The virulence and antimicrobial resistance of *Salmonella* spp. isolates from rodents inhabiting chicken farms in Mafikeng, South Africa

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DECLARATION

I, Tsepo A Ramatla, declare that the thesis entitled "**The virulence and antimicrobial resistance of *Salmonella* spp. isolates from rodents inhabiting chicken farms in Mafikeng, South Africa**", submitted for the degree of *Doctor of Philosophy in Animal Health*, has not previously been submitted by me for a degree at this or any other University. I further declare that this is my work in design and execution and that all materials contained herein have been duly acknowledged.

Signed: _____ Date: _____

Tsepo A Ramatla (Candidate)

As supervisors, we agree to the submission of this thesis.

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Prof. M. Syakalima (Promoter)

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Prof. O.M.M. Thekisoe (Co-Promoter)

GENERAL ABSTRACT

Rodents are known to carry a number of zoonotic pathogens of importance causing both human and animal diseases. Rodents inhabiting poultry houses have been shown to carry disease causative agents for salmonellosis, fowl pox, erysipelas and leptospirosis. The main aim of the current study was to determine the types and virulence of *Salmonella* spp. carried by rodents captured at poultry farms around Mafikeng, North West Province, South Africa. In order to achieve this, rodents were captured around poultry houses and identified. A total of 154 rodents were humanely captured from six selected poultry farms, processed and identified molecularly using the *Cytochrome oxidase subunit 1* (*COI*) and the *Cytochrome-b* (*Cyt-b*) barcoding genes for species identification. Two rodent pest species namely; *Rattus rattus* known as the black rat and *Rattus tanezumi* (Asian Rat/Asian House Rat) were identified. Out of 154 rodents captured, the dominant population, 99 (64.3%), were identified as *R. rattus* and 55 (35.7%) were *R. tanezumi*. Of the two barcoding genes, *Cyt-b* gene was only able to identify 40 (25.97%) of the total samples while *COI* was more efficient and amplified all the samples.

After identifying the types of rodents found in these farms, these rats were then checked for the presence of *Salmonella*. Fecal samples were collected from their caeca and analyzed for *Salmonella* using cultural methods and conventional PCR targeting the *16S rDNA* gene. Sixty eight *Salmonella* spp. were detected, identified and confirmed by PCR. Overly, 38.2% were identified as *S.* typhimurium, 11.8% as *S. newport*, 17.6% as *S. enteritidis*, 10.3% as *S. heidelberg*, 8.8% as *S. bongori*, 5.9% as *S. enteric* serovar paratyphi B, 4.4% as *S. tennessee* and 2.9% as *S. pullorum*. Most of the *Salmonella* isolates were from *R. rattus* (63.3%) species and the rest were from *R. tanezumi* (36.8%). This, to our knowledge, is the first study to have isolated

and determined *Salmonella* spp. in rats around poultry farms in South Africa, particularly from *R. tanezumi*.

The *Salmonella* isolates were then checked for their virulence by detection of documented virulence genes. Isolates were screened for the presence of eleven (n=11) virulence genes that are known to confer pathogenicity to *Salmonella,* namely; *inv*A, *Sdf I*, *Spy*, *Spv*C, *hil*A, *spi*C, *mis*L, *orf*L, *Ppb23, fli*B and *fli*C. The virulence genes were detected by PCR using published primers. Out of the 68 *inv*A positive *Salmonella* strains, 12 (18%), 25 (37%), 14 (21%), 34 (50%), 44 (65%), 32 (47%), 39 (57%) were positive for *SdfI*, *spy*, *Spv*C, *hil*A, *mis*L, *Orf*L *spi*C genes, respectively. There were *Salmonella* serotypes which were carrying multi-virulence genes i.e. *S.* typhimurium, *S. enteritidis, S. newport*, *S. heidelberg*, *S. bongori*, and *S. pullorum*, with 7 (10.3%), 6 (8.8%), 2 (2.9%), 3 (4.4%), 2 (2.9%) and 3 (4.4%), respectively. The more the number of virulent genes detected in an isolate, the higher the risk of pathogenicity the isolate was likely to be, and so most of these strains were of high pathogenic potential.

Finally, the isolates were assessed for antibiotic resistance by both phenotypic and genotypic methods. Most of the *Salmonella* isolates showed resistance to Rifampicin 68 (100%), Tetracycline 32 (47.1%), Ciprofloxacin 21 (30.9%), Sulphonamides 12 (17.6%), Cephalothin 12 (17.6%), Chloramphenicol 9 (13.2%), Streptomycin 8 (11.8%), Enrofloxacin 6 (8.8%), Ampicillin 3 (4.4%), Amoxicillin/clavulanic Acid 2 (2.9%) and Nalidixic acid 1 (1.5%). All *Salmonella* isolates were, however, susceptible to gentamicin. Several *Salmonella* serovars showed multiple drug resistance of up to four different antibiotics. Using molecular means, antibiotic resistance genes assessed included the following resistance genes; *tet*, *cat*, *bla*TEM, *sul, qnrA* and *aadA.* Each of the genes represents resistance to the antibiotics: Tetracycline, Chloramphenicol, *β*-lactams, Sulfonamide, Quinolones and Aminoglycoside, respectively. All

these genes were detected from some of *Salmonella* isolates at varying levels. Seventy-seven percent (n=52) of the isolates were also confirmed as harbouring class 1 integrons, the presence of which indicates that these isolates were containing one/more genes that encode antibiotic resistance.

In conclusion, this study has shown that two rodent types, namely, *R. tanezumi* and *R. rattus* are the common rodent species in poultry farms around Mafikeng. These rat types carry *Salmonella* spp. some of which are known for causing disease outbreaks in animals and humans. Their pathogenic potential is represented by the virulent genes that were detected. These *Salmonellae* spp. had varying levels of antibiotic responsiveness with some showing multiple drug resistance. These findings are very important in the control and treatment of *Salmonella* in poultry farms as well as its management at public health level. The findings of this study also highlight the significance of rodent control in order to control the occurrence of *Salmonella* in poultry farms.

Keywords: Rodents, *Salmonella* spp., virulence genes, antibiotic resistance pattern.

DEDICATION

I dedicate this study to God and my family.

"Bless the Lord, O my soul and all that is within me, bless his holy name.

Bless the Lord, O my soul and forget not all his benefits.

(Psalm 103:1-2)"

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LIST OF ABBREVIATIONS AND ACRONYMS

API	Analytical profile index
BGS	Brilliant Green Agar with Sulfadiozine
bp	base pairs
BPLS	brilliant-green phenol-red lactose sucrose
BPW	buffered peptone water
COI	Cytochrome Oxydase I
Cyt-*b*	Cytochrome *b*
DNA	Deoxyribonucleic acid
FAO	Food and Agricultural Organization
*hil*A	Hyper-invasive locus A
*hil*B	Hyper-invasive locus B
*hil*C	Hyper-invasive locus C
*inv*A	Invasion A gene
ISO	International Organization for Standardization
LPS	Lipopolysaccharide

MDR	Multidrug resistance
MH	Muller-Hinton Agar
MKTT	Muller-Kauffmann Tetrathionet with novobiocinnin
NCCLS	National Committee for Clinical Laboratory Standards
NTS	Non-typhoid Salmonellosis
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
RV	Rapport-Vassiliadis Broth
*sip*C	*Salmonella* invasion protein C
SPI	*Salmonella* pathogenicity island
*spi*C	*Salmonella* pathogenicity island protein C
spp.	Species
spv	*Salmonella* plasmid virulence
TSI	Triple Sugar Iron agar
USA	United States of America
VP	Voges-Proskauer
WHO	World Health Organization

XLD	Xylose lactose deoxycholate agar
3'	(Reverse Primer) an oligonucleotide that flanks the 3'end of the Amplicon
5'	**(**Forward Primer) or an oligonucleotide that flanks the 5'end of the Amplicon

LIST OF UNITS

±	Plus or minus
:	Is to
>	Greater than
<	Less than
%	Percentage
/	Per
°C	Degree Celsius
g	Gram
L	Liter
Mg	Milligram
µ/g	Microgram/gram
µ/mL	Microgram/milliliter
mL	Milliliter
mm	Millimeter
nm	Nanometer

μg	Micro gram
μL	Micro litre
μm	Micro metre
Kb	Kilo base pairs

CHAPTER 1− GENERAL BACKGROUND

1.1 BACKGROUND

Rodents are major vectors and reservoirs (carriers) of many important pathogens of importance which cause both animal and human diseases (El-Sharkawy *et al.*, 2017; Inoue *et al.*, 2008). Their role in the spread and transmission of human diseases has been documented in historical archives. For example, the *Rattus rattus* (black roof-rat) is a known carrier of bubonic plague (also known as the Black Death), a disease that destroyed a part of Europe's population in the 14th century, and killed 25 million people. Furthermore, the disease invaded South Africa's harbor regions in the 19th century. The other rat species, the *Rattus norvegicus* (brown rat), on the other hand, has been a known carrier of Weil's disease, Hantavirus pulmonary syndrome and viral hemorrhagic fever (Kidanemariam *et al.*, 2010).

Rodents are now recognized as vectors of all classes of pathogens in the bacterial, viral, protozoa and helminthic groups that are important in poultry. Some of the important and notable bacterial pathogens are *Pasteurella multocida*, *Salmonella* typhimurium, and *S. enteritidis* (Amk, 2015). Others include the causes of *Leptospirosis, Fowl typhoid, Salmonellosis, Pseudo tuberculosis, Fowl cholera* and *Erysipelas* (Meerburg & Kijlstra, 2007; Meerburg *et al.*, 2009). As for the viral diseases, they have been implicated in the spreading of Newcastle disease virus whereas for the protozoan infections they carry *Toxoplasma gondii* and *Eimeria* spp. which cause *Toxoplasmosis* and *Coccidiosis*, respectively (Chaisiri *et al.*, 2012). The later diseases impact seriously on poultry production (Criste *et al.*, 2011) as well as on other livestock. Moreover, they are also known as disease-causing agents of food animals and humans (Jemilehin *et al.*, 2016).

Of particular concern in this study amongst the bacteria carried by rodents is *Salmonella* that causes salmonellosis, which is a serious disease in humans and livestock (Feng *et al.*, 2012; Hong *et al.*, 2018; Liu *et al.*, 2013). The risks posed by the presence of rodents with regards to *Salmonella* persistence in poultry houses have been evaluated in different studies; in the UK (Davies & Wray, 1995), USA (Meerburg & Kijlstra, 2007), Japan (Lapuz *et al.*, 2008), and Nigeria (Jemilehin *et al.*, 2016). *Salmonella* serotypes detected from these studies were important for poultry and human infections. *Salmonella* serotypes detected from rats infesting poultry farms included *Salmonella* typhimurium, *Salmonella montivideo*, *Salmonella derby* and *Salmonella enteritidis* all of which can be associated with human diseases as well (McKiel *et al.*, 1970; Meerburg & Kijlstra, 2007).

Salmonella is a main food-borne disease and studies elsewhere have indicated that it is the second most vital food-borne disease, especially in the Western World (Martinson *et al.*, 2007; Taylor *et al.*, 2010; Van Nierop *et al.*, 2005; Van *et al.*, 2012). *Salmonella* strains have been grouped as typhoidal and non-typhoidal organisms based on their disease propagation dynamics. Non-typhoidal salmonellosis (NTS) is the most important public health problem worldwide and mostly in sub-Saharan Africa (Olobatoke & Mulugeta, 2015) and accounts for food-borne illnesses with an estimated 94 million cases (Control & Prevention, 2016). NTS is generally a self-limiting enteric disease caused by different serovars of *Salmonella enteric* subspecies *enterica*, including serovar *enteridis* and typhimurium (Health & Welfare, 2013). Most of *Salmonella* cases globally are caused by *Salmonella serovar enteritidis*, of which the major sources are from poultry meat and eggs (Backhans & Fellström, 2012). On the other hand, typhoidal *Salmonella*, namely, *Salmonella enteric serovar typhi*, is accountable for 22 million

cases of typhoid fever globally and approximately 200,000 associated deaths annually (Backhans & Fellström, 2012; Imanishi *et al.*, 2015).

The link between rodents and disease transmission in general and *Salmonella* transmission in particular has been reported in previous studies from different countries (Lapuz *et al.*, 2008; Umali *et al.*, 2012). However, few if any similar studies have been found in South Africa even though many parts of the country have reported increased rat infestations in many towns. A Rapport newspaper report of 25th September 2012, pointed out "that the province of Gauteng suffers from unusually high levels of rat infestations by three biotypes of rats; the black roof-rat (*R. rattus*), the brown rat (*R. norvegicus*) and a mysterious black/white species which an animal expert believes could be a new hybrid". Of these, the black roof rat and the brown rat are known to be carriers of diseases. However, the black/white rat species that may be a hybrid is so far not a known vector of any diseases and therefore needs to be investigated. It is therefore important to investigate the rodent species present in each particular area, their vector potential and more specifically their ability to transmit and maintain virulent strains of pathogens such as *Salmonella*.

Among the rodents, rats and mice are known to carry *Salmonella* to chickens and maintain it when chickens have been cleared (Trampel *et al.*, 2014). They can act as disease reservoirs and controlling these rats and mice effectively controls the pathogens as well. The *Salmonella* in rats and mice may also have been exposed to certain antibiotics used to treat chickens in the feed. This exposure may give rise to antibiotic-resistant strains selected and maintained in the rats and mice found in a particular farm or geographical location. This poses a great danger to future antibiotic use at farm or region level as well as public health level and thus should always be tested and properly monitored as part of health control strategies.

1.2 PROBLEM STATEMENT

A study which was conducted here in North West Province (South Africa) has shown that chicken samples which were obtained from different retail outlets were contaminated with Salmonella (Olobatoke & Mulugeta, 2015). Therefore, this shows that there is a presence of these bacteria (*Salmonella*) in poultry products and rodents may play a vital role in distribution of these bacteria. Studies have shown that *Salmonella* contamination in poultry farms can be attributed to different factors that include infected rodents, especially rats (Lapuz *et al.*, 2008); contaminated feed (Murase *et al.*, 2004; Shirota *et al.*, 2000); unhygienic poultry management practices (Holt *et al.*, 1994), and chicks (Kinde *et al.*, 1996). The rat inhabitants provide the chance for environment–rat–chicken interaction during ingestion of *Salmonella*-contaminated rodent fecal droppings by the new substitute flocks thus increasing the risk of re-introducing *Salmonella* contamination in the poultry farm after the flock is cleared and area disinfected (Lapuz *et al.*, 2008; Umali *et al.*, 2012). A farmer can practice "all in all out" system, cleaning and disinfection of infected poultry houses promptly after removal of spent poultry as a critical step to avoid infection of replacement flock moving into an earlier contaminated house. However, if the rats are not controlled; there is a high possibility of introducing these rat-borne pathogenic bacteria to the same poultry house. The scale at which this is happening in South African poultry farms and even extends to households infested with rats still needs to be considered.

Additionally, in South Africa, there is an unusual increase in rat infestations by three biotypes of rats the *R. rattus*, *R. norvegicus* and a mysterious black/white species which an animal expert believes could be a new hybrid (Lakshminarayanan *et al.*, 2015). It is not known if all or only some of these rats and mice infest poultry houses. If they do, it is important to know which ones

do, whether they are vectors of *Salmonella* and how virulent the *Salmonella* strains they carry are to both animals and humans.

1.3 JUSTIFICATION OF THE STUDY

Salmonellosis is among the most important foodborne diseases worldwide. Most of the infections by this pathogen can be traced back to chicken involvement. Chickens themselves harbor the pathogens in their gut without showing any clinical signs but when these pathogens contaminate chicken products, consumers can get infected and become diseased. However, not all strains are known to cause human infections so identifying what strains are present in a selected area gives an idea of what risks may arise. Furthermore, knowing the infection dynamics that help to maintain the bacteria in any given area is very important for risk assessment.

Rodents have been implicated in many studies as sources of contaminations to chickens and maintenance of the pathogenic organisms in the chicken surroundings. However, the studies relating *Salmonella* carriage in rats are not always directly comparable and thus there is need to proactively have a mechanism of updating this data to reflect area-specific differences (Premaalatha *et al.,* 2010). Geographical and regional differences have been observed in reports of the pathogens involved and the risks that rodents pose in the transmission of *Salmonella* infections between chickens and humans. Very few such studies relating to the involvement of rodents in salmonellosis have been undertaken in South Africa and more so in the North West Province. The current study intends to provide this bio-data in Mafikeng, Ngaka Modiri Molema district for purposes of disease dynamic awareness in rodents and pathogen control.

Apart from providing bio-data on strains of *Salmonella* that are found in rodents, the current study intends to establish the virulence and antibiotic responsiveness of the *Salmonella* isolates from these rodents. This information is important for the treatment of the pathogen as well as understanding how the rodents are involved in the choice of antibiotic resistant strains in the environment.

1.4 RESEARCH AIMS AND OBJECTIVES

1.4.1 The Aim

The main aim of this study was to determine the species, virulence and antibiotic susceptibility characteristics of *Salmonella* isolates from rodents captured in poultry farms in Mafikeng, Ngaka Modiri Molema District, North West Province, South Africa

1.4.2 Objectives

The objectives of the current study were to:

1. To identify rodent species that infest poultry houses in Mafikeng, Ngaka Modiri Molema District, North West Province, South Africa.

2. To detect and characterise the *Salmonella* spp. from rodents captured in poultry farms; around Mafikeng, North West Province, South Africa

3. To determine the virulence of the *Salmonella* isolates from rats using documented virulent gene markers of the *Salmonella* strains

4. To document antimicrobial resistance profiles of *Salmonella* isolates from rats using disk diffusion as well as through resistance genes known for each of the antibiotics

1.5 REFERENCES

Amk, M.R., Sakthivel, P. 2015. Role of rodents in poultry environs and their management. *Journal of Dairy, Veterinary & Animal Research*, 2(3): 107-114.

Backhans, A. & Fellström, C. 2012. Rodents on pig and chicken farms-a potential threat to human and animal health. *Infection Ecology & Epidemiology*, *2*(1), p.17093

Chaisiri, K., Chaeychomsri, W., Siruntawineti, J., Ribas, A., Herbreteau, V. & Morand, S. 2012. Diversity of gastrointestinal helminths among murid rodents from northern and northeastern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 43(1):21-8.

Control, C.f.D. & Prevention. 2016. Core elements of hospital antibiotic stewardship programs. Atlanta, GA: US Department of Health and Human Services, CDC; 2014.

Criste, V.I., Arama, M. & Ciurascu, C. 2011. Implementation of food safety management system in a production unit of feed and poultry. *The Environment and the Industry*, 95-105.

Davies, R. & Wray, C. 1995. Mice as carriers of *Salmonella enteritidis* on persistently infected poultry units. *The Veterinary Record*, 137(14):337-341.

El-Sharkawy, H., Tahoun, A., El-Gohary, A.E.-G.A., El-Abasy, M., El-Khayat, F., Gillespie, T., Kitade, Y., Hafez, H.M., Neubauer, H. & El-Adawy, H. 2017. Epidemiological, molecular characterization and antibiotic resistance of *Salmonella* enterica serovars isolated from chicken farms in Egypt. *Gut Pathogens*, 9(1):8.

Feng, Y., Liu, J., Li, Y.-G., Cao, F.-L., Johnston, R.N., Zhou, J., Liu, G.-R. & Liu, S.-L. 2012. Inheritance of the *Salmonella* virulence plasmids: mostly vertical and rarely horizontal. *Infection, Genetics and Evolution*, 12(5):1058-1063.

Health, E.P.o.A. & Welfare. 2013. Scientific Opinion on Review of the European Union Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks-Terms of reference 2 to 7. *EFSa Journal*, 11(1):3074.

Holt, P.S., Buhr, R., Cunningham, D. & Porter Jr, R. 1994. Effect of two different molting procedures on a *Salmonella enteritidis* infection. *Poultry Science*, 73(8):1267-1275.

Hong, Y.-P., Wang, Y.-W., Huang, I.-H., Liao, Y.-C., Kuo, H.-C., Liu, Y.-Y., Tu, Y.-H., Chen, B.-H., Liao, Y.-S. & Chiou, C.-S. 2018. Genetic relationships among multidrug-resistant *Salmonella enterica* Serovar typhimurium Strains from Humans and Animals. *Antimicrobial Agents and Chemotherapy*: AAC. 00213-00218.

Imanishi, M., Newton, A., Vieira, A., Gonzalez-Aviles, G., Scott, M.K., Manikonda, K., Maxwell, T., Halpin, J., Freeman, M. & Medalla, F. 2015. Typhoid fever acquired in the United States, 1999–2010: epidemiology, microbiology, and use of a space-time scan statistic for outbreak detection. *Epidemiology & Infection*, 143(11):2343-2354.

Inoue, K., Maruyama, S., Kabeya, H., Yamada, N., Ohashi, N., Sato, Y., Yukawa, M., Masuzawa, T., Kawamori, F. & Kadosaka, T. 2008. Prevalence and genetic diversity of *Bartonella* species isolated from wild rodents in Japan. *Applied and Environmental Microbiology*, 74(16):5086-5092.

Jemilehin, F., Ogunleye, A., Okunlade, A. & Ajuwape, A. 2016. Isolation of *Salmonella* species and some other gram negative bacteria from rats cohabitating with poultry in Ibadan, Oyo State, Nigeria. *African Journal of Microbiology Research*, 10(29):1104-1110.

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(1):37-44.

Kinde, H., Read, D., Chin, R., Bickford, A., Walker, R., Ardans, A., Breitmeyer, R., Willoughby, D., Little, H. & Kerr, D. 1996. *Salmonella enteritidis*, phage type 4 infection in a commercial layer flock in Southern California: bacteriologic and epidemiologic findings. *Avian Diseases*, 40(3):665-671.

Lapuz, R., Tani, H., Sasai, K., Shirota, K., Katoh, H. & Baba, E. 2008. The role of roof rats (*Rattus rattus*) in the spread of *Salmonella enteritidis* and *S. infantis* contamination in layer farms in eastern Japan. *Epidemiology and Infection*, 136(09):1235-1243.

Liu, C.-C., Yeung, C.-Y., Chen, P.-H., Yeh, M.-K. & Hou, S.-Y. 2013. *Salmonella* detection using *16S* ribosomal DNA/RNA probe-gold nanoparticles and lateral flow immunoassay. *Food Chemistry*, 141(3):2526-2532.

Martinson, N.A., Karstaedt, A., Venter, W.F., Omar, T., King, P., Mbengo, T., Marais, E., McIntyre, J., Chaisson, R.E. & Hale, M. 2007. Causes of death in hospitalized adults with a premortem diagnosis of tuberculosis: an autopsy study. *Aids*, 21(15):2043-2050.

McKiel, J., Rappay, D., Cousineau, J., Hall, R. & McKenna, H. 1970. Domestic rats as carriers of *Leptospires* and *Salmonellae* in Eastern Canada. *Canadian Journal of Public Health/Revue Canadienne de Sante'e Publique*, 61(4):336-340.

Meerburg, B.G. & Kijlstra, A. 2007. Role of rodents in transmission of *Salmonella* and *Campylobacter*. *Journal of the Science of Food and Agriculture*, 87(15):2774-2781.

Meerburg, B.G., Singleton, G.R. & Kijlstra, A. 2009. Rodent-borne diseases and their risks for public health. *Critical Reviews in Microbiology*, 35(3):221-270.

Murase, T., Nagato, M., Shirota, K., Katoh, H. & Otsuki, K. 2004. Pulsed-field gel electrophoresis-based subtyping of DNA degradation-sensitive *Salmonella enterica* subsp. enterica serovar *livingstone* and serovar *cerro* isolates obtained from a chicken layer farm. *Veterinary Microbiology,* 99(2):139-143.

Olobatoke, R.Y. & Mulugeta, S.D. 2015. Incidence of non-typhoidal *Salmonella* in poultry products in the North West Province, South Africa. *South African Journal of Science,* 111(11-12):1-7.

Premaalatha, B., Nurulaini, R., Zawida, Z., Norakmar, I., Imelda Lynn, V., Adnan, M., Zaini, C., Jamnah, O., Tan, L. & Zainab, Z. 2010. A survey of bacterial and parasitic infections of rats caught in the Veterinary Research Institute (VRI), Ipoh. *Malaysian Journal of Veterinary Research*, 1(1):45-50.

Shirota, K., Katoh, H., ITO, T. & Otsuki, K. 2000. *Salmonella* contamination in commercial layer feed in Japan. *Journal of Veterinary Medical Science*, 62(7):789-791.

Taylor, S., Wakem, M., Dijkman, G., Alsarraj, M. & Nguyen, M. 2010. A practical approach to RT-qPCR—publishing data that conform to the MIQE guidelines. *Methods*, 50(4):S1-S5.

Umali, D.V., Lapuz, R.R.S.P., Suzuki, T., Shirota, K. & Katoh, H. 2012. Transmission and shedding patterns of *Salmonella* in naturally infected captive wild roof rats (*Rattus rattus*) from a *Salmonella*-contaminated layer farm. *Avian Diseases*, 56(2):288-294.

