256
76. SCHULZE, T., LÜDTKE, A., RAHLFF, I., TUNN, P.-. & HOHENBERGER, P. 2009. *Salmonella* osteomyelitis in an immune-compromised patient presenting as a primary lymphoma of the bone. *International Journal of Infectious Diseases*, 13:e67-e70
77. SCHWARZ, S., KEHRENBERG, C. & WALSH, T.R. 2001. Use of antimicrobial agents in veterinary medicine and food animal production. *International journal of antimicrobial agents*, 17:431-437
78. SHAW, K.J., RATHER, P.N., HARE, R.S. & MILLER, G.H. 1993. Molecular-Genetics of Aminoglycoside Resistance Genes and Familial Relationships of the Aminoglycoside-Modifying Enzymes. *Microbiological Reviews*, 57:138-163
79. SOUFI, L., SAENZ, Y., DE TORO, M., ABBASSI, M.S., ROJO-BEZARES, B., VINUE, L., BOUCHAMI, O., TOUATI, A., BEN HASSEN, A., HAMMAMI, S. & TORRES, C. 2012. Phenotypic and Genotypic Characterization of *Salmonella enterica* Recovered from

Poultry Meat in Tunisia and Identification of New Genetic Traits. *Vector-Borne and Zoonotic Diseases*, 12:10-16
80. SVARM. 2001. *Swedish Veterinary Antimicrobial Resistance Monitoring*, ISSN - 1650-6332 ed. Uppsala, Sweden: National Veterinary Institute Uppsala, Sweden
81. THRELFALL, E.J. 2000. Epidemic *Salmonella* Typhimurium DT 104--a truly international multiresistant clone. *The Journal of Antimicrobial Chemotherapy*, 46:7-10
82. THRELFALL, E., WARD, L. & ROWE, B. 1998. Multi-resistant *Salmonella* Typhimurium DT 104 and *Salmonella* bacteraemia. *The Lancet*, 352:287-288
83. TRAN, J.H., JACOBY, G.A. & HOOPER, D.C. 2005. Interaction of the plasmid-encoded quinolone resistance protein Qnr with *Escherichia coli* DNA gyrase. *Antimicrobial Agents and Chemotherapy*, 49:118-125
84. TRAVIS, R.M., GYLES, C.L., REID-SMITH, R., POPPE, C., MCEWEN, S.A., FRIENDSHIP, R., JANECKO, N. & BOERLIN, P. 2006. Chloramphenicol and kanamycin resistance among porcine *Escherichia coli* in Ontario. *Journal of Antimicrobial Chemotherapy*, 58:173-177
85. VAN SORGE, N.M., ZIALCITA, P.A., BROWNE, S.H., QUACH, D., GUINEY, D.G. & DORAN, K.S. 2011. Penetration and activation of brain endothelium by *Salmonella enterica* serovar Typhimurium. *Journal of Infectious Diseases*, 203:401-405
86. VELDMAN, K., CAVACO, L.M., MEVIUS, D., BATTISTI, A., FRANCO, A., BOTTELDOORN, N., BRUNEAU, M., PERRIN-GUYOMARD, A., CERNY, T., DE FRUTOS ESCOBAR, C., GUERRA, B., SCHROETER, A., GUTIERREZ, M., HOPKINS, K., MYLLYNIEMI, A., SUNDE, M., WASYL, D. & AARESTRUP, F.M. 2011. International collaborative study on the occurrence of plasmid-mediated quinolone resistance in *Salmonella enterica* and *Escherichia coli* isolated from animals, humans, food and the environment in 13 European countries. *Journal of Antimicrobial Chemotherapy*, 66:1278-1286
87. WALL, P.G., MORGAN, D., LAMDEN, K., RYAN, M., GRIFFIN, M., THRELFALL, E.J., WARD, L.R. & ROWE, B. 1994. A case control study of infection with an epidemic strain of multi-resistant *Salmonella* Typhimurium DT104 in England and Wales. *Communicable Disease Report Review*, 4:R130-5
88. WHITE, P.A., MCIVER, C.J. & RAWLINSON, W.D. 2001. Integrons and gene cassettes in the Enterobacteriaceae. *Antimicrobial Agents and Chemotherapy*, 45:2658-2661
89. YU, F., CHEN, Q., YU, X., LI, Q., DING, B., YANG, L., CHEN, C., QIN, Z., PARSONS, C., ZHANG, X., HUANG, J., LUO, Y., WANG, L. & PAN, J. 2011. High Prevalence of Extended-Spectrum Beta Lactamases among *Salmonella enterica* Typhimurium Isolates from Paediatric Patients with Diarrhoea in China. *Plos One*, 6:e16801
90. ZHAO, S., MCDERMOTT, P.F., WHITE, D.G., QAIYUMI, S., FRIEDMAN, S.L., ABBOTT, J.W., GLENN, A., AYERS, S.L., POST, K.W., FALES, W.H., WILSON, R.B., REGGIARDO, C. & WALKER, R.D. 2007. Characterization of multidrug resistant *Salmonella* recovered from diseased animals. *Veterinary Microbiology*, 123:122-132

CHAPTER 5: GENERAL DISCUSSION

Salmonella Typhimurium isolates are important foodborne pathogens which have been implicated in human gastroenteritis (EFSA 2008, Kariuki *et al.* 2005). They can also be invasive and cause bacteraemia especially in immune-deficient patients (Keddy *et al.* 2009, Feasey *et al.* 2010). The nature and severity of *S.* Typhimurium infections depends on both the virulence makeup, and resistance of bacteria to the external environments. The latter includes the immunity status of the host and bacteriostatic or bactericide effects of antimicrobial agents (Foley *et al.* 2013). Poultry products are among the potential sources of transmission of *Salmonella* infections and antimicrobial resistance to humans (Fearnley *et al.* 2011). The main aim of this study was to investigate the molecular characterization and antimicrobial resistance profiles of *S.* Typhimurium isolated from organs and environments of diseased poultry between 1995 and 2002 in South Africa and the objectives to investigate this aim are indicated in Chapter 1 with relevant literature in Chapter 2.

In Chapter 3 the genetic diversity among *S.* Typhimurium isolates (n=141) was evaluated using PFGE and isolates were screened for the carriage of SPIs, bacteriophages and plasmids-encoded virulence genes. Plasmids profiling was performed for 43 isolates that were representative of different pulsotypes. Eighty five per cent (85%) of the poultry *S.* Typhimurium isolates carried at least 5 different combinations of virulence genes that are involved in invasion and survival. That was expected since the isolates were isolated from diseased chickens. The findings of this study indicate that poultry *S.* Typhimurium isolates carry virulence genes that are incriminated in *Salmonella* infections consistent with studies carried out elsewhere on *S.* Typhimurium isolates from animals, food of animal origin and humans (Huehn *et al.* 2010, Borriello *et al.* 2012, Capuano *et al.* 2013). All isolates carried SPIs-encoded virulence genes; 75.4% of the isolates carried 5 bacteriophage-encoded virulence genes (*gtgB, gtgE, sodC1,*

sspH1, and *sspH2*) while 43% of isolates carried all five plasmid-encoded virulence genes (*spvC, pefA, mig5, srgA,* and *rck*) that were tested. That was expected since SPIs are conserved chromosomal loci (Amavisit *et al.* 2003) while bacteriophages and plasmids are mobile genetic elements (Heuer, Abdo & Smalla 2008). The understanding of these pathogenicity factors that interact with the eukaryotic host cells by evading the host defence system and establishing pathogenesis will be important for proper disease management. Virulotyping clustered 141 isolates into 59 distinct virulotypes of which only 6 were considered predominant (Table 3.4) while 47 were single isolate distinct virulotypes (Appendix 3.4). Predominant clusters included isolates that were mostly recovered from common sources during the same period. Although, the genes located on SPIs were found in each virulotype, the high variation observed among virulotypes was related to the presence or absence of virulence genes encoded on bacteriophages (*gip A* and *sopE*) and plasmids. This was expected since these mobile genetic elements are characterized by high level of insertion and deletion of genes as a rapid mode of evolution in *S.* Typhimurium strains (Heuer *et al.* 2008, Brussow, Canchaya & Hardt 2004). Acquisition of knowledge about SPIs, bacteriophages, plasmids and the pathogenicity factors encoded is helpful in a better understanding of bacterial evolution and the interaction of pathogen with eukaryotic host cells. PFGE differentiated 140 isolates into 55 distinct pulsotypes with 5 major pulsotypes, smaller clusters comprising less than 5 isolates and 33 single isolate distinct pulsotypes were also observed. Five major pulsotypes (X25, X5, X13, X45 and X3) were represented by 22.6%, 9.3%, 7.1%, 4.3% and 3.6% of isolates respectively (Figure 3.4). PFGE revealed also that *S.* Typhimurium isolates from poultry were genetically diverse consistent with previous studies (Benacer *et al.* 2010, Kariuki *et al.* 1999, Huang *et al.* 2014), indicating that they were collected from several farms for a long period spanning 8 years. Virulotyping and PFGE showed the same discriminatory index (D = 0.93) and thus, virulotyping can be recommended for subtyping of *Salmonella* serovars in laboratories where PFGE is not available. Pulsotypes, virulotypes also corresponded mainly to the plasmid profiles. For instance, all the 3 (100%) isolates (ST112,

ST116 and ST123) that belonged to pulsotype X3 and closely related virulotypes fell into the same plasmid profile D (90kb and 2kb) together with other 3 isolates that belonged to pulsotype X5 (Appendix 3.5). Pulsotypes X3 and X5 were genetically similar at 96.7% and were separated by only one node meaning that at certain time in the past; only one mutation gave rise to a separate line of evolution. Surprisingly, one isolate belonging to the pulsotype X5 contained additional plasmid 10kb; this plasmid could be acquired during conjugation. To the best of our knowledge, there is no study that combined virulotyping, PFGE, and plasmid profiling among poultry *S.* Typhimurium isolates. Although, there were small clones with same pulsotype, virulotypes, and plasmid profiles (Appendix 3.5), in overall the detailed characterization that combined virulotyping, PFGE, and plasmid profiling demonstrated the genetic diversity amongst *S.* Typhimurium isolates from South African poultry productions.

In Chapter 4 the susceptibility of *S.* Typhimurium isolates (n=141) to 16 antimicrobials were determined by disk diffusion and further screened for the presence of 27 antimicrobial resistance genes, and integrons. The multi-resistant *S.* Typhimurium definitive phage type 104 (DT104) were identified by disk diffusion and confirmed by PCR. *Salmonella* Typhimurium isolates were resistant to multiple antimicrobials especially those that are commonly used in poultry operations for prophylaxis and growth promotion (streptomycin: 94.3%, sulphonamides: 87.2% and tetracycline: 61%) at sub-inhibitory concentrations which promotes antimicrobial resistance via selection pressure (Hofacre, Fricke & Inglis 2013, Aarestrup 2000) and those used for therapy in human salmonellosis: ciprofloxacin (74.5%), cefotaxime (55.3%), ampicillin (46.4%), and ceftazidime (34.7%) (Chen 2013). Ninety seven per cent (97.2%) of the isolates were resistant to two or multiple drugs while 75.8% of isolates were resistant to at least 5 different combinations of antimicrobial agents (Table 4.1). These isolates were identified from post mortem carcasses and the death of chickens could have been a result of treatment failure due to high level of resistance to antimicrobials administered for treatment and growth promotion. These results are consistent

with other studies (Ahmed & Shimamoto 2012, Zhao *et al.* 2007) that demonstrated a high prevalence of multi-drug resistant *S.* Typhimurium isolates from diseased poultry. The most common resistance genes that were recorded in phenotypically resistant isolates included *qnrA* 79.4% (112/141) for fluoroquinolones, *sul1* 66% (92/141) for sulphonamides, *ant3'Ia* 60.3% (85/141) for streptomycin, and *blaSHV* 57.4% (81/141) for beta lactams, and *str* 50.4% (71/141) for streptomycin (Figure 4.3). For each phenotypic resistance recorded in this study, there was at least one corresponding resistance gene. There was also a perfect agreement between phenotypic and genotypic results except for few susceptible isolates which carried resistance genes (Table 4.6). These resistance genes were not expressed in susceptible isolates probably due to mutations. The high rates of class 1 integrons (83.3%) that was recorded in this study poses a big threat to public health since integrons are implicated in the wide dissemination of resistance genes thereby contributing to the emergence of pathogenic and resistant bacteria affecting animals and humans. The high prevalence of class 1 integrons is common in clinical *S.* Typhimurium isolates (Recchia & Hall 1995, Lindstedt *et al.* 2003). The high proportion (34.7%) of *S.* Typhimurium DT104 is the first one to be reported in *S.* Typhimurium isolates in South Africa. The role played by poultry DT104 in the occurrence of human disease will be worth investigating due to the previous discovery of resistance type (ACSSuT) of DT104 strains (13%) among human *S.* Typhimurium in South Africa (Keddy *et al.* 2009). This is a public health concern as DT104 has been associated with severe epidemics characterized by long period of hospitalizations and deaths (Threlfall 2000, Mather *et al.* 2013, Helms *et al.* 2002). Therefore, DT104 requires enhanced monitoring and surveillance systems to protect human and animal health.

The combined results of Chapters 3 and 4 of the *S.* Typhimurium isolates (n=141) from poultry in South Africa will enable insight, recommendations and conclusions on the relationship between virulence and antimicrobial resistance as well as DT104 isolates, PFGE and virulotyping.

5.1. Genetic diversity of *S.* Typhimurium isolates demonstrated by detailed characterization (virulotyping, PFGE, plasmid profiles and antimicrobial resistance profiles).

Virulotyping clustered 141 isolates into 59 distinct virulotypes with 5 predominant and 47 single isolate virulotypes. Likewise, PFGE differentiated 140 isolates into 55 distinct pulsotypes. Among these 55 pulsotypes, four major pulsotypes, smaller pulsotypes comprising less than 6 isolates and 33 single isolate pulsotypes were also observed. Ten plasmid profiles were observed in 43 *S.* Typhimurium isolates. The total number of phenotypic antimicrobial resistance profiles was 108 of which 21 were considered major resistance profiles (≥ 2 isolates) while 87 were single isolate profiles. All experiments (virulotyping, PFGE, plasmid profiling, and antimicrobial resistance profiling) showed a high level of genetic diversity among *S.* Typhimurium isolates. This is not surprising since the isolates were collected from multiple sources for long period of time (8 years). However, the small number of clones that were observed in each experiment included isolates that were mostly recovered from same sources during the same period. The diversity of *S.* Typhimurium isolates may be explained by the presence of mobile genetic elements that are characterized by high level of gain and deletion of genes as a rapid mode of evolution in *S.* Typhimurium strains (Heuer *et al.* 2008, Brussow *et al.* 2004). This high level of genetic variation could also be ascribed to genetic adaptation of *S.* Typhimurium strains to different environmental changes and their ability to invade a wide range of hosts. The genetic diversity of *S.* Typhimurium is in agreement with other studies that have been carried out worldwide (Huang *et al.* 2014, Lukinmaa *et al.* 2006).

5.2. Relationship between virulence characterization and antimicrobial resistance

Virulence is closely associated with development of antimicrobial resistance in the fact that antimicrobial therapy is administered after apparition of an infection (presence of a pathogen)

whereas in the absence of infection, the development of resistance is lower due to the lack of exposure to antimicrobials (Beceiro, Tomas & Bou 2013). Furthermore, the expression of virulence genes produces proteins which are essential for adaptation and survival of the pathogen against host immune systems while antimicrobial resistance genes are essential for survival of the pathogen against antimicrobial effects (Burrus & Waldor 2004).