Van Nierop, W., Duse, A., Marais, E., Aithma, N., Thothobolo, N., Kassel, M., Stewart, R., Potgieter, A., Fernandes, B. & Galpin, J. 2005. Contamination of chicken carcasses

in Gauteng, South Africa, by *Salmonella*, *Listeria monocytogenes* and *Campylobacter*. *International Journal of Food Microbiology*, 99(1):1-6.

Van, T.T.H., Nguyen, H.N.K., Smooker, P.M. & Coloe, P.J. 2012. The antibiotic resistance characteristics of non-typhoidal *Salmonella enterica* isolated from food-producing animals, retail meat and humans in South East Asia. *International Journal of Food Microbiology*, 154(3):98-106.

CHAPTER 2-LITERATURE REVIEW

2.1 RODENT SPECIES

2.1.1 Rodents in general

The word rodent originated from the Latin verb ‘’Rodere’’ meaning to gnaw (Carleton, 1984). The order Rodentia represents the most diverse mammalian both in terms of the number of species and individuals (Kidanemariam *et al.*, 2010), representing approximately 43% of the entire mammalian species (Huchon *et al.*, 2002). They are characterized by pairs of incisors which grow continuously from the upper and lower jaws. The majority of rodents are small animals with long tails, short limbs, and robust bodies (Stein, 2000). Some of the common rodents known are; rats, mice, prairie dogs, chipmunks, capybaras, porcupines and beavers. The majority, if not all species, of rodents have different behavioural characteristics and they occupied different habitats apparently in order to minimize competition. They are all generally known to be problematic in the agricultural sector because they damage stored grains, crops and infrastructure (Bastos *et al.*, 2005).

2.1.2. Identification of rodents

Majority of the rodents are relatively small, having a dense body with short legs (Beck *et al.*, 2006). They also have a number of morphological variations during their development thus making identification very difficult. Meehan (1984), suggested that the small rodent’s species could be identified easily by their shape and the shape of their droppings. However, recent studies have disagreed with this study indicating that many small rodents (rats and mice) are

difficult to identify morphologically even from whole carcasses and therefore accurate identification of these rodents is still a big challenge (Robins *et al.*, 2007).

To circumvent the difficulties resulting from the limitation of morphological identification, molecular methods have increasingly been adopted as the methods of choice for identifying different animal species globally (Mubita *et al.*, 2008; Robins *et al.*, 2007; Syakalima *et al.*, 2016). Over the last ten years, the DNA barcoding has emerged as a dependable molecular method especially for species identification (Ali *et al.*, 2015). DNA barcoding relies on an identical region of the mitochondrial gene being amplified, sequenced and analyzed by comparison to an open-access database. Using molecular taxonomy to create a biological barcode that identifies organisms is the central goal of DNA barcoding, as well as creating a standardized reference library for the DNA based identification of target species (Kerr *et al.*, 2007). DNA barcoding has a number of steps: 1, DNA extraction, 2, PCR amplification, 3, DNA sequencing and lastly analysis. DNA extraction is a very important step because, without high-quality DNA, the PCR amplification will not be optimal. The PCR is done to amplify a fragment, which is then sequenced and compared to a database of known organisms (Ali *et al.*, 2015; Hebert *et al.*, 2004; Lakshminarayanan *et al.*, 2015; Syakalima *et al.*, 2016). Mitochondrial DNA is the most frequently used for species identification, mainly targeting the *Cyt-b* and *COI* genes (Lakshminarayanan *et al.*, 2015; Syakalima *et al.*, 2016).

Robins *et al.* (2007) have successfully used two DNA barcoding genes to identify *Rattus* species namely; *Cyt-b* and *COI* and also using a tree-based method using D-loop. These methods, especially targeting the DNA barcoding genes, gave very dependable results and have been adopted for our rodent studies. Furthermore, among the special molecular methods that have been used for rodent's identification, Hebert *et al.* (2003) had previously recommended that the

mitochondrial gene *COI* apart from serving as a genetic barcode for all animal life can be employed to differentiate individuals not just at taxonomic levels but at the species level. They suggested that taxonomic revisions that recognize polytypic or cryptic species would lead to even better accuracy of DNA-based identification methods.

2.1.3. Importance of rodents in poultry houses

The presence of rodents, especially rats, can lead to a number of adverse effects in chickens and the farm as a whole. Rodents contribute to significant physical losses to poultry farms globally (Amori & Gippoliti, 2003; Fraschina *et al.*, 2014; Inoue *et al.*, 2008). They cause damage to the poultry structures by eating of eggs, chicks and poultry feed also by their burrowing nature (Amori & Gippoliti, 2003; Singleton *et al.*, 2010). They primarily feed in storage areas and then leave their feces and urine there, which leads to spreads of pathogenic organisms such as *Salmonella* (Franssen *et al.*, 2016; Jemilehin *et al.*, 2016). They can also damage the building and equipment which leads to losses in terms of fixation (Fraschina *et al.*, 2014). They gnaw the support structures and burrowing under walls and concrete thus causing a lot of structural damage or instability (Inoue *et al.*, 2008). Rodents can cause a serious damage to poultry buildings by creating holes in the roofs which results in water leakage during rainy seasons, gnawing, nibbling of wooden doors and windows, also they can cause fire by nibbling electricity cables. Furthermore, they can carry and maintain diseases; and the type of disease will depend on a particular rodent species (Franssen *et al*., 2016). Therefore, their presence in a poultry setting is always bound to have serious economic consequences.

2.1.4 Diseases transmitted by rodents

Rodents, especially rats, live in close contact with domestic animals and human beings, so they can transmit diseases through the environment or via contaminated rodent saliva, feces, hair remnants, and urine (Villafañe *et al.*, 2004). According to Franssen *et al.*, 2016, rodents can pick pathogens such as bacteria from the environment and multiply them thus maintaining them ready for transmission. As a result rodents, especially rats, have been linked with different bacterial diseases such as salmonellosis, plague, leptospirosis, tularemia and rat-bite fever (Amatre *et al.*, 2009; Roomaney *et al.*, 2012). They may also carry important protozoa which cause opportunistic diseases for immune-compromised patients such as cryptosporidiosis, toxoplasmosis and coccidiosis (Meerburg *et al.*, 2009). They are known carriers of trematodes, cestodes and nematodes (Franssen *et al.*, 2016), and also they play a role of spreading of *Coxiella burnetii* (Reusken *et al.*, 2011). They also act as vectors for numerous disease-carrying arthropods like fleas, lice and mites and consequently the diseases that these arthropods carry (Buckle & Smith, 2015). They have also been implicated as reservoirs of viruses such as the Lymphocytic choriomeningitis virus (Bonthius, 2012; Knust *et al.*, 2014) and Lassa virus (Agbonlahor *et al.*, 2017; Bonwitt *et al.*, 2017; Fichet-Calvet, 2014). Rodents are therefore an important concern in any health control strategy in poultry houses and in the general environment as a whole.

2.2 *SALMONELLA*

2.2.1. *Salmonella* spp. in general

The genus *Salmonella* was named in 1885 after D. E. Salmon, an American bacteriologist and veterinarian, jointly with T. Smith isolated the "hog cholera bacillus" (Salmon & Smith, 1885). It belongs to the family of Enterobacteriaceae and is facultatively anaerobic and gram-negative (Johannes, 2015), oxidase-negative, rod-shaped bacteria having peritrichous flagellation and mobility (Coburn *et al.*, 2007; Johannes, 2015). It causes disease in humans and also in animals (Coburn *et al.*, 2007; Torpdahl *et al.*, 2013).

Salmonella spp. ferment mannose and glucose with no gas production but do not ferment sucrose or lactose. The majority of the isolates produce hydrogen sulphide gas in triple sugar iron agar (TSI). *Salmonella* spp. which exist in livestock intestines are some of the vital hazardous pathogens causing food poisoning (Nair *et al.*, 2015). *Salmonella* is classified into two major divisions namely: *Salmonella bongori* and *Salmonella enterica* (Figure 2.1**)**. The *S. enterica* is also divided into *S. enterica* subsp. *houtenae, S. arizonae, S. enterica* subsp. *diarizonae*, and *S. enterica* subsp. More than 2400 serotypes have been identified (Kidanemariam *et al.*, 2010).

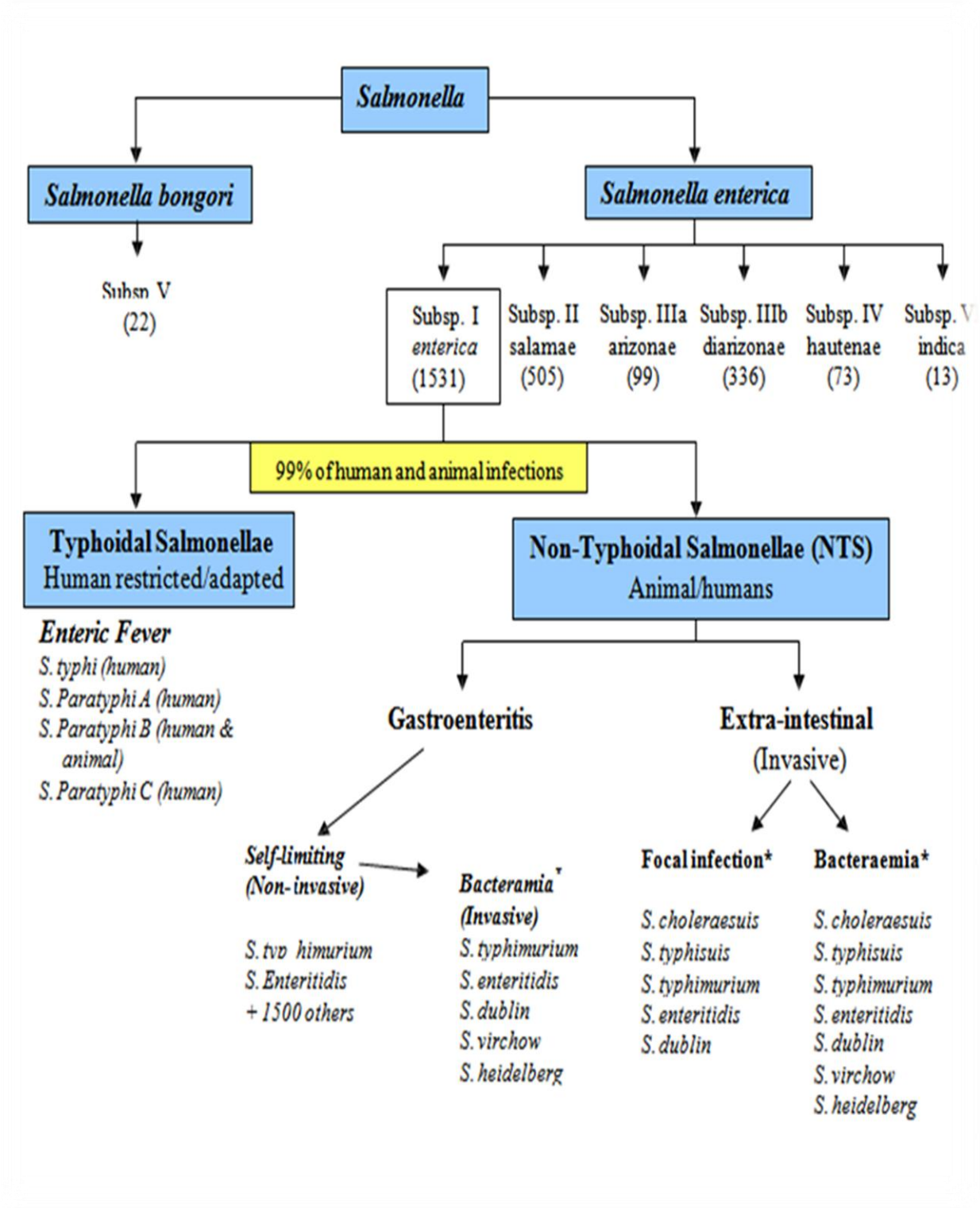


Figure 2. 1: Classification of the genus *Salmonella* (Akyala & Alsam, 2015)

2.2.2. *Salmonella* in chicken

Commercial poultry production is growing rapidly globally to meet the needs of the growing human population (Olobatoke & Mulugeta, 2015). Most middle-class farmers in developing countries are taking up poultry to increase their profits and this industry has become the main income source to them. Therefore, the presence of pathogenic organisms such as *Salmonella* in chickens, as a major food-borne infection in humans, can have an adverse impact (Imanishi *et al.*, 2015; McKiel *et al.*, 1970; Taylor *et al.*, 2008). Chicken products have been implicated in most of *Salmonella* outbreaks because they act as carriers of the pathogens in their gut (Black, 2008). Moreover, it is known that *Salmonella* primarily colonizes the caeca of the birds (Desmidt *et al.*, 1997). The chickens that harbor this kind of bacterium in their gut are, however, not harmed and show no clinical symptoms. However, through unhygienic slaughtering processes, the carrier chickens are able to contaminate the environment, the equipment and personnel. Subsequently, the end result is the transmission of the bacteria and disease to the consumers (Folster *et al.*, 2010).

Salmonella that can be traced to chickens are classified into three groups (Hafez, 2013). The first group contains highly host-adapted and invasive serotype such *S. typhi* in humans, *S. gallinarum* and *S. pullorum* in poultry. The second group is non-host adapted which includes invasive serotypes such as *S. enteritidis*, *S.* typhimurium and *S. arizonae*. The third group contains non-host adapted which are noninvasive serotypes and the majority of these serotypes are harmless to animals and humans (Andino & Hanning, 2015; Umali *et al.*, 2012). *Salmonella* colonization of the chicken gut is established within the two caeca, which symbolize a reservoir of infection of *Salmonella* in poultry. The level and duration of bacterial colonization are usually under genetic control of the chicken host (Sadeyen *et al.*, 2004). Understanding the mechanisms of *Salmonella*

infection, intestinal colonization, persistence and excretion in poultry are essential to determine appropriate measures to be taken to decrease both contaminations of flocks and public health risk (Andino & Hanning, 2015; Sadeyen *et al.*, 2004). Most of the *Salmonella* serovars including *S. infantis* and *S. enteritidis* are not really serious pathogens in chickens, although they are of public health concern (Lapuz *et al.*, 2012).

Chickens, apart from being normal carriers, can also suffer from *Salmonella* diseases like Pullorum disease (PD) caused by *Salmonella pullorum* and Fowl typhoid (FT) which is caused by *S. gallinarum* (Ahmed *et al.*, 2008; Barrow, 1990; Barrow & Neto, 2011; Lee *et al.*, 2005; Pan *et al.*, 2009). Pullorum disease affects mostly young chickens (Barrow & Neto, 2011), aged 2−3 weeks, whereas, Fowl typhoid affects mostly adult chickens (Barrow, 1990). Pullorum is a fatal septicemia disease-causing clinical signs in layers that include low fertility, and hatchability (Andino & Hanning, 2015). On the other hand, Fowl Typhoid results in dejected ruffled birds with yellow diarrhoea.

2.2.3. The role played by rodents in transmission of *Salmonella*

Studies carried out in different countries such as the UK have revealed an obvious array of pathogenic organisms isolated from rats captured in urban areas. Some of these pathogens were the causative agents of food-borne disease including salmonellosis (Battersby, 2002; Jemilehin *et al.*, 2016). Most rodents, especially rats that carry *Salmonella* spp., play a vital role in terms of spreading diseases (Jemilehin *et al.*, 2016; Lapuz *et al.*, 2008; Lapuz *et al.*, 2012). In poultry farms, rats infected with *Salmonella* have been reported (Henzler & Opitz, 1992). However, *Salmonella* is not continually encountered in rodents around farms. This is acknowledged by

previous studies where *Salmonella* was not detected from such rodents samples (Pocock *et al.*, 2001; Healing & Greenwood, 1991).

Few studies of *Salmonella* in rodents, especially rats, have been undertaken but there is still uncertainty in their role in the maintenance and transmission of infection (Roomaney *et al.*, 2012). Probably this could be because even though the rats captured from farms in previous studies were *Salmonella*-infected; they still appeared healthy (normal) or not showing any clinical signs of illness (Table 2.1). This could, therefore, create a wrong impression that the *Salmonella* they carry may not be of any importance. This impression should however not be supported and more studies are still required.

Table 2. 1: Studies on *Salmonella* in rodents

Rodent's species	Location	Reference
M. musculus	Mixed farms	(Pocock *et al.*, 2001)
M. musculus and *R. norvegicus*	Pig and poultry farms	(Meerburg & Kijlstra, 2007)
Species not specified	Laying farms	(Davies & Wray, 1995)
Species not specified	Laying farms	(Davies & Breslin, 2003)
Mus musculus	Not specified	(Shimi *et al.*, 1979)
Rattus norvegicus	Urban	(Hilton *et al.*, 2002)
Rattus (species not specified)	Urban	(Singh *et al.*, 1980)
M. musculus	chicken layer farms	(Henzler & Opitz, 1992)
A. sylvaticus M. musculus, and R. norvegicus	Organic pig farms	(Jensen *et al.*, 2004)
Species not specified	Slum	(Gakuya *et al.*, 2001)

Studies have shown that transmission of *Salmonella* from the rodents can be spread by means of bites, faeces and contamination of food with rodent urine (Meerburg & Kijlstra, 2007; Villafañe *et al.*, 2004). Therefore, these studies have been able to show that different *Salmonella* can be detected from rodents which include: *Salmonella* typhimurium, *Salmonella montivideo*, *Salmonella derby* and *Salmonella enteritidis* (Henzler & Opitz, 1992; McKiel *et al.*, 1970; Meerburg & Kijlstra, 2007). Studies have also established that rodent control measures can

successfully reduce *Salmonella* in poultry houses (Henzler *et al.*, 1998; Meerburg & Kijlstra, 2007; Rodenburg *et al.*, 2004) thereby highlighting the importance of rodents in the transmission and maintenance of the pathogen. Furthermore, studies have also shown that not only do rodents carry the *Salmonella* but the pathogens they carry are pathogenic and exhibit *Salmonella* strains that are resistant to antibiotics (Nkogwe *et al.*, 2011).

2.3 *SALMONELLA* VIRULENCE AND PATHOGENESIS

2.3.1 Virulence and *Salmonella* Pathogenicity Islands (SPIs)

Bacterial virulence factors are essential for invading, adhering and replicating inside host cells (Majowicz *et al.*, 2010; Yap *et al.*, 2014). Virulence genes (factors) assist bacteria to invade and causes disease, also to overcome the host defenses (Baron, 1996). Several virulence genes in *Salmonella* are known and most are situated in *Salmonella* pathogenicity islands (SPIs), prophages, plasmids and fimbrial clusters (Prasanna Kumar, 2016). There are about twenty one identified pathogenicity island (SPIs-protein coding) ranging from SPI1 to SPI21 for *Salmonella* but only twelve are known to contain virulence factors (López *et al.*, 2012). They consist of areas of genomic DNA ranging from 10 to 200 kb (Saroj *et al.*, 2008).

The majority of the virulence genes are gathered within *Salmonella* pathogenicity islands 1 (SPI-1) and *Salmonella* pathogenicity islands (SPI-2) (Marcus *et al.*, 2000). The SPI-1 encodes factors essential for cell adhesion whilst, SPI-2 encodes factors essential for replication and intracellular survival (Majowicz *et al.*, 2010; Wisner *et al.*, 2010). The SPIs play significant tasks in the invasion, antibiotic resistance and adhesion (Kim & ju Lee, 2017; Majowicz *et al.*, 2010). Most genes like *inv*A, *sop*B, *sop*E and *sip*A attributed for invasion are positioned within SPI-1 (López

et al., 2012). Current investigations have revealed that *Salmonella* spp. applies its type3 secretion systems, encoded by SPI-1 and SPI-2 to encourage intestinal and reproductive tract colonization in animal species (Hur *et al.*, 2011). Effectors proteins translocated through SPI-1, T3SSs are significant in eliciting inflammation from the intestines (Card *et al.*, 2016; Haneda *et al.*, 2012). Therefore, *Salmonella* strains without SPI-1 and SPI-2 cannot elicit inflammation from the intestines (Card *et al.*, 2016; Haneda *et al.*, 2012). A study conducted by Saroj and co-authors (2008) found that there is absence of SPI-8 and SPI-10 in some *Salmonella* serovars; *S. dublin*, *S. worthington*, *S. paratyphi* C and *S. paratyphi* B despite them being virulent.

2.3.1.1 Role of virulence genes in Salmonella species

Based on *Salmonella* concern in the invasion of cultured epithelial cells, the *Sip* proteins, which contain *Sip*ABCD were the primary virulent genes used to characterized *Salmonella* spp. (Zishiri *et al.*, 2016). They enhance multiplication and aid in replication systemically (Hur *et al.*, 2011; Prasanna Kumar, 2016). They are also capable of inducing apoptosis in macrophages (Kaur & Jain, 2012).

The operon *spv* (*Salmonella* plasmid virulence) is considered as one of the virulence plasmids of numerous *Salmonella* serotypes that generate systemic diseases (Castilla *et al.*, 2006). It harbors five genes *spv*RABCD (Rotger & Casadesús, 1999) which have been identified to contribute to pathogenesis (Card *et al.*, 2016).

The presence of *Hil*A gene in *Salmonella* is essential for the expression of the type III secretion system (TTSS) components and it encodes the central regulator *Hil*A (Borges *et al.*, 2013). This

gene (*Hil*A) is required to induce apoptosis of macrophages and invade epithelial cells (Borges *et al.*, 2013). However, the secreted effectors *sop*ABD, and *sop*E act collectively to stimulate diarrhoea (Zhang *et al.*, 2003).

The *sip*C gene acts as a translocase, mediating bacterial entry into epithelial (Prasad, 2012). On the other hand, *spi*C acts to modulate invasion gene expression (Hayward & Koronakis, 1999). Previous studies suggested that *sip*A, *sip*C and *sip*B form a translocation complex that distributes effectors proteins into the host cells (Prasad, 2012; Zhang *et al.*, 2003).

The *inv*A gene affects the host cell by delivery of type III secreted effectors, for mutant phenotype and it is also essential for invasion of epithelial cells (Darwin & Miller, 2000; Dione *et al.*, 2011; El-Sharkawy *et al.*, 2017; Marcus *et al.*, 2000). Different studies have been using *inv*A to detect/confirm *Salmonella* spp. (Li *et al.*, 2018; Refai *et al.*, 2017; Sunar *et al.*, 2014). The *inv*A gene has been confirmed to be present in *Salmonella* species only and hence is used in genetic diagnosis of *Salmonella* species (Fekry *et al.*, 2018; Refai *et al.*, 2017). Some of the most common virulent genes which may cause disease are shown in Table 2.2.

Table 2. 2: *Salmonella* secreted effector proteins and their possible significance in disease

Location	gene	Possible significance	References
SPI-3	*pef*A	Plasmid-encoded fimbriae	(McWhorter *et al.*, 2014)
SPI-1	*inv*A	Invasion of epithelial cells	(Ekwanzala *et al.*, 2017)
SPI-1	*sip*C	Translocase mediating bacteria entry into epithelial cells	(Zishiri *et al.*, 2016)
SPI-1	*hil*A	Central transcriptional regulator of the invasion genes	(Modarressi & Thong, 2010)
SPI-5	*sop*B	Invasion and intracellular replication	(McWhorter *et al.*, 2014)
SPI-2	*spi*C	Modulate invasion gene expression	(Zishiri *et al.*, 2016)
SPI-4	*orf*L	Adhesion, auto transportation and colonization	(Hughes *et al.*, 2008)
SPI-3	*mis*L	Chronic infection and host specificity	(Zishiri *et al.*, 2016)
SPI-3	*avr*A	Modulation of host immune response	(McWhorter *et al.*, 2014)
SPI-1	*Spt*P	Disruption of acting cytoskeleton	(McWhorter *et al.*, 2014)
SPI-5	*pip*D	Induction of fluid secretion	(Kingsley *et al.*, 2009)

2.4 *SALMONELLA* DETECTION METHODS

2.4.1 *Salmonella* isolation

Isolation of *Salmonella* spp. engages a nonselective pre-enrichment followed by a selective enrichment, plating onto selective agars. Different broths have been (Trypticase Soy Broth RV Medium, Rappaport-Vassiliadis Soy Broth, Tetrathionate broth (Müller-Kauffman) used as official *Salmonella* enrichment media in the official standard method. Commonly used plating media include Salmonella-Shigella agar (SSA), Bismuth-Sulfite agar (BSA), Brilliant Green agar (BGA), MacConkey agar, and Xylose-lysine-deoxycholate agar (XLD) have been adapted to enhance *Salmonella* growth and thus to amplify the sensitivity and selectivity. The *Salmonella* isolation and identification is recommended by International Organization for Standardization (ISO-6579, 2002) (ISO, 2002) and the procedure is summarized in Figure 2.2.

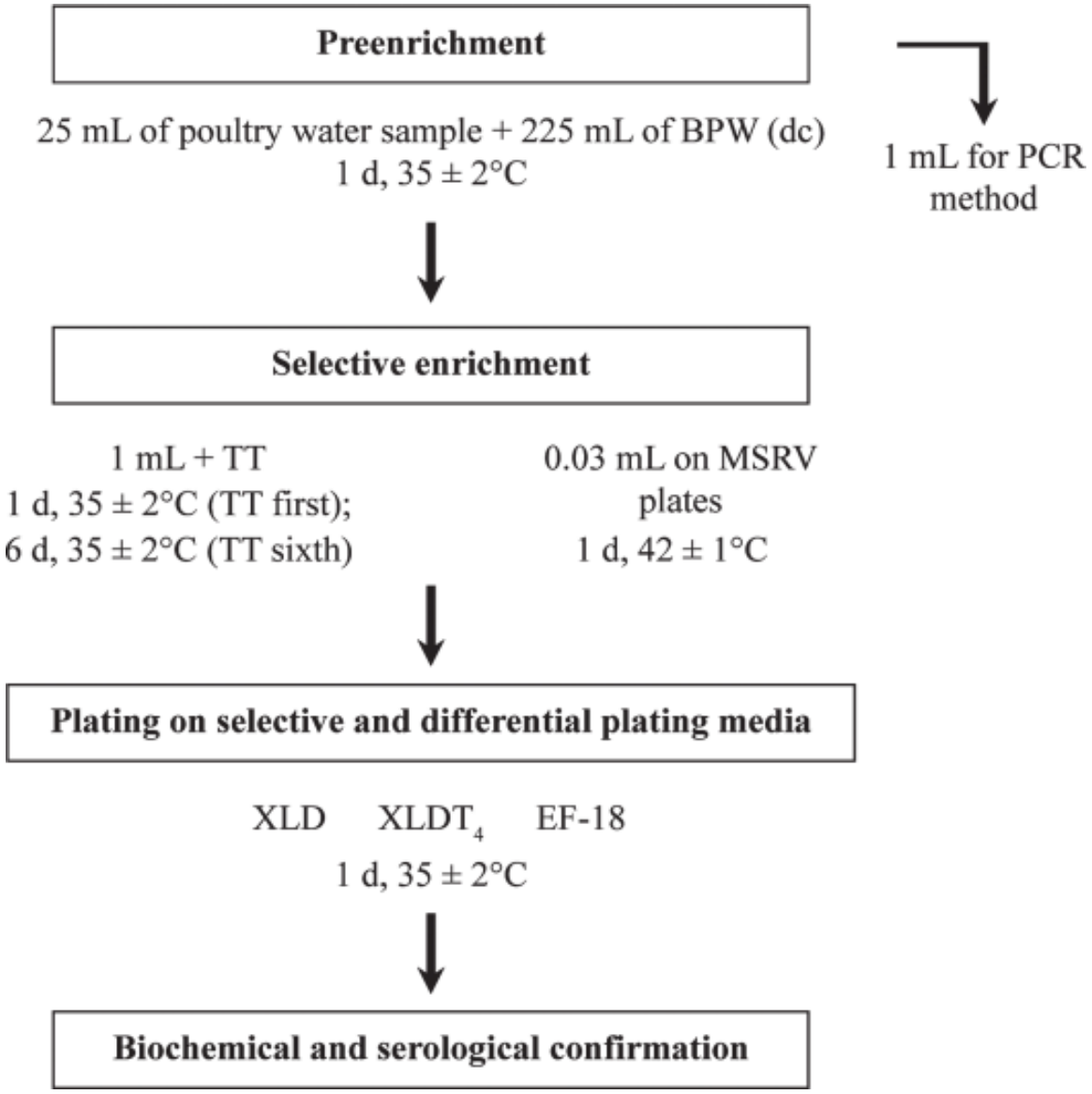


Figure 2. 2: *Salmonella* isolation procedure conventional cultural enrichment (ISO-6579, 2002)

However, previous studies (Pal & Marshall, 2009; Tang *et al.*, 2018) affirmed that culturing can be time-consuming, labour-intensive and low in sensitivity which makes it inappropriate for regular testing of great numbers of samples.

2.4.2 Biochemical confirmation

The *Salmonellae* are catalase positive thus the test is considered positive when gas bubbles appear on the surface of the culture material. For Triple Sugar Iron agar (TSI), distinctive *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas formation and formation of hydrogen sulfide, and when lactose positive *Salmonella* is isolated the TSI agar slant is yellow (Phokela *et al.,* 2011). The entire *Salmonella* genus is urea negative; the positive reaction urea test shows a splitting of urea which liberated ammonia, with changes of the color from phenol red to rose pink, and later to deep cerise (moderate red). However, for the negative reaction, the color of the Urea media remains unchanged.

Salmonellae are indole negative. After mixing a suspected *Salmonella* colony with Indole mixture, it forms a red ring which indicates a positive; but the yellow-brown ring indicates a negative reaction (Ateba & Mochaiwa, 2014; Brown, 2007; Samaxa *et al.*, 2012).

Analytical Profile Index (API) 20E has been used as standardized identification for members belonging to the genus *Salmonella* (Fekry *et al.*, 2018; Odumeru & León-Velarde, 2012). Table 2.3 shows the summary of biochemical reactions of *Salmonella*.

Table 2. 3: The summary of biochemical tests on *Salmonella* species (Brown, 2007)

Test Name	Result Observed	Expected Result
Oxidase	No color change to red on the filter paper	−
Catalase	Bubbles formed	+
Indole	No red ring at the top of the broth	−
Methyl Red	Red reagent liquid in test tube	+
Voges-Proskauer	Dark brown liquid	−
Simmons-Citrate	The indicator liquid turned from sea green to dark blue	+
H_2S	Black in the butt of the test tube	+
Urease	Test tube broth remained yellow	−
Motility	Vertical "tornado" in the semi-solid agar that was punctured showed red cloudiness	+
Sucrose	Red in test tube	−
D-Glucose, Acid Production	Yellow in test tube	+
D-Glucose, Gas Production	Large bubble in the Durham tube	+
Lactose	Red in test tube	−
D-Mannitol	Yellow in test tube	+

2.4.3 Serotyping

Salmonella serotyping is a subtyping method helpful in differentiating *Salmonella* isolates (Yan *et al.*, 2003). Generally, the method is based on agglutination by means of specific sera to recognize antigenic variants of the O-antigen of the Lipopolysaccharides (LPS), capsular (Vi) antigens and flagellar antigen (H) (Broadbent *et al.*, 2010).

Since the antigenic makeup of the H1, H2 along with O antigens are an indication of their distinctive DNA sequence alleles (Hong *et al.*, 2008; Joys, 1985; Samuel & Reeves, 2003) *Salmonella* expresses flagellar (H), polysaccharide (O) and capsular (Vi) antigens which verify strain pathogenicity (Nataro *et al.*, 2011). Generally, the flagella surface polysaccharides function to inhibit agglutination by homologous "O" anti-sera (Chart *et al.*, 2007). The H antigens are heat labile proteins contained in the flagella and the surface polysaccharide antigens, and often occur in two forms, phase I and phase II. The VI antigen, the surface antigen of the typhoid organism, is the most important example of heat sensitive surface polysaccharide antigens. These VI antigens surface polysaccharides inhibit agglutination by homologous "O" anti-sera. It only occurs in three *Salmonella* species such as *S. paratyphi C, S. typhi* and *S. dublin* (Shilangale, 2014a; Todar, 2008).

The auto-agglutination method for *Salmonella* serotyping has practical limitations, these include difficult to standardize and time-consuming (Rasschaert *et al.*, 2005; Shilangale, 2014a). However, the advantages are that they are stable and reproducible (Bohaychuk *et al.*, 2007; Mortimer *et al.*, 2004).

2.4.4 Molecular methods

Established techniques for microbiology such as the ISO 6579 for detecting pathogen require several days (4−5 days) to obtain a result. However, the polymerase chain reaction (PCR) has revolutionized quantification and detection of pathogens such as *Salmonella*. The method is a useful device to conquer time-consuming measures (Özbey *et al.*, 2008). Furthermore, the PCR technique is specific, rapid and more sensitive (Ateba & Mochaiwa, 2014). PCR assays are now extensively used in the identification and detection of *Salmonella* species (Ekwanzala *et al.*, 2017; Goodman *et al.*, 2017; Li *et al.*, 2018; Sunar *et al.*, 2014).

In the last decade, real-time PCR (qPCR) systems have been used for the specific quantification of *Salmonella* spp. (Kurowski *et al.*, 2002; Siala *et al.*, 2017). Real-time PCR has a better dynamic variety for quantification of target sequences (Bagóné Vántus *et al.*, 2018; Matsuki *et al.*, 2004). It focuses on the logarithmic phase of result accumulation unlike on the end product abundance. It is accurate as it is less affected by amplification depletion of a reagent (Blessmann *et al.*, 2002; Huijsdens *et al.*, 2002). Real-time PCR possesses sensitivity, reproducibility and specificity (Song *et al.*, 2004).

2.5 ANTIMICROBIAL RESISTANCE OF *SALMONELLA*

2.5.1 Antimicrobial resistance of *Salmonella* species

A bacterial isolate is classified as resistant when it is no longer inhibited by a concentration of antibiotics that would otherwise inhibit the growth of susceptible members of such a group (Ateba & Bezuidenhout, 2008; Esaki *et al.*, 2004; Phokela *et al.*, 2011). Some of bacteria species produce antibiotics such as Streptomyces, Cephalosporium and Penicillium. They produce this

antibiotic as part of defending or protection mechanism (Aavitsland, 2008). Sometimes, resistance can occur as an outcome of mutations in the genome (DNA) (Ochman & Groisman, 1996).

The antimicrobial resistance of *Salmonella* to a particular antibiotic was reported in the early 1960s (Montville & Matthews, 2008). Antibiotic resistance in *Salmonella* strains is generally encoded by resistance plasmids acquired as a consequence of antibiotic selective pressure in veterinary medicine. Byrd *et al.* (2003) maintain that the multi-drug resistant on *Salmonella* (*S. typhimurium*) definitive type DT104 in the United Kingdom (UK) and the USA is an example of how an extremely resistant clone of *Salmonella* has the ability to effectively spread among animals also to humans.

In a number of countries, resistant strains of *Salmonella* have been identified and have been accountable for severe outbreaks. For example, in Asia in the late 1990s, large outbreaks of *typhi* were caused by multidrug-resistant (MDR) strains resistant to the first-line antimicrobials Chloramphenicol, Co-trimoxazole and Ampicillin (Nwiyi & Erumaka, 2012; Parry *et al.*, 2011). There have been reports of Ciprofloxacin-resistant isolates being found in various countries like Spain, India, Pakistan, Malawi and Vietnam (Gokul *et al.*, 2010; Kariuki *et al.*, 2015; Menezes *et al.*, 2010). In a study conducted by El-Sharkawy *et al.* (2017) in Egypt, all *Salmonella* isolates were resistant to Chloramphenicol, Ampicillin, and Tetracycline. In some studies conducted in South Africa, *Salmonella* isolates were resistance to Tetracycline, Trimethoprim-sulfamethoxazole, Trimethoprim, Kanamycin, Gentamicin, Ampicillin, Amoxicillin and Chloramphenicol, Erythromycin and Streptomycin (Manie *et al.*, 1998; Mathole *et al.*, 2017; Olobatoke & Mulugeta, 2015; Zishiri *et al.*, 2016).

There is an indication that there is an increase of antibiotic resistance in typhoidal and non-typhoidal *Salmonella* isolates especially in developing countries (Chart *et al.*, 2007; Kariuki *et al.*, 2005; Ridley & Threlfall, 1998; Threlfall, 2002 Mossoro-Kpinde et al., 2015). This might be due to the availability of drugs without prescriptions (Shilangale, 2014b). The use of antibiotics, such as Tetracyclines, for example, as growth promoters in animal husbandry in most African countries is also a factor in the rising prevalence of resistance (Mossoro-Kpinde *et al*., 2015).

2.5.2 Antibiotic resistance genes and integrons

A number of genes which confer resistance to antibiotics in *Salmonella* serovars have been detected (Abatcha *et al.*, 2018; Chuah *et al.*, 2018; Moawad *et al.*, 2017; Qiao *et al.*, 2017; Zhang *et al.*, 2014). Most cassettes contain genes that present resistance to a variety of antimicrobial agents and genes that confer resistance to disinfectants and antiseptics (Antunes *et al.*, 2006). Resistance genes acquisition has been regarded as the main factor in the distribution and spread of antibiotic resistance, through either vertical transfer or horizontal transfer. The horizontal transfer involves mobile genetic elements like transposons and plasmids (Deng *et al.*, 2015; Xu *et al.*, 2011). Antibiotic-producing microorganisms, which produce natural antimicrobial antibiotics, carry genes encoding resistance in order to protect themselves, hence they spread their resistance genes to some of the nonpathogenic bacteria and they become the sources for resistance genes (Courvalin, 2005; Davies & Davies, 2010; Wright, 2007). Bacteria may attain resistance via intention modification site either by a single step or multiple steps (Schwarz *et al.*, 2001).

Different antibiotics are encoded by specific genes i.e. Chloramphenicol resistance is occurred due to the presence of Chloramphenicol transacetylase [*cat*] (*cat1, cat2, and cat3, cmlA, cmlB, floR)* (Olobatoke & Mulugeta, 2015); Sulfonamides is encoded by *sul1, sul2, sul3, dfrA1, dfrB*, Tetracycline resistance is encoded by *tet*ABCDEGH, *tetL-1, tetL-2* (Briggs & Fratamico, 1999; Carattoli *et al.*, 2002; Cosby *et al.*, 2015) and Aminoglycoside resistance has also been described by Frana *et al.* (2001) and is encoded by *rmtB, rmtC, armA, rmt*A,D *aadB [ant(20)-la], aacC4* [*aac*(3)-*Iva*], *aacC2* [*aac*(3)-*Iic*], *aadA1*,*aac*(6)-31, Macrolides by *ermA,B,C, mefA/E* and *sul1*,*2*, and *β*-lactams ($bla_{\text{TEM-1,}}$, *CTX-M-1G, 9G, 2G, 64, CTX-M-25 DHA, VIM-1, 2, SPM-1, CMY-2*, and many more), Quinolones or Fluoroquinolones encoded by *Qnr*ABSCD and the integrons coded by *intl1, intl2, intl3* genes.

Integrons are genetic elements that identify species (Ranjbar *et al.*, 2011) and capture mobile gene cassettes species (Kim *et al.*, 2011), which generally encode antimicrobial drug resistance determinants (Carattoli *et al.*, 2002). Four classes of integrons have been identified which includes Class 1, 2, 3 and 4, which have been also detected from *Salmonella* species (Antunes *et al.*, 2006; Kim *et al.*, 2011; Odoch *et al.*, 2018; Povilonis *et al.*, 2010). They have been identified containing one/more genes that encode antibiotic resistance (Gillings, 2014; Stokes & Hall, 1989). Class 1 integron has been identified as mercury resistance transposon Tn*21* (Goldstein *et al.*, 2001; Grinsted & De La Cruz, 1990), carrying the integrase gene *(Intll)* (Carattoli *et al*., 2001) and usually found in pathogenic bacteria. The high percentage of transposon Tn*21* family encode resistance to Sulphonamides (*sull*) and to mercuric ion (Stokes & Hall, 1989). Class 2 integron carry transponson Tn*7* and gene cassette called dihydrofolate reductase (Daly *et al.*, 2000; Rådström *et al.*, 1994). The third one is Class 3 integrons; this class has been associated with *bla1MP* gene cassette which encodes resistance mostly to broad-spectrum beta-lactam

(Goldstein *et al.*, 2001). The last one is Class 4 integrons encoded in the chromosome of *Vibrio cholera* and it contains the *intI4* gene (Mazel, 2006; Mazel *et al.*, 1998). Majority of genes which accounts for *Salmonella* resistance have been characterized, most of them are mainly found as a part of class 1 integrons (Faldynova *et al.*, 2003).

2.6 METHODS USED TO DETECT ANTIMICROBIAL RESISTANCE

2.6.1 Phenotypic methods

There are several phenotypic tests to indicate antibiotic resistance for different bacteria. The most commonly used one is the Minimal Inhibitory Concentration (MIC) method. The method classifies inhibition in three ways: Susceptible where bacterial strain is said to be susceptible to a given antibiotic when it is inhibited in vitro by a concentration of this drug associated with a high likelihood of therapeutic success; intermediate where the sensitivity of a bacterial strain to a given antibiotic is said to be intermediate when it is inhibited *in vitro* by a concentration of this drug associated with an uncertain therapeutic effect; and resistant where a bacterial strain is said to be resistant to a given antibiotic when it is inhibited *in vitro* by a concentration of a drug associated with a high likelihood of therapeutic failure (Kagambèga *et al.*, 2018; Kiehlbauch *et al.*, 2000; Odoch *et al.*, 2018; Olobatoke & Mulugeta, 2015; Rodloff *et al.*, 2008; Thung *et al.*, 2017). Double Disc Synergy test is another test for resistance which is employed for the detection of beta-lactamases that are inhibited by beta-lactamase inhibitors like clavulanic acid (Drieux *et al.*, 2008; Kaur *et al.*, 2013).

2.6.2 Molecular methods to detect antibiotic resistance

Among different molecular methods used for the detection of antimicrobial resistance, PCR has been employed to detect antibiotic resistance genes. It has been used for detection of resistance genes in numerous bacterial species (Aarestrup *et al.*, 2003; Akiyama & Khan, 2011; Chagas *et al.*, 2011; Olobatoke & Mulugeta, 2015; Özgen, 2007; Ploy *et al.*, 2000). Conventional PCR contains three steps: the first one is denaturing of the double-stranded DNA, second, annealing of the PCR primers and extension of the DNA (Anjum *et al.*, 2017). It requires agarose gel to visualize the amplified DNA fragments which indicate the presence of the resistance genes.

Recently, scientists have developed different methods; isothermal amplifications such as loop-mediated isothermal amplification (LAMP) (Anjum *et al.*, 2013; Glais & Jacquot, 2015), and the recombinase polymerase amplification (RPA) (García-Fernández *et al.*, 2014). They have also developed advanced PCR techniques like real-time PCR (qPCR) (Anjum *et al.*, 2017) and multiplex PCR (Poirel *et al.*, 2011), which are also used for antimicrobial resistance genes detection. The qPCR is different from conventional PCR in such a way that it does not require agarose gel electrophoresis and also safer as it does not require use of ethidium bromide, which is a carcinogen. Multiplex PCR, is a technique whereby numerous target DNA fragments can be amplified concurrently using different primers (Anjum *et al.*, 2017; Poirel *et al.*, 2011). Multiplex PCR is more advanced compared to other methods as several resistance genes can be detected at once (Anjum *et al.*, 2017). It has been designed to detect numerous genes, especially the ones that fall under the same resistance phenotype (Dallenne *et al.*, 2010; Poirel *et al.*, 2011; Solanki *et al.*, 2014). PCR targets genes encoding resistance to the major antibiotic families such as Sulfonamides, β-lactams, Aminoglycoside, Tetracyclines, Chloramphenicol,

Fluoroquinolones, Macrolides including the Integrons (Adesiji *et al.*, 2014; Aziz *et al.*, 2018; Nabi, 2017; Thong & Modarressi, 2011).

2.7 REFERENCES

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(4):715-718.

Aavitsland, P. 2008. Antimicrobial resistance-still our joint responsibility. *EpiNorth-Journal of the Network for Communicable Disease Control in Northern and Eastern Europe*, 9(3):67-69.

Abatcha, M.G., Effarizah, M.E. & Rusul, G. 2018. Prevalence, antimicrobial resistance, resistance genes and class 1 integrons of *Salmonella* serovars in leafy vegetables, chicken carcasses and related processing environments in Malaysian fresh food markets. *Food Control*, 91:170-180.

Adesiji, Y.O., Deekshit, V.K. & Karunasagar, I. 2014. Antimicrobial-resistant genes associated with *Salmonella* spp. isolated from human, poultry, and seafood sources. *Food Science & nutrition*, 2(4):436-442.

Agbonlahor, D., Erah, A., Agba, I., Oviasogie, F., Ehiaghe, A., Wankasi, M., Eremwanarue, O., Ehiaghe, I., Ogbu, E. & Iyen, R. 2017. Prevalence of Lassa virus among rodents trapped in three South-South States of Nigeria. *Journal of Vector Borne Diseases*, 54(2):146.

Ahmed, A., Islam, M., Haider, M. & Hossain, M. 2008. Seroprevalence and pathology of naturally infected Salmonellosis in poultry with isolation and identification of causal agents. *J. Bangladesh Agric. Univ*, 6(2):327-334.

Akiyama, T. & Khan, A.A. 2011. Molecular characterization of strains of fluoroquinolone-resistant *Salmonella enterica* serovar *schwarzengrund* carrying multidrug resistance isolated from imported foods. *Journal of Antimicrobial Chemotherapy*, 67(1):101-110.

Akyala, A.I. & Alsam, S. 2015. Extended spectrum beta lactamase producing strains of *Salmonella* species-A Systematic Review. *Journal of Microbiology Research*, 5(2):57-70.

Ali, M.E., Razzak, M.A., Hamid, S.B.A., Rahman, M.M., Al Amin, M. & Rashid, N.R.A. 2015. Multiplex PCR assay for the detection of five meat species forbidden in Islamic foods. *Food Chemistry*, 177:214-224.

Almeida, C., Cerqueira, L., Azevedo, N. & Vieira, M. 2013. Detection of *Salmonella enterica* serovar *enteritidis* using real time PCR, immunocapture assay, PNA FISH and standard culture methods in different types of food samples. *International Journal of Food Microbiology*, 161(1):16-22.

Amatre, G., Babi, N., Enscore, R.E., Ogen-Odoi, A., Atiku, L.A., Akol, A., Gage, K.L. & Eisen, R.J. 2009. Flea diversity and infestation prevalence on rodents in a plague-endemic region of Uganda. *The American Journal of Tropical Medicine and Hygiene*, 81(4):718-724.

Amori, G. & Gippoliti, S. 2003. How do rodent systematics affect conservation priorities? *Aciar Monograph Series*, 96:112-114.

Andino, A. & Hanning, I. 2015. *Salmonella enterica*: survival, colonization, and virulence differences among serovars. *The Scientific World Journal*, 2015:1-16.

Anjum, M., Lemma, F., Cork, D., Meunier, D., Murphy, N., North, S., Woodford, N., Haines, J. & Randall, L. 2013. Isolation and detection of extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae from meat using chromogenic agars and isothermal loop-mediated amplification (LAMP) assays. *Journal of Food Science*, 78(12):M1892-M1898.

Anjum, M.F., Zankari, E. & Hasman, H. 2017. Molecular methods for detection of antimicrobial resistance. *Microbiology*, 5(6:) ARBA-0011-2017. doi:10.1128/microbiolspec.ARBA-0011-2017.

Antunes, P., Machado, J. & Peixe, L. 2006. Characterization of antimicrobial resistance and class 1 and 2 integrons in *Salmonella* enterica isolates from different sources in Portugal. *Journal of Antimicrobial Chemotherapy*, 58(2):297-304.

Aplin, K.P., Brown, P.R., Jacob, J., Krebs, C.J. & Singleton, G.R. 2003. Field methods for rodent studies in Asia and the Indo-Pacific. *Monographs*. EconPapers.repec.org/RePEc:ags:aciarm:114053.

Arakawa, Y., Murakami, M., Suzuki, K., Ito, H., Wacharotayankun, R., Ohsuka, S., Kato, N. & Ohta, M. 1995. A novel integron-like element carrying the metallo-beta-lactamase gene blaIMP. *Antimicrobial agents and Chemotherapy*, 39(7):1612-1615.

Ateba, C. & Mochaiwa, B. 2014. Use of *inv*A gene specific PCR analysis for the detection of virulent *Salmonella* species in beef products in the north west province, South Africa. *Journal of Food and Nutrition Research*, 2(6):294-300.

Ateba, C.N. & Bezuidenhout, C.C. 2008. Characterisation of *Escherichia coli* O157 strains from humans, cattle and pigs in the North-West Province, South Africa. *International Journal of Food Microbiology*, 128(2):181-188.

Aziz, S.A.A., Abdel-Latef, G.K., Shany, S.A. & Rouby, S.R. 2018. Molecular detection of integron and antimicrobial resistance genes in multidrug resistant *Salmonella* isolated from poultry, calves and human in Beni-Suef governorate, Egypt. *Beni-Suef University Journal of Basic and Applied Sciences*. https://doi.org/10.1016/j.bjbas.2018.06.005

Bagóné Vántus, V., Dalle Zotte, A., Cullere, M., Bónai, A., Dal Bosco, A., Szendrő, Z., Tornyos, G., Pósa, R., Bóta, B. & Kovács, M. 2018. Quantitative PCR with *16S rRNA*-gene-targeted specific primers for analysis of caecal microbial community in growing rabbits after dietary supplementation of thyme (*Thymus vulgaris*) and spirulina (*Arthrospira platensis*). *Italian Journal of Animal Science*, 17(3):657-665.