Most of virulence and resistance genes are transmitted and disseminated between bacterial species via horizontal gene transfer which is facilitated by mobile genetic elements such as bacteriophages, plasmids and integrons (Burrus & Waldor 2004). In this study 97.2% of isolates that were resistant to ≥ 2 antimicrobial agents also carried SPIs – encoded virulence genes (*invA, sopB,* and *sifA*) involved in invasion and survival of *S.* Typhimurium in the host (Table 3.2). In addition, more than 50% of the isolates that were resistant to each of the antimicrobial agents tested in this study also carried at least 12 virulence genes: *invA, sopB, sifA, gtgB, gtgE, sodC1, sspH1, sspH2, spvC, pefA, mig5,* and *srgA* (Appendix 5.1). This is not surprising since multi-drug resistant bacteria have been associated with increased virulence which leads to increased illness and death (Helms *et al.* 2002). Furthermore, the short term exposure of *S.* Typhimurium DT104 to a sub-therapeutic dose of tetracycline was followed by over-expression of virulence genes associated with early *Salmonella* invasion of host epithelial cells (Weir *et al.* 2008). The adaptation or fight of bacteria against antimicrobial effects or other environmental challenges may be accompanied with over-expression and activation of other defence mechanisms including virulence genes. In addition, there was also a certain relationship between antimicrobial resistance profiles (AMRP) and virulotypes (Appendix 5.2). Most of the isolates that belonged to the same antimicrobial resistance profile (phenotype and genotype) carried at least 8 common virulence genes (Appendix 5.2; Appendix 5.3). The presence of more than 8 common virulence genes in multi-drug resistant isolates highlights the contribution of *S.* Typhimurium isolates in causing death in poultry. In addition, most of the isolates that were clustered in

antimicrobial resistance profiles were resistant to ciprofloxacin and harboured genes essential for invasion, and survival of the pathogen in the host. This was expected since quinolones resistance has been associated with increased risk of invasive illness or death in *S.* Typhimurium infections (Helms, Simonsen & Molbak 2004). In this study, the majority of poultry *S.* Typhimurium isolates carried virulence genes that are encoded on mobile geneelements including bacteriophages (*Gisfy-1; Gisfy-2, Gisfy-3* and *sopE*), and plasmids *(spvC, pefA, rck, mig5, and srgA).* The isolates that carried mobile genetic elements such as plasmids and integrons also carried a certain number of resistance genes. All isolates that carried at least a plasmid carried also ≥ 4 resistance genes agents (Appendix 5.4). The majority of isolates that carried class 1 integrons also carried at least 5 resistance genes (*sul1, PSE-1, flo, tetG, ant(3')-Ia*) which encode resistance to sulphonamides, ampicillin, chloramphenicol and florfenicol, tetracycline, and streptomycin and spectinomycin respectively (Appendix 5.4). Different studies also have demonstrated the presence of virulence and resistance genes on mobile genetic elements (Capuano *et al.* 2013, Ahmed & Shimamoto 2012, Chen *et al.* 2004). The findings of this study showed that *S.* Typhimurium isolates from diseased poultry carried virulence and resistance genes on mobile genetic elements (bacteriophages, plasmids and integrons). These mobile genetic elements constitute a public health concern due the risk of emergence of new hyper-virulent and multi-drug resistant bacterial species.

5.3. Relationship between identification of DT104 isolates, PFGE and virulotyping

There was a relationship between identification of DT104 isolates, PFGE, and virulotyping. The major pulsotype X25 clustered 21.9% (31/141) of the strains that were confirmed as *S.* Typhimurium DT104. A significant number 44.9% (22/49) of the DT104 isolates that shared the same pulsotype X25 also belonged to virulotype V3a which contained 13 virulence genes: *invA, sopB, sifA, gipA, gtgB, gtgE, sodC1, sspH1, sspH2, spvC, pefA, mig5,* and *srgA* (Table 4.7; Appendix 3.4). Most of these isolates (20) were recovered in 1998 from unknown farms but some

of the isolates possessed the same case number (219, 221, 127, and 151) suggesting that they might be recovered from the same farm (Figure 3.2; Table 3.4). All the DT104 isolates under study harboured the invasion gene (*invA*) and a virulence plasmid (*spvC*) consistent with the findings reported by (Khan *et al.* 2000, Ebner & Mathew 2001). SpvC is responsible for survival within macrophages and contribute to systemic infections (Gulig & Doyle 1993). Therefore, the *spvC* gene contributes significantly to the increased virulence of DT104 isolates. On top of ACSSuT resistance type, DT104 isolates showed also additional resistance to clinically recommended antimicrobials (ciprofloxacin) against human salmonellosis. This is consistent with previous studies carried out elsewhere (Helms *et al.* 2004, Mindlin *et al.* 2013, Ruiz *et al.* 2008). This clone of DT104 strains deserves a particular attention due to its pathogenicity, antimicrobial resistance and widespread.

5.4. Perspectives

Although, there was a lack of full information on the origin (farms, and provinces) of 72 isolates, the findings of the present study contributes towards the current knowledge of the role played by poultry *S.* Typhimurium isolates in human infections. There is also a need for further studies on the origin of *S.* Typhimurium isolates resistant to antimicrobials which are exclusively used to treat human salmonellosis (ciprofloxacin, ampicillin, cefotaxime, ceftazidime) and their introduction into poultry productions. In Chapter 4, we suggested that the high level of resistance to ciprofloxacin observed in this study may be associated with cross - resistance between ciprofloxacin and norfloxacin or olaquindox. These antimicrobials (norfloxacin, and olaquindox) are used in poultry operations in South Africa but cross-resistance hypothesis needs further investigation. However, our findings allow us to formulate some recommendations to all stakeholders of poultry production system in South Africa.

5.5. Recommendations

Despite the South African Antimicrobial Resistance Strategy Framework (Brink A. 2014) which focuses on three main pillars: enhance antimicrobial surveillance, antimicrobial stewardship and improved infection prevention and control, antimicrobial resistance still exists in South Africa. Thus the control of antimicrobial resistance in foodborne pathogens such as *S.* Typhimurium requires a particular attention since animals are considered reservoirs of several foodborne zoonotic pathogens. We recommend the use of gentamycin to treat poultry salmonellosis to Veterinarians, South African Poultry Association (SAPA), South African Veterinary Council (SAVC), National Veterinary Surveillance and Monitoring for Resistance to Antimicrobial Drugs (SANVAD). The low level of resistance to gentamycin (14.1%) found in this study was consistent with that previously recorded from poultry *Salmonella* spp. isolates in South Africa: 6.3% (Manie *et al.* 1998) and 5.1% (Gouws & Brozel 2000). In these studies gentamycin was the most active antimicrobial agent recommended for treatment of poultry salmonellosis. Gentamycin is also approved for treatment of poultry salmonellosis worldwide (Hofacre *et al.* 2013). Therefore, we recommend the use of gentamycin in the treatment of salmonellosis in South African poultry productions. However, our study, Manie *et al. (*1988) and Gouws & Brozel (2000) were investigations before 2002; therefore, the current situation need to be assessed. Our recommendations to policy makers (Ministry of agriculture, forestry and fisheries as well as health, SAPA, SAVC, SANVAD, South African Bureau of standards (SABS) includes:

- consolidation of stringent policies aiming at reducing contamination at poultry farm level;
- enhancement of Hazard Analysis and Critical Control Points: HACCP along the poultry chain production;
- control of informal market of poultry meat; respect of poultry husbandry standard practices including standardized poultry houses, density etc;

- enhancement of regular rapid diagnostic methods at each critical control point including even at farm level;
- control of counterfeit medicines, prescription and administration of all antimicrobials by the legally and qualified personnel;
- improvement of educational campaigns on farm practices and emergence of antimicrobial resistance to all citizens through all communication channels;
- management of wastes from houses, health clinics, hospitals, pharmacies and other institutions to avoid exposure of antimicrobial waste to environmental bacteria;
- more research on the role of food animals in development of antimicrobial resistance and their transmission to humans;
- prudent antimicrobials use and enhanced *Salmonella* monitoring and surveillance systems to mitigate antimicrobials abuse and ensure food safety for consumers.

5.6. General conclusion

The detailed characterization that combined virulotyping, PFGE, plasmid profiling and antimicrobial resistance profiling showed that *S.* Typhimurium isolates are genetically diverse. This genetic diversity was associated with 1) the adaptation of *S.* Typhimurium to hostile environments, 2) the multiple sources of isolates and 3) the long period of isolation spanning 8 years. However, the isolates recovered from the same farms or during the same period were closely related. Poultry *S.* Typhimurium isolates carried virulence genes, plasmids, resistance genes and integrons. The virulence genes that were identified in this study have been incriminated in human outbreaks and were found located on SPIs, bacteriophages and plasmids. The resistance genes that were associated with phenotypic antimicrobial resistance are known to be located on plasmids and integrons which were also identified in the majority of the isolates under study. More than 50% of the isolates resistant to each antimicrobial tested carried also at least 12 virulence genes. The mobile genetic elements (bacteriophages, plasmids, and integrons) which

carry virulence and resistance genes constitute a public health concern as mobile genetic elements contribute to the emergence and propagation of virulence and antimicrobial resistance in bacterial species affecting both animals and humans. A significant number of the multi-drug DT104 isolates (44.9%) belonged to the same pulsotype, and virulotype which contained 13 virulence genes. The increased rate of multi-resistant DT104 strains which were also hyper-virulent was observed for the first time in South Africa. This is also a problem of concern in South Africa since DT104 isolates are ~~is~~ associated with hospitalizations and deaths in animals and humans. Prudent antimicrobials use and enhanced *Salmonella* monitoring and surveillance systems with rapid diagnostic methods are recommended to mitigate antimicrobials abuse and ensure food safety to consumers.

5.7. References

1. AARESTRUP, F.M. 2000. Occurrence, selection and spread of resistance to antimicrobial agents used for growth promotion for food animals in Denmark. *APMIS.Supplementum*, 101:1-48
2. AHMED, A.M. & SHIMAMOTO, T. 2012. Genetic analysis of multiple antimicrobial resistance in *Salmonella* isolated from diseased broilers in Egypt*. Microbiology and Immunology*, 56:254-261
3. AMAVISIT, P., LIGHTFOOT, D., BROWNING, G.F. & MARKHAM, P.F. 2003. Variation between pathogenic serovars within *Salmonella* pathogenicity islands. *Journal of Bacteriology*, 185:3624-3635
4. BECEIRO, A., TOMAS, M. & BOU, G. 2013. Antimicrobial Resistance and Virulence: a Successful or Deleterious Association in the Bacterial World?*. Clinical Microbiology Review*s, 26:185-230
5. BENACER, D., THONG, K.L., WATANABE, H. & PUTHUCHEARY, S.D. 2010. Characterization of Drug-Resistant *Salmonella enterica* Serotype Typhimurium by Antibiograms, Plasmids, Integrons, Resistance Genes, and PFGE*. Journal of Microbiology and Biotechnology*, 20:1042-1052
6. BORRIELLO, G., LUCIBELLI, M.G., PESCIAROLI, M., CARULLO, M.R., GRAZIANI, C., AMMENDOLA, S., BATTISTONI, A., ERCOLINI, D., PASQUALI, P. & GALIERO, G. 2012. Diversity of *Salmonella* spp. serovars isolated from the intestines of water buffalo calves with gastroenteritis. *BMC Veterinary Research*, 8:201
7. BRINK A. 2014. *The South African Antimicrobial Resistance Strategy Framework*, http://www.fidssa.co.za/Content/Documents/01_The_South_African_Antimicrobial_Resistance_Strategy_Framework_Adrian_Brink.pdf ed. Johannesburg, South Africa: Ampath National Laboratory Services, Milpark Hospital, Johannesburg

8. BRUSSOW, H., CANCHAYA, C. & HARDT, W. 2004. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiology and Molecular Biology Reviews*, 68:560-602tabeofontents
9. BURRUS, V. & WALDOR, M.K. 2004. Shaping bacterial genomes with integrative and conjugative elements. *Research in Microbiology*, 155:376-386
10. CAPUANO, F., MANCUSI, A., CAPPARELLI, R., ESPOSITO, S. & PROROGA, Y.T.R. 2013. Characterization of drug resistance and virulotypes of *Salmonella* strains isolated from food and humans. *Foodborne Pathogens and Disease*, 10:963-968
11. CHEN, S., ZHAO, S., WHITE, D.G., SCHROEDER, C.M., LU, R., YANG, H., MCDERMOTT, P.F., AYERS, S. & MENG, J. 2004. Characterization of multiple-antimicrobial-resistant *Salmonella* serovars isolated from retail meats. *Applied and Environmental Microbiology*, 70:1-7
12. EBNER, P.D. & MATHEW, A.G. 2001. Three molecular methods to identify *Salmonella enterica* serotype Typhimurium DT104: PCR fingerprinting, multiplex PCR and rapid PFGE. *FEMS Microbiology Letters*, 205:25-29
13. EFSA, . 2008. Scientific Opinion of the Panel on Biological Hazards on a request from the European Commission on a quantitative microbiological risk assessment on *Salmonella* in meat: Source attribution for human salmonellosis from meat. *The EFSA Journal*, 625:1-32
14. FEARNLEY, E., RAUPACH, J., LAGALA, F. & CAMERON, S. 2011. *Salmonella* in chicken meat, eggs and humans; Adelaide, South Australia, 2008. *International Journal of Food Microbiology*, 146:219-227
15. FEASEY, N.A., ARCHER, B.N., HEYDERMAN, R.S., SOOKA, A., DENNIS, B., GORDON, M.A. & KEDDY, K.H. 2010. Typhoid fever and invasive nontyphoid salmonellosis, Malawi and South Africa. *Emerging Infectious Diseases*, 16:1448-1451
16. FOLEY, S.L., JOHNSON, T.J., RICKE, S.C., NAYAK, R. & DANZEISEN, J. 2013. *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. *Microbiology and Molecular Biology Reviews*, 77:582-607
17. GOUWS, P.A. & BROZEL, V.S. 2000. Antimicrobial resistance of *Salmonella* isolates associated with retail chicken and a poultry abattoir. *South African Journal of Science*, 96:254-256
18. GULIG, P.A. & DOYLE, T.J. 1993. The *Salmonella* Typhimurium Virulence Plasmid Increases the Growth-Rate of Salmonellae in Mice. *Infection and Immunity*, 61:504-511
19. HELMS, M., SIMONSEN, J. & MOLBAK, K. 2004. Quinolone resistance is associated with increased risk of invasive illness or death during infection with *Salmonella* serotype Typhimurium. *Journal of Infectious Diseases*, 190:1652-1654
20. HELMS, M., VASTRUP, P., GERNER-SMIDT, P. & MOLBAK, K. 2002. Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. *Emerging Infectious Diseases*, 8:490-495
21. HEUER, H., ABDO, Z. & SMALLA, K. 2008. Patchy distribution of flexible genetic elements in bacterial populations mediates robustness to environmental uncertainty. *FEMS Microbiology Ecology*, 65:361-371
22. HOFACRE, C.L., FRICKE, J.A. & INGLIS, T. 2013. Antimicrobial Drug Use in Poultry, in *Antimicrobial Therapy in Veterinary Medicine*: John Wiley & Sons, Inc: 569-587
23. HUANG, Y., KE, B., SUN, J., HE, D., CHEN, Q., KE, C. & YU, S. 2014. [Molecular typing and surveillance on *Salmonella* Typhimurium strain in Guangdong province, 2009-2011]. *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi*, 35:917-924
24. HUEHN, S., LA RAGIONE, R.M., ANJUM, M., SAUNDERS, M., WOODWARD, M.J., BUNGE, C., HELMUTH, R., HAUSER, E., GUERRA, B., BEUTLICH, J., BRISABOIS, A., PETERS, T., SVENSSON, L., MADAJCZAK, G., LITRUP, E., IMRE, A., HERRERA-LEON, S., MEVIUS, D., NEWELL, D.G. & MALORNY, B. 2010. Virulotyping and antimicrobial resistance typing of