Baron, S. 1996. Protozoa: Structure, classification, growth, and development-Medical Microbiology: University of Texas Medical Branch at Galveston, USA (1996).

Barrow, P. 1990. Immunity to experimental fowl typhoid in chickens induced by a virulence plasmid-cured derivative of *Salmonella gallinarum*. *Infection and Immunity*, 58(7):2283-2288.

Barrow, P. & Neto, O.F. 2011. Pullorum disease and fowl typhoid—new thoughts on old diseases: a review. *Avian Pathology*, 40(1):1-13.

Bastos, A., Chimimba, C., Von Maltitz, E., Kirsten, F. & Belmain, S. 2005. Identification of rodent species that play a role in disease transmission to humans in South Africa. *Proceedings of the South African Society for Veterinary Epidemiology and Preventive Medicine*:78-83.

Battersby, S. 2002. Urban rat infestations: society's response and the public health implications. University of Surrey. (Doctoral dissertation, University of Surrey).

Beck, R.M., Bininda-Emonds, O.R., Cardillo, M., Liu, F.-G.R. & Purvis, A. 2006. A higher-level MRP supertree of placental mammals. *BMC evolutionary biology*, 6(1):93.

Beckmann, I., Subbaiah, T. & Stocker, B. 1964. Rough mutants of *Salmonella* typhimurium (2) Serological and chemical investigations. *Nature*, 201(4926):1299.

Bélanger, S.D., Boissinot, M., Clairoux, N., Picard, F.J. & Bergeron, M.G. 2003. Rapid detection of *Clostridium* difficile in feces by real-time PCR. *Journal of Clinical Microbiology*, 41(2):730-734.

Black, J.G. 2008. Microbiology: principles and explorations: John Wiley & Sons.

Blessmann, J., Buss, H., Nu, P.A.T., Dinh, B.T., Ngo, Q.T.V., Le Van, A., Alla, M.D.A., Jackson, T.F., Ravdin, J.I. & Tannich, E. 2002. Real-time PCR for detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in fecal samples. *Journal of Clinical Microbiology*, 40(12):4413-4417.

Bohaychuk, V., Gensler, G., McFall, M., King, R. & Renter, D. 2007. A real-time PCR assay for the detection of *Salmonella* in a wide variety of food and food-animal matrices. *Journal of Food Protection*, 70(5):1080-1087.

Bonthius, D.J. 2012. Lymphocytic choriomeningitis virus: an underrecognized cause of neurologic disease in the fetus, child, and adult. (*In*. Seminars in pediatric neurology organised by: Elsevier. p. 89-95).

Bonwitt, J., Sáez, A.M., Lamin, J., Ansumana, R., Dawson, M., Buanie, J., Lamin, J., Sondufu, D., Borchert, M. & Sahr, F. 2017. At home with *Mastomys* and *Rattus*: human-rodent

interactions and potential for primary transmission of Lassa virus in domestic spaces. *The American Journal of Tropical Medicine and Hygiene*, 96(4):935-943.

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

Briggs, C.E. & Fratamico, P.M. 1999. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella typhimuriumDT104*. *Antimicrobial Agents and Chemotherapy*, 43(4):846-849.

Broadbent, S., Davies, M. & Van Der Woude, M. 2010. Phase variation controls expression of *Salmonella* lipopolysaccharide modification genes by a DNA methylation-dependent mechanism. *Molecular microbiology*, 77(2):337-353.

Brown, A. 2007. Benson's Microbiological Applications: Laboratory Manual in General Microbiology, Short Version: McGraw-Hill HigherEducation.

Buckle, A. & Smith, R., 2015. Rodent pests and their control. *Rodent pests and their control.*, (Ed. 2).

Bugarel, M., Granier, S.A., Weill, F.-X., Fach, P. & Brisabois, A. 2011. A multiplex real-time PCR assay targeting virulence and resistance genes in *Salmonella enterica* serotype Typhimurium. *BMC Microbiology*, 11(1):151.

Byrd, J., Anderson, R., Callaway, T., Moore, R., Knape, K., Kubena, L., Ziprin, R. & Nisbet, D. 2003. Effect of experimental chlorate product administration in the drinking water on *Salmonella* Typhimurium contamination of broilers. *Poultry Science*, 82(9):1403-1406.

Carattoli, A., Filetici, E., Villa, L., Dionisi, A.M., Ricci, A. & Luzzi, I. 2002. Antibiotic resistance genes and *Salmonella* genomic island 1 in *Salmonella* enterica serovar

Typhimurium isolated in Italy. *Antimicrobial Agents and Chemotherapy*, 46(9):2821-2828.

Carattoli, A., Villa, L., Pezzella, C., Bordi, E. & Visca, P. 2001. Expanding drug resistance through integron acquisition by IncFI plasmids of *Salmonella* enterica typhimurium. *Emerging Infectious Diseases*, 7(3):444.

Card, R., Vaughan, K., Bagnall, M., Spiropoulos, J., Cooley, W., Strickland, T., Davies, R. & Anjum, M.F. 2016. Virulence characterisation of *Salmonella enterica* isolates of differing antimicrobial resistance recovered from UK livestock and imported meat samples. *Frontiers in Microbiology*, 7:640.

Carleton, M. 1984. Introduction to rodents. Pp, 255-265. *Orders and families of recent mammals of the world (S. Anderson and JK Jones, Jr., eds.). John Wiley and Sons, New York*, 686 pp.

Castilla, K.S., Ferreira, C.S.A., Moreno, A.M., Nunes, I.A. & Ferreira, A.J.P. 2006. Distribution of virulence genes sefC, pefA and spvC in *Salmonella enteritidis* phage type 4 strains isolated in Brazil. *Brazilian Journal of Microbiology*, 37(2):135-139.

Chagas, T.P.G., Alves, R.M., Vallim, D.C., Seki, L.M., Campos, L.C. & Asensi, M.D. 2011. Diversity of genotypes in CTX-M-producing *Klebsiella pneumoniae* isolated in different hospitals in Brazil. *Brazilian Journal of Infectious Diseases*, 15(5):420-425.

Chart, H., Cheasty, T., de Pinna, E., Siorvanes, L., Wain, J., Alam, D., Nizami, Q., Bhutta, Z. & Threlfall, E.J. 2007. Serodiagnosis of *Salmonella enterica* serovar *Typhi* and *S. enterica* serovars Paratyphi A, B and C human infections. *Journal of Medical Microbiology*, 56(9):1161-1166.

Chuah, L.-O., Syuhada, A.-K.S., Suhaimi, I.M., Hanim, T.F. & Rusul, G. 2018. Genetic relatedness, antimicrobial resistance and biofilm formation of *Salmonella* isolated from naturally contaminated poultry and their processing environment in northern Malaysia. *Food Research International*, 105:743-751.

Coburn, B., Grassl, G.A. & Finlay, B. 2007. *Salmonella*, the host and disease: a brief review. *Immunology and Cell Biology*, 85(2):112-118.

Cosby, D.E., Cox, N.A., Harrison, M.A., Wilson, J.L., Buhr, R.J. & Fedorka-Cray, P.J. 2015. *Salmonella* and antimicrobial resistance in broilers: a review. *Journal of Applied Poultry Research*, 24(3):408-426.

Courvalin, P. 2005. Antimicrobial drug resistance:" Prediction is very difficult, especially about the future". *Emerging Infectious Diseases*, 11(10):1503.

Dagnra, A.-Y., Akolly, K., Gbadoe, A., Aho, K. & David, M. 2007. Émergence des souches de *Salmonelles* multirésistantes aux antibiotiques à Lomé (Togo). *Médecine et maladies infectieuses*, 37(5):266-269.

Dallenne, C., Da Costa, A., Decré, D., Favier, C. & Arlet, G. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy*, 65(3):490-495.

Daly, M., Buckley, J., Power, E., O'hare, C., Cormican, M., Cryan, B., Wall, P. & Fanning, S. 2000. Molecular Characterization of Irish *Salmonella enterica* Serotype typhimurium: Detection of Class I integrons and Assessment of Genetic Relationships by DNA Amplification Fingerprinting. *Applied and Environmental Microbiology*, 66(2):614-619.

Darwin, K.H. & Miller, V.L. 1999. InvF is required for expression of genes encoding proteins secreted by the SPI1 type III secretion apparatus in *Salmonella* typhimurium. *Journal of Bacteriology,* 181(16):4949-4954.

Darwin, K.H. & Miller, V.L. 2000. The putative invasion protein chaperone SicA acts together with InvF to activate the expression of *Salmonella* typhimurium virulence genes. *Molecular Microbiology,* 35(4):949-960.

Davies, J. & Davies, D. 2010. Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews*, 74(3):417-433.

Davies, R. & Breslin, M. 2003. Observations on *Salmonella* contamination of. *The Veterinary Record,* 152:283-287.

Davies, R. & Wray, C. 1995. Mice as carriers of *Salmonella enteritidis* on persistently infected poultry units. *The Veterinary Record*, 137(14):337-341.

Deng, Y., Bao, X., Ji, L., Chen, L., Liu, J., Miao, J., Chen, D., Bian, H., Li, Y. & Yu, G. 2015. Resistance integrons: class 1, 2 and 3 integrons. *Annals of Clinical Microbiology and Antimicrobials*, 14(1):45.

Desmidt, M., Ducatelle, R. & Haesebrouck, F. 1997. Pathogenesis of *Salmonella enteritidis* phage type four after experimental infection of young chickens. *Veterinary Microbiology,* 56(1-2):99-109.

DiMarzio, M., Shariat, N., Kariyawasam, S., Barrangou, R. & Dudley, E.G. 2013. Antibiotic resistance in *Salmonella* typhimurium associates with CRISPR sequence type. *Antimicrobial Agents and chemotherapy*:AAC. 00913-00913.

Dione, M.M., Ikumapayi, U., Saha, D., Mohammed, N.I., Adegbola, R.A., Geerts, S., Ieven, M. & Antonio, M. 2011. Antimicrobial resistance and virulence genes of non-typhoidal**

*Salmonella*** isolates in The Gambia and Senegal. *Journal of Infection in Developing Countries*, 5(11):765-775.

Drieux, L., Brossier, F., Sougakoff, W. & Jarlier, V. 2008. Phenotypic detection of extended-spectrum β-lactamase production in Enterobacteriaceae: review and bench guide. *Clinical microbiology and infection*, 14:90-103.

Ekwanzala, M.D., Abia, A.L.K., Keshri, J. & Momba, M.N.B. 2017. Genetic characterization of *Salmonella* and *Shigella* spp. isolates recovered from water and riverbed sediment of the Apies River, South Africa. *Water SA*, 43(3):387-397.

El-Sharkawy, H., Tahoun, A., El-Gohary, A.E.-G.A., El-Abasy, M., El-Khayat, F., Gillespie, T., Kitade, Y., Hafez, H.M., Neubauer, H. & El-Adawy, H. 2017. Epidemiological, molecular characterization and antibiotic resistance of *Salmonella* enterica serovars isolated from chicken farms in Egypt. *Gut Pathogens*, 9(1):8.

Esaki, H., Morioka, A., Ishihara, K., Kojima, A., Shiroki, S., Tamura, Y. & Takahashi, T. 2004. Antimicrobial susceptibility of *Salmonella* isolated from cattle, swine and poultry (2001–2002): report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. *Journal of Antimicrobial Chemotherapy*, 53(2):266-270.

Faldynova, M., Pravcova, M., Sisak, F., Havlickova, H., Kolackova, I., Cizek, A., Karpiskova, R. & Rychlik, I. 2003. Evolution of antibiotic resistance in *Salmonella enterica* serovar typhimurium strains isolated in the Czech Republic between 1984 and 2002. *Antimicrobial Agents and Chemotherapy*, 47(6):2002-2005.

Fekry, E., Ammar, A.M. & Hussien, A.-e. 2018. Molecular Detection of InvA, OmpA and Stn Genes in *Salmonella* Serovars from Broilers in Egypt. *Alexandria Journal for Veterinary Sciences*, 56(1).

Fichet-Calvet, E. 2014. Lassa Fever: A rodent-human interaction. The Role of Animals in Emerging Viral Diseases. Elsevier. p. 89-123).

Folster, J.P., Pecic, G., Bolcen, S., Theobald, L., Hise, K., Carattoli, A., Zhao, S., McDermott, P.F. & Whichard, J.M. 2010. Characterization of extended-spectrum cephalosporin–resistant *Salmonella* enterica serovar *heidelberg* isolated from humans in the United States. *Foodborne Pathogens and Disease*, 7(2):181-187.

Frana, T.S., Carlson, S.A. & Griffith, R.W. 2001. Relative distribution and conservation of genes encoding aminoglycoside-modifying enzymes in *Salmonella* enterica serotype *typhimurium* phage type DT104. *Applied and Environmental Microbiology*, 67(1):445-448.

Frank, T., Gautier, V., Talarmin, A., Bercion, R. & Arlet, G. 2007. Characterization of sulphonamide resistance genes and class 1 integron gene cassettes in Enterobacteriaceae, Central African Republic (CAR). *Journal of Antimicrobial Chemotherapy*, 59(4):742-745.

Franssen, F., Swart, A., van Knapen, F. & van der Giessen, J. 2016. Helminth parasites in black rats (*Rattus rattus*) and brown rats (*Rattus norvegicus*) from different environments in the Netherlands. *Infection Ecology & Epidemiology*, 6, 31413.

Fraschina, J., León, V.A. & Busch, M. 2014. Role of Landscape Scale in the Distribution of Rodents in an Agroecosystem of Argentina. *Journal of Agricultural Science*, 6(12):22.

Gakuya, F., Kyule, M., Gathura, P. & Kariuki, S. 2001. Antimicrobial resistance of bacterial organisms isolated from rats. *East African Medical Journal*, 78(12):646-649.

García-Fernández, S., Morosini, M.-I., Marco, F., Gijón, D., Vergara, A., Vila, J., Ruiz-Garbajosa, P. & Cantón, R. 2014. Evaluation of the eazyplex® SuperBug CRE system

for rapid detection of carbapenemases and ESBLs in clinical Enterobacteriaceae isolates recovered at two Spanish hospitals. *Journal of Antimicrobial Chemotherapy*, 70(4):1047-1050.

Gillings, M.R. 2014. Integrons: past, present, and future. *Microbiology and Molecular Biology Reviews*, 78(2):257-277.

Glais, L. & Jacquot, E. 2015. Detection and characterization of viral species/subspecies using isothermal recombinase polymerase amplification (RPA) assays. Plant Pathology. Springer. p. 207-225).

Gokul, B.N., Menezes, G.A. & Harish, B.N. 2010. Acc-1 β-lactamase–producing *Salmonella* enterica serovar *Typhi*, India. *Emerging Infectious Diseases*, 16(7):1170.

Goldstein, C., Lee, M.D., Sanchez, S., Hudson, C., Phillips, B., Register, B., Grady, M., Liebert, C., Summers, A.O. & White, D.G. 2001. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. *Antimicrobial Agents and Chemotherapy*, 45(3):723-726.

Goodman, L.B., McDonough, P.L., Anderson, R.R., Franklin-Guild, R.J., Ryan, J.R., Perkins, G.A., Thachil, A.J., Glaser, A.L. & Thompson, B.S. 2017. Detection of *Salmonella* spp. in veterinary samples by combining selective enrichment and real-time PCR. *Journal of Veterinary Diagnostic Investigation*, 29(6):844-851.

Grinsted, J. & De La Cruz, F. 1990. The Tn21 subgroup of bacterial transposable elements. *Plasmid*, 24(3):163-189.

Hafez, H.M. 2013. 10 *Salmonella* Infections in Turkeys. *Salmonella in domestic animals*:193.

Haneda, T., Ishii, Y., Shimizu, H., Ohshima, K., Iida, N., Danbara, H. & Okada, N. 2012. *Salmonella* type III effector SpvC, a phosphothreonine lyase, contributes to reduction in

inflammatory response during intestinal phase of infection. *Cellular Microbiology*, 14(4):485-499.

Hayward, R.D. & Koronakis, V. 1999. Direct nucleation and bundling of actin by the SipC protein of invasive *Salmonella*. *The EMBO journal*, 18(18):4926-4934.

Healing, T. & Greenwood, M. 1991. Frequency of isolation of *Campylobacter* spp., *Yersinia* spp. and *Salmonella* spp. from small mammals from two sites in southern Britain. *International Journal of Environmental Health Research*, 1(1):54-62.

Hebert, P.D., Ratnasingham, S. & de Waard, J.R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B: Biological Sciences,* 270(Suppl 1):S96-S99.

Hebert, P.D., Stoeckle, M.Y., Zemlak, T.S. & Francis, C.M. 2004. Identification of birds through DNA barcodes. *PLoS biology*, 2(10):e312.

Henzler, D., Kradel, D. & Sischo, W. 1998. Management and environmental risk factors for *Salmonella enteritidis* contamination of eggs. *American Journal of Veterinary Research*, 59(7):824-829.

Henzler, D. & Opitz, H. 1992. The role of mice in the epizootiology of *Salmonella enteritidis* infection on chicken layer farms. *Avian diseases*:625-631.

Hilton, A.C., Willis, R.J. & Hickie, S.J. 2002. Isolation of *Salmonella* from urban wild brown rats (Rattus norvegicus) in the West Midlands, UK. *International Journal of Environmental Health Research*, 12(2):163-168.

Hong, Y., Liu, T., Lee, M.D., Hofacre, C.L., Maier, M., White, D.G., Ayers, S., Wang, L., Berghaus, R. & Maurer, J.J. 2008. Rapid screening of *Salmonella* enterica serovars

enteritidis, *hadar*, *heidelberg* and typhimurium using a serologically-correlative allelotyping PCR targeting the O and H antigen alleles. *BMC Microbiology*, 8(1):178.

Hossain, M., Chowdhury, E., Islam, M., Haider, M. & Hossain, M. 2006. Avian *Salmonella* infection: isolation and identification of organisms and histopathological study. *Bangladesh Journal of Veterinary Medicine*, 4(1):7-12.

Huchon, D., Madsen, O., Sibbald, M.J., Ament, K., Stanhope, M.J., Catzeflis, F., De Jong, W.W. & Douzery, E.J. 2002. Rodent phylogeny and a timescale for the evolution of Glires: evidence from an extensive taxon sampling using three nuclear genes. *Molecular Biology and Evolution*, 19(7):1053-1065.

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. 2008. Characterisation of *Salmonella* enterica serotype typhimurium isolates from wild birds in northern England from 2005–2006. *BMC Veterinary Research*, 4(1):4.

Huijsdens, X.W., Linskens, R.K., Mak, M., Meuwissen, S.G., Vandenbroucke-Grauls, C.M. & Savelkoul, P.H. 2002. Quantification of bacteria adherent to gastrointestinal mucosa by real-time PCR. *Journal of Clinical Microbiology*, 40(12):4423-4427.

Hur, J., Kim, J.H., Park, J.H., Lee, Y.-J. & Lee, J.H. 2011. Molecular and virulence characteristics of multi-drug resistant *Salmonella enteritidis* strains isolated from poultry. *The Veterinary Journal*, 189(3):306-311.

Imanishi, M., Newton, A., Vieira, A., Gonzalez-Aviles, G., Scott, M.K., Manikonda, K., Maxwell, T., Halpin, J., Freeman, M. & Medalla, F. 2015. Typhoid fever acquired in the United States, 1999–2010: epidemiology, microbiology, and use of a space–time scan statistic for outbreak detection. *Epidemiology & Infection*, 143(11):2343-2354.

Inoue, K., Maruyama, S., Kabeya, H., Yamada, N., Ohashi, N., Sato, Y., Yukawa, M., Masuzawa, T., Kawamori, F. & Kadosaka, T. 2008. Prevalence and genetic diversity of *Bartonella* species isolated from wild rodents in Japan. *Applied and Environmental Microbiology*, 74(16):5086-5092.

ISO, P. 2002. 6579: 2002. Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Salmonella* spp. Switzerland: International Standards Organization.

Jemilehin, F., Ogunleye, A., Okunlade, A. & Ajuwape, A. 2016. Isolation of *Salmonella* species and some other gram negative bacteria from rats cohabitating with poultry in Ibadan, Oyo State, Nigeria. *African Journal of Microbiology Research*, 10(29):1104-1110.

Jensen, A.N., Lodal, J. & Baggesen, D.L. 2004. High diversity of *Salmonella* serotypes found in an experiment with outdoor pigs. *NJAS Wageningen Journal of Life Sciences*, 52(2):109-117.

Johannes, N.S. 2015. Identification and characterisation of potential virulence genes of *Salmonella* from rooibos tea. (Doctoral dissertation, University of the Western Cape)

Joys, T.M. 1985. The covalent structure of the phase-1 flagellar filament protein of *Salmonella* typhimurium and its comparison with other flagellins. *Journal of Biological Chemistry*, 260(29):15758-15761.

Kagambèga, A., Lienemann, T., Frye, J.G., Barro, N. & Haukka, K. 2018. Whole genome sequencing of multidrug-resistant *Salmonella* enterica serovar typhimurium isolated from humans and poultry in Burkina Faso. *Tropical Medicine and Health*, 46(1):4.

Kariuki, S., Gordon, M.A., Feasey, N. & Parry, C.M. 2015. Antimicrobial resistance and management of invasive *Salmonella* disease. *Vaccine*, 33:C21-C29.

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(1):38-43.

Kaur, J., Chopra, S. & Sheevani, G.M. 2013. Modified double disc synergy test to detect ESBL production in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *Journal of Cinical and Diagnostic Research: JCDR*, 7(2):229.

Kaur, J. & Jain, S. 2012. Role of antigens and virulence factors of *Salmonella* enterica serovar Typhi in its pathogenesis. *Microbiological Research*, 167(4):199-210.

Kerr, K.C., Stoeckle, M.Y., Dove, C.J., Weigt, L.A., Francis, C.M. & Hebert, P.D. 2007. Comprehensive DNA barcode coverage of North American birds. *Molecular Ecology Resources*, 7(4):535-543.

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(1):37-44.

Kiehlbauch, J.A., Hannett, G.E., Salfinger, M., Archinal, W., Monserrat, C. & Carlyn, C. 2000. Use of the National Committee for Clinical Laboratory Standards guidelines for disk diffusion susceptibility testing in New York state laboratories. *Journal of Clinical Microbiology*, 38(9):3341-3348.

Kim, J.E. & ju Lee, Y. 2017. Molecular characterization of antimicrobial resistant non-typhoidal *Salmonella* from poultry industries in Korea. *Irish Yeterinary Journal*, 70(1):20.

Kim, S., Kim, S.-H., Kim, J., Shin, J.-H., Lee, B.-K. & Park, M.-S. 2011. Occurrence and distribution of various genetic structures of class 1 and class 2 integrons in Salmonella enterica isolates from foodborne disease patients in Korea for 16 years. *Foodborne Pathogens and Disease*, 8(2):319-324.

Kingsley, R.A., Msefula, C.L., Thomson, N.R., Kariuki, S., Holt, K.E., Gordon, M.A., Harris, D., Clarke, L., Whitehead, S. & Sangal, V. 2009. Epidemic multiple drug resistant *Salmonella* typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype. *Genome Research*. 19: 2279-87.

Knust, B., Ströher, U., Edison, L., Albariño, C.G., Lovejoy, J., Armeanu, E., House, J., Cory, D., Horton, C. & Fowler, K.L. 2014. Lymphocytic choriomeningitis virus in employees and mice at multipremises feeder-rodent operation, United States, 2012. *Emerging Infectious Diseases*, 20(2):240.

Kurowski, P.B., Traub-Dargatz, J.L., Morley, P.S. & Gentry-Weeks, C.R. 2002. Detection of *Salmonella* spp in fecal specimens by use of real-time polymerase chain reaction assay. *American Journal of Veterinary Research*, 63(9):1265-1268.

Lakshminarayanan, R.R., Shanmugam, A. & Govindaraju, A. 2015. Sequencing of *COI* gene in four rodent pests for species identification. *DNA Barcodes*, 3(1).

Lapuz, R., Tani, H., Sasai, K., Shirota, K., Katoh, H. & Baba, E. 2008. The role of roof rats (Rattus rattus) in the spread of *Salmonella enteritidis* and *S. infantis* contamination in layer farms in eastern Japan. *Epidemiology and Infection*, 136(09):1235-1243.

Lapuz, R.R.S.P., Umali, D.V., Suzuki, T., Shirota, K. & Katoh, H. 2012. Comparison of the prevalence of *Salmonella* infection in layer hens from commercial layer farms with high and low rodent densities. *Avian Diseases*, 56(1):29-34.

Lee, Y.J., Mo, I.P. & Kang, M.S. 2005. Safety and efficacy of *Salmonella gallinarum* 9R vaccine in young laying chickens. *Avian Pathology*, 34(4):362-366.

Li, X., Liu, L., Li, Q., Xu, G. & Zheng, J. 2018. *Salmonella* Contamination in Layer Farms in China: Detection and Genetic Analysis. *The Journal of Poultry Science*, 55(1):1-9.

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(2):842-851.

Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., Jones, T.F., Fazil, A. & Hoekstra, R.M. 2010. International Collaboration on Enteric Disease "Burden of Illness" Studies 2010. *The global burden of nontyphoidal Salmonella gastroenteritis. Clininical Infectious Diseases*, 50(6):882-889.

Manie, T., Khan, S., Brözel, V., Veith, W. & Gouws, P. 1998. Antimicrobial resistance of bacteria isolated from slaughtered and retail chickens in South Africa. *Letters in applied microbiology*, 26(4):253-258.

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

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

Matsuki, T., Watanabe, K., Fujimoto, J., Kado, Y., Takada, T., Matsumoto, K. & Tanaka, R. 2004. Quantitative PCR with 16S rRNA-gene-targeted species-specific primers for analysis of human intestinal bifidobacteria. *Applied and Environmental microbiology*, 70(1):167-173.

Mazel, D. 2006. Integrons: agents of bacterial evolution. *Nature Reviews Microbiology*, 4(8):608.

Mazel, D., Dychinco, B., Webb, V.A. & Davies, J. 1998. A distinctive class of integron in the Vibrio cholerae genome. *Science*, 280(5363):605-608.

McKiel, J., Rappay, D., Cousineau, J., Hall, R. & McKenna, H. 1970. Domestic rats as carriers of *Leptospires* and *Salmonellae* in Eastern Canada. *Canadian Journal of Public Health/Revue Canadienne de Sante'e Publique*, 61(4):336-340.

McWhorter, A.R., Davos, D. & Chousalkar, K. 2014. Pathogenicity of *Salmonella* strains isolated from egg shell and layer farm environment in Australia. *Applied and environmental microbiology*:AEM. 02931-02914.

Meehan, A.P. 1984. Rats and mice. Their biology and control: Rentokil Ltd.

Meerburg, B.G. & Kijlstra, A. 2007. Role of rodents in transmission of *Salmonella* and *Campylobacter*. *Journal of the Science of Food and Agriculture*, 87(15):2774-2781.

Meerburg, B.G., Singleton, G.R. & Kijlstra, A. 2009. Rodent-borne diseases and their risks for public health. *Critical Reviews in Microbiology*, 35(3):221-270.

Menezes, G.A., Khan, M.A., Harish, B.N., Parija, S.C., Goessens, W., Vidyalakshmi, K., Baliga, S. & Hays, J. 2010. Molecular characterization of antimicrobial resistance in non-typhoidal *Salmonellae* associated with systemic manifestations from India. *Journal of Medical Microbiology*, 59(12):1477-1483.

Moawad, A.A., Hotzel, H., Awad, O., Tomaso, H., Neubauer, H., Hafez, H.M. & El-Adawy, H. 2017. Occurrence of *Salmonella enterica* and *Escherichia coli* in raw chicken and beef meat in northern Egypt and dissemination of their antibiotic resistance markers. *Gut Pathogens*, 9(1):57.

Modarressi, S. & Thong, K.L. 2010. Isolation and molecular sub typing of *Salmonella* enterica from chicken, beef and street foods in Malaysia. *Scientific Research and Essays*, 5(18):2713-2720.

Montville, T. & Matthews, K. 2008. Food microbiology: An introduction . 428 p: United States of America: ASM Press, Washington, DC. ISBN.

Mortimer, C.K., Peters, T.M., Gharbia, S.E., Logan, J.M. & Arnold, C. 2004. Towards the development of a DNA-sequence based approach to serotyping of *Salmonella* enterica. *BMC Microbiology*, 4(1):31.

Mossoro-Kpinde, C.D., Manirakiza, A., Mbecko, J.-R., Misatou, P., Le Faou, A. & Frank, T. 2015. Antimicrobial Resistance of *enteric Salmonella* in Bangui, Central African Republic. *Journal of Tropical Medicine*, 2015:483974.

Mubita, C., Syakalima, M., Chisenga, C., Munyeme, M., Bwalya, M., Chifumpa, G., Hang ombe, B.M., Sinkala, P., Simuunza, M. & Fukushi, H. 2008. Antibiograms of faecal *Escherichia coli* and *Enterococci* species isolated from pastoralist cattle in the interface areas of the Kafue basin in Zambia. *Veterinarski arhiv*, 78(2):179.

Musser, G.G., Smith, A.L., Robinson, M. & Lunde, D.P. 2005. Description of a new genus and species of rodent (Murinae, Muridae, Rodentia) from the Khammouan Limestone National Biodiversity Conservation Area in Lao PDR. *American Museum Novitates*:1-31.

Nabi, A.Q. 2017. Molecular study on some antibiotic resistant genes in *Salmonella* spp. isolates. (*In*. AIP Conference Proceedings organised by: AIP Publishing. p. 020037).

Nair, A., Rawool, D.B., Doijad, S., Poharkar, K., Mohan, V., Barbuddhe, S.B., Kolhe, R., Kurkure, N.V., Kumar, A. & Malik, S. 2015. Biofilm formation and genetic diversity of

Salmonella isolates recovered from clinical, food, poultry and environmental sources. *Infection, Genetics and Evolution*, 36:424-433.

Nataro, J.P., Bopp, C.A., Fields, P.I., Kaper, J.B. & Strockbine, N.A. 2011. *Escherichia, shigella*, and *salmonella*. Manual of Clinical Microbiology, 10th Edition. *American Society of Microbiology*. p. 603-626).

Nkogwe, C., Raletobana, J., Stewart-Johnson, A., Suepaul, S. & Adesiyun, A. 2011. Frequency of detection of *Escherichia coli*, *Salmonella* spp., and *Campylobacter* spp. in the faeces of wild rats (*Rattus* spp.) in Trinidad and Tobago. *Veterinary medicine international*, 2011: 1-7.

Nwiyi, P. & Erumaka, G. 2012. Frequency of detection of *Salmonella* spp. and *Esherichia coli* in the faeces of house rat in Abia State, Nigeria. *Research Opinions in Animal & Veterinary Sciences*, 2(10): 686923.

Ochman, H. & Groisman, E.A. 1996. Distribution of pathogenicity islands in *Salmonella* spp. *Infection and immunity*, 64(12):5410-5412.

Odoch, T., Sekse, C., L'Abee-Lund, T.M., Høgberg Hansen, H.C., Kankya, C. & Wasteson, Y. 2018. Diversity and Antimicrobial Resistance Genotypes in Non-Typhoidal *Salmonella* Isolates from Poultry Farms in Uganda. *International journal of environmental research and public health*, 15(2):324.

Odumeru, J.A. & León-Velarde, C.G. 2012. *Salmonella* detection methods for food and food ingredients. *Salmonella*-A Dangerous Foodborne Pathogen. InTech, 450.

Olobatoke, R.Y. & Mulugeta, S.D. 2015. Incidence of non-typhoidal *Salmonella* in poultry products in the North West Province, South Africa. *South African Journal of Science*, 111(11-12):1-7.

Özbey, G., Seven, P.T., Muz, A., Ertaş, H. & ÇERÇI, H. 2008. Isolation of *Salmonella* spp. from faecal samples of cracked egg fed hens and polymerase chain reaction (PCR) confirmation. *Bulgarian Journal of Veterinary Medicine*, 11(2):103-112.

Özgen, C. 2007. Isolation, molecular characterization of food-borne drug resistant *Salmonella* spp. and detection of class 1 integrons. Citeseer. Doctoral dissertation, Middle East Technical University.

Pal, A. & Marshall, D.L. 2009. Comparison of culture media for enrichment and isolation of *Salmonella* spp. from frozen Channel catfish and Vietnamese basa fillets. *Food Microbiology*, 26(3):317-319.

Pan, Z., Wang, X., Zhang, X., Geng, S., Chen, X., Pan, W., Cong, Q., Liu, X., Jiao, X. & Liu, X. 2009. Changes in antimicrobial resistance among *Salmonella* enterica subspecies enterica serovar *pullorum* isolates in China from 1962 to 2007. *Veterinary Microbiology*, 136(3-4):387-392.

Parry, C.M., Wijedoru, L., Arjyal, A. & Baker, S. 2011. The utility of diagnostic tests for enteric fever in endemic locations. *Expert review of anti-infective therapy*, 9(6):711-725.

Phokela, P.T., Ateba, C.N. & Kawadza, D.T. 2011. Assessing antibiotic resistance profiles in *Escherichia coli* and *Salmonella* species from groundwater in the Mafikeng area, South Africa. *African Journal of Microbiology Research*, 5(32):5902-5909.

Ploy, M.-C., Denis, F., Courvalin, P. & Lambert, T. 2000. Molecular Characterization of Integrons in Acinetobacter baumannii: Description of a Hybrid Class 2 Integron. *Antimicrobial Agents and Chemotherapy*, 44(10):2684-2688.

Pocock, M., Searle, J., Betts, W. & White, P. 2001. Patterns of infection by Salmonella and Yersinia spp. in commensal house mouse (*Mus musculus domesticus*) populations. *Journal of Applied Microbiology*, 90(5):755-760.

Poirel, L., Walsh, T.R., Cuvillier, V. & Nordmann, P. 2011. Multiplex PCR for detection of acquired carbapenemase genes. *Diagnostic Microbiology and Infectious Disease*, 70(1):119-123.

Povilonis, J., Šeputienė, V., Ružauskas, M., Šiugždinienė, R., Virgailis, M., Pavilonis, A. & Sužiedėlienė, E. 2010. Transferable class 1 and 2 integrons in *Escherichia coli* and S*almonella* enterica isolates of human and animal origin in Lithuania. *Foodborne Pathogens and Disease*, 7(10):1185-1192.

Prasad, K. 2012. Clinical Significance HIV-positive patients appear to be at an increased risk of acquiring *Salmonella* infection. In AIDS patients, *Salmonella* gastroenteritis is most often due to the nontyphoidal species (*Salmonella* serotype. *Textbook-Atlas of Intestinal Infections in AIDS*:271.

Prasanna Kumar, V. 2016. Studies on virulence, antimicrobial resistance and genetic diversity of *Salmonella* typhimurium isolates from North india. GB Pant University of Agriculture and Technology, Pantnagar-263145 (Uttarakhand).

Qiao, J., Zhang, Q., Alali, W.Q., Wang, J., Meng, L., Xiao, Y., Yang, H., Chen, S., Cui, S. & Yang, B. 2017. Characterization of extended-spectrum β-lactamases (ESBLs)-producing *Salmonella* in retail raw chicken carcasses. *International Journal of Food Microbiology*, 248:72-81.

Rådström, P., Bäckman, A., Qian, N., Kragsbjerg, P., Påhlson, C. & Olcén, P. 1994. Detection of bacterial DNA in cerebrospinal fluid by an assay for simultaneous detection of

Neisseria meningitidis, Haemophilus influenzae, and streptococci using a seminested PCR strategy. *Journal of Clinical Microbiology*, 32(11):2738-2744.

Ranjbar, R., Giammanco, G.M., Farshad, S., Owlia, P., Aleo, A. & Mammina, C. 2011. Serotypes, antibiotic resistance, and class 1 integrons in *Salmonella* isolates from pediatric cases of enteritis in Tehran, Iran. *Foodborne Pathogens and Disease*, 8(4):547-553.

Rasschaert, G., Houf, K., Imberechts, H., Grijspeerdt, K., De Zutter, L. & Heyndrickx, M. 2005. Comparison of five repetitive-sequence-based PCR typing methods for molecular discrimination of *Salmonella* enterica isolates. *Journal of Clinical Microbiology*, 43(8):3615-3623.

Refai, M., Hatem, M.E., Elhariri, M., Salem, G.H. & Mohamed, A.H. 2017. Using of molecular biology techniques compared with conventional detection methods for detection of *Salmonella* in cattle in Egypt. *Journal of American Science*, 13(10).

Reusken, C., van der Plaats, R., Opsteegh, M., de Bruin, A. & Swart, A. 2011. *Coxiella burnetii* (Q fever) in *Rattus norvegicus* and *Rattus rattus* at livestock farms and urban locations in the Netherlands; could Rattus spp. represent reservoirs for (re) introduction? *Preventive Veterinary Medicine*, 101(1-2):124-130.

Ridley, A. & Threlfall, E.J. 1998. Molecular epidemiology of antibiotic resistance genes in multiresistant epidemic *Salmonella* typhimurium DT 104. *Microbial Drug Resistance*, 4(2):113-118.

Rivoal, K., Protais, J., Quéguiner, S., Boscher, E., Chidaine, B., Rose, V., Gautier, M., Baron, F., Grosset, N. & Ermel, G. 2009. Use of pulsed-field gel electrophoresis to characterize the heterogeneity and clonality of *Salmonella* serotype e*nteritidis*, typhimurium and *infantis*

isolates obtained from whole liquid eggs. *International Journal of Food Microbiology*, 129(2):180-186.

Robins, J.H., Hingston, M., Matisoo-smith, E. & Ross, H.A. 2007. Identifying *Rattus* species using mitochondrial DNA. *Molecular Ecology Resources*, 7(5):717-729.

Rodenburg, T., Van Der Hulst-Van Arkel, M. & Kwakkel, R. 2004. *Campylobacter* and *Salmonella* infections on organic broiler farms. *NJAS wageningen journal of life sciences*, 52(2):101-108.

Rodloff, A., Leclercq, R., Debbia, E., Cantón, R., Oppenheim, B. & Dowzicky, M. 2008. Comparative analysis of antimicrobial susceptibility among organisms from France, Germany, Italy, Spain and the UK as part of the tigecycline evaluation and surveillance trial. *Clinical Microbiology and Infection*, 14(4):307-314.

Roomaney, R., Ehrlich, R. & Rother, H.-A. 2012. The acceptability of rat trap use over pesticides for rodent control in two poor urban communities in South Africa. *Environmental Health*, 11(1):32.

Rotger, R. & Casadesús, J. 1999. The virulence plasmids of *Salmonella*. *International Microbiology*, 2:177-184.

Sadeyen, J.-R., Trotereau, J., Velge, P., Marly, J., Beaumont, C., Barrow, P.A., Bumstead, N. & Lalmanach, A.-C. 2004. *Salmonella* carrier state in chicken: comparison of expression of immune response genes between susceptible and resistant animals. *Microbes and Infection*, 6(14):1278-1286.

Sahilah, A., Laila, R., Sallehuddin, H.M., Osman, H., Aminah, A. & Azuhairi, A.A. 2014. Antibiotic resistance and molecular typing among cockle (*Anadara granosa*) strains of

Vibrio parahaemolyticus by polymerase chain reaction (PCR)-based analysis. *World Journal of Microbiology and Biotechnology*, 30(2):649-659.

Salmon, D. & Smith, T. 1885. Report on swine plague. US Bureau of Animal Industries. *2nd Ann. Rep. US Gov't. Printing Office, Washington*, 184.

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

Samuel, G. & Reeves, P. 2003. Biosynthesis of O-antigens: genes and pathways involved in nucleotide sugar precursor synthesis and O-antigen assembly. *Carbohydrate Research*, 338(23):2503-2519.

Saroj, S.D., Shashidhar, R., Karani, M. & Bandekar, J.R. 2008. Distribution of *Salmonella* pathogenicity island (SPI)-8 and SPI-10 among different serotypes of *Salmonella*. *Journal of Medical Microbiology*, 57(4):424-427.

Schwarz, S., Kehrenberg, C. & Walsh, T. 2001. Use of antimicrobial agents in veterinary medicine and food animal production. *International Journal of Antimicrobial Agents*, 17(6):431-437.

Shilangale, R. 2014a. Prevalence, Serotypes and Antimicrobial Resistance of *Salmonella* Isolated from Beef and Animal Feed in Namibia. Ph. D. thesis, The University of Namibia, Omusati Region, Namibia, 2014.[Google Scholar]. Retrieved on the 06- 04- 2018

Shilangale, R.P. 2014b. Prevalence, Serotypes and antimicrobial resistance os *Salmonella* isolated from beef and small animal feed in Namibia. (Doctoral dissertation). The University of Namibia, Omusati Region, Namibia

Shimi, A., Keyhani, M. & Hedayati, K. 1979. Studies on salmonellosis in the house mouse, Mus musculus. *Laboratory Animals*, 13(1):33-34.

Siala, M., Smaoui, S., Taktak, W., Hachicha, S., Ghorbel, A., Marouane, C., Kammoun, S., Gamara, D., Slim, L. & Gdoura, R. 2017. First-time detection and identification of the Mycobacterium tuberculosis Complex members in extrapulmonary tuberculosis clinical samples in south Tunisia by a single tube tetraplex real-time PCR assay. *PLoS Neglected Tropical Diseases*, 11(5):e0005572.

Singh, S., Sethi, M. & Sharma, V. 1980. The occurrence of *Salmonellae* in rodent, shrew, cockroach and ant. *International Journal of Zoonoses*, 7(1):58-61.

Singleton, G.R., Belmain, S., Brown, P.R., Aplin, K. & Htwe, N.M. 2010. Impacts of rodent outbreaks on food security in Asia. *Wildlife Research*, 37(5):355-359.

Solanki, R., Vanjari, L., Sreevidya Subramanian, A.B., Nagapriyanka, E. & Lakshmi, V. 2014. Comparative evaluation of multiplex PCR and routine laboratory phenotypic methods for detection of carbapenemases among Gram negative bacilli. *Journal of clinical and Diagnostic Research: JCDR*, 8(12):DC23.

Song, Y., Liu, C. & Finegold, S.M. 2004. Real-time PCR quantitation of clostridia in feces of autistic children. *Applied and environmental microbiology*, 70(11):6459-6465.

Stein, B.R. 2000. Morphology of subterranean rodents. *Life underground: the biology of subterranean rodents (EA Lacey, JL Patton, and GN Cameron, eds.). University of Chicago Press, Chicago, Illinois*:19-61.

Stokes, H.t. & Hall, R.M. 1989. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. *Molecular Microbiology*, 3(12):1669-1683.

Sunar, N., Stentiford, E., Stewart, D. & Fletcher, L. 2014. Molecular techniques to characterize the *inv*A genes of *Salmonella* spp. for pathogen inactivation study in composting. *arXiv preprint arXiv:1404.5208*.

Swanson, S.J., Snider, C., Braden, C.R., Boxrud, D., Wünschmann, A., Rudroff, J.A., Lockett, J. & Smith, K.E. 2007. Multidrug-resistant *Salmonella* enterica serotype typhimurium associated with pet rodents. *New England Journal of Medicine*, 356(1):21-28.

Syakalima, M., Munyeme, M. & Yasuda, J. 2016. *Cytochrome c Oxidase* Sequences of Zambian Wildlife Helps to Identify Species of Origin of Meat. *International Journal of Zoology*, 2016: 1-6.

Tang, X.-J., Yang, Z., Chen, X.-B., Tian, W.-F., Tu, C.-N. & Wang, H.-B. 2018. Verification and large scale clinical evaluation of a national standard protocol for *Salmonella* spp./*Shigella* spp. screening using real-time PCR combined with guided culture. *Journal of microbiological methods*, 145:14-19.

Taylor, P.J., Arntzen, L., Hayter, M., Iles, M., Frean, J. & Belmain, S. 2008. Understanding and managing sanitary risks due to rodent zoonoses in an African city: beyond the Boston Model. *Integrative Zoology*, 3(1):38-50.

Thong, K.L. & Modarressi, S. 2011. Antimicrobial resistant genes associated with *Salmonella* from retail meats and street foods. *Food Research International*, 44(9):2641-2646.

Threlfall, E.J. 2002. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food-and water-borne infections. *FEMS Microbiology Reviews*, 26(2):141-148.

Thung, T.Y., Radu, S., Mahyudin, N.A., Rukayadi, Y., Zakaria, Z., Mazlan, N., Tan, B.H., Lee, E., Yeoh, S.L. & Chin, Y.Z. 2017. Prevalence, virulence genes and antimicrobial

resistance profiles of *Salmonella* serovars from retail beef in Selangor, Malaysia. *Frontiers in Microbiology*, 8:2697.

Todar, K. 2008. Todar's Online Textbook of Bacteriology *Salmonella* and Salmonellosis. Retrieved from http://www. textbookofbacteriology.net/index.html.[2018-06-11].

Torpdahl, M., Lauderdale, T.-L., Liang, S.-Y., Li, I., Wei, S.-H. & Chiou, C.-S. 2013. Human isolates of *Salmonella* enterica serovar *typhimurium* from Taiwan displayed significantly higher levels of antimicrobial resistance than those from Denmark. *International Journal of Food Microbiology*, 161(2):69-75.

Umali, D.V., Lapuz, R.R.S.P., Suzuki, T., Shirota, K. & Katoh, H. 2012. Transmission and shedding patterns of *Salmonella* in naturally infected captive wild roof rats (*Rattus rattus*) from a *Salmonella*-contaminated layer farm. *Avian Diseases*, 56(2):288-294.

Villafañe, I.E.G., Miñarro, F., Ribicich, M., Rossetti, C.A., Rossotti, D. & Busch, M. 2004. Assessment of the risks of rats (*Rattus norvegicus*) and opossums (*Didelphis albiventris*) in different poultry-rearing areas in Argentina. *Brazilian Journal of Microbiology*, 35(4):359-363.

Wattiau, P., Boland, C. & Bertrand, S. 2011. Methodologies for *Salmonella* enterica ssp enterica subtyping: gold standards and alternatives. *Applied and Environmental microbiology*:AEM. 05527-05511.

Wisner, A.L., Desin, T.S., Koch, B., Lam, P.-K.S., Berberov, E.M., Mickael, C.S., Potter, A.A. & Köster, W. 2010. *Salmonella* enterica subspecies enterica serovar *Enteritidis Salmonella* pathogenicity island 2 type III secretion system: role in intestinal colonization of chickens and systemic spread. *Microbiology*, 156(9):2770-2781.

Wright, G.D. 2007. The antibiotic resistome: the nexus of chemical and genetic diversity. *Nature Reviews Microbiology*, 5(3):175.

Xu, Z., Li, L., Shirtliff, M., Peters, B., Li, B., Peng, Y., Alam, M., Yamasaki, S. & Shi, L. 2011. Resistance Class 1 integron in clinical methicillin-resistant *Staphylococcus aureus* strains in southern China, 2001–2006. *Clinical Microbiology and Infection*, 17(5):714-718.

Yan, J.-J., Ko, W.-C., Chiu, C.-H., Tsai, S.-H., Wu, H.-M. & Wu, J.-J. 2003. Emergence of ceftriaxone-resistant *Salmonella* isolates and rapid spread of plasmid-encoded CMY-2–like cephalosporinase, Taiwan. *Emerging infectious diseases*, 9(3):323.

Yap, K.-P., Gan, H.M., Teh, C.S.J., Chai, L.C. & Thong, K.L. 2014. Comparative genomics of closely related *Salmonella* enterica serovar *Typhi* strains reveals genome dynamics and the acquisition of novel pathogenic elements. *BMC Genomics*, 15(1):1007.

Zhang, S., Kingsley, R.A., Santos, R.L., Andrews-Polymenis, H., Raffatellu, M., Figueiredo, J., Nunes, J., Tsolis, R.M., Adams, L.G. & Bäumler, A.J. 2003. Molecular pathogenesis of *Salmonella* enterica serotype typhimurium-induced diarrhea. *Infection and Immunity*, 71(1):1-12.

Zhang, Z., Meng, X., Wang, Y., Xia, X., Wang, X., Xi, M., Meng, J., Shi, X., Wang, D. & Yang, B. 2014. Presence of qnr, aac (6′)-Ib, qep A, oqx AB, and Mutations in Gyrase and Topoisomerase in Nalidixic Acid–Resistant *salmonella* Isolates Recovered from Retail Chicken Carcasses. *Foodborne pathogens and Disease*, 11(9):698-705.

Zishiri, O.T., Mkhize, N. & Mukaratirwa, S. 2016. Prevalence of virulence and antimicrobial resistance genes in *Salmonella* spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. *Onderstepoort Journal of Veterinary Research*, 83(1):1-11.

Zou, W., Lin, W.-J., Foley, S.L., Chen, C.-H., Nayak, R. & Chen, J.J. 2010. Evaluation of pulsed-field gel electrophoresis profiles for identification of *Salmonella* serotypes. *Journal of Clinical Microbiology*, 48(9):3122-3126.

Zulkifli, Y., Alitheen, N., Son, R., Raha, A., Samuel, L., Yeap, S. & Nishibuchi, M. 2009. Random amplified polymorphic DNA-PCR and ERIC PCR analysis on Vibrio parahaemolyticus isolated from cockles in Padang, Indonesia. *International Food Research Journal*, 16(2):141-150.