Salmonella enterica serovars relevant to human health in Europe. *Foodborne Pathogens and Disease*, 7:523-535
25. KARIUKI, S., GILKS, C., KIMARI, J., MUYODI, J., WAIYAKI, P. & HART, C.A. 1999. Analysis of *Salmonella enterica* serotype Typhimurium by phage typing, antimicrobial susceptibility and pulsed-field gel electrophoresis. *Journal of Medical Microbiology*, 48:1037-1042
26. KARIUKI, S., REVATHI, G., KARIUKI, N., MUYODI, J., MWITURIA, J., MUNYALO, A., KAGENDO, D., MURUNGI, L. & HART, C.A. 2005. Increasing prevalence of multidrug-resistant non-typhoidal salmonellae, Kenya, 1994-2003. *International journal of antimicrobial agents*, 25:38-43
27. KEDDY, K.H., DWARIKA, S., CROWTHER, P., PEROVIC, O., WADULA, J., HOOSEN, A., SOOKA, A., CREWE-BROWN, H.H. & SMITH, A.M. 2009. Genotypic and demographic characterization of invasive isolates of *Salmonella* Typhimurium in HIV co-infected patients in South Africa. *Journal of infection in Developing Countries*, 3:585-592
28. KHAN, A.A., NAWAZ, M.S., KHAN, S.A. & CERNIGLIA, C.E. 2000. Detection of multidrug-resistant *Salmonella* Typhimurium DT104 by multiplex polymerase chain reaction. *FEMS Microbiology Letters*, 182:355-360
29. LINDSTEDT, B., HEIR, E., NYGARD, I. & KAPPERUD, G. 2003. Characterization of class I integrons in clinical strains of *Salmonella enterica* subsp. enterica serovars Typhimurium and Enteritidis from Norwegian hospitals. *Journal of Medical Microbiology*, 52:141-149
30. LUKINMAA, S., NAKARI, U.M., LIIMATAINEN, A. & SITTONEN, A. 2006. Genomic diversity within phage types of *Salmonella enterica* ssp enterica serotypes Enteritidis and Typhimurium. *Foodborne Pathogens and Disease*, 3:97-105
31. MANIE, T., KHAN, S., BROZEL, V.S., VEITH, W.J. & GOUWS, P.A. 1998. Antimicrobial resistance of bacteria isolated from slaughtered and retail chickens in South Africa. *Letters in Applied Microbiology*, 26:253-258
32. MATHER, A.E., REID, S.W.J., MASKELL, D.J., PARKHILL, J., FOOKES, M.C., HARRIS, S.R., BROWN, D.J., COIA, J.E., MULVEY, M.R., GILMOUR, M.W., PETROVSKA, L., DE PINNA, E., KURODA, M., AKIBA, M., IZUMIYA, H., CONNOR, T.R., SUCHARD, M.A., LEMEY, P., MELLOR, D.J., HAYDON, D.T. & THOMSON, N.R. 2013. Distinguishable Epidemics of Multidrug-Resistant *Salmonella* Typhimurium DT104 in Different Hosts. *Science*, 341:1514-1517
33. MINDLIN, M.J., LANG, N., MAGUIRE, H., WALSH, B., VERLANDER, N.Q., LANE, C., TAYLOR, C., BISHOP, L.A. & CROOK, P.D. 2013. Outbreak investigation and case-control study: penta-resistant *Salmonella* Typhimurium DT104 associated with biltong in London in 2008. *Epidemiology and Infection*, 141:1920-1927
34. RECCHIA, G.D. & HALL, R.M. 1995. Gene cassettes: A new class of mobile element. *Microbiology-UK*, 141:3015-3027
35. RUIZ, J., HERRERA-LEON, S., MANDOMANDO, I., MACETE, E., PUYOL, L., ECHEITA, A. & ALONSO, P.L. 2008. Detection of *Salmonella enterica* serotype Typhimurium DT104 in Mozambique. *The American Journal of Tropical Medicine and Hygiene*, 79:918-920
36. THRELFALL, E.J. 2000. Epidemic *Salmonella* Typhimurium DT 104--a truly international multiresistant clone. *The Journal of Antimicrobial Chemotherapy*, 46:7-10
37. WEIR, E.K., MARTIN, L.C., POPPE, C., COOMBES, B.K. & BOERLIN, P. 2008. Subinhibitory concentrations of tetracycline affect virulence gene expression in a multi-resistant Salmonella enterica subsp. enterica serovar Typhimurium DT104. *Microbes and infection / Institut Pasteur*, 10:901-907
38. ZHAO, S., MCDERMOTT, P.F., WHITE, D.G., QAIYUMI, S., FRIEDMAN, S.L., ABBOTT, J.W., GLENN, A., AYERS, S.L., POST, K.W., FALES, W.H., WILSON, R.B., REGGIARDO, C. & WALKER, R.D. 2007. Characterization of multidrug resistant *Salmonella* recovered from diseased animals. *Veterinary Microbiology*, 123:122-132

APPENDIXES

Chapter 3

Appendix 3.1. Information of *Salmonella* Typhimurium isolates used in this study

Lab code	Date	Farms	Province	Sources and Type of samples	Case No
ST1	2000	F0	NA	Organ - inside lung	-
ST2	2002	F21	NA	Organ -swab	-
ST3	2002	F20	Kwazu-Natal	Envir. -litter & dust	378
ST4	2000	F7	Limpopo	organs & intestines	966/00
ST5	2000	F7	Limpopo	organs & intestines	966/00
ST6	2000	F7	Limpopo	Organ - G13 lung	709/00
ST7	2002	F20	Kwazu-Natal	Envir - litter	169
ST8	2002	F20	Kwazu-Natal	Envir - litter	169
ST9	2002	F20	Kwazu-Natal	Envir - litter	169
ST10	2002	F20	Kwazu-Natal	Envir - litter & dust	378
ST11	2002	F20	Kwazu-Natal	Envir -litter & dust	378
ST12	2002	F20	Kwazu-Natal	Envir - litter & dust	378
ST13	2002	F20	Kwazu-Natal	Envir - litter & dust	378
ST14	2002	F20	Kwazu-Natal	Envir - litter & dust	378
ST15	2002	F20	Kwazu-Natal	Envir - litter & dust	378
ST16	2002	F20	Kwazu-Natal	Envir - litter & dust	378
ST17	2002	F20	Kwazu-Natal	Envir - litter & dust	378
ST18	2002	F20	Kwazu-Natal	Envir - litter & dust	378

ST19	2002	F20	Kwazu-Natal	Envir - litter & dust	378
ST20	2002	F20	Kwazu-Natal	Envir - litter	436
ST21	2002	F20	Kwazu-Natal	Envir - litter	436
ST22	2002	F20	Kwazu-Natal	Envir - litter	436
ST23	2002	F20	Kwazu-Natal	Envir - litter	436
ST24	2000	F7	Limpopo	Organ - Peritoneum	390/00
ST25	2000	F5	Gauteng	Organ-outside lung	731/00
ST26	2002	F20	Kwazu-Natal	Envir -litter & dust	518
ST27	2001	F0	NA	organ	315
ST28	2002	F20	Kwazu-Natal	Envir -litter & dust	518
ST29	2002	F20	Kwazu-Natal	Envir- litter & dust	518
ST30	2002	F20	Kwazu-Natal	Envir - litter & dust	518
ST31	2002	F20	Kwazu-Natal	Envir - litter & dust	518
ST32	2002	F21	NA	Organ - swab	522
ST33	2002	F14	Limpopo	Organ- swab	646
ST34	2002	F20	Kwazu-Natal	Envir - Dust	1468
ST35	2002	F20	Kwazu-Natal	Envir - Dust	1468
ST36	1995	F20	Gauteng	Organ	D707
ST37	1995	F20	Kwazu-Natal	Organ	D624
ST38	1995	F10	North West	Organ -swab	D266
ST39	1995	F20	Gauteng	Organ	D707
ST40	1995	F0	NA	organ	D302
ST41	1995	F20	Gauteng	Organ	D707

ST42	1995	F22	North West	Organ	
ST43	1995	F0	NA	organ	D871
ST44	1995	F17	NA	Organ	D902
ST45	1995	F20	Gauteng	Organ	D707
ST46	1995	F10	Gauteng	swab	D299
ST47	1995	F0	NA	organ	D785
ST48	1995	F5	NA	organ	D47
ST49	1995	F2	NA	organ	D469
ST50	1995	F9	Free States	organ	D87ii
ST51	1995	F0	NA	organ	D192
ST52	1995	F15	Gauteng	organ	D169
ST53	1995	F22	NA	organ	D906
ST54	1995	F9	Free States	organ	D532
ST55	1995	F0	NA	organ	D30
ST56	1995	F15	Gauteng	organ	D169
ST57	1995	F7	Limpopo	organ	D670
ST58	1995	F13	Gauteng	organ	D410
ST59	1995	F0	NA	organ	D505
ST60	1996	F0	NA	organ	
ST61	1996	F12	Mpalanga	organ	E 397
ST62	1996	F8	Gauteng	organ	D 1821
ST63	1996	F0	NA	organ	E 98
ST64	1996	F0	NA	organ	E 226

ST65	1996	F8	Gauteng	organ	E 147
ST66	1996	F12	Mpalanga	organ	E 397
ST67	1997	F3	Gauteng	organ	D 1835
ST68	1996	F0	NA	organ	149
ST69	1996	F15	Gauteng	organ	D 1273
ST70	1996	F8	Gauteng	organ	D 1821
ST71	1996	F1	NA	organ	E 127
ST72	1996	F2	North West	organ	E 126
ST73	1996	F11	NA	organ	E255
ST74	1995	F0	NA	organ	12310
ST75	1996	F6	Gauteng	organ	E 201
ST76	1995	F0	NA	organ	D 1716
ST77	1996	F8	Gauteng	organ	D 1821
ST78	1996	F8	Gauteng	organ	D 1821
ST79	1996	F0	NA	litter	E 789
ST80	1996	F8	Gauteng	organ	D 1821
ST81	1996	F0	NA	organ	E 359
ST82	1996	F12	Mpalanga	organ	E 397
ST83	1997	F0	NA	organ	F 753
ST84	1997	F0	NA	organ	F 582
ST85	1997	F0	NA	organ	F 581
ST86	1995	F18	Gauteng	organ	D 1836
ST87	1997	F0	NA	organ	F 753

ST88	1997	F0	NA	organ	F 98
ST89	1998	F0	NA	organ	151
ST90	1998	F0	NA	organ	153
ST91	1998	F0	NA	organ	151
ST92	1998	F0	NA	organ	190
ST93	1998	F0	NA	organ	221
ST94	1998	F0	NA	organ	221
ST95	1998	F0	NA	organ	219
ST96	1998	F0	NA	organ	221
ST97	1998	F19	NA	organ	E 19
ST98	1998	F0	NA	organ	219
ST99	1998	F0	NA	organ	221
ST100	1998	F0	NA	organ	127
ST101	1998	F0	NA	organ	219
ST102	1998	F0	NA	organ	219
ST103	1998	F0	NA	organ	219
ST104	1998	F0	NA	organ	127
ST105	1998	F0	NA	organ	151
ST106	1998	F0	NA	organ	190
ST107	1998	F0	NA	organ	897
ST108	1998	F0	NA	organ	149
ST109	1998	F0	NA	organ	149
ST110	1998	F0	NA	organ	549

ST111	1998	F0	NA	organ	1395
ST112	1998	F0	NA	organ	1395
ST113	1998	F0	NA	organ	1395
ST114	1999	F0	NA	organ	205
ST115	1999	F0	NA	organ	205
ST116	1999	F0	NA	organ	205
ST117	1999	F0	NA	organ	283
ST118	1999	F0	NA	organ	283
ST119	1999	F0	NA	organ	205
ST120	1999	F0	NA	organ	283
ST121	1999	F0	NA	organ	283
ST122	1999	F0	NA	organ	283
ST123	1999	F0	NA	organ	205
ST124	1999	F0	NA	organ	-
ST125	1999	F0	NA	organ	-
ST126	1999	F0	NA	organ	205
ST127	1999	F0	NA	organ	367
ST128	1997	F0	NA	organ	-
ST129	1998	F0	NA	organ	-
ST130	1998	F0	NA	organ	-
ST131	1999	F0	NA	organ	742
ST132	1999	F0	NA	organ	92
ST133	1999	F0	NA	organ	459

ST134	1999	F0	NA	organ	742
ST135	1999	F0	NA	organ	860
ST136	1999	F0	NA	organ	683
ST137	1999	F0	NA	organ	860
ST138	1999	F0	NA	organ	1037
ST139	1999	F0	NA	organ	1419
ST140	1999	F0	NA	organ	742
ST141	1999	F0	NA	organ	609

NA: not applicable

Appendix 3. 2. Pairwise correlation between virulence genes of *Salmonella* Typhimurium recovered from diseased poultry in South Africa

Location of virulence genes		**Correlations of virulence genes**	**Proportions (%)**
SPIs	SPIs -SPIs	*invA - sopB*	-
		invA - sifA	-
		sopB - sifA	-
Phages	(Gisfy2 -Gisfy2)	*sodC1 - gtgB*	62.9
		gtgE - gtgB	75.2
		gtgE - sodC1	69.8
	Gisfy2 - remnant phage	*sodC1 - sspH2*	43
	Gisf1 - Gisfy2	*gipA - sodC1*	19
		gipA - gtgE	29
		gipA-gtgB	28
	Gisf1 - Gisfy3	*gipA - sspH1*	-3
	Gisfy1 - remnant phage	*gipA - sspH2*	22
	Gisfy3- Gisfy2	*sspH1 - sodC1*	14
		sspH1 - gtgB	4
		sspH1 - gtgE	10
	Gisfy3 - remnant phage	*sspH1 - sspH2*	13
Plasmid	Plasmid - plasmid	*spvC - srgA*	72.5
		spvC - rck	64.1
		spvC - mig5	48
		spvC - pefA	36

		srgA -pefA	47
		srgA - mig5	55
		srgA - rck	66.7
		rck - mig5	52
		rck - pefA	52
		mig5 - pefA	19
Phages - Plasmids	Gisfy1 -plasmid	*sspH1 - pefA*	9
		sspH1 - rck	14
	Gisfy2-plasmid	*sodC1 - pefA*	18.6
		gtgB - mig5	-4.2
		gtgE - spvC	41.8
		sodC1 - mig5	4
		gtgB - spvC	35
	Gisfy3 -plasmid	*sspH2- pefA*	0.21
		sspH2 - mig5	-0.01
		sspH2 - spvC	0.15
		sspH2 - rck	0.18
		sspH2 - srgA	0.11

Appendix 3.3. Raw data on frequencies of virulence genes of *Salmonella* Typhimurium isolated between 1995 and 2002 from diseased poultry in South Africa