CHAPTER 3− IDENTIFICATION OF RODENT SPECIES THAT INFEST POULTRY HOUSES IN MAFIKENG, NGAKA MODIRI MOLEMA DISTRICT, NORTH WEST PROVINCE, SOUTH AFRICA

ABSTRACT

Rodents can cause serious adverse effects on farm production due to damage of food, contamination of feed and through spreading of diseases. The extent of damage or diseases spread will depend on the type of rodents that are invading the farm. The first part of this study investigated the species of rodents infesting poultry farms around Mafikeng. The study was an initial part of a project that was investigating *Salmonella* in rodents found on poultry farms around the province. The study trapped 154 rodents from selected farms and used the *COI* and the *Cyt-b* barcoding genes for species identification. Two rodent pest species namely; *Rattus tanezumi* and *Rattus rattus* were identified. A total of 99 (64%) of these were identified as *R. rattus* and 55 (36%) were *R. tanezumi*. Between the two target genes, *Cyt-b* gene was only able to identify 40 (26%) of the total samples (n=154) while *COI* was more efficient and amplified all the samples (n=154) thus was recommended as a better target gene for this kind of identification. The two rat species identified are known vectors of serious diseases thus their presence should be regarded as an indication of high risk for diseases. Despite having been detected in the country before, the findings of *R. tanezumi* as the second largest rat species in the area was unexpected since this species is known to be indigenous to Asia.

Keywords: Rodents, *COI*, *Cyt-b*, poultry farms, PCR

3.1. INTRODUCTION

Rodents are relatively small mammals that belong to the order Rodentia, which includes porcupines, rats, mice, squirrels and marmots (Meerburg *et al.*, 2009). They are famously known to cause huge losses to stored food, crops, property and also to transmit many pathogens that cause diseases to humans and animals (Wakawa *et al.*, 2015). The *Mus musculus*, *R. norvegicus* and *R. rattus* are the three main species of rodents usually found worldwide (Backhans & Fellström, 2012). The genus *Rattus* is the most common rodent found in poultry houses.

The genus *Rattus* consists mainly of *R. rattus, R. norvegicus, R. tanezumi* and *R. mulium* (Musser *et al.*, 2005). The genus has some of the most adaptable rodents in the world and most of them have their origins in Asia where they migrated from to other parts of the world following the development of agriculture which provided food and shelter for their survival. Their intricate association with farms makes them very important vectors of pathogens some of which are zoonotic. For instance, the brown rat is a famous carrier of mites responsible for plague and gastrointestinal helminthes, the Black rat is a carrier of trematode species, cestode species and *Salmonella* spp. and the Asian rat is also a source of gastrointestinal helminthes (Chaisiri *et al.*, 2012; Chaisiri *et al.*, 2015; Franssen *et al.*, 2016; Reusken *et al.*, 2011).

Identifying the rodent species in a farm set-up is, therefore, important in determining the specific rat species' impact on disease transmission as well as damage to the farming inputs. Unfortunately, rodents are not very easy to distinguish by the routine methods available that use physical attributes (Baumas, 2005). Therefore, molecular identification has been offering the best option for rat speciation. Molecular identification can be attained by different methods but DNA barcoding, which is a taxonomic method that exercises a short genetic marker in an organism's DNA to recognize it to a particular species, has been found easy and particularly effective for

this purpose (West, 2016). The target gene used for barcoding is the *COI* gene which is a very common gene among species and has been fairly conserved over generations (Dalton & Kotze, 2011; Syakalima *et al.*, 2016). Another gene commonly used is the *Cyt-b* gene which is also a very good discriminatory gene for species identification (Balakirev & Rozhnov, 2010; West, 2016). These two genes were, therefore, used in this study to identify rodent's species in poultry houses from selected farms around Mafikeng, in North West Province.

3.2. MATERIAL AND METHODS

3.2.1 Study Area

The study was carried out in Mafikeng, the North West Province. This Province is referred to as one of the biggest agricultural production areas in South Africa, with some of the largest cattle herds in the country found at Vryburg and mixed crop farming land. The province is also the second largest chicken producer in South Africa at 21.3 % after the Western Cape with 21.9 % (SAPA, 2014). The province has four districts namely: Dr Ruth Segomotsi Mompati, Bojanala Platinum, Ngaka Modiri Molema, and Dr Kenneth Kaunda. This study was conducted around Mafikeng in The Ngaka Modiri Molema district (Figure 3.1). The city lies between 25 and 28°C South of the Equator and 22 and 28°C longitude east of the Greenwich meridian. It shares an international border with the Republic of Botswana in the North and is 260 km West of Johannesburg. Mafikeng is built on the open veld at an elevation of 1500 m along the banks of the Upper Molopo River. Climatic conditions of the province differ significantly from West to East. The Western region receives less than 300 mm of rain per annum, the central region around

550 mm per annum, while the Eastern and South Eastern regions receive over 600 mm per annum (Ramatla *et al.*, 2017).

Figure 3. 1: Map showing the sampling area in Mafikeng located in North West

3.2.2. Collection of samples

A list of poultry farms in the Mafikeng area was compiled using the Department of Agriculture records. Six farms in the south, east, north, and west were randomly selected, the farmers were approached and those that agreed were included in the study. Rodents were captured using Sherman traps rats (Dizney *et al.*, 2008) baited with peanut butter plus cheese and placed where the rats frequently visit. The traps were checked each morning for the period of three consecutive days. The target number of rodents (rats) was between 150 − 200 based on previous studies (Franssen *et al.*, 2016; Nwiyi & Erumaka, 2012; Umali *et al.*, 2012; Wakawa *et al.*, 2015). Live rats will be euthanized humanely using Chloroform inhalation. Their body surface was disinfected with 70% Ethyl alcohol before dissection. Dissection of the abdominal cavity was done using a pair of forceps, a surgical blade, and tissues (kidneys and muscles) were harvested then placed in 4°C until processing or −80°C if processing would be delayed. Extra care was taken to avoid cross-contamination by use of new disposable utensils like scalpels, forceps, Petri-dishes and gloves for every one sample. After collecting the samples, carcasses were placed in carcass containers located within designated carcass refrigerators/freezers in the post-mortem room and then incinerated.

3.2.3. Extraction of genomic DNA.

Before DNA extraction, frozen samples were thawed at room temperature. DNA was extracted from skeletal muscle using QIAamp DNA Blood and Tissue Kit [Qiagen, Hilden, Germany (No. 69504)]. The procedure was performed according to the manufacturer's instructions. Briefly, samples were thawed at room temperature for 30 minutes before extraction. Approximately 20−24 mg of skeletal muscle placed into 1.5 mL Eppendorf tube. ATL Buffer (180 mL) and

proteinase K (20 µL) were added followed by lyses buffers 200 µL and the mixture was incubated at 56°C for 3 hours. About 200 µL of alcohol was added then vortex. The mixture was transferred to column (Dneasy Mini Spin column) then centrifuged for 1 minute. Subsequently, 500 µL of AW1 in Dneasy Mini Spin column was added and the mixture was vigorously rotated then, followed by 500 µL of AW2 then centrifuged for 3 minutes. The elution volume was 200 mL into a 1.5 mL Eppendorf tube. Then DNA was eluted from the nucleospin column in a 200 µL Buffer AE and stored at −70 C until tested by PCR.

3.2.4. Evaluation of the quantity and quality of isolated DNA.

The quantity of DNA extracted from the samples was determined by spectrophotometry with a NanoDrop ND-1000 system (NanoDrop Technologies, Inc., Wilmington, DE, USA). The purity of DNA was determined spectrophotometrically from the ratio of absorbance at 260 and 280 nm (A_{260}/A_{280}). A ratio of between 1.7 and 2 indicates excellent quality of DNA (Ali *et al.*, 2015; Parchami *et al.*, 2014).

3.2.5. PCR using *COI* and *Cyt-b* genes of captured rats.

Cytochrome Oxidase I (*COI*) (Lakshminarayanan *et al.*, 2015; West, 2016), as well as Cytochrome b (Cyt-b) genes (Balakirev & Rozhnov, 2010; West, 2016), were targeted for species identification. The final reaction mixture for both gene reactions was 25 µL and consisted of two µL of template DNA, 8.5 µL double distilled water, 2X Dream *Taq* Green PCR Master Mix (2X Dream *Taq* Green buffer, 4 mM $MgCl_2$, 0.4 mM of each dNTP and 1 unit/µL of thermo stable *Taq* polymerase (Thermo Scientific, USA), the primer mix contained 1 µM of each

oligonucleotide primer. To amplify the 750 bp product of *COI*, the primers used were: BatL5310 (CCT ACT CRG CCA TTT TAC CTA TG) and R6036R (ACT TCT GGG TGT CCA AAG AAT CA) using a PCR Engine T100 Thermal^{TM} cycler (BioRad, *Singapore*). The PCR conditions were as followed; 35 cycles, denaturation step of 94°C for 2 min; denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec and the first extension at 72°C for 1 min and a final extension step of 72°C for 5 min with the holding temperature at 4°C (Lakshminarayanan *et al.*, 2015).

To amplify the 762 bp product of *Cyt-b* gene, the primers used were: RGu2L (CAG CAT TTA ACT GTG ACT AAT GAC) and RCb9H (TAC ACC TAG GAG GTC TTT AAT TG), and the following PCR conditions were used: denaturation step of 94°C, 3 min, 35 cycles of: denaturation at 94°C, 30 sec, annealing at 60°C, 30sec, the first extension at 72°C, 1min, followed by a final extension at 72°C, 5 min and cooling down to 4 °C and storage (Robins *et al.*, 2010).

The PCR amplicons in both PCR reactions were analysed by electrophoresis in 1.5% (w/v) agarose gel containing Ethidium bromide (EtBr) (10 μg ml^{-1}) then viewed using U.V light at 420nm wavelength. A ChemiDoc Imaging System (Bio-Rad ChemiDocTM MP Imaging System, UK) was used to capture the image using Gene Snap (version 6.00.22) software.

3.2.6 Sequencing.

Seventeen micro liters of all positive PCR products were sent for sequencing at Inqaba Biotechnical Industries (Pty) Ltd in Pretoria, South Africa. The acquired sequence was aligned against GenBank data base using Basic Local Alignment Search Tool (BLAST)

(www.ncbi.nlm.nih.gov/BLAST) from the National Center for Biotechnology Information (NCBI) to identify sequences with high similarity (Syakalima *et al.,* 2016).

3.2.7. Phylogenetic analysis

Gene sequences obtained from all positively tested amplicons were edited using BioEdit (Moyane *et al.*, 2013) to remove any degenerate base pairs and saved as FASTA format. To confirm sequences obtained from *CO1* and *Cyt-b* analysis, the nucleotide basic local alignment search tool (BLASTn) was used. Only gene sequences with 97%-100% similarity match score were considered as significant.

The phylogenic tree was constructed to illustrate the evolutionary relationships among *Rattus* spp. Multiple alignments of the sequences were carried out by MAFFT program 6.864 against corresponding nucleotide sequences retrieved from Gen-Bank. Evolutionary distance matrices were generated (Hendrickson *et al.,* 2002). The aligned *Cyt-b* sequences was used to construct a phylogenetic tree as implemented in the MEGA 7 package and the neighbor-joining (NJ) and distance matrix methods were used (Hendrickson *et al.,* 2002). A bootstrap confidence analysis was performed with 1000 replicates. A putative chimeric sequence was identified using the Chimera Buster 1.0 software. Manipulation and tree editing were carried out using Tree View (Moyane *et al.*, 2013).

For *CO1* analysis, multiple and pair-wise alignments were done by Clustal W on Mega 7 (Kumar *et al.,* 2016). Subsequently, the evolutionary history was inferred based on the Hasegawa-Kishino-Yano model (Hasegawa *et al.*, 1985) with 1000 bootstrap support values. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pair-wise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log-likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 69.02% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 54 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 604 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.*, 2016).

3.2.8 Ethics Committee Approval

Prior to the commencement of the study, the research proposal was approved based on Animal Research Ethical Committee (NWU-00274-18-A5) guidelines by North-West University Research Ethics Regulatory Committee (NWU-RERC).

3.3. RESULTS

Out of the 200 target rodent samples, only 154 rat samples were collected and examined using *Cyt-b* and *COI* genes to identify the rodent species. Of the 154 samples, 99 (64%) were identified by the *COI* gene primers as *R. rattus* and 55 (36%) were *R. tanezumi* (Table 3.1). Figure 3.2 shows how the fragments appeared on a gel after amplification with of the *COI* gene primers.

Table 3. 1: Identification of rodents from different farms and their species using both *cytochrome oxidase 1* and *cytocrome b* gene

Farm	No. of rats	Species	*Cytochrome oxidase 1*	*Cytocrome b*
A	25	➢ *R. rattus*	19	14
		➢ *R. tanezumi*	6	2
B	3	➢ *R. rattus*	3	–
		➢ *R. tanezumi*	–	–
C	21	➢ *R. rattus*	15	4
		➢ *R. tanezumi*	6	–
D	17	➢ *R. rattus*	10	1
		➢ *R. tanezumi*	7	2
E	68	➢ *R. rattus*	46	10
		➢ *R. tanezumi*	22	5
H	20	➢ *R. tanezumi*	14	1
		➢ *R. rattus*	6	1
Total	**154**		**154**	**40**

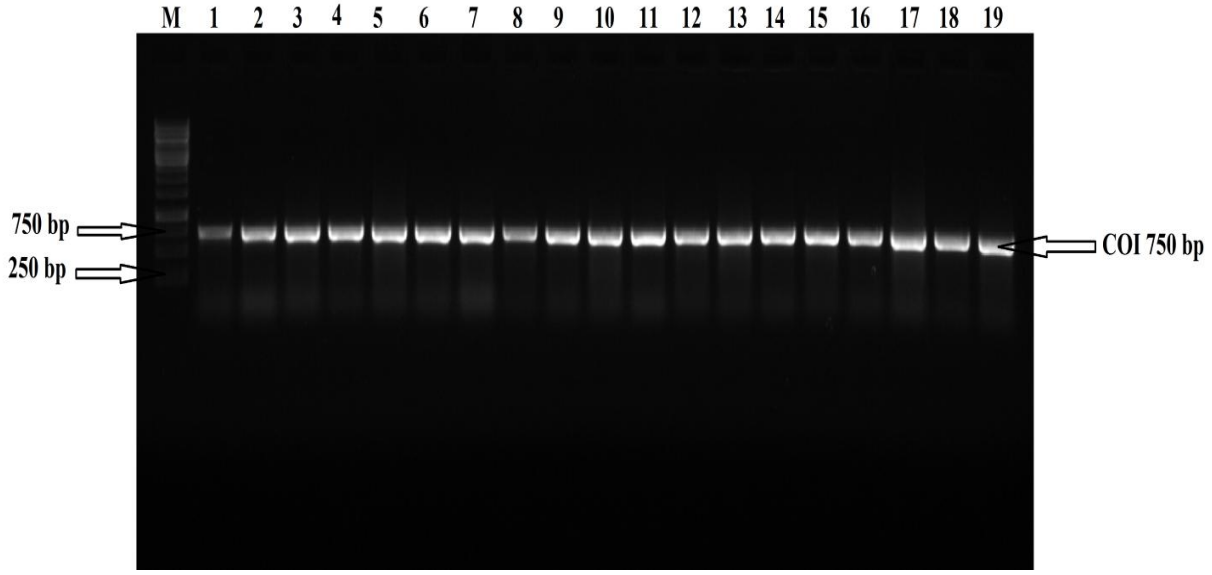


Figure 3. 2: PCR amplification of *COI* gene. Lane M: Molecular weight marker (1kb); Lane 1-19 *COI* gene fragments from DNA extracted from Rodents.

Using the *Cyt-b* gene primer only 40 samples amplified the required PCR products from a total of 154 as follows; *R. rattus* 30 (10%) and *R. tanezumi* 10 (7%) as shown in Table 3.1. Many samples did not show any amplification products for *Cyt-b* as shown in Figure 3.3.

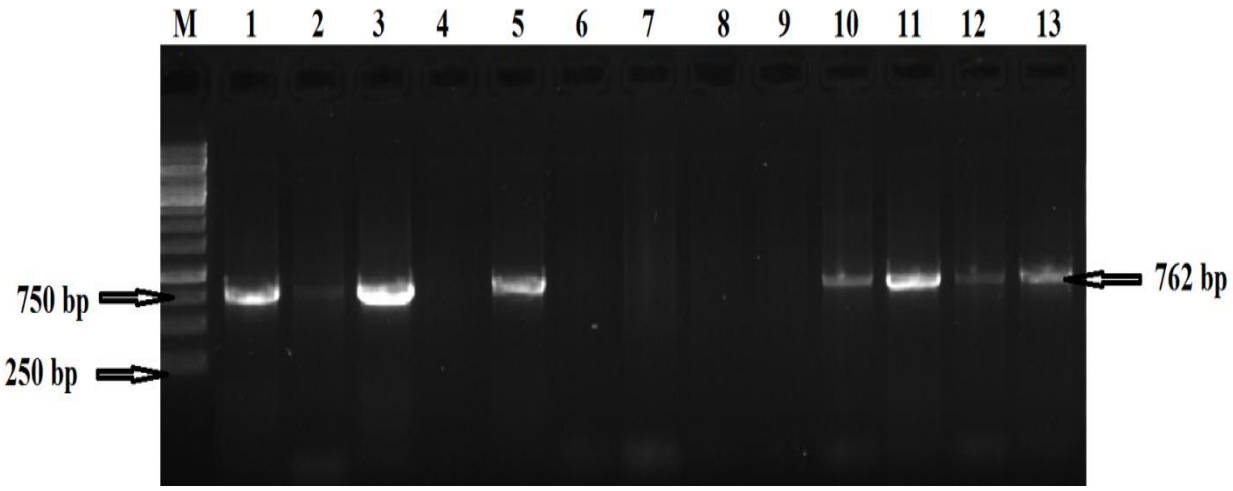


Figure 3. 3: Lane M: Molecular weight marker (1kb); Lanes 1, 2, 3, 5, 10, 11, 12, 13; amplified genes for *Cyt-b*; Lane 4, 6, 7, 8, 9; samples which were not amplified.

3.3.1. Phylogeny of *R. Tanezumi* and *R. rattus.*

The samples were subjected to sequencing and phylogenetic analysis using reference sequences obtained from GenBank. The derived Phylogenetic tree had two main clusters. Cluster I consisted of *R. rattus* and the second cluster consisted of *R. tanezumi*. The *R. norvengicus* was used as an out-group for *cyt-b* (Figure 3.4). The *R. tanezumi* clustered closely with the strains from China and others from South Africa and Swaziland.

For *CO1*, KF999094 *Micromys erythrotis*, KF999093 *Micromys erythrotis*, JQ667697 *Hylomscus simus* and JQ667694 *Hylomscus simus* were used as an out group (Figure 3.5). The results from the different approaches showed totally resolved, well supported phylogeny of the two species with high bootstrap support values in internal nodes.

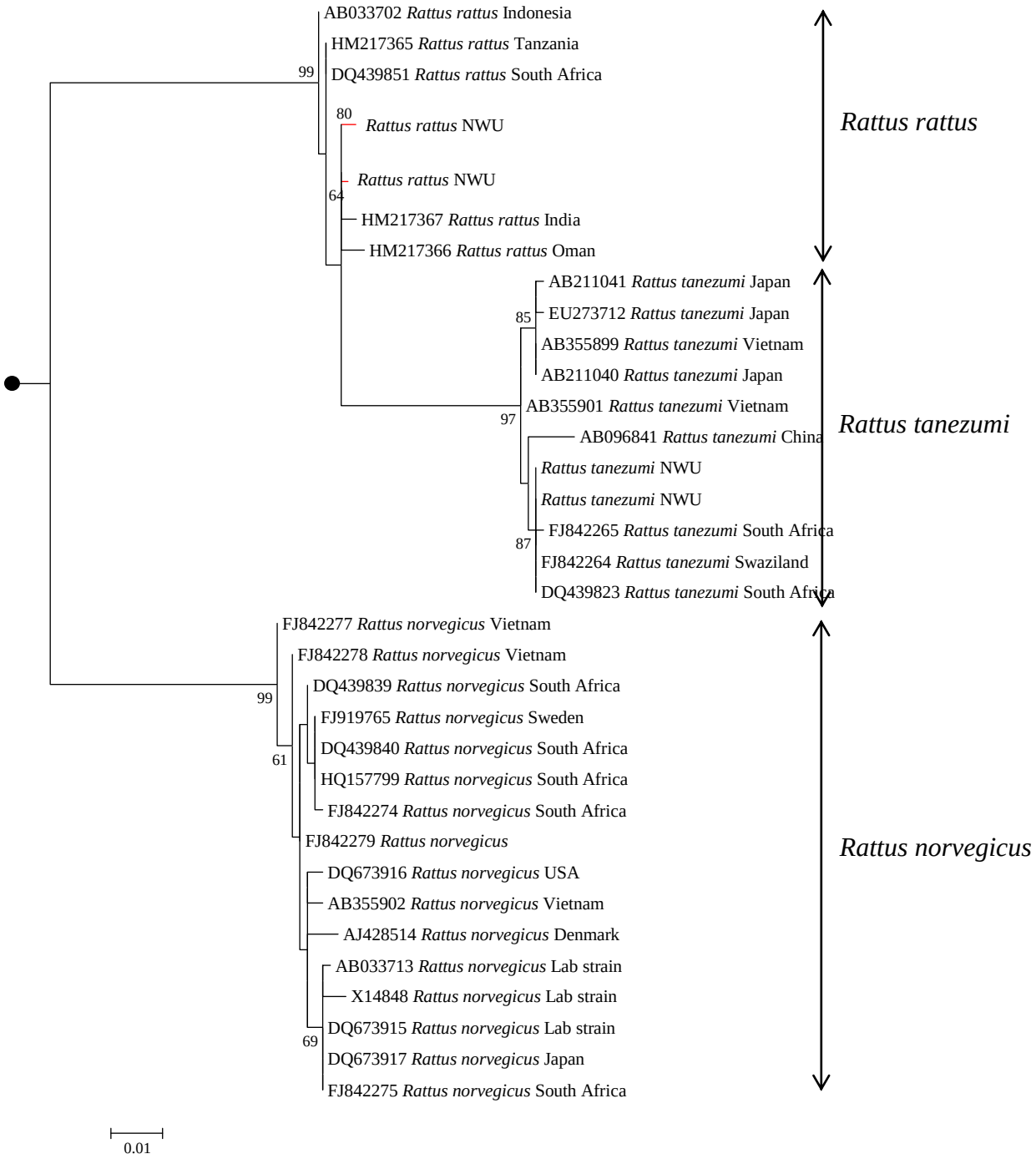


Figure 3. 4: Neighbour-joining tree of the rats sing *Cyt-b* gene sequences from *R. tanezumi* and *R. rattus*. Only values greater than 60% are shown.

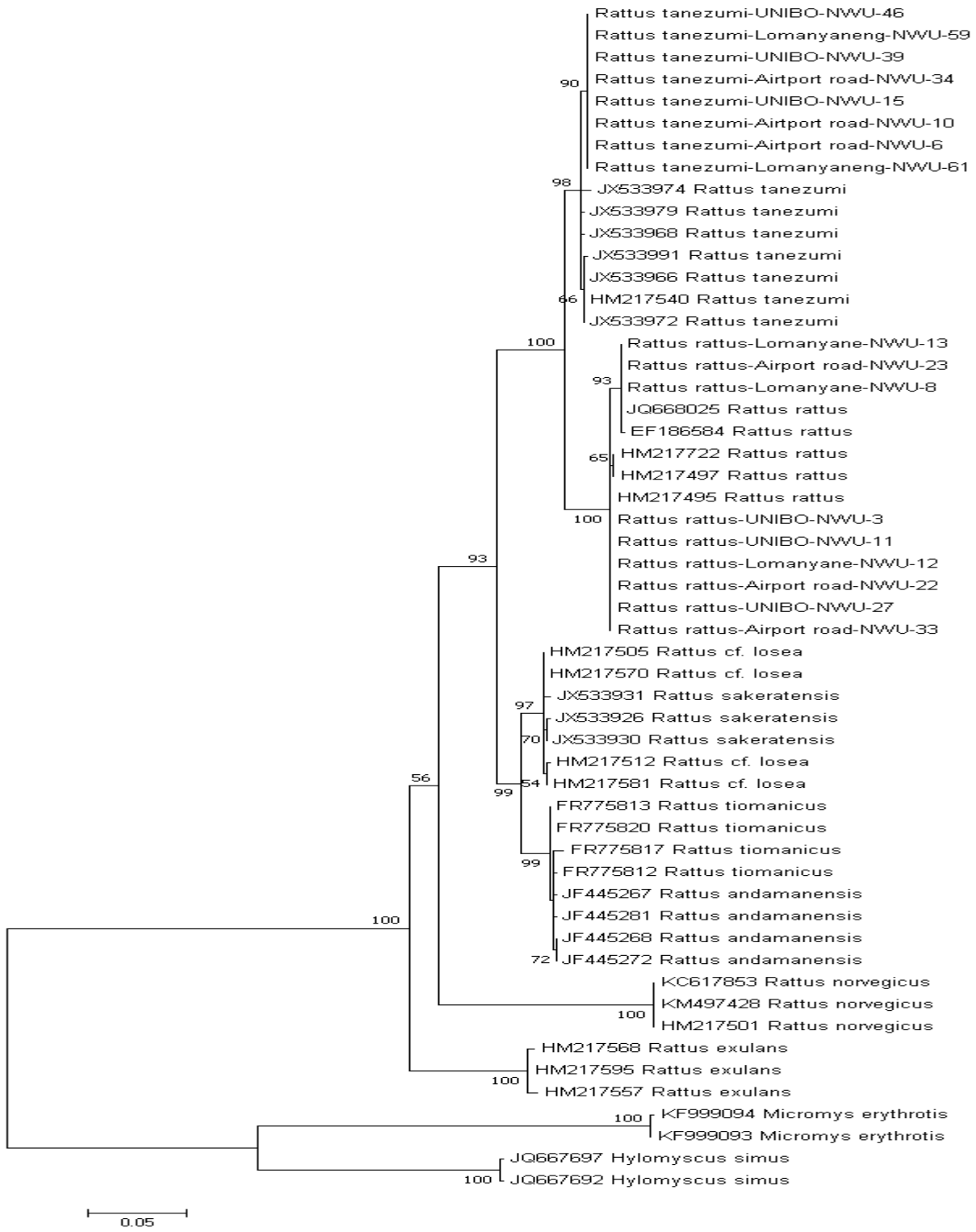


Figure 3. 5: Neighbour-joining phylogenetic tree based on distance matrix analysis of *COI* gene sequences from *R. tanezumi* and *R. rattus* based on the Hasegawa-Kishino-Yano model with 1,000 bootstrap support values.