					0:ABSENCE					1: PRESENCE									
Lab Code	**Date**	**Clients**	**Sources**	**Case No**	***sopB***	***sifA***	***invA***	***spvC***	***pefA***	***mig5***	***srgA***	***rck***	***gipA***	***gtgB***	***sodC***	***gtgE***	***sspH1***	***sspH2***	***sopE***
ST1	2000	F0	org	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ST2	2002	F21	org	-	1	1	1	1	0	0	0	0	0	1	1	1	1	1	0
ST3	2002	F20	envi	378	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST4	2000	F7	org	966	1	1	1	0	0	0	0	0	1	1	1	1	1	1	0
ST5	2000	F7	org	966	1	1	1	0	0	0	0	0	1	1	1	1	1	1	0
ST6	2000	F7	org	709	1	1	1	1	0	0	0	0	1	1	1	1	1	1	0
ST7	2002	F20	envi	169	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST8	2002	F20	envi	169	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ST9	2002	F20	envi	169	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST10	2002	F20	envi	378	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST11	2002	F20	envi	378	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST12	2002	F20	envi	378	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST13	2002	F20	envi	378	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST14	2002	F20	envi	378	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST15	2002	F20	envi	378	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST16	2002	F20	envi	378	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST17	2002	F20	envi	378	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST18	2002	F20	envi	378	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST19	2002	F20	envi	378	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST20	2002	F20	envi	436	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST21	2002	F20	envi	436	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ST22	2002	F20	envi	436	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST23	2002	F20	envi	436	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1

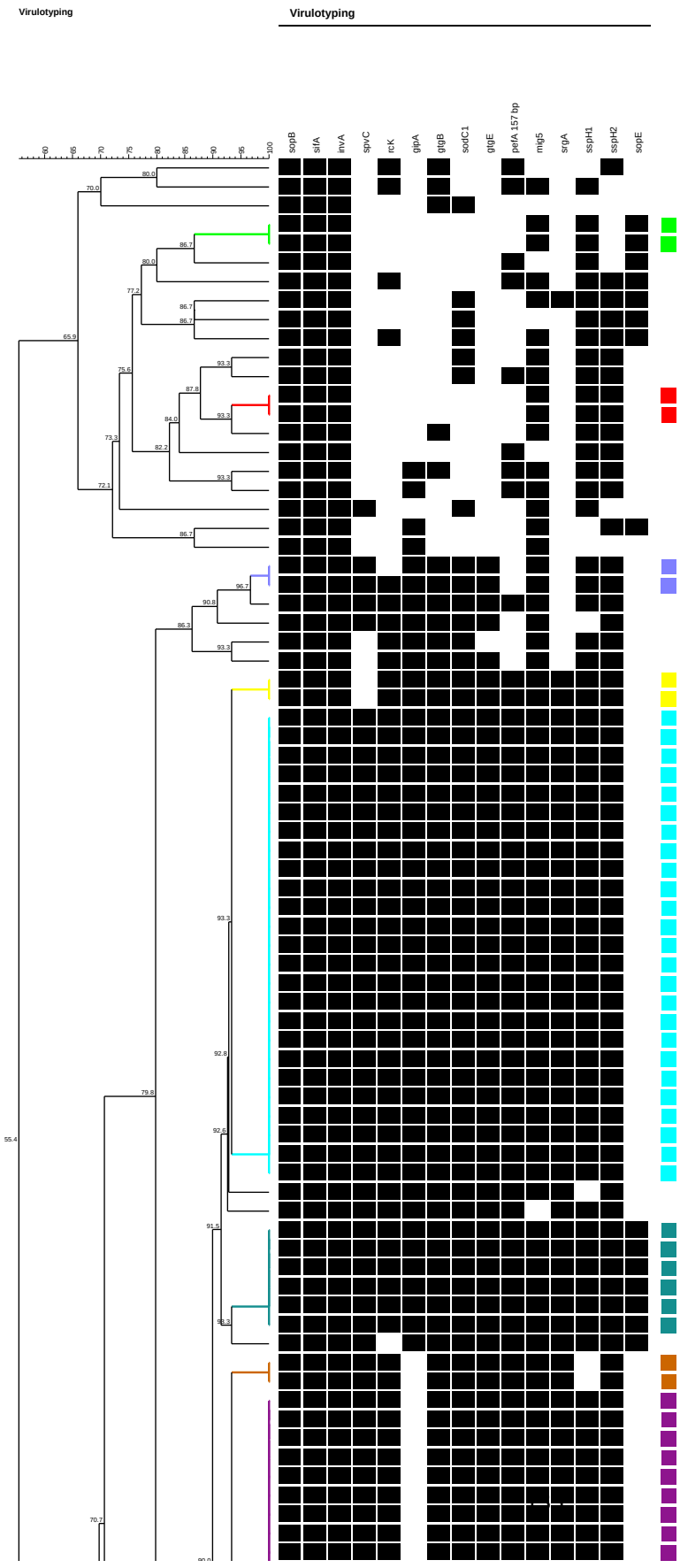
ST24	2000	F7	org	390/0	1	1	1	1	0	0	0	0	1	1	1	1	1	1	0
ST25	2000	F5	org	731/0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0
ST26	2002	F20	envi	518	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST27	2001		org	315	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST28	2002	F20	envi	518	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST29	2002	F20	envi	518	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ST30	2002	F20	envi	518	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST31	2002	F20	envi	518	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST32	2002	F21	org	522	1	1	1	1	0	0	0	0	1	1	1	1	1	1	0
ST33	2002	F14	org	646	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0
ST34	2002	F20	envi	1468	1	1	1	1	0	0	0	0	0	1	1	1	1	1	0
ST35	2002	F20	envi	1468	1	1	1	0	0	0	0	0	0	1	1	0	1	1	1
ST36	1995	F20	org	D707	1	1	1	0	1	0	0	1	0	1	1	1	1	1	1
ST37	1995	F20	org	D624	1	1	1	0	0	1	1	0	0	0	1	0	1	1	1
ST38	1995	F10	org	D266	1	1	1	0	0	1	1	0	0	0	0	0	1	0	1
ST39	1995	F20	org	D707	1	1	1	0	1	0	0	0	0	0	0	0	1	0	1
ST40	1995	F0	org	D302	1	1	1	1	0	1	0	0	0	0	1	0	1	0	0
ST41	1995	F20	org	D707	1	1	1	0	0	0	0	0	0	1	1	1	1	1	0
ST42	1995	F22	org	-	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0
ST43	1995	F0	org	D871	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1
ST44	1995	F17	org	D902	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
ST45	1995	F20	org	D707	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST46	1995	F10	org	D299	1	1	1	0	0	0	1	0	0	0	1	0	1	1	1
ST47	1995	F0	org	D785	1	1	1	0	0	0	0	0	0	1	1	1	1	1	0
ST48	1995	F5	org	D47	1	1	1	0	0	1	0	0	0	1	1	1	1	1	1
ST49	1995	F2	org	D469	1	1	1	0	0	1	0	0	0	1	1	1	0	1	0
ST50	1995	F9	org	D87ii	1	1	1	0	0	1	0	0	0	0	0	0	1	0	1
ST51	1995	F0	org	D192	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST52	1995	F15	org	D169	1	1	1	0	1	1	0	1	1	1	1	1	1	1	0
ST53	1995	F22	org	D906	1	1	1	1	0	1	0	1	1	1	1	1	1	1	0

ST54	1995	F9	org	D532	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0
ST55	1995	F0	org	D30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST56	1995	F15	org	D169	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST57	1995	F7	org	D670	1	1	1	1	0	1	0	1	1	1	1	1	0	1	0
ST58	1995	F13	org	D410	1	1	1	0	0	1	0	1	0	0	1	0	1	1	1
ST59	1995	F0	org	D505	1	1	1	1	0	1	0	1	1	1	1	1	1	1	0
ST60	1996	F0	org	-	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1
ST61	1996	F12	org	E 397	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ST62	1996	F8	org	D 1821	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST63	1996	F0	org	E 98	1	1	1	0	0	1	0	0	1	0	0	0	0	1	1
ST64	1996	F0	org	E 226	1	1	1	0	0	1	0	0	1	0	0	0	0	0	0
ST65	1996	F8	org	E 147	1	1	1	0	0	1	0	0	1	1	1	0	1	1	1
ST66	1996	F12	org	E 397	1	1	1	0	0	1	0	1	1	1	1	0	1	1	0
ST67	1997	F3	org	D 1835	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0
ST68	1996	F0	org	149	1	1	1	0	0	1	0	0	0	0	0	0	1	1	0
ST69	1996	F15	org	D 1273	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0
ST70	1996	F8	org	D 1821	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0
ST71	1996	F1	org	E 127	1	1	1	0	1	1	0	0	1	1	1	1	1	1	1
ST72	1996	F2	org	E 126	1	1	1	0	1	1	0	0	1	0	0	0	1	1	0
ST73	1996	F11	org	E255	1	1	1	0	0	1	0	0	0	1	0	0	1	1	0
ST74	1995	F0	org	12310	1	1	1	0	1	1	0	1	0	0	0	0	1	1	1
ST75	1996	F6	org	E 201	1	1	1	0	0	1	0	1	1	1	1	1	1	1	0
ST76	1995	F0	org	D 1716	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST77	1996	F8	org	D 1821	1	1	1	0	0	1	0	0	0	0	0	0	1	1	0
ST78	1996	F8	org	D 1821	1	1	1	0	0	1	0	0	0	0	1	0	1	1	0
ST79	1996	F0	envi	E 789	1	1	1	0	0	1	0	0	1	1	1	1	1	1	0
ST80	1996	F8	org	D 1821	1	1	1	0	1	1	0	0	0	0	1	0	1	1	0
ST81	1996	F0	org	E 359	1	1	1	0	1	1	0	0	1	1	1	1	1	1	0
ST82	1996	F12	org	E 397	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST83	1997	F0	org	F 753	1	1	1	0	0	1	0	0	0	1	1	1	1	1	0

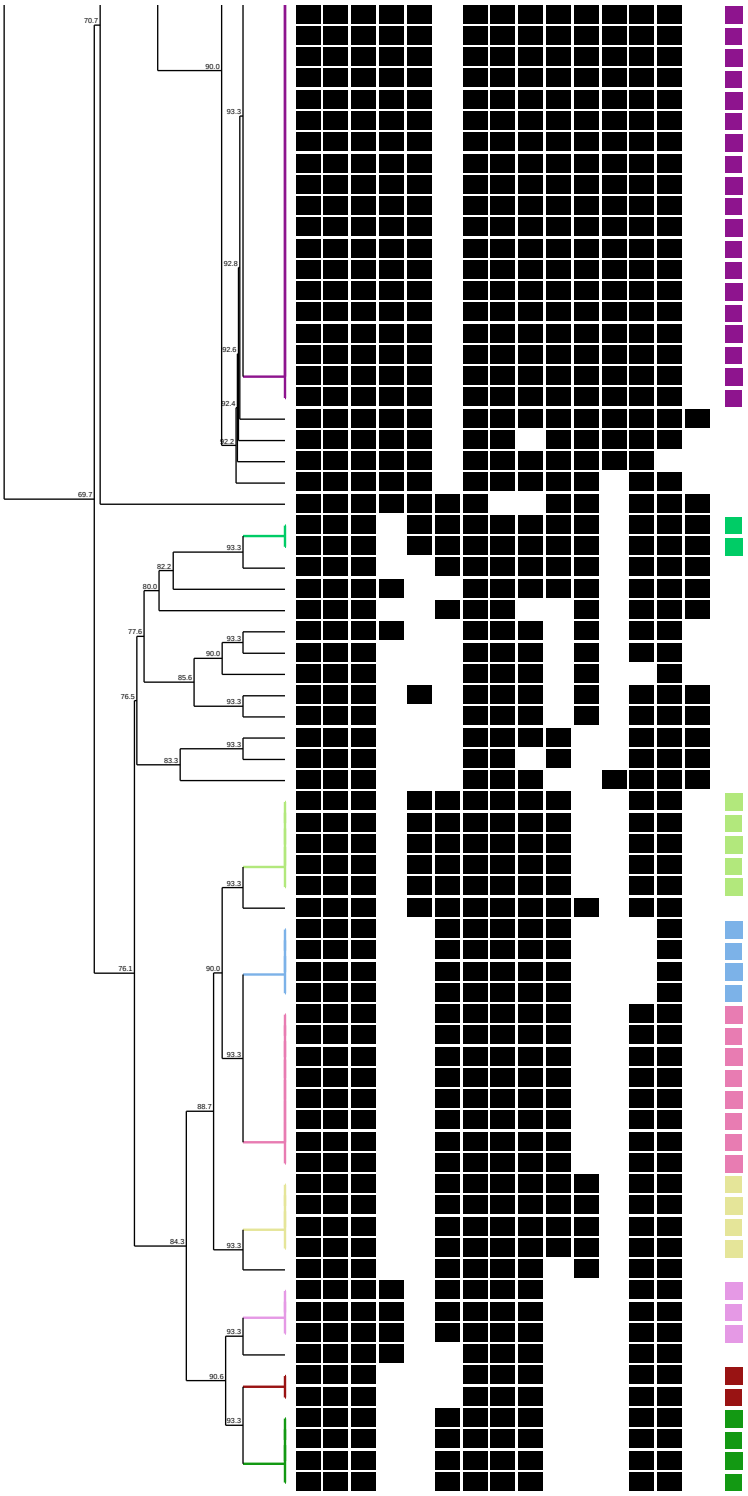
ST84	1997	F0	org	F 582	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ST85	1997	F0	org	F 581	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0
ST86	1995	F18	org	D 1836	1	1	1	1	1	1	0	1	0	1	1	1	1	1	0
ST87	1997	F0	org	F 753	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1
ST88	1997	F0	org	F 98	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST89	1998	F0	org	151	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST90	1998	F0	org	153	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST91	1998	F0	org	151	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST92	1998	F0	org	190	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST93	1998	F0	org	221	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST94	1998	F0	org	221	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0
ST95	1998	F0	org	219	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST96	1998	F0	org	221	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST97	1998	F19	org	E 19	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST98	1998	F0	org	219	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST99	1998	F0	org	221	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST100	1998	F0	org	127	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST101	1998	F0	org	219	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST102	1998	F0	org	219	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST103	1998	F0	org	219	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST104	1998	F0	org	127	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST105	1998	F0	org	151	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST106	1998	F0	org	190	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST107	1998	F0	org	897	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST108	1998	F0	org	149	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST109	1998	F0	org	149	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST110	1998	F0	org	549	1	1	1	0	1	0	0	1	0	1	0	0	0	1	0
ST111	1998	F0	org	1395	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0
ST112	1998	F0	org	1395	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0
ST113	1998	F0	org	1395	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0

ST114	1999	F0	org	205	1	1	1	0	1	0	0	0	1	1	1	1	1	1	0
ST115	1999	F0	org	205	1	1	1	0	1	0	0	0	1	1	1	1	1	1	0
ST116	1999	F0	org	205	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0
ST117	1999	F0	org	283	1	1	1	0	1	1	0	0	1	1	1	1	1	1	0
ST118	1999	F0	org	283	1	1	1	0	1	0	0	0	1	1	1	1	0	1	0
ST119	1999	F0	org	205	1	1	1	0	0	0	0	0	1	1	1	1	1	1	0
ST120	1999	F0	org	283	1	1	1	0	1	0	0	0	1	1	1	1	1	1	0
ST121	1999	F0	org	283	1	1	1	0	1	0	0	0	1	1	1	1	1	1	0
ST122	1999	F0	org	283	1	1	1	0	1	0	0	0	1	1	1	1	1	1	0
ST123	1999	F0	org	205	1	1	1	0	1	0	0	0	1	1	1	1	1	1	0
ST124	1999	F0	org	-	1	1	1	0	1	0	0	0	1	1	1	1	1	1	0
ST125	1999	F0	org	-	1	1	1	0	1	0	0	0	1	1	1	1	1	1	0
ST126	1999	F0	org	205	1	1	1	0	1	1	0	0	1	1	1	1	1	1	1
ST127	1999	F0	org	367	1	1	1	0	1	0	0	0	0	0	0	0	1	1	0
ST128	1997	F0	org	-	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
ST129	1998	F0	org	-	1	1	1	1	1	1	0	1	0	1	0	0	1	0	0
ST130	1998	F0	org	-	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST131	1999	F0	org	742	1	1	1	0	0	0	0	0	1	1	1	1	1	1	0
ST132	1999	F0	org	92	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1
ST133	1999	F0	org	459	1	1	1	0	1	0	0	0	1	1	1	1	0	1	0
ST134	1999	F0	org	742	1	1	1	0	1	1	0	0	1	1	1	1	1	1	0
ST135	1999	F0	org	860	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0
ST136	1999	F0	org	683	1	1	1	0	1	0	0	0	0	1	1	1	1	1	1
ST137	1999	F0	org	860	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
ST138	1999	F0	org	1037	1	1	1	1	1	0	0	0	1	1	1	1	0	1	0
ST139	1999	F0	org	1419	1	1	1	0	1	0	0	0	1	1	1	1	1	1	0
ST140	1999	F0	org	742	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0
ST141	1999	F0	org	609	1	1	1	0	1	0	0	0	1	1	1	1	0	1	0

Appendix 3.4. Virulotyping dendrogram of *Salmonella* Typhimurium isolates from diseased poultry in South Africa



Key	Case No	Date	Clients	Sources	Virulotypes
ST110	549	1998	F0	org	S
ST129	182	1998	F0	org	S
ST42	D492	1995	F22	org	S
ST38	D266	1995	F10	org	V7b
ST50	D87ii	1995	F9	org	V7b
ST39	D707	1995	F20	org	S
ST74	12310	1995	F0	org	S
ST37	D624	1995	F20	org	S
ST46	D299	1995	F10	org	S
ST58	D410	1995	F13	org	S
ST78	D 1821	1996	F8	org	S
ST80	D 1821	1996	F8	org	S
ST68	149	1996	F0	org	V7a
ST77	D 1821	1996	F8	org	V7a
ST73	E255	1996	F11	org	S
ST127	367	1999	F0	org	S
ST70	D 1821	1996	F8	org	S
ST72	E 126	1996	F2	org	S
ST40	D302	1995	F0	org	S
ST63	E 98	1996	F0	org	S
ST64	E 226	1996	F0	org	S
ST 59	D505	1995	F0	Organ	V8c
ST53	D906	1995	F22	org	V8c
ST69	D 1273	1996	F15	org	S
ST57	D670	1995	F7	org	S
ST66	E 397	1996	F12	org	S
ST75	E 201	1996	F6	org	S
ST25	731/00	2000	F5	org	V3b
ST33	646	2002	F14	org	V3b
ST10	378	2002	F20	envi	V2
ST11	378	2002	F20	envi	V2
ST12	378	2002	F20	envi	V2
ST13	378	2002	F20	envi	V2
ST14	378	2002	F20	envi	V2
ST15	378	2002	F20	envi	V2
ST16	378	2002	F20	envi	V2
ST17	378	2002	F20	envi	V2
ST18	378	2002	F20	envi	V2
ST19	378	2002	F20	envi	V2
ST20	436	2002	F20	envi	V2
ST22	436	2002	F20	envi	V2
ST26	518	2002	F20	envi	V2
ST27	315	2001		org	V2
ST28	518	2002	F20	envi	V2
ST3	378	2002	F20	envi	V2
ST30	518	2002	F20	envi	V2
ST31	518	2002	F20	envi	V2
ST51	D192	1995	F0	org	V2
ST55	D30	1995	F0	org	V2
ST62	D 1821	1996	F8	org	V2
ST7	169	2002	F20	envi	V2
ST82	E 397	1996	F12	org	V2
ST84	F 582	1997	F0	org	V2
ST9	169	2002	F20	envi	V2
ST94	221	1998	F0	org	S
ST45	D707	1995	F20	org	S
ST1	0	2000	F0	org	V1
ST21	436	2002	F20	envi	V1
ST29	518	2002	F20	envi	V1
ST44	D902	1995	F17	org	V1
ST61	E 397	1996	F12	org	V1
ST8	169	2002	F20	envi	V1
ST23	436	2002	F20	envi	S
ST135	860	1999	F0	org	V8a
ST54	D532	1995	F9	org	V8a
ST100	127	1998	F0	org	V3a
ST101	219	1998	F0	org	V3a
ST102	219	1998	F0	org	V3a
ST103	219	1998	F0	org	V3a
ST104	127	1998	F0	org	V3a
ST105	151	1998	F0	org	V3a
ST106	190	1998	F0	org	V3a
ST107	897	1998	F0	org	V3a
ST108	149	1998	F0	org	V3a



ST106	190	1998	F0	org	V3a
ST107	897	1998	F0	org	V3a
ST108	149	1998	F0	org	V3a
ST109	149	1998	F0	org	V3a
ST130	220	1998	F0	org	V3a
ST137	860	1999	F0	org	V3a
ST56	D169	1995	F15	org	V3a
ST76	D 1716	1995	F0	org	V3a
ST88	F 98	1997	F0	org	V3a
ST89	151	1998	F0	org	V3a
ST90	153	1998	F0	org	V3a
ST91	151	1998	F0	org	V3a
ST92	190	1998	F0	org	V3a
ST93	221	1998	F0	org	V3a
ST95	219	1998	F0	org	V3a
ST96	221	1998	F0	org	V3a
ST97	E 19	1998	F19	org	V3a
ST98	219	1998	F0	org	V3a
ST99	221	1998	F0	org	V3a
ST128	F534	1997	F0	org	S
ST67	D 1835	1997	F3	org	S
ST85	F 581	1997	F0	org	S
ST86	D 1836	1995	F18	org	S
ST60	0	1996	F0	org	S
ST132	92	1999	F0	org	V3c
ST71	E 127	1996	F1	org	V3c
ST126	205	1999	F0	org	S
ST87	F 753	1997	F0	org	S
ST65	E 147	1996	F8	org	S
ST34	1468	2002	F20	envi	S
ST83	F 753	1997	F0	org	S
ST49	D469	1995	F2	org	S
ST36	D707	1995	F20	org	S
ST48	D47	1995	F5	org	S
ST136	683	1999	F0	org	S
ST35	1468	2002	F20	envi	S
ST43	D871	1995	F0	org	S
ST111	1395	1998	F0	org	V4a
ST112	1395	1998	F0	org	V4a
ST113	1395	1998	F0	org	V4a
ST116	205	1999	F0	org	V4a
ST140	742	1999	F0	org	V4a
ST52	D169	1995	F15	org	S
ST118	283	1999	F0	org	V6a
ST133	459	1999	F0	org	V6a
ST138	1037	1999	F0	org	V6a
ST141	609	1999	F0	org	V6a
ST114	205	1999	F0	org	V5a
ST115	205	1999	F0	org	V5a
ST120	283	1999	F0	org	V5a
ST121	283	1999	F0	org	V5a
ST122	283	1999	F0	org	V5a
ST124	0	1999	F0	org	V5a
ST125	0	1999	F0	org	V5a
ST139	1419	1999	F0	org	V5a
ST117	283	1999	F0	org	V5b
ST123	205	1999	F0	org	V5b
ST134	742	1999	F0	org	V5b
ST81	E 359	1996	F0	org	V5b
ST79	E 789	1996	F0	envi	S
ST24	390/00	2000	F7	org	V4b
ST32	522	2002	F21	org	V4b
ST6	709/00	2000	F7	org	V4b
ST2	775	2002	F21	org	S
ST41	D707	1995	F20	org	V9
ST47	D785	1995	F0	org	V9
ST119	205	1999	F0	org	V6b
ST131	742	1999	F0	org	V6b
ST4	966/00	2000	F7	org	V6b
ST5	966/00	2000	F7	org	V6b

Appendix 3.5. Raw data of plasmid profiles of *Salmonella* Typhimurium isolated between 1995 and 2002 from diseased poultry in South Africa

						0: ABSENCE					1: PRESENCE		
Code	**Date**	**Clients**	**Sources**	**Province**	**No of plasmids**	**Estimation of size in Kilobase (Kb)**							
						90	**75**	**10**	**8**	**4**	**3.5**	**3**	**2**
ST1	2000	F0	organ	NA	4	1	1	0	0	0	0	1	1
ST2	2002	F21	organ	NA	2	0	0	0	0	0	0	0	0
ST5	2000	F7	organ	Limpopo	0	1	1	0	0	0	0	0	1
ST7	2002	F20	environment	Kwazulu-Natal	1	1	0	0	0	0	0	0	0
ST8	2002	F20	Environment	Kwazulu-Natal	1	1	0	0	0	0	0	0	0
ST13	2002	F20	environment	Kwazulu-Natal	1	1	0	0	0	0	0	0	0
ST16	2002	F20	environment	Kwazulu-Natal	1	1	0	0	0	0	0	0	0
ST19	2002	F20	environment	Kwazulu-Natal	2	0	0	0	1	1	0	0	0
ST22	2002	F20	environment	Kwazulu-Natal	0	0	0	0	0	0	0	0	0
ST24	2000	F7	organ	Limpopo	1	0	0	0	0	1	0	0	0
ST28	2002	F20	Environment	Kwazulu-Natal	2	0	0	0	1	1	0	0	0
ST30	2002	F20	Environment	Kwazulu-Natal	1	1	0	0	0	0	0	0	0
ST37	1995	F20	organ	Kwazulu-Natal	0	0	0	0	0	0	0	0	0
ST38	1995	F10	organ	North West	0	0	0	0	0	0	0	0	0
ST39	1995	F20	Organ	Gauteng	0	0	0	0	0	0	0	0	0
ST42	1995	F22	organ	North West	1	1	0	0	0	0	0	0	0
ST47	1995	F0	Organ	NA	1	0	0	0	0	0	0	0	1

ST54	1995	F9	organ	Free states	1	1	0	0	0	0	0	0	0
ST55	1995	F0	organ	NA	1	1	0	0	0	0	0	0	0
ST57	1995	F7	organ	Limpopo	1	1	0	0	0	0	0	0	0
ST65	1996	F8	organ	Gauteng	0	0	0	0	0	0	0	0	0
ST66	1996	F12	organ	Mpumalanga	0	0	0	0	0	0	0	0	0
ST76	1995	F0	organ	NA	0	0	0	0	0	0	0	0	0
ST85	1997	F0	organ	NA	0	0	0	0	0	0	0	0	0
ST87	1997	F0	organ	NA	1	0	0	0	0	0	1	0	0
ST93	1998	F0	organ	NA	2	1	0	0	0	0	1	0	0
ST97	1998	F19	organ	NA	2	1	0	0	0	0	1	0	0
ST99	1998	F0	organ	NA	2	1	0	0	0	0	1	0	0
ST102	1998	F0	organ	NA	2	1	0	0	0	0	1	0	0
ST107	1998	F0	organ	NA	2	1	0	0	0	0	1	0	0
ST111	1998	F0	organ	NA	1	0	0	0	0	0	1	0	0
ST112	1998	F0	organ	NA	2	1	0	0	0	0	0	0	1
ST114	1999	F0	organ	NA	2	1	0	0	0	0	0	0	1
ST116	1999	F0	organ	NA	2	1	0	0	0	0	0	0	1
ST119	1999	F0	organ	NA	1	1	0	0	0	0	0	0	0
ST122	1999	F0	organ	NA	2	1	0	0	0	0	0	0	1
ST123	1999	F0	organ	NA	2	1	0	0	0	0	0	0	1
ST126	1999	F0	organ	NA	2	1	0	0	0	0	0	0	1
ST129	1998	F0	organ	NA	3	1	0	1	0	0	0	0	1
ST131	1999	F0	organ	NA	0	0	0	0	0	0	0	0	0
ST133	1999	F0	organ	NA	1	1	0	0	0	0	0	0	0
ST137	1999	F0	organ	NA	1	1	0	0	0	0	0	0	0
ST140	1999	F0	organ	NA	3	1	0	1	0	0	0	0	1

Appendix 3.6. Relationship between pulsotypes, virulotypes and plasmid profiles among *S.* Typhimurium isolates from diseased poultry in South Africa.

Code	Date	Clients/farms	Sources	Pulsotypes	Virulotypes	Plasmid profiles
ST112	1998	F0	organ	X3	V4a	D
ST116	1999	F0	organ	X3	V4a	D
ST123	1999	F0	organ	X3	V4b	D
ST114	1999	F0	organ	X5	V5a	D
ST122	1999	F0	organ	X5	V5a	D
ST126	1999	F0	organ	X32	S	D
ST8	2002	F20	Envir	X11	V1	E
ST13	2002	F20	Envir	X13	V2	E
ST16	2002	F20	Envir	X13	V2	E
ST7	2002	F20	Envir	X14	V2	E
ST30	2002	F20	Envir	X15	V2	E
ST28	2002	F20	Envir	X17	V2	G
ST19	2002	F20	Envir	X18	V2	G
ST42	2002	F22	organ	X20	S	E
ST57	1995	F7	organ	X41	S	E
ST54	1995	F9	organ	X21	V8a	E
ST97	1998	F19	organ	X24	V3a	C
ST102	1998	F0	organ	X25	V3a	C
ST107	1998	F0	organ	X25	V3a	C
ST93	1998	F0	organ	X25	V3a	C
ST99	1998	F0	organ	X25	V3a	C

Chapter 4

Appendix 4.1: List of genes and primers, primers concentration and PCR conditions used in this study