3.4. DISCUSSION

The first objective of the current study was the identification of rodent species that invade poultry houses in the North West Province in particular and in South Africa in general as part of the bigger study on whether rodents in these poultry houses carry *Salmonella*. This information is important for risk assessment and for pest disease risk assessment (Mengak, 2009; Stuart *et al.*, 2015). This study has therefore shown that of the 154 rodents, 99 (64%) were *R. rattus* and the other 55 (36%) were *R. tanezumi*.

Rattus rattus was the most dominant species among the farms that were investigated and this was to be expected since other studies have shown that the rat species has been known to be present in South Africa for many years and in large numbers (Bastos *et al.*, 2011). *Rattus rattus* is an important rat species because it is the most damaging invasive rodent in the world (Meerburg *et al.*, 2009). Furthermore, the rat has also been known in South Africa as a source of diseases communicable to humans (Bastos *et al.*, 2005). The rat is a carrier of trematodes, cestodes and nematodes (Franssen *et al.*, 2016). Reusken *et al.* (2011) also implicated the rat in its role in spreading of *Coxiella burnetii*. It may also carry important protozoa which are mainly dangerous for immune-compromised patients (Meerburg *et al.*, 2009). Bacterial pathogens like *Salmonella* that are important both to humans and livestock have also been isolated from *R. rattus* in studies from various other countries such as Japan, (Umali *et al.*, 2012), Reunion Island (Erwan *et al.*, 2016), Pakistan (Mushtaq-ul-Hassan *et al.*, 2008), and Canada (Himsworth *et al.*, 2015). In the phylogenetic tree, the rat species observed in the study area clustered well with the species found in India, Jordan, Tanzania and other South African studies. The rat's occurrence, especially in livestock farms is, therefore, a significant health risk factor.

The other *Rattus* species found in this study, *R. tanezumi*, has also been detected in South Africa before (Chaisiri *et al.*, 2012; Miller, 2007). *Rattus tanezumi* was first identified in Limpopo Province (Bastos *et al.*, 2005) but this is probably the first report of its detection in the North West Province. What is also significant was the finding that it is the second most dominant species in the poultry farms meaning it is getting more prolific and invasive all over the country. This rat species is predominantly found in Asia and wherever it is discovered it will always have its origins from Asia hence the name the Asian House rat. It is a carrier of hantaviruses (Iliev *et al.*, 2017) and it has also been found to carry important mites as well as helminths Chaisiri *et al.*, 2015). Apart from diseases, the rat plays an important role by causing serious damage to field crops, destroying food stores and also causing infrastructural damage (Al-Gendy *et al.*, 2017; Singleton *et al.*, 2010). It is obvious, therefore that its increasing presence in South Africa brings with it these negative traits in the farm and human environment.

Of the two mitochondrial DNA genes used for this study; *cyt-b* and *COI*, the *Cyt-b* could only amplify DNA from 40 (26%) samples out of 154. However, *COI* gene amplified all the samples thus indicating that *COI* is a better gene for this purpose. The effectiveness of *COI* gene in species classification has also been reported before in wildlife (Syakalima *et al.*, 2016), nematodes (Callejón *et al.*, 2009), reedbuck (Dalton & Kotze, 2011), birds (Hebert *et al.*, 2004), rodents (Lakshminarayanan *et al.*, 2015) and lepidoptera (Hajibabaei *et al.*, 2006). *COI* genes have been the most frequent methods used for species identification in animal biological studies due to its high degree of phylogenetic species differentiation compared to other *mtDNA* genes (Hebert *et al.*, 2004). This study confirmed these findings and recommends its superior use over *Cyt-b*.

3.5. CONCLUSION

This study established that the two common rodent species found in poultry houses around Mafikeng were: *R. tanezumi* and *R. rattus*. The finding that *R. tanezumi* is the second most prominent rat species in these farms was unexpected because the rat species was reported for the first time in South Africa not so long ago and that the species is not indigenous to Africa but Asia. It is thus important to expect it to increase and become a prominent species in the years to come together with the health and economic risks it brings. The study also confirmed that *COI* genes serve as a reliable and more precise target for identification of these rodent species.

3.6 REFERENCES

Adhikari, P., Han, S.-H., Kim, Y.-K., Kim, T.-W., Thapa, T.B., Subedi, N., Kunwar, A., Banjade, M. & Oh, H.-S. 2018. New record of the Oriental house rat, *Rattus tanezumi*, in Nepal inferred from mitochondrial *Cytochrome B* gene sequences. *Mitochondrial DNA Part B*, 3(1):386-390.

Al-Gendy, A., Baghdadi, S., Malek, S.S. & Dongol, E. 2017. Incidence and damage assessment of rats (*Mammalia: Rodentia*) infesting poultry and animal farms and their stores in Upper Egypt. *Journal of Phytopathology and Pest Management*, 4(1):69-77.

Ali, M.E., Razzak, M.A., Hamid, S.B.A., Rahman, M.M., Al Amin, M. & Rashid, N.R.A. 2015. Multiplex PCR assay for the detection of five meat species forbidden in Islamic foods. *Food Chemistry*, 177:214-224.

Aplin, K.P., Chesser, T. & Have, J.t. 2003. Evolutionary biology of the genus *Rattus*: profile of an archetypal rodent pest. *Aciar Monograph Series*, 96:487-498.

Backhans, A. & Fellström, C. 2012. Rodents on pig and chicken farms-a potential threat to human and animal health. *Infection Ecology & Epidemiology*, 2(1), p. 17093.

Balakirev, A. & Rozhnov, V. 2010. Phylogenic relationships and species composition in the genus Niviventer (*Rodentia, Muridae*) based on studies of the cytochrome b gene of mtDNA. *Moscow University Biological Sciences Bulletin*, 65(4):170-173.

Bastos, A., Chimimba, C., Von Maltitz, E., Kirsten, F. & Belmain, S. 2005. Identification of rodent species that play a role in disease transmission to humans in South Africa. *Proceedings of the South African Society for Veterinary Epidemiology and Preventive Medicine*:78-83.

Bastos, A.D., Nair, D., Taylor, P.J., Brettschneider, H., Kirsten, F., Mostert, E., Von Maltitz, E., Lamb, J.M., Van Hooft, P. & Belmain, S.R. 2011. Genetic monitoring detects an overlooked cryptic species and reveals the diversity and distribution of three invasive Rattus congeners in South Africa. *BMC Genetics*, 12(1):26.

Callejón, R., de Rojas, M., Ariza, C., Ubeda, J., Guevara, D. & Cutillas, C. 2009. *Cytochrome oxidase subunit 1* and mitochondrial *16S rDNA* sequences of Trichuris skrjabini (Tricocephalida: Trichuridae). *Parasitology Research*, 104(3):715-716.

Chaisiri, K., Chaeychomsri, W., Siruntawineti, J., Ribas, A., Herbreteau, V. & Morand, S. 2012. Diversity of gastrointestinal helminths among murid rodents from northern and northeastern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 43(1):21.

Chaisiri, K., Siribat, P., Ribas, A. & Morand, S. 2015. Potentially zoonotic helminthiases of murid rodents from the Indo-Chinese peninsula: impact of habitat and the risk of human infection. *Vector-Borne and Zoonotic Diseases*, 15(1):73-85.

Dalton, D.L. & Kotze, A. 2011. DNA barcoding as a tool for species identification in three forensic wildlife cases in South Africa. *Forensic Science International*, 207(1-3):e51-e54.

Dizney, L., Jones, P.D. & Ruedas, L.A. 2008. Efficacy of three types of live traps used for surveying small mammals in the Pacific Northwest. *Northwestern Naturalist*, 89(3):171-180.

Erwan, C.T.L.P.A., Denis, L.G.L.M.M., Cardinale, E., Erwan, M., Le, G., Denis, M. & Cardinale, E. 2016. Wild fauna as a carrier of *Salmonella* in Reunion Island: impact on pig farms.

Franssen, F., Swart, A., van Knapen, F. & van der Giessen, J. 2016. Helminth parasites in black rats (*Rattus rattus*) and brown rats (*Rattus norvegicus*) from different environments in the Netherlands. *Infection Ecology & Epidemiology*, 6(1), p.31413.

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. & Hebert, P.D. 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America*, 103(4):968-971.

Hasegawa, M., Kishino, H. & Yano, T.-a. 1985. Dating of the human-ape splitting by a molecular clock of *mitochondrial DNA*. *Journal of Molecular evolution*, 22(2):160-174.

Hebert, P.D., Stoeckle, M.Y., Zemlak, T.S. & Francis, C.M. 2004. Identification of birds through DNA barcodes. *PLoS Biology*, 2(10):e312.

Hendrickson, H., Slechta, E.S., Bergthorsson, U., Andersson, D.I. & Roth, J.R. 2002. Amplification–mutagenesis: evidence that "directed" adaptive mutation and general hypermutability result from growth with a selected gene amplification. *Proceedings of the National Academy of Sciences*, 99(4):2164-2169.

Himsworth, C.G., Zabek, E., Desruisseau, A., Parmley, E.J., Reid-Smith, R., Jardine, C.M., Tang, P. & Patrick, D.M. 2015. Prevalence and characteristics of *Escherichia coli* and *Salmonella* spp. in the feces of wild urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) from an inner-city neighborhood of Vancouver, Canada. *Journal of Wildlife Diseases*, 51(3):589-600.

Iliev, P.T., Georgiev, G.Z., Kirkova, Z.T. & Chakarova, B.G. 2017. A Survey of Helminth Infections in the Black Rat from Stara Zagora District, Bulgaria. *Macedonian Veterinary Review*, 40(2):177-182.

Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7):1870-1874.

Lakshminarayanan, R.R., Shanmugam, A. & Govindaraju, A. 2015. Sequencing of COI gene in four rodent pests for species identification. *DNA Barcodes*, 3(1).

Meerburg, B.G., Singleton, G.R. & Kijlstra, A. 2009. Rodent-borne diseases and their risks for public health. *Critical Reviews in Microbiology*, 35(3):221-270.

Mengak, M.T. 2009. Rats and mice: get them out of your house and yard. Retrieved from https://athenaeum.libs.uga.edu/bitstream/handle/10724/12234/C970.pdf? sequence=1 [2018-04-12].

Miller, R.W. 2007. *Rattus tanezumi* in the upland rice terraces of Banaue, Philippines: Demography, habitat use, crop damage and yield assessment [M. Sc. thesis]. *Sydney: The University of New South Wales*.

Moyane, J., Jideani, A. & Aiyegoro, O. 2013. Antibiotics usage in food-producing animals in South Africa and impact on human: Antibiotic resistance. *African Journal of Microbiology Research*, 7(24):2990-2997.

Mushtaq-ul-Hassan, M., Hussain, I., Shehzadi, B., Shaheen, M., Mahmood, M., Rafique, A. & Mahmood-ul-Hassan, M. 2008. Occurrence of some zoonotic microorganisms in faecal matter of house rat (*Rattus rattus*) and house mouse (*Musmusculus*) trapped from various structures. *Pakistan Veterinary Journal*, 28(4): 171-174.

Musser, G.G., Smith, A.L., Robinson, M. & Lunde, D.P. 2005. Description of a new genus and species of rodent (Murinae, Muridae, Rodentia) from the Khammouan Limestone National Biodiversity Conservation Area in Lao PDR. *American Museum Novitates,* 3497:1-31.

Nwiyi, P. & Erumaka, G. 2012. Frequency of detection of *Salmonella* spp. and *Esherichia coli* in the faeces of house rat in Abia State, Nigeria. *Research Opinions in Animal & Veterinary Sciences*, 2(10): 521-524.

Parchami, A., Sadeghpour-Gildeh, B., Nourbakhsh, M. & Mashinchi, M. 2014. A new generation of process capability indices based on fuzzy measurements. *Journal of Applied Statistics*, 41(5):1122-1136.

Ramatla, T., Ngoma, L., Adetunji, M. & Mwanza, M. 2017. Evaluation of Antibiotic Residues in Raw Meat Using Different Analytical Methods. *Antibiotics*, 6(4):34.

Reusken, C., van der Plaats, R., Opsteegh, M., de Bruin, A. & Swart, A. 2011. *Coxiella burnetii* (Q fever) in *Rattus norvegicus* and *Rattus rattus* at livestock farms and urban locations in the Netherlands; could *Rattus* spp. represent reservoirs for (re) introduction? *Preventive Veterinary Medicine*, 101(1-2):124-130.

Robins, J.H., McLenachan, P.A., Phillips, M.J., McComish, B.J., Matisoo-Smith, E. & Ross, H.A. 2010. Evolutionary relationships and divergence times among the native rats of Australia. *BMC Evolutionary Biology*, 10(1):375: 1-16.

Singleton, G.R., Belmain, S., Brown, P.R., Aplin, K. & Htwe, N.M. 2010. Impacts of rodent outbreaks on food security in Asia. *Wildlife Research*, 37(5):355-359.

Stuart, A.M., Singleton, G.R. & Prescott, C.V. 2015. Population ecology of the Asian house rat (*Rattus tanezumi*) in complex lowland agroecosystems in the Philippines. *Wildlife Research*, 42(2):165-175.

Syakalima, M., Munyeme, M. & Yasuda, J. 2016. C*ytochrome c Oxidase* Sequences of Zambian wildlife helps to identify species of origin of meat. *International Journal of Zoology*, 2016: 1-6.

Umali, D.V., Lapuz, R.R.S.P., Suzuki, T., Shirota, K. & Katoh, H. 2012. Transmission and shedding patterns of *Salmonella* in naturally infected captive wild roof rats (*Rattus rattus*) from a *Salmonella*-contaminated layer farm. *Avian Diseases*, 56(2):288-294.

Wakawa, A., Mohammed, F. & Mamman, H. 2015. Isolation and antibiotic susceptibility of *Escherichia coli* and *Salmonella gallinarum* isolated from rats in commercial poultry farms with recurrent Colibacillosis and Fowl typhoid cases in Zaria, Nigeria. *Journal. Veterinary Advance*, 5(11):1147-1152.

West, K.M. 2016. Investigating patterns of prehistoric dispersal in Eastern Polynesia: a commensal approach using complete ancient and modern mitochondrial genomes of the Pacific rat, Rattus exulans. University of Otago. Retrieved on the 2018-04-18.

CHAPTER 4− DETECTION OF *SALMONELLA* SPP. FROM RODENTS CAPTURED IN POULTRY FARMS AROUND MAFIKENG

ABSTRACT

Rodents are a serious nuisance in poultry farms and are also known to be major reservoirs of disease agents. One of the disease agents that these rodents are known to carry is *Salmonella*. This study's main focus was to investigate the *Salmonella* species carried by rodents from poultry farms around Mafikeng in South Africa. A total of 154 rats were caught and humanely processed from six poultry farms. Fecal samples were collected from the caeca and investigated for the occurrence of *Salmonella* using conventional culture methods and PCR, targeting the *16S rDNA* gene. The results showed that 19 (12.3%) rats were infected with *Salmonella* species. Out of 68 confirmed isolates; *S.* typhimurium 26 (38.2%), *S. enteritidis* 12 (17.6%), *S. newport* 8 (11.8%), *S. heidelberg* 7 (10.3%), *S. bongori* 6 (8.8%), *S. enteric* Serovar paratyphi B 4 (5.9%), *S. tennessee* 3(4.4%) and *S. pullorum* 2 (2.9%). Most of the *Salmonella* isolates were from *R. rattus* 12 (63.3%) species and the rest were from *R. tanezumi* 7 (36.8%). The *Salmonella* species that were detected are important public health pathogens throughout the world. According to literature, this is the first study to have isolated and determined *Salmonella* species in rats around poultry farms in South Africa and more especially from *R. tanezumi*. These findings, therefore, provide a basis for understanding the importance of rats in the transmission and maintenance of the *Salmonella* contamination in poultry premises and to the human environment. There is need to put in place official measures to control the rat population in poultry houses in the Mafikeng and the rest of South Africa

Keywords: *Salmonella, R. rattus, R. tanezumi, 16S rDNA*, South Africa

4.1 INTRODUCTION

Rodents are an economic menace in poultry farms throughout the world. Estimates have established that rodents in a poultry farm might consume between 2 and 50 kg of poultry feed each day and also damage the bags used for feed storage (Hezler and Opitz, 1992). A study in India (Clapperton, 2006), found that rats were responsible for 2.5% loss of food grain and thus had a serious economic impact on the poultry farm.

Rodents are also known vectors and reservoirs of important pathogens responsible for diseases in poultry and humans. These pathogens include Erysipelas, Fowl cholera, Salmonellosis, Pasteurellosis multocida, and Influenza (Hilton *et al.*, 2002; Judy, 2010; Meerburg & Kijlstra, 2007; Nwiyi & Erumaka, 2012). These diseases are transmitted via rodent bites, food contamination with feces and via rodent urine (Roomaney *et al.*, 2012). These diseases impact seriously on poultry production (Meerburg & Kijlstra, 2007) as well as on other livestock health. Furthermore, some of these pathogens e.g. *Salmonella* spp. are zoonotic disease agents and so have added public health implications (Rocha-e-Silva *et al.*, 2014).

Salmonella pathogens have been isolated in rats and mice captured in poultry farms by a number of studies elsewhere and the species isolated included: *Salmonella* typhimurium, *Salmonella montivideo, Salmonella derby* and *Salmonella enteritidis,* most of which can be linked to human diseases (Meerburg & Kijlstra, 2007; Nkogwe *et al.*, 2011; Umali *et al.*, 2012). Although the link between rodents and diseases transmission in general and *Salmonella* transmission in particular, has been reported widely, it is still difficult to conclude whether rodents bring the pathogenic bacteria into a poultry operation or, if they get the bacteria from previously infected poultry house (Judy, 2010). However, knowing the pathogens that are in rodents is a sure indication of what can be spread in the poultry environment. Furthermore, it also gives an indication after

cleaning/disinfection of a poultry house that as long as rodents (rats and mouse) remain, these pathogens will persist in the poultry farms (Lapuz *et al.,* 2012). Determining the pathogens, therefore, has long-term economic and public health implications (Nwiyi & Erumaka, 2012).

Among the different methods available for detecting *Salmonella* spp. in the chicken environment, PCR has been found to be the most reliable (Liu *et al.*, 2013; Trkov & Avguštin, 2003). The use of PCR coupled with *16S rDNA* gene has been widely successful in identifying *Salmonella* species (Liu *et al.*, 2013; Trkov & Avguštin, 2003). According to literature, no study has been done to establish the occurrence of *Salmonella* species from rodents in Mafikeng, North West Province. The current study was, therefore, intended to isolate *Salmonella* species from rats sharing the poultry house environment around Mafikeng in North West Province in South Africa.

4.2 MATERIAL AND METHODS

4.2.1 Study site and sample collection

Study site and sample collection were described in details under section 3.2.1 to 3.2.2, respectively.

4.2.2 Sample preparation

Rodent's body surfaces were disinfected with 70% ethyl alcohol to avoid cross-contamination. Dissection of the abdominal cavity was done using sterilized a pair of forceps, a surgical blade,

and the samples were harvested from the cecum (Appendix F1) of rats and placed in disposable Petri-dishes (Lapuz *et al.*, 2008) while preparing for enrichment and stored at −20 until analysis.

4.2.3 *Salmonella* non-selective pre-enrichment

Salmonella was isolated from the caecal content following the International Organization for Standardization method (ISO-6579: 2002) (Rodriguez *et al.*, 2015). Briefly, fecal content (1 g) was weighed and transferred into a sterile container. Approximately, 10 mL of peptone water (BPW Oxoid, Biolab, South Africa) was added into the samples then homogenized by vortexing for about 2 minutes followed by incubation at 37 °C for 18 to 24 hours. Following pre-enrichment, 1 mL of sample was transferred to 10 mL of Mueller-Kauffmann Tetrathionate Novobiocin (MKTTn) broth (Sigma-aldrich, S.A. Barcelona, Spain) which was incubated at 45°C for six hours. Thereafter, 1 mL volume was then transferred from MKTTn to 10 mL of Rappaport-Vassiliadis medium with soya (RVS) broth (Sigma-aldrich, S.A. India) and incubated at 37°C for 24 hours. One mL aliquot from the RV broth was then transferred to a 30% glycerol solution (EMD chemicals, USA) and stored at -20°C for later use.

4.2.4 Culture and identification

A loopful of the enriched cultures of RVS broth was streaked separately onto two selective agar plates: XLD (Merck, Wadeville, South Africa) and BGA (Scharlau Chemie S.A. Barcelona, Spain). The plates were incubated in an overturned position at 37 °C for 18 to 24 hours. Following incubation, the black and pink colonies with or without black center on XLD agar

[Appendix F2 (A)], the colorless or opaque-white colonies surrounded by red or pink zone and the red colonies [Appendix F2 (B)] on BGA were identified as suspected *Salmonella*. Suspected colonies of *Salmonella* were confirmed according to the guidelines of ISO 6579: 2002. About 3 to 5 colonies were picked out and purified on Nutrient agar (NA) (Merck, Wadeville, South Africa) by incubation at 37°C for 18 to 24 hours.

4.2.5 Gram staining

The *Salmonella* colonies were characterized morphologically by means of Gram's stain according to the method explained by Parvej (2013). A slide smear was prepared by picking a drop of the suspended culture from Nutrient Agar (NA) (Merck, Wadeville, South Africa) and transferred on a slide, smeared, air dried, and heat fixed. After performing the Gram stain, the smear was examined under a light microscope. All Gram-negative rod-shaped (Appendix F5) bacteria were further subjected to preliminary biochemical tests (Catalase, Triple Sugar Iron, Agar Test Urease and Indole test).

4.2.6 Preliminary biochemical tests

4.2.6.1 Indole test

A tube containing 5 mL of peptone water was inoculated with a pure colony of culture under examination and the medium was then incubated at 37 °C for 24 hours. Following incubation, 0.5 mL Kovac's reagent was added, shaken well and examined after one minute. The positive

reaction was indicated by a red ring. A negative reaction was indicated by the yellow-brown ring. The negative reaction was suspected to be *Salmonella*.

4.2.6.2 Catalase Test

A solution containing a drop of 3% aqueous hydrogen peroxide was placed on a clean microscope slide. A colony of test culture from nutrient agar (Merck, Wadeville, South Africa) was then placed on the hydrogen peroxide drop. *Salmonella* was considered positive when bubbles appeared on the surface of the culture material.

4.2.6.3 Triple Sugar Iron Agar

The TSI agar test (Biolab, Merck - South Africa) slant was inoculated by streaking slant and stabbing the butt with a pure culture of typical suspicious *Salmonella* colonies from the Nutrient agar. After inoculation, the TSI agar was incubated at 37 °C ±1 for 18 to 24 hours. The inoculated tubes were capped loosely to maintain aerobic conditions while incubating in order to prevent excessive hydrogen sulphide (H_2S) production. For interpretation of the TSI results, typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and (in about 90% of the cases) formation of hydrogen sulfide (blackening of the agar). When lactose-positive *Salmonella* is isolated the TSI agar slant is yellow.

4.2.6.4 Urea agar test

The urea agar slant surface was inoculated by streaking the agar slope surface and stabbing the butt with a pure culture of typical suspicious *Salmonella* spp. from the Nutrient agar (Merck, Wadeville, South Africa). The Urea agar slants were then incubated at 37 °C ±1 °C for 18 to 24 hours and then followed by results interpretation. The positive reaction is expected to show changes in the color from phenol red to rose pink, then later to deep cerise (moderate red). This

occurs as a result of splitting of urea indicated by liberated ammonia. For a negative reaction, the color of the Urea media remains unchanged and suspected to be *Salmonella*.