List of genes and primers, primers concentration and PCR conditions used in this study								
ANTIMICROBIAL RESISTANCE GENES								
m PCR	**Class**	**Genes**	**Primer**	**Primer sequence (5' to 3')**	**[primer] (µM)**	**Tm (°C)**	**size (bp)**	**Reference**
1	beta Lactams	*blaTEM*	GKTEMF	TTA ACT GGC GAA CTA CTT AC	0.2	55	247	(Kozak *et al.* 2009)
			GKTEMR	GTC TAT TTC GTT CAT CCA TA				
		blaCMY-2	CMYF	GAC AGC CTC TTT CTC CAC A	0.2	55	1,000	
			CMYR	TGG ACA CGA AGG CTA CGT A				
2		*blaSHV*	SHV-F	AGG ATT GAC TGC CTT TTT G	0.4	55	393	(S. Chen *et al.* 2004)
			SHV-R	ATT TGC TGA TTT CGC TCG				
		blaPSE-1	BlaPSE-1-F	TGC TTC GCA ACT ATG ACT AC	0.2	55	438	
			BlaPSE-1-R	AGC CTG TGT TTG AGC TAG AT				
	Phenicols	*cat1*	Cat1-F	CTT GTC GCC TTG CGT ATA AT	0.4	55	508	
			Cat1-R	ATC CCA ATG GCA TCG TAA AG				
		flo	flo-F	CTG AGG GTG TCG TCA TCT AC	0.3	55	673	
			flo-R	GCT CCG ACA ATG CTG ACT AT				
		cmlA	cmlA-F	CGC CAC GGT GTT GTT GTT AT	0.3	55	394	
			CmlA-R	GCG ACC TGC GTA AAT GTC AC				
3	Tetracyclines	*tet A*	TetA-L	GGC GGT CTT CTT CAT CAT CAT GC	0.1	63	502	(Lanz *et al.* 2003)
			TetA-R	CGG CAG GCA GAG CAA GTA GA				
		tetB	TetBGK-F2	CGC CCA GTG CTG TTG TTG TC	0.2	63	173	(Goswami *et al.* 2008)
			TetBGK-R2	CGC GTT GAG AAG AAG CTG AGG TG				
		tetC	TetC-L	GCT GTA GGC ATA GGC TTG GT	0.5	63	888	(Lanz *et al.* 2003)
			TetC-R	GCC GGA AGC GAG AAG AAT CA				
single PCR		*tetG*	tetG-F	AGCAGCCTCAACCATTGCCGAT	0.2	55	391	(Chiu *et al.* 2006)
			tetG-R	GGTGTTCCACTGAAAACGGTCCT				

single PCR	Aminoglycosides	*ant3'-Ia*	ant3'Ia-F	CGC CGA AGT ATC GAC TCA AC	0.2	55	559	(S. Chen *et al.* 2004)
			Ant3'Ia-R	GCG GGA CAA CGT AAG CAC TA				
single PCR		*str*	STR-F	AGACGCTCCGCGCTATAGAAGT	0.2	63	203	(Chiu *et al.* 2006)
			STR-R	CGGACCTACCAAGGCAACGCT				
single PCR		*aac(3)-IVa*	aac(3)IVa-F	GAT GGG CCA CCT GGA CTG AT	0.2	55	462	(S. Chen *et al.* 2004)
			aac(3)IVa-R	GCG CTC ACA GCA GTG GTC AT				
4		*aadB*	aadB-L	GAG GAG TTG GAC TAT GGA TT	0.2	55	208	(Travis *et al.* 2006)
			aadB-R	CTT CAT CGG CAT AGT AAAAG				
		aphA1	aph(3')-Ia F	ATG GGC TCG CGA TAA TGT C	0.4	55	600	(Maynard *et al.* 2003)
			aph(3')-Ia R	CTC ACC GAG GCA GTT CCA T				
		aphA2	aphA2-L	GAT TGA ACA AGA TGG ATT GC	0.1	55	347	(Travis *et al.* 2006)
			aphA2-R	CCA TGA TGG ATA CTT TCT CG				
single PCR		*aphA(3')-IIa*	aphA(3')IIa-F	TCC GGT GCC CTG AAT GAA CT	0.2	55	519	(S. Chen *et al.* 2004)
			aphA(3')IIa-R	ACG GGT AGC CAA CGC TAT GT				
5	Quinolones	*qnrA*	qnrA-F	ATTTCTCACGCCAGGATTTG	0.3	53	516	(Gay *et al.* 2006)
			qnrA-R	GATCGGCAAAGGTTAGGTCA				
		qnrB	qnrB-F	GATCGTGAAAGCCAGAAAGG	0.3	53	469	
			qnrB-R	ACGATGCCTGGTAGTTGTCC				
		qnrS	qnrS-F	ACGACATTCGTCAACTGCAA	0.3	53	417	
			qnrS-R	TAAATTGGCACCCTGTAGGC				
6	Trimethoprim	*dfrI*	dfrI-F	CGG TCG TAA CAC GTT CAA GT	0.2	55	220	(S. Chen *et al.* 2004)
			dfrI-R	CTG GGG ATT TCA GGA AAG TA				
		dfrXII	dfrXII-F	AAA TTC CGG GTG AGC AGA AG	0.2	55	429	
			dfrXII-R	CCC GTT GAC GGA ATG GTT AG				
		dfrXIII	dfrXIII-F	GCA GTC GCC CTA AAA CAA AG	0.2	55	294	
			dfrXIII-R	GAT ACG TGT GAC AGC GTT GA				
7	Sulfonamides	*sul1*	sul1-F	CGG CGT GGG CTA CCT GAA CG	0.2	66	433	(Kerrn *et al.* 2002)
			sul1-B	GCC GAT CGC GTG AAG TTC CCG				

		sul3	sul3-Gka-F	CAA CGG AAG TGG GCG TTG TGG A	0.2	66	244	(Kozak *et al.* 2009)
			sul3-Gka-R	GCT GCA CCA ATT CGC TGA ACG				
single PCR		*sul2*	sul2-F	GCG CTC AAG GCA GAT GGC ATT	0.3	69	293	(Aarestrup *et al.* 2003)
			sul2-B	GCG TTT GAT ACC GGC ACC CGT				
INTEGRONS								
8	Integrons	*int1*	Lev1-F	GCC TTG CTG TTC TTC TAC GG	0.4	55	Variable	(Levesque *et al.* 1995)
			Lev1-R	GAT GCC TGC TTG TTC TAC GG				
		int2	Maz-F	CAC GGA TAT GCG ACA AAA AGG T	0.4	55	Variable	(Mazel *et al.* 2000)
			Maz-R	GTA GCA AAC GAG TGA CGA AAT G				
9		*int2*	White-F	CGG GAT CCC GGA CGG CAT GCACGA TTT GTA	0.4	65	Variable	(White *et al.* 2001)
			White-R	GAT GCC ATC GCA AGT ACG AG				
		int3	int3-F	GCC TCC GGC AGC GAC TTT CAG	0.4	65	Variable	(Mazel *et al.* 2000)
			int3-R	ACG GAT CTG CCA AAC CTG ACT				
***Salmonella* Typhimurium DT104**								
10	Phenicol	*flost*	FloF	ACC CGC CCT CTG GAT CAA GTC AAG	0.3	60	584	(Khan *et al.* 2000)
			FloR	CAA ATC ACG ACG GGC CAC GCT GTA TC				
	Virulence plasmid	*spvC*	VirF	GGG GCG GAA ATA CCA TCT ACA	0.3	60	392	
			VirR	GCG CCC AGG CTA ACA CG				
	Virulence invasion	*invA*	InvF	CGC GGC CCG ATT TTC TCT GGA	0.3	60	321	
			InvR	AAT GCG GGG ATC TGG GCG ACA AG				
	Integron	*int*	IntF	GCC CTC CCG CAC GAT GAT	0.3	60	265	
			IntR	ATT GGC GGC CTT GCT GTT CTT CTA				

mPCR: Multiplex PCR; Tm: melting temperature; [primer]: final concentration of primer

Appendix 4. 2: Antimicrobial resistance profiles (Phenotype and Genotype), and integrons

Code	Date	Farms	Source	Resistance profiles (phenotype)	Resistance genes profiles	Integrons
ST1	2000	-	org	AM-CF-CTX-S10-GM-K-CT-CIP-ENR-NA-S3	*blaTEM, str, aaC3-Iva, aphA1, aph3-IIa, qnrA,sul2*	int1
ST2	2002	F21	org	AM-CF-CTX-CAZ-S10-CT-CIP-ENR-NA-S3	*blaTEM, blaSHV,str, qnrA, sul2*	int1
ST3	2002	F20	envi	AM-CF-CTX-S10-K-CT-CIP-ENR-NA-S3	*blaTEM, blaSHV, ant3''-Ia, str, aphA1, aph3-IIa, qnrA, sul2*	int1
ST4	2000	F7	org	[Penta]-CF-CTX-CAZ-K-CT-CIP-SXT-NA	*blaTEM, cmlA, tetA, ant3''-Ia, str, aphA1,qnrA, dhfrI, sul13*	int1
ST5	2000	F7	org	[Penta]-CF-CTX-CAZ-K-CT-CIP-SXT	*blaTEM, cmlA, tetA, ant3''-Ia, str, aphA1,qnrA, dhfrI, dhfrXII, dhfrXIII, sul1, sul3*	int1
ST6	2000	F7	org	[Penta]-CF-CTX-GM-K-CT-CIP-SXT	*blaTEM, blaSHV, cmlA, tetA, ant3''-Ia, str, aaC3-Iva, aphA1, qnrA, dhfrI, sul1, sul3*	int1
ST7	2002	F20	envi	CF-CTX-S10-K-CT-CIP-ENR-NA-S3	*blaTEM, ant3''-Ia, str, aphA1, aph3-IIa, qnrA, sul2*	int1
ST8	2002	F20	envi	CF-CTX-S10-K-CT-CIP-S3	*blaTEM, ant3''-Ia, aphA1, aph3-IIa, qnrA, sul3*	int1
ST9	2002	F20	envi	AM-CF-CTX-CAZ-S10-K-CT-CIP-NA-S3	*blaTEM, blaSHV, str, aphA1, aph3-IIa, qnrA, sul1, sul3*	int1
ST10	2002	F20	envi	AM-CF-CTX-CAZ-S10-K-CT-CIP-ENR-NA-S3	*blaTEM, blaSHV, ant3''-Ia, aphA1, qnrA, sul1, sul3*	0
ST11	2002	F20	envi	CF-S10-CT-CIP-S3	*blaTEM, ant3''-Ia, str, qnrA, sul1*	0
ST12	2002	F20	envi	S10	*ant3''-Ia, str, aphA1, sul1, sul3*	int1
ST13	2002	F20	envi	CF-CTX-S10-CIP-S3	*blaTEM, blaSHV, ant3''-Ia, qnrA, sul2*	int1
ST14	2002	F20	envi	CF-CTX-CAZ-S10-CT-CIP-ENR-S3	*blaTEM, blaSHV, ant3''-Ia, qnrA, sul1, sul3*	int1
ST15	2002	F20	envi	S10-CT-CIP-S3	*blaTEM, blaSHV, ant3''-Ia, aphA1, qnrA, sul3*	int1
ST16	2002	F20	envi	CTX-S10-CT-CIP-S3	*ant3''-Ia, qnrA, sul2*	int1
ST17	2002	F20	envi	CTX-S10-CT-CIP-S3	*blaTEM, blaSHV, ant3''-Ia, qnrA, sul3*	int1
ST18	2002	F20	envi	CF-CTX-CAZ-S10-GM-K-CIP-NA-S3	*blaTEM,blaSHV,ant3''-Ia,aaC3-Iva,aphA1,aph3-IIa,qnrA,sul1,sul3*	int1

ST19	2002	F20	envi	CTX-CAZ-S10-CT-CIP-S3	*BlaTEM, blaSHV,ant3'-Ia,qnrA,sul2*	int1
ST20	2002	F20	envi	CF-CTX-S10-CT-CIP-S3	*BlaTEM,ant3'-Ia,qnrA,sul1*	int1
ST21	2002	F20	envi	CAZ-S10-K-CIP-S3	*BlaTEM,blaSHV,ant3'-Ia,aphA1,aph3-IIa,qnrA,sul2*	int1
ST22	2002	F20	envi	AM-CF-CTX-CAZ-S10-K-CT-CIP-NA-S3	*blaTEM,blaSHV,ant3'-Ia,aphA1,aph3-IIa,qnrA,sul2*	int1
ST23	2002	F20	envi	CF-CTX-CAZ-S10-GM-CIP-NA-S3	*blaTEM,blaSHV,ant3'-Ia,aaC3-Iva,qnrA,sul2*	0
ST24	2000	F7	org	CTX-C-TE-S10-K-CT-CIP-SXT-S3	*blaSHV,cmlA,tetA,str,aphA1,qnrA,dhfrI,sul1,sul3*	int1
ST25	2000	F5	org	CF-CTX-CAZ-TE-S10-CT-CIP-S3	*blaSHV,tetA,tetB,ant3'-Ia,qnrA,sul1,sul3*	int1
ST26	2002	F20	envi	CF-CTX-CAZ-S10-GM-K-CT-CIP-NA-S3	*blaTEM,blaSHV,ant3'-Ia,aaC3-Iva,aphA1,aph3-Iia,qnrA,sul2*	int1
ST27	2001	-	org	CTX-S10-K-CIP-S3	*blaTEM,blaSHV,ant3'-Ia,aphA1,aph3-IIa,qnrA,sul2*	int1
ST28	2002	F20	envi	CTX-CAZ-S10-CT-S3	*blaTEM,blaSHV,ant3'-Ia,qnrA,sul1*	int1
ST29	2002	F20	envi	CF-CTX-CAZ-S10-K-CIP-S3	*BlaTEM,blaSHV,ant3'-Ia,aphA1,aph3-IIa,qnrA,sul1*	int1
ST30	2002	F20	envi	CF-CTX-CAZ-S10-K-CIP-S3	*blaTEM,blaSHV,ant3'-Ia,aphA1,aph3-IIa,qnrA,sul2*	int1
ST31	2002	F20	envi	CTX-CAZ-S10-K-CIP-S3	*blaTEM,blaSHV,ant3'-Ia,aphA1,aph3-IIa,qnrA,sul2*	int1
ST32	2002	F21	org	CTX-C-TE-S10-K-CIP-SXT-S3	*blaSHV,cmlA,tetA,tetB,ant3'-Ia,str,aphA1,aph3-IIa,qnrA,dhfrI,dhfrXIII,sul1,sul3*	int1
ST33	2002	F14	org	CTX-S10-CIP	*blaSHV,str,qnrA,sul3*	int1
ST34	2002	F20	envi	AM-CF-CTX-CAZ-S10-GM-K-CT-CIP-S3	*blaSHV,ant3'-Ia,aaC3-Iva,aphA1,aph3-IIa,qnrA,sul2*	int1
ST35	2002	F20	envi	CF-CTX-S10-K-S3	*blaTEM,ant3'-Ia,aphA1,sul2*	0
ST36	1995	F20	org	CF-CTX-CAZ-S10-K	*blaTEM,ant3'-Ia,aphA1,sul1*	int1
ST37	1995	F20	org	TE-S10-GM-K	*tetA,tetB,ant3'-Ia,aphA1,aph3-IIa*	int1
ST38	1995	F10	org	CTX-S10-CIP	*blaSHV,tetA,ant3'-Ia,qnrA*	int1
ST39	1995	F20	org	CTX-CAZ-S10-S3	*blaSHV,ant3'-Ia,sul2*	int1
ST40	1995	-	org	CTX-CAZ-S10-CIP-S3	*blaSHV,ant3'-Ia,qnrA,sul1*	int1
ST41	1995	F20	org	-	-	int1
ST42	1995	F22	org	AM-CF-C-CIP-S3	*blaSHV,cmlA,aaC3-Iva,qnrA,sul1*	0

ST43	1995		org	[Penta]-AMC-CIP	*blaPSE,blaSHV,flo,tetG,str,aaC3-Iva,qnrA,sul1*	int1
ST44	1995	F17	org	S10-GM-S3	*ant3'-Ia, aaC3-Iva, sul1*	int1
ST45	1995	F20	org	S10-GM-CIP-S3	*blaSHV,tetB,ant3'-Ia,qnrA,sul1*	int1
ST46	1995	F10	org	S10-CIP	*ant3'-Ia,qnrA*	int1
ST47	1995	-	org	CTX-C-S10-CIP-ENR-NA-S3	*blaSHV,cmlA,str,qnrA,sul2,sul3*	int1
ST48	1995	F5	org	S10-K-S3	*ant3'-Ia,aphA1,aph3-IIa,sul1,sul3*	int1
ST49	1995	F2	org	CTX-CIP-S3	*blaSHV,qnrA,sul2*	0
ST50	1995	F9	org	-	*tetA,sul1,sul3*	int1
ST51	1995	-	org	S10-S3	*tetG,ant3'-Ia,sul2*	0
ST52	1995	F15	org	CTX-S10-CIP-S3	*blaTEM,blaSHV,ant3'-Ia,qnrA,sul1*	0
ST53	1995	F22	org	[Penta]-CF-CTX-CAZ-CIP	*blaPSE, blaTEM,blaSHV,flo,tetG,ant3'-Ia,qnrA,sul1*	0
ST54	1995	F9	org	CTX-S10-CIP-S3	*blaSHV,ant3'-Ia,qnrA,sul1*	int1
ST55	1995	-	org	S10-K-CIP-S3	*STR, aphA1, aph3-Iia, qnrA, sul1, sul3*	int1
ST56	1995	F15	org	S10-CIP-S3	*ant3'-Ia,qnrA,sul1*	int1
ST57	1995	F7	org	AM-AMC-CF-CTX-S10-CIP-S3	*blaTEM,str,qnrA,sul2*	0
ST58	1995	F13	org	[Penta]-CF-K-CIP-SXT	*blaTEM,cat1,tetB,str,aphA1,qnrA,dhfrXII,dhfrXIII,sul1*	0
ST59	1995	-	org	TE-CT	*blaTEM,tetA,tetB,sul1*	int1
ST60	1996	-	org	[Penta]-AMC-CF-CIP	*blaPSE, blaTEM,tetA,flo,tetA,ant3'-Ia,str,qnrA,sul1,sul3*	int1, int2
ST61	1996	F12	org	[Penta]-AMC-CF-CIP	*blaPSE, blaTEM,blaSHV,flo,tetG,str,qnrA,sul1*	int1
ST62	1996	F8	org	[Penta]-CF-CTX-K-CIP-ENR-SXT	*blaTEM,blaSHV,flo,tetA,tetB,tetG,str,aphA1,qnrA,dhfrI,sul1,sul3*	int1
ST63	1996	-	org	TE-S10-S3	*tetB,ant3'-Ia,sul1*	int1
ST64	1996	-	org	TE-S10-K-CIP-S3	*tetB, str, aphA1, aph3-Iia, qnrA, sul1*	0
ST65	1996	F8	org	[Penta]-CF-CTX-K-CIP-SXT	*blaTEM,cmlA,tetA,tetB,str,aphA1,aph3-IIa,qnrA,dhfrI,dhfrXIII,sul1,sul3*	int1
ST66	1996	F12	org	[Penta]	*blaPSE, blaTEM,cmlA,tetB,ant3'-Ia,sul1,sul3*	int1
ST67	1997	F3	org	CF-CTX-C-TE-S10-K-CIP-SXT-S3	*blaTEM,flo,tetA,tetB,tetG,str,aphA1,aphA2,qnrA,dhfrI,dhfrXIII,sul1,sul3*	int1

ST68	1996	-	org	[Penta]-AMC-K-CIP	*blaPSE, blaTEM,cmlA,tetB,ant3'-Ia,aphA1,aph3-Iia,qnrA,sul1*	int1
ST69	1996	F15	org	[Penta]-CIP-SXT-NA	*blaTEM,flo, tetB,str,qnrA,dhfrXIII,sul1*	int1
ST70	1996	F8	org	CAZ-C-TE-S10-K-CIP-SXT-S3	*blaTEM,blaSHV,cmlA,tetA,tetB,str,aphA1,qnrA,dhfrXIII,sul1,sul3*	int1
ST71	1996	F1	org	[Penta]-CF-CTX-K-CIP-STX	*blaTEM,blaSHV,cmlA,tetA,tetB,str,aphA1,qnrA,dhfrI,dhfrXIII,sul1,sul3*	int1
ST72	1996	F2	org	C-S10-CIP-S3	*cmlA, tetB, ant3'-Ia, str, qnrA, sul1*	int1
ST73	1996	F11	org	-	-	0
ST74	1995	-	org	S10-S3	*tetA,STR,aphA1,sul1,sul3*	int1
ST75	1996	F6	org	CTX-S10-K-CIP-S3	*blaTEM,blaSHV,ant3'-Ia,str,aphA1,aph3-IIa,qnrA,sul3*	int1
ST76	1995	-	org	CTX-S10-CIP-S3	*blaSHV,str,qnrA,sul1*	0
ST77	1996	F8	org	CTX-C-TE-S10-K-CIP-SXT-S3	*blaTEM,cmlA,tetA,tetB,ant3'-Ia,aphA1,qnrA,dhfrI,sul1,sul3*	int1
ST78	1996	F8	org	[Penta]-CF-CTX-K-CIP-SXT	*blaTEM,blaSHV,cmlA,tetA,str,aphA1,qnrA,dhfrI,dhfrXIII,sul1,sul3*	int1
ST79	1996	-	envi	CTX-S10-S3	*blaTEM,ant3'-Ia,str,sul2*	int1
ST80	1996	F8	org	C-TE-S10-K-CIP-SXT-S3	*blaTEM,blaSHV,cmlA,tetA,ant3'-Ia,str,aphA1,aphA2,qnrA,dhfrI,sul1,sul3*	int1
ST81	1996	-	org	CTX-S10-CIP-S3	*blaSHV,ant3'-Ia,str,qnrA,sul2,sul3*	int1
ST82	1996	F12	org	[Penta]-CIP	*blaPSE, blaSHV,flo,tetG,str,qnrA,sul1*	int1
ST83	1997	-	org	[Penta]-AMC-CF-CTX-CAZ-CIP-SXT	*blaPSE, blaSHV,flo,tetG,ant3'-Ia,qnrA,dhfrXIII,sul1,sul3*	int1
ST84	1997	-	org	[Penta]-CIP-NA	*blaPSE,flo,tetG,ant3'-Ia,str,qnrA,sul1,sul2*	int1
ST85	1997	-	org	[Penta]-CF-CTX-CAZ-CIP	*blaPSE, blaSHV,flo,tetG,ant3'-Ia,str,qnrA,sul1*	int1
ST86	1995	F18	org	[Penta]-CIP	*blaPSE,flo,tetG,ant3'-Ia,str,aphA1,aphA2,qnrA,sul1,sul3*	int1
ST87	1997	-	org	[Penta]-CIP	*blaPSE,flo,tetG,ant3'-Ia,str,aphA1,qnrA,sul1,sul2,sul3*	int1
ST88	1997	-	org	[Penta]-AMC-CIP	*blaPSE, blaTEM,flo,tetG,ant3'-Ia,str,aphA1,qnrA,sul1,sul3*	int1
ST89	1998	-	org	[Penta]-CIP	*blaPSE,flo,tetG,ant3'-Ia,qnrA,sul1*	int1
ST90	1998	-	org	[Penta]-CIP	*blaPSE,flo,cmlA,tetB,tetG,ant3'-Ia,qnrA,sul1*	int1

ST91	1998	-	org	[Penta]-AMC-CIP	*blaPSE,blaTEM,blaCMY,flo,tetA,tetB,tetG,str,aphA1,qnrA,sul1,sul2,sul3*	0
ST92	1998	-	org	[Penta]-CF-CAZ-K-CIP	*blaPSE, blaCMY,flo,tetB,tetG,ant3'-Ia,str,aphA1,aph3-Iia,qnrA,sul1,sul3*	0
ST93	1998	-	org	[Penta]-AMC-K-CIP	*blaPSE,flo,tetB,tetG,ant3'-Ia,str,aphA1,aph3-Iia,qnrA,sul1*	int1
ST94	1998	-	org	[Penta]-AMC-CIP	*blaPSE,flo,cmlA,tetG,ant3'-Ia,str,qnrA,sul1,sul3*	int1
ST95	1998	-	org	[Penta]-AMC-CIP	*blaPSE,flo,tetG,ant3'-Ia,str,qnrA,sul3*	int1
ST96	1998	-	org	[Penta]-AMC-CIP	*blaPSE,flo,tetG,ant3'-Ia,qnrA,sul1*	int1
ST97	1998	F19	org	[Penta]-AMC-CF-CIP	*blaSHV,blaPSE,flo,tetG,ant3'-Ia,qnrA,sul1*	int1
ST98	1998	-	org	[Penta]-AMC-CAZ-CIP	*blaPSE,flo,tetG,ant3'-Ia,qnrA,sul1*	int1
ST99	1998	-	org	[Penta]-AMC-CAZ-CIP	*blaPSE, blaSHV,flo,tetG,ant3'-Ia,qnrA,sul1*	int1
ST100	1998	-	org	[Penta]-AMC-CF-CTX-CAZ-GM-K-CT-CIP-ENR-SXT-NA	*blaPSE, blaSHV,flo,tetG,ant3'-Ia,aaC3-Iva,aphA1,aph3-Iia,qnrA,dhfrXIII,sul1*	int1
ST101	1998	-	org	[Penta]-AMC-CF-CTX-CAZ-GM-K-CT-CIP-ENR-SXT	*blaPSE, blaTEM,blaSHV,flo,tetG,ant3'-Ia,aaC3-Iva,aphA1,qnrA,dhfrXIII,sul1*	int1
ST102	1998	-	org	[Penta]-AMC-CF-CTX-CAZ-GM-K-CT-CIP-ENR-SXT	*blaPSE, blaTEM,blaCMY,flo,tetG,ant3'-Ia,aaC3-Iva,aphA1,qnrA,dhfrXIII,sul1,sul2,sul3*	int1
ST103	1998	-	org	[Penta]-AMC-CF-CTX-CAZ-GM-K-CT-CIP-ENR-SXT-NA	*blaPSE, blaSHV,Flo,tetB,tetG,ant3'-Ia,aaC3-Iva,aphA1,qnrA,dhfrXIII,sul1*	int1
ST104	1998	-	org	[Penta]-AMC-CF-CTX-CAZ-K-CIP-SXT	*blaPSE,blaSHV,flo,tetG,ant3'-Ia,aphA1,aph3-IIa,qnrA,dhfrXIII,sul1*	int1
ST105	1998	-	org	[Penta]-AMC-CTX-CAZ-CT-CIP-ENR	*blaPSE, blaSHV,flo,tetG,ant3'-Ia,qnrA,sul1*	int1
ST106	1998	-	org	[Penta]-CTX-CAZ-K-CT-CIP	*blaPSE,blaSHV,flo,tetG,ant3'-Ia,aphA1,aph3-Iia,qnrA,sul1*	int1
ST107	1998	-	org	[Penta]-AMC-CF-CAZ-GM-K-CIP	*blaPSE,blaSHV,flo,tetG,ant3'-Ia,aaC3-Iva,aphA1,qnrA,sul1*	int1
ST108	1998	-	org	[Penta]-CTX-CAZ-CT-CIP	*blaPSE,blaSHV,flo,tetB,tetG,ant3'-Ia,qnrA,sul1*	int1
ST109	1998	-	org	[Penta]-AMC-CF-CTX-CAZ-GM-CT-CIP-NA	*blaPSE, blaSHV,flo,tetB,tetG,ant3'-Ia,aaC3-Iva,qnrA,sul1*	int1
ST110	1998	-	org	[Penta]-CTX-C-CT-CIP	*blaPSE, blaSHV,flo,tetB,tetG,str,qnrA,qnrB,sul1*	int1
ST111	1998	-	org	[Penta]-CIP	*blaPSE, blaSHV,flo,tetB,tetG,qnrA,sul1*	int1
ST112	1998	-	org	[Penta]-CTX-CT-CIP	*blaTEM,blaSHV,flo,tetB,str,qnrA,sul1*	int1
ST113	1998	-	org	TE-CIP	*tetB,qnrA*	0

ST114	1999	-	org	CF-CAZ-TE-S10-CT-CIP	*blaTEM,blaSHV,tetB,str,qnrA,sul1*	0
ST115	1999	-	org	CTX-TE-S10-CT-CIP	*blaSHV,tetB,str,qnrA*	int1
ST116	1999	-	org	CTX-TE-S10-GM-K-CT-CIP-S3	*blaSHV,tetB,str,aaC3-Iva,aphA1,aph3-IIa,qnrA*	int1
ST117	1999	-	org	CTX-CAZ-TE-S10-CT-CIP-S3	*blaSHV,tetB,str,qnrA,sul2*	0
ST118	1999	-	org	TE-S10-CIP	*tetB, str,qnrA*	int1
ST119	1999	-	org	CTX-CAZ-TE-S10-CIP	*blaSHV,tetB,str,qnrA*	int1
ST120	1999	-	org	TE-S10-CIP-S3	*tetB,str,qnrA,sul2*	0
ST121	1999	-	org	TE-S10-GM-CIP-S3	*tetB,str,aaC3-Iva,qnrA,sul2*	0
ST122	1999	-	org	AM-CF-CTX-CAZ-TE-S10-K-CIP-S3	*blaSHV,tetB,str,aphA1,aph3-IIa,qnrA,sul2*	0
ST123	1999	-	org	AM-CF-CTX-CAZ-TE-S10-GM-K-CT-CIP-S3	*blaSHV,tetB,str,aaC3-Iva,aphA1,aph3-IIa,qnrA,sul2*	0
ST124	1999	-	org	CF-CTX-CAZ-TE-S10-GM-K-CIP-S3	*blaSHV,tetB,str,aaC3-Iva,aphA1,aph3-IIa,qnrA,sul1*	int1
ST125	1999	-	org	CTX-CAZ-TE-S10-K-CT-CIP-S3	*blaSHV,tetB,str,aphA1,aph3-IIa,qnrA,sul2*	0
ST126	1999	-	org	AM-CF-CTX-TE-K-CIP	*blaTEM,blaSHV,tetA,tetB,tetC,tetG,aphA1,aph3-IIa,qnrA,qnrB,sul1,sul2,sul3*	int1
ST127	1999	-	org	AM-CF-CTX-CAZ-TE-S10-K-CT-S3	*blaSHV,tetA,STR,aphA1,aphA2,aph3-IIa,sul3*	0
ST128	1997	-	org	[Penta]-AMC-CF-CTX-CAZ-K-CIP	*blaPSE,blaTEM,blaSHV,Flo,tetG,str,aphA1,aph3-Iia,qnrA,sul1*	int1
ST129	1998	-	org	[Penta]-AMC	*blaPSE,Flo,tetG,str,sul1*	int1
ST130	1998	-	org	[Penta]-AMC-CTX-CAZ-GM-K	*blaPSE,blaSHV,Flo,tetG,ant3'-Ia,aaC3-Iva,aphA1,aph3-Iia,sul1*	int1
ST131	1999	-	org	[Penta]-CF-K-SXT	*blaTEM,blaSHV,cat1,tetB,str,aadB,aaC3-Iva,aphA1,aphA2,dhfrXII,dhfrXIII,sul1,sul2*	int1
ST132	1999	-	org	CTX-CAZ-TE-S10-S3	*blaSHV,tetB,ant3'-Ia,sul1*	int1
ST133	1999	-	org	TE-S10-K-S3	*tetB,str,aphA1,aph3-IIa,sul2*	int1
ST134	1999	-	org	[Penta]-CF-GM-K-STX	*blaTEM,blaSHV,cat1,tetB,str,aadB,aaC3-Iva,aphA1,aphA2,dhfrXII,dhfrXIII,sul2*	int1
ST135	1999	-	org	TE-S10-SXT-S3	*tetA,ant3'-Ia,dhfr,sul1*	int1
ST136	1999	-	org	TE-S10	*tetB,str,sul1*	int1
ST137	1999	-	org	TE-S10-K-CT-SXT-S3	*tetA, ant3'-Ia, aphA1, dhfrI, sul1*	int1

ST138	1999	-	org	C-TE-S10-SXT-S3	*cmlA, tetA, ant3'-Ia, str, aadB, aaC3-Iva, dhfrI, sul1, sul3*	int1
ST139	1999	-	org	CTX-TE-S10-CIP	*blaSHV,tetB,str,qnrA,sul1*	int1
ST140	1999	-	org	[Penta]-CF-K-SXT	*blaTEM,blaSHV,cat1,tetB,str,aphA1,aphA2,dhfrXII, dhfrXIII,sul1,sul2*	int1
ST141	1999	-	org	TE-S10-S3	*tetB,str,sul2*	int1

[Penta]= [ACSSuT] or [AM-C-S10-S3-TE]

Chapter 5

Appendix 5.1. Distribution of virulence genes among *Salmonella* Typhimurium isolates resistant to particular antimicrobial agents

AMR		*sopB*	*sifA*	*invA*	*spvC*	*pefA*	*mig5*	*srgA*	*rck*	*gipA*	*gtgB*	*sodC1*	*gtgE*	*sspH1*	*sspH2*	*sopE*
		%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
No of positive isolates		141	141	141	79	105	103	68	81	81	125	127	115	128	133	27
AM	65	65	65	65	44	49	50	34	46	28	61	61	55	61	62	10
		100	100	100	67.7	75.4	76.9	52.3	70.8	43.1	93.8	93.8	84.6	93.8	95.4	15.4
AMC	26	26	26	26	23	22	25	20	23	4	25	24	23	24	25	4
		100	100	100	88.5	84.6	96.2	76.9	88.5	15.4	96.2	92.3	88.5	92.3	96.2	15.4
CF	56	56	56	56	36	42	41	31	36	35	53	55	48	54	54	13
		100	100	100	64.3	75	73.2	55.4	64.3	62.5	94.6	98.2	85.7	96.4	96.4	23.2
CTX	78	78	78	78	45	57	56	40	47	49	72	73	68	74	74	13
		100	100	100	57.7	73.1	71.8	51.3	60.3	62.8	92.3	93.6	87.2	94.9	94.9	16.7
CAZ	49	49	49	49	43	41	36	30	32	26	46	46	45	49	46	7
		100	100	100	87.8	83.7	73.5	61.2	65.3	53.1	93.9	93.9	91.8	100	93.9	14.3
C	62	62	62	62	40	45	48	30	41	24	56	56	48	58	59	8
		100	100	100	64.5	72.6	77.4	48.4	66.1	38.7	90.3	90.3	77.4	93.5	95.2	12.9

TE	86	86	86	86	43	66	57	34	48	45	77	78	70	77	83	13
		100	100	100	50	76.7	66.3	39.5	55.8	52.3	89.5	90.7	81.4	89.5	96.5	15.1
S10	133	133	133	133	78	103	98	68	83	78	118	121	110	122	127	25
		100	100	100	58.6	77.4	73.7	51.1	62.4	58.6	88.7	91	82.7	91.7	95.5	18.8
GM	21	21	21	21	15	18	15	13	13	12	20	21	20	21	21	4
		100	100	100	71.4	85.7	71.4	61.9	61.9	57.1	95.2	100	95.2	100	100	19
K	60	60	60	60	33	43	43	30	34	36	52	55	48	58	59	13
		100	100	100	55	71.7	71.7	50	56.7	60	86.7	91.7	80	96.7	98.3	21.7
CT	41	41	41	41	29	34	27	25	29	28	40	39	39	40	41	2
		100	100	100	70.7	82.9	65.9	61	70.7	68.3	97.6	95.1	95.1	97.6	100	4.9
CIP	112	112	112	112	70	87	85	62	72	68	102	105	96	104	107	16
		100	100	100	62.5	77.7	75.9	55.4	64.3	60.7	91.1	93.8	85.7	92.9	95.5	14.3
ENR	13	13	13	13	12	11	11	11	11	6	13	13	13	13	13	1
		100	100	100	92.3	84.6	84.6	84.6	84.6	46.2	100	100	100	100	100	7.7
NA	17	17	17	17	15	14	14	13	13	12	17	17	17	17	17	2
		100	100	100	88.2	82.4	82.4	76.5	76.5	70.6	100	100	100	100	100	11.8
SXT	27	27	27	27	14	16	19	9	12	14	23	25	20	25	27	3
		100	100	100	51.9	59.3	70.4	33.3	44.4	51.9	85.2	92.6	74.1	92.6	100	11.1
S3	123	123	123	123	77	95	95	63	80	71	111	112	102	111	117	20
		100	100	100	62.6	77.2	77.2	51.2	65	57.7	90.2	91.1	82.9	90.2	95.1	16.3

Appendix 5.2. Relationship between phenotypic antimicrobial resistance profiles and virulotypes of *Salmonella* Typhimurium isolated between 1995 and 2002 from diseased poultry in South Africa

Profiles	lab code	Date	Clients / Farms	Source	Case No	Antimicrobial resistance profiles	Virulotypes	
P1	ST 82	1996	F12	organ	E 395	AM,C,TE,S10,CIP,S3	V2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*

	ST 86	1995	F18	organ	D 1836		S	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5*
	ST 87	1997	F0	organ	F 753		S	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-sopE-spvC-pefA-mig5*
	ST 89	1998	F0	organ	151		V3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 90	1998	F0	organ	153		v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 111	1998	F0	organ	1395		v5a	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-pefA*
P2	ST 98	1998	F0	organ	219	AM,CAZ,C,TE,S10,CIP,S3	V3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 99	1998	F0	organ	221		V3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
P3	ST 43	1995	F0	organ	D 871	AM,AMC,C,TE,S10,CIP,S3	s	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-sopE*
	ST 88	1997	F0	organ	F 98		v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 91	1998	F0	organ	151		v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 94	1998	F0	organ	221		s	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 95	1998	F0	organ	219		v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 96	1998	F0	organ	221		v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
P4	ST68	1996	F0	organ	149	AM,AMC,C,TE,S10,K,CIP,S3	v7a	*invA-sopB-sifA-sspH1-sspH2-sopE-mig5*
	ST93	1998	F0	organ	221		v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
P5	ST60	1996	F0	organ	0	AM,AMC,CF,C,TE,S10,CIP,S3	s	*invA-sopB-sifA-gipA-gtgB-sodC1-sspH1-sspH2-sopE-spvC-rck-pefA-mig5*
	ST61	1996	F12	organ	E397		v1	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-sopE-spvC-rck-pefA-mig5-srgA*
	ST97	1998	F19	organ	E 19		v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
P6	ST110	1998	F0	organ	1395	AM,CTX,C,TE,S10,CT,CIP,S3	s	*invA-sopB-sifA-gtgB-sspH2-rck-pefA*
	ST112	1998	F0	organ	149		v4a	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-rck-pefA*
P7	ST53	1995	F22	organ	F 581	AM,CF,CTX,CAZ,C,TE,S10,CIP,S3	v8c	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-mig5*
	ST85	1997	F0	organ	190		s	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
P8	ST 65	1996	F8	organ	E 127	AM,CF,CTX,C,TE,S10,K,CIP,SXT,S3	v8b	*invA-sopB-sifA-gipA-gtgB-sodC1-sspH1-sspH2-sopE-mig5*
	ST 71	1996	F1	organ	D 1821		s	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-sopE-pefA-mig5*
	ST 78	1996	F8	organ	D 410		s	*invA-sopB-sifA-sodC1-sspH1-sspH2-mig5*
P9	ST 131	1999	F0	organ	742	AM,CF,C,TE,S10,K,STX,S3	v6b	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2*
	ST 140	1999	F0	organ	742		v4a	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-rck-pefA*
P10	ST 32	2002	F 21	organ	522	CTX,C,TE,S10,K,CIP,SXT,S3	v4b	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC*
	ST 77	1996	F 8	organ	D1821		v7a	*invA-sopB-sifA-sspH1-sspH2-mig5*

P11	ST 51	1995	F0	organ	D47	S10,S3	v2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 74	1995	F0	organ	12310		s	*invA-sopB-sifA-gtgE-sspH1-sspH2-sopE-rck-pefA-mig5*
P12	ST141	1999	F0	organ	609	TE,S10,S3	v6a	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH2-mig5*
	ST63	1996	F0	organ	E 98		s	*invA-sopB-sifA-gipA-sspH2-sopE-mig5*
P13	ST 52	1995	F15	organ	D 169	CTX,S10,CIP,S3	s	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-rck-pefA-mig5*
	ST 54	1995	F9	organ	D 532		v8a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-mig5*
	ST 76	1995	F0	organ	D 1716		v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 81	1996	F0	organ	E 359		v5b	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-pefA-mig5*
P14	ST 33	2002	F14	organ	646	CTX,S10,CIP	v3b	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-rck-pefA-mig5-srgA*
	ST 38	1995	F10	organ	D 266		v7b	*invA-sopB-sifA-sspH1-sopE-mig5-srgA*
P15	ST28	2002	F20	Env	518	CTX,CAZ,S10,CIP,S3	v2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST40	1995	F0	organ	D 302		s	*invA-sopB-sifA-gtgB-sspH1-spvC-mig5*
P16	ST 27	2001	F0	organ	315	CTX,S10,K,CIP,S3	v2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 75	1996	F6	organ	E 201		s	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-rck-mig5*
P17	ST29	2002	F20	Env	518	CF,CTX,CAZ.S10,K,CIP,S3	v1	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-sopE-spvC-rck-pefA-mig5-srgA*
	ST30	2002	F20	Env	518		v2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
P18	ST16	2002	F20	Env	378	CTX,S10,CT,CIP,S3	v2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST17	2002	F20	Env	378		v2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
P19	ST22	2002	F20	Env	169	AM,CF,CTX,CAZ,S10,K,CT,CIP,NA,S3	v2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST9	2002	F20	Env	378		v2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
P20	ST 101	1998	F0	organ	219	AM,AMC,CF,CTX,CAZ,C,TE,S10,GM,K,CT,CIP,ENR,SXT,S3	v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 102	1998	F0	organ	219		v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
P21	ST 100	1998	F0	organ	219	AM,AMC,CF,CTX,CAZ,C,TE,S10,GM,K,CT,CIP,ENR, NA,SXT,S3	v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 103	1998	F0	organ	149		v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*

Appendix 5.3. Relationship between genotypic antimicrobial resistance profiles and virulotypes of *Salmonella* Typhimurium isolated between 1995 and 2002 from diseased poultry in South Africa

Profiles	lab code	Date	Clients / Farms	Source	Case No	Antimicrobial resistance profiles		Virulotypes
	ST21	2002	F20	Env	436		v1	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-sopE-spvC-rck-pefA-mig5-srgA*
	ST22	2002	F20	Env	436		v2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST27	2001	F0	Organ	315		v2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST30	2002	F20	Env	518	*blaTEM, blaSHV,ant3'-Ia,aphA1,aph3-Iia,qnrA,sul2*	v2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
G1	ST31	2002	F20	Env	518		v2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST28	2002	F20	Env	518	*blaTEM,blaSHV,ant3'-Ia,qnrA,sul1*	v2	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
G2	ST52	1995	F15	Organ	D169		s	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-rck-pefA-mig5*
	ST40	1995	F0	Organ	D302		s	*invA-sopB-sifA-gtgB-sspH1-spvC-mig5*
G3	ST54	1995	F9	Organ	D532	*blaSHV,ant3'-Ia,qnrA,sul1*	v8a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-mig5*
	ST89	1998	F0	Organ	151		v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST96	1998	F0	Organ	221	*blaPSE,flo,tetG,ant3'-Ia,qnrA,sul1*	v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
G4	ST98	1998	F0	Organ	219		v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 99	1998	F0	Organ	221		v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
G5	ST 105	1998	F0	Organ	151	*blaPSE,blaSHV,flo,tetG,ant3'-Ia,qnrA,sul1*	v3a	*invA-sopB-sifA-gtgB-sodC1-gtgE-sspH1-sspH2-spvC-rck-pefA-mig5-srgA*
	ST 115	1999	F0	Organ	205		v5a	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-pefA*
G6	ST 119	1999	F0	Organ	205	*blaSHV,tetB,str,qnrA*	v6b	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2*
	ST122	1999	F0	Organ	283	*blaSHV,tetB,str,aphA1,aph3'-Iia,qnrA,sul2*	v5a	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-pefA*
G7	ST125	1999	F0	Organ	0		v5a	*invA-sopB-sifA-gipA-gtgB-sodC1-gtgE-sspH1-sspH2-pefA*

In color: virulence genes that are shared by respective antimicrobial resistance profile (phenotype and genotype);

Yellow: SPIs-encoded virulence genes;

Green: bacteriophages encoded virulence genes;

Blue: plasmid-encoded virulence genes.

Appendix 5.4. Relationship between Virulotyping, PFGE, Plasmid profiling, resistance genes and class 1 integrons

Code	Date	Farms	Sources	Pulsotypes	Virulotypes	Plasmid profiles	Resistance genes	Int 1
ST112	1998	F0	organ	X3	V4a	D	*blaTEM,blaSHV,flo,tetB,str,qnrA,sul1*	*Int 1*
ST116	1999	F0	organ	X3	V4a	D	*blaSHV,tetB,str,aaC3-Iva,aphA1,aph3-IIa,qnrA*	Int 1
ST123	1999	F0	organ	X3	V4b	D	*blaSHV,tetB,str,aaC3-Iva,aphA1,aph3-IIa,qnrA,sul2*	-
ST114	1999	F0	organ	X5	V5a	D	*blaTEM,blaSHV,tetB,str,qnrA,sul1*	-
ST122	1999	F0	organ	X5	V5a	D	*blaSHV,tetB,str,aphA1,aph3 IIa,qnrA,sul2*	-
ST126	1999	F0	organ	X32	S	D	*blaTEM,blaSHV,tetA,tetB,tetC,tetG,aphA1,aph3IIa,qnrA,qnrB,sul1,sul2,sul3*	Int1
ST8	2002	F20	Envir	X11	V1	E	*blaTEM, ant3'-Ia, aphA1, aph3-IIa, qnrA, sul3*	Int1
ST13	2002	F20	Envir	X13	V2	E	*blaTEM, blaSHV, ant3'-Ia, qnrA, sul2*	Int1
ST16	2002	F20	Envir	X13	V2	E	*ant3'-Ia, qnrA, sul2*	Int1
ST7	2002	F20	Envir	X14	V2	E	*blaTEM, ant3'-Ia, str, aphA1, aph3-IIa, qnrA, sul2*	Int1
ST30	2002	F20	Envir	X15	V2	E	*blaTEM,blaSHV,ant3'-Ia,aphA1,aph3-IIa,qnrA,sul2*	Int1

ST28	2002	F20	Envir	X17	V2	G	*blaTEM,blaSHV,ant3'-Ia,qnrA,sul1*	Int1
ST19	2002	F20	Envir	X18	V2	G	*BlaTEM, blaSHV,ant3'-Ia,qnrA,sul2*	Int1
ST42	2002	F22	organ	X20	S	E	*blaSHV,cmlA,aaC3-Iva,qnrA,sul1*	-
ST57	1995	F7	organ	X41	S	E	*blaTEM,str,qnrA,sul2*	-
ST54	1995	F9	organ	X21	V8a	E	*blaSHV,ant3'-Ia,qnrA,sul1*	Int1
ST97	1998	F19	organ	X24	V3a	C	*blaSHV,blaPSE,flo,tetG,ant3'-Ia,qnrA,sul1*	Int1
ST102	1998	F0	organ	X25	V3a	C	*blaPSE,blaTEM,blaCMY,flo,tetG,ant3'Ia,aaC3Iva,aphA1,qnrA,dhfrXIII,sul1,sul2,sul3*	Int1
ST107	1998	F0	organ	X25	V3a	C	*blaPSE,blaSHV,flo,tetG,ant3'-Ia,aaC3-Iva,aphA1,qnrA,sul1*	Int1
ST93	1998	F0	organ	X25	V3a	C	*blaPSE,flo,tetB,tetG,ant3'-Ia,str,aphA1,aph3-Iia,qnrA,sul1*	Int1
ST99	1998	F0	organ	X25	V3a	C	*blaPSE, blaSHV,flo,tetG,ant3'-Ia,qnrA,sul1*	Int1

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Molecular epidemiology and antimicrobial resistance profiling of *Salmonella Typhimurium* isolates from poultry and poultry environments in South Africa**
PROJECT NUMBER	**V020-15**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Mr. J B Ntivuguruzwa**

STUDENT NUMBER (where applicable)	**12360377**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**n/a**	
NUMBER OF ANIMALS	**n/a**	
Approval period to use animals for research/testing purposes		**1 May 2015 – 1 May 2016**
SUPERVISOR	**Dr. M Karama**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure /s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date	25 May 2015
CHAIRMAN: UP Animal Ethics Committee	Signature	

S***4285-15***