4.2.7 Confirmatory biochemical tests for isolates

The API 20E (BioMerieux, Marcy l'Etoile, France) is developed for identification of Enterobacteriaceae and it includes various tests such as: o-nitrophenile-β-D-galactosidase, lysine decarboxylase, arginine dihydrolase, citrate utilisation, ornithine decarboxylase, hydrogen sulphide production, urease, indole production, tryptophan deaminase, acetoin production by the Voges-Proskauer test, gelitinase and fermentation of mannose, inositol, glucose, sorbitol, rhamnose, mannose, inositol, arabinose sucrose, mannose, and inositol. One colony representing a presumptive *Salmonella* isolate was re-suspended in deionized water and this was inoculated into the API 20E (BiomerieuX) test strips.

The wells including: mannose, inositol, sorbitol, rhamnose, indole, voges proskauer test, gelatinase, arabinose, sucrose, amygdalin, glucose, melibose, and dehydrated enzymes that include O-nitrophenyl-Dgalactopyranoside, citrate, urease, tryptophan deaminase, indole, voges proskauer test, gelatinase were allowed to react aerobically. However, some biochemical reactions like: Arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), Hydrogen Sulphide (H_2S) and Urea (URE) were allowed to occur under anaerobic conditions by overlaying the wells with a drop of mineral oil. The strips were placed into trays hydrated with 5 ml distilled water to create a humid atmosphere. The strips were incubated using an aerobic incubator for 24 hours. This was done following the manufacturer's commands to identify the Gram-negative organism (Ateba & Mochaiwa, 2014; Johannes, 2015).

Indices were generated for the diverse isolates and used to verify their identities using the API web^{TM} identification software.

4.2.8 Serological confirmation and identification of *Salmonella*

To confirm *Salmonella* isolates, serovars were determined by using slide agglutination with commercial *Salmonella* antisera (Mast Assure, Davies Diagnostics Pty. Ltd., Randburg, South Africa). The test was performed as per the directions provided by the manufacturer using the slide agglutination technique for both flagella (H) somatic and (O) antigens. The test was performed following manufacturer's orders. Briefly, 10 μL volumes of sterile 0.85% saline solution were placed on sterile microscope slides in duplicate. A colony was picked and emulsified on both drops to obtain identical turbidity. Then 10 μL aliquots of each polyvalent antiserum were added to one of the emulsions though a drop of saline was added to the other emulsion (control).

4.2.9 Molecular identification

4.2.9.1 Extraction of genomic DNA

For the extraction of genomic DNA, the procedure was done according to manufacturer's recommendations (Zymo-Research Fungal/Bacterial Soil Microbe DNA Mini Prep kit, Catalog No. D6005 USA supplied by Biolab, South Africa). Briefly, pure which were confirmed biochemically and serologically were subcultered on Nutrient agar then pure colonies were

inoculated into 10 mL of peptone water and incubated aerobically at 37°C for 24 hours while shaking the mixture. After incubation, the tubes were centrifuged in order to get the pellets.

Pellets were suspended in 750 µL lysis solution, disrupted with a disruptor gene (Inqaba Biotech mode No, SI D258, USA) and vortexed at 14.000 rpm for 5 minutes followed by centrifugation at 10 000 rpm for 1 minute. About 400 µL of the upper aqueous phase was liquated into a new Eppendorf tube and centrifuged at 7000 rpm for 1 minute. 1200 µL of buffer was added to the filtrate and 800 µL of the mixture transferred to the new collection tube and centrifuged at 10.000 rpm for 1 minute. The filtered DNA was pre-washed by adding 200 µL DNA pre-wash buffer and centrifuged at 10.000 rpm for 1 minute. 500 µL of DNA wash buffer was added to the new collection tube and centrifuged at 10.000 rpm for 1minute. Finally, 100 µL of DNA elution buffer was added to elude the DNA in a clean 1.5 mL micro-centrifuge tube and stored at -20°C for molecular confirmation of the *Salmonella* species and for virulence and antimicrobial resistance genes screening.

4.2.9.2 Determination of the successful extraction of genomic DNA

The concentration of DNA fragment for *Salmonella* spp. was measured by use of Nano-Drop ND-1000 UV spectrophotometer (Thermo-Fisher Scientific Inc., USA). This process is required before the amplification of *16S-rDNA* gene. A 260/280 ratio between 1.8 and 2.0 is indicative of pure DNA, while a ratio above 2.0 indicates phenol and contamination and a ratio below 1.8 indicates contamination by proteins (Psifidi *et al.*, 2015; Taylor *et al.*, 2010).

4.2.9.3 Identification of Salmonella

PCR was conducted using the Universal primers; forward (AGA GTT TGA TCC TGG CTC AG) and the Reverse (ACG GCT ACC TTG TTA CGA CTT) with the reaction volume of 25

µL, containing: 12.5 µL PCR Master Mix, 2 µL template DNA, 8.5 µL nuclease-free water and 1 µL of each oligonucleotide primer using an Engine T100 Thermal™ cycler (BioRad, *Singapore*). The thermo cycling conditions consisted of an initial denaturation step at 95°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 61°C for 30 seconds and extension at 72°C for 5 minutes, finally a single and final extension step at 72°C for 7 minutes (Ngoma *et al.*, 2013).

4.2.9.4 Electrophoresis of PCR Products

To check for the successful amplification of *16S-rDNA,* the agarose gel was prepared as follows; 100 g was measured and mixed with 100 mL of TE buffer, and then agarose was dissolved by heating using microwave, then gel was allowed to cool to about 40°C and stained with ethidium bromide (EtBr) (Özbey *et al.*, 2008). After the gel had set inside the electrophoresis chamber, 5 µL PCR products was transferred to each wells in the gel electrophoresis tank. Electrophoresis conditions were 43 minutes at 80 Voltage. A ChemiDoc Imaging System (Bio-Rad ChemiDocTM MP Imaging System, UK) was employed to capture the image using Gene Snap (version 6.00.22) software to check for the presence of DNA fragment.

4.2.9.5 DNA Sequencing

PCR products were sequenced using an ABI PRISM® 3500XL DNA Sequencer (Applied Biosystems) at Inqaba Biotechnical Industrial (Pty) Ltd. (Pretoria, South Africa). The acquired sequences were aligned against GenBank data base using Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST) from the National Center for Biotechnology Information (NCBI) to identify sequences with high similarity (Hendrickson *et al.*, 2002).

4.2.10 Phylogenetic analysis

All confirmed sequencing results were edited by using Bio-Edit software (Hall, 1999) and saved as FASTA format. The sequences were used to search the GenBank database with the BLASTn algorithm to find out the relative Phylogenetic positions. The sequences were aligned by using the multiple alignment fast Fourier transform (MAFFT) program 6.8464 to conduct multiple and pair-wise sequence alignments against corresponding nucleotide sequences retrieved from GenBank. Evolutionary distance matrices were generated as described previously by Jukes and Cantor (Jukes & Cantor, 1969). Phylogenetic analysis was performed using the program MEGA version 7 (Kumar *et al.*, 2016) and neighbor joining, maximum-parsimony, maximum likelihood analyses were obtained for each gene then the tree was constructed. Bootstrap analyzes were performed using 1000 replications for neighbor-joining, maximum-parsimony, maximum likelihood. Recognized chimeric sequences were identified using the Chimera Buster 1.0 software. Manipulation and tree editing were carried out using Tree View (Timme *et al.*, 2013).

4.2.11 Accession numbers

The *16S-rDNA* sequences obtained from the current study were placed into the GenBank database and were given accession numbers from MH352147 to MH352214. All assigned accession numbers are indicated in Appendix T2.

4.3 RESULTS

4.3.1 Isolation of *Salmonella* species

Of the 154 rats examined, 19 (12.3%) were infected with *Salmonella* spp. (Appendix T1). Of these rats 12 (63.2%) were *Rattus rattus* and 7 (36.8%) were *Rattus tanezumi*. Out of 19 positive rats, 4 (21 %) were from farm A, 2 (10.5%) from farm B, 1 (5.3%) from farm C, 2 (10.5%) from farm D, 1 (5.3%) from farm H and lastly 9 (47.4%) were from farm E.

4.3.2 Preliminary and confirmatory biochemical tests for *Salmonella*

After screening all the samples, 120 presumptive isolates were suspected to be *Salmonella* i.e. based on preliminary tests (catalase test, Indole test, Urease test, Tripe Sugar Iron test) and only 68 isolates were confirmed by API 20E (Appendix F3), serology and conventional PCR. All the results for preliminary and confirmatory biochemical test are shown in Table 4.1. All the suspected isolates were Gram-negative and catalase positive. Some of these isolates (56%) were indole positive. Of these isolates only 85% resulted in sugar fermentation and hydrogen sulfide production. However, for the Urease test, all the isolates were unable to break down urea in the broth in order to form both carbon dioxide and ammonia. Out of 120 suspected isolates, only 68 isolates were identified as *Salmonella* species using both confirmatory biochemical test (API 20E and serotyping). A certain proportion of the isolates (27%) were positive for poly H (b, d, e, r) antiserum whereas a large percentage of the isolates (41%) were positive for *Salmonella* Poly O (A-G) antiserum. Most of these *Salmonella* serotypes were from farm E, A, D, B, C and H, respectively.

Table 4. 1: Results for preliminary and confirmatory biochemical tests (API E20 and Serotyping)

Farms	GN Rods	Catalase	Indole	Urease	TSI	API 20E	Serotyping	
							O (A−G)	H (b, d, e, r)
A	26	26(100%)	13(50%)	0(0%)	15(57%)	13(50%)	3(11%)	10(38%)
B	15	15(100%)	5(33%)	0(0%)	8(53%)	5(33%)	0(0%)	5(33%)
C	12	12(100%)	4(33%)	0(0%)	8(66%)	4(33%)	2(16%)	2(16%)
D	11	11(100%)	7(63%)	0(0%)	7(6%)	7(63%)	4(36%)	3(27%)
E	50	50(100%)	5(70%)	0(0%)	41(82%)	35(70%)	29(58%)	6(12%)
H	8	8(100%)	4(50%)	0(0%)	6(75%)	4(50%)	3(37%)	1(12%)
Total	**122**	**122(100%)**	**68(56%)**	**0(0%)**	**85(71%)**	**68(56%)**	**41(34%)**	**27(22%)**

GN= Gram Negative

4.3.3 The detection of *Salmonella* species using *16S rRNA* gene

Salmonella isolates were further reconfirmed by use of conventional *16S rDNA* gene PCR. Figure 4.1 shows how the fragments appeared on a gel after amplification. A total of 68 were confirmed by PCR as *Salmonella* species distributed as follows: *Salmonella* typhimurium 26 (38.2%), *Salmonella enteritidis* 12 (17.6%), *Salmonella newport* 8 (11.8%), *Salmonella heidelberg* 7 (10.3%), *Salmonella bongori* 6 (8.8%), *Salmonella enteric* serovar paratyphi B 4 (5.9%), *Salmonella tennessee* 3 (4.4%) and *Salmonella pullorum* 2 (2.9%), (Table 4.2). The high

percentage of the isolates were from *R. rattus* (Black rat) 12 (63.3%) while *R. tanezumi* (Asian Rat/Asian House Rat) had 7 (36.8%). The results further showed that the majority of infections were in male rats 13 (68.4%) compared to 6 (31.6%) in the female rats (Appendix T1).

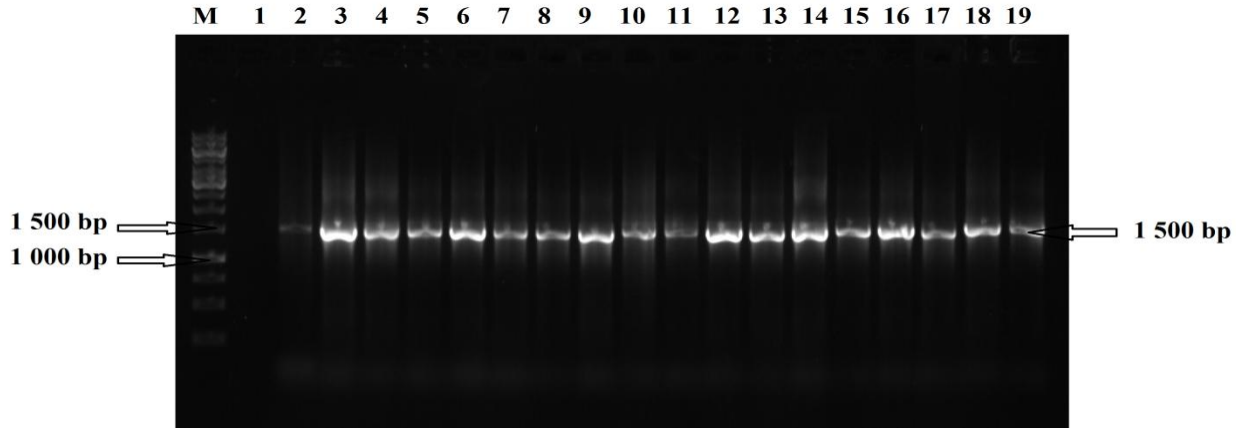


Figure 4. 1: Electrophoresis in a 1% agarose gel of PCR amplified *16S rDNA* of *Salmonella* strains; molecular weight marker (1kb DNA ladder Lane M); (Lane 1) distilled water, Lane 2-19 (*Salmonella* species)

Table 4. 2: Confirmation of *Salmonella* spp. (n=68) from rodents captured in poultry farms around Mafikeng using PCR.

Farms	*Rattus spp*	*S.* typhimurium (n=26)	*S. enteritidis* (n=12)	*S. newport* (n=8)	*S. heidelberg* (n=7)	*S. bongori* (n=6)	*S. paratyphi* B (n=4)	*S. pullorum* (n=2)	*S. tennessee* (n=3)
Farm A	*R. tanazumi*	–	1(8%)	–	1(14.3%)	–	–	–	–
	R. rattus	4(15%)	3(25%)	1(13%)	2(28.6%)	1(17%)	–	–	–
Farm B	*R. tanazumi*	–	–	–	–	–	–	–	–
	R. rattus	2(8%)	1(8%)	–	–	2(33%)	–	–	–
Farm C	*R. tanazumi*	2(78%)	–	–	1(14%)	–	1(25%)	–	–
	R. rattus	–	–	–	–	–	–	–	–
Farm D	*R. tanazumi*	1(3.8%)	2(17%)	–	–	–	–	–	–
	R. rattus	–	–	3(38%)	–	1(17%)	–	–	–
Farm E	*R. tanazumi*	10(38%)	–	2(25%)	1(14%)	–	2 (50%)	1(50%)	1(33%)
	R. rattus	6(23%)	5(42%)	2(25%)	2(28.6%)	1(17%)	1 (25%)	–	1(33%)
Farm H	*R. tanazumi*	–	–	–	–	–	–	–	–
	R. rattus	1(4%)	–	–	–	1(17%)	–	1(50%)	1(33%)

4.3.4 Phylogenetic analysis of the *Salmonella* isolates from rats

A phylogenetic tree for *Salmonella* isolates from rats was constructed to understand the genetic closeness of *Salmonella* strains with other related strains from different countries in and outside the African continent. *Shigella fleneri* [KY199565.1] from the family *Shigella* was used as an out-group for the *16S-rDNA* gene. The resulting neighbour-joining (NJ) revealed that strain *S. heidelburg* clustered closely with sequences of the following *S. heidelberg*: CP004086.1, originating from retail meats, humans, and animals; CP016586.1, originating from food sources human and animal; *S. tennessee*; CP024164.1, originating from laboratory control strains and CP025217.1, originating from the food industry. *S. bongori*; MF289161.1; originating from the crops, MG663480.1, originating from chicken faeces; *Salmonella* paratyphi; CP006575.1 originating from bacteria strain obtained from source laboratory of Zoonosis; *S.* typhimurium; CP014971.2, originating from humans and cattle; *S. enteritidis*; CP018651.1, originating from the historical *S. enteritidis* isolated between 1940s and 1990s. The phylogenetic tree is shown below in Figure 4.2.

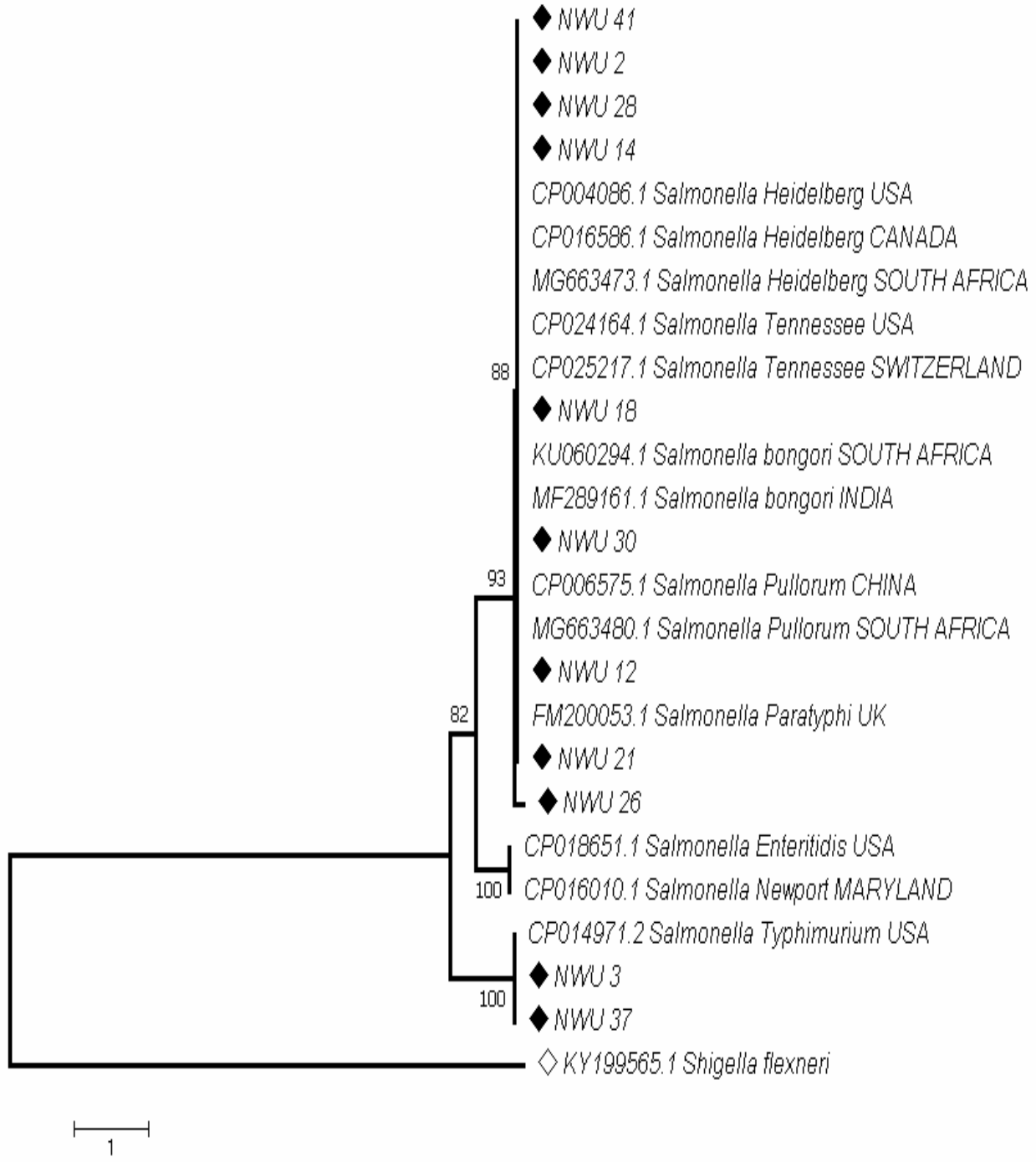


Figure 4. 2: Phylogenetic relationship of *Salmonella* detected in the faeces from *Rattus* species (*R. tanazumi* and *R. Rattus*). Neighbour-joining tree of *Salmonella* spp. based on partial *16S rDNA* gene sequences. The reliability of the tree was evaluated by the bootstrap method with 1000 replications. All position containing gaps and missing data were eliminated from the dataset (complete deletion option). KY199565.1 *Shigella flexneri* was used as an out-group.

4.4 DISCUSSION

The secondary objective of the study was to isolate and characterise *Salmonella* spp. from rodents that invade poultry farms in Mafikeng, North West, South Africa in an effort to understand their livestock and public health significance. This is because rats are known to carry *Salmonella* to poultry or vice versa and maintain it when poultry have been cleared thus acting as reservoirs for the disease (Lapuz *et al.*, 2012; Umali *et al.*, 2012). Controlling these rats can thus help to effectively control not only the resulting economic damage but also the pathogens and consequently the diseases that they carry. Knowing the pathogens present also helps to target control measures to cover all carriers or vectors of those pathogens.

Among *Salmonella* spp. detected in this study, *S. enteritidis* and *S.* typhimurium are the two majority commonly reported serotypes in South Africa (Jambalang *et al.*, 2017). A third serotype, *S. newport*, has also previously been isolated from ready-to-eat (RTE) chicken products (Olobatoke & Mulugeta, 2015) in the North West Province of South Africa. The previous retrospective study on the incidence of *Salmonella* isolations in animals in South Africa (Kidanemariam *et al.*, 2010), which analyzed diagnostic data collected from laboratory since 1996−2006 found that ten *Salmonella* serotypes are frequently isolated in the country and these include: *S. muenchen, S. newport, S. dublin, S.* typhimurium, *S. enteritidis, S. chester, S. hadar, S. schwarzengrund, S. heidelberg* and *S. mbandaka*. The above-mentioned study investigated isolates collected from birds, cattle, pigs and sheep species. However, according to literature, no such investigation has been done on rodents. The fact that rodents in our study have the same *Salmonella* serotypes may indicate that these serotypes are circulating and maintained in these hosts also in the area.

Four of the serotypes; *S. enteritidis*, *S.* typhimurium, *S. heidelberg* and *S. newport* in this study are in the top 5 most commonly isolated serotypes in poultry meat and in human disease in South Africa and elsewhere. The *S. heidelberg* was associated with a 1996-1999 salmonellosis outbreak in USA (CP004086.1) (Hoffmann *et al.*, 2014). The *S* typhimurium was responsible for human salmonellosis outbreaks in Australia and in Germany; from 2001 to 2005 (de Freitas Neto *et al.*, 2010), and 63 *Salmonella* outbreaks in Italy (de Freitas Neto *et al.*, 2010). Phylogenetic analysis of *S. typhimurium* (CP014971.2) from those outbreaks clusters well with our isolates. This is significant as it points to the possibility that our strains can also play a significant role in human disease. The other serotype, *S. newport* has been linked to 510 patients in Virginia State (USA) (Kretsinger *et al.*, 2003); whereas *S. enterica* serovar paratyphi B has been associated with human outbreaks in the USA (Harris *et al.*, 2009), in France (Desenclos *et al.*, 1996), in Australia (Levings *et al.*, 2006), Canada (Stratton *et al.*, 2001), and European countries (Miko *et al.*, 2002). This is important data for public health risk assessments where rats will be at the center as maintenance or reservoir hosts.

Our phylogenetic analysis also revealed that the *Salmonella* isolates found in the rats of this study were very similar to other genotypes previously identified in several other sources, including chickens, human, milk and other animals as shown in Figure 4.2. This, therefore, indicates that the strains that were found in rats in this study could easily be circulating in other animals and their products and thus be responsible for both livestock and human diseases. This corroborates well with, for example, a previous study (Henzler & Opitz, 1992) that established that the high occurrences of *S. enteritidis* food poisoning were associated with the occurrence of *S. enteritidis* infected rodents. It is therefore evident that any salmonellosis outbreaks should be investigated from all likely sources including rodents. Furthermore, control of rats will need to

be done as part of a disease management strategy for farms and not only because rats are a nuisance economically.

This study further agrees with previous studies that have also found out that *R. rattus* is a very important carrier of *Salmonella* infections (Himsworth *et al.*, 2015; Mushtaq-ul-Hassan *et al.*, 2008; Mushtaq *et al.*, 2014; Umali *et al.*, 2012). Nevertheless, the proportion of 12.3% for *Salmonella* spp. isolated from rats in the present study is much lower than that observed in other countries such as; 32% in Nigeria (Oboegbulem & Okoronkwo, 1990), 18.0% also in another study in Nigeria (Wakawa *et al.*, 2015), 16.2% in the USA (Henzler & Opitz, 1992), 46.0% in France (Seguin *et al.*, 1986), 31.8% and 41.2% in Japan (Lapuz *et al.*, 2008), and 17% in Iran (Shimi *et al.*, 1979), to mention but a few. However, the findings of this study are higher than those found in other countries; 10.0% in the UK (Hilton *et al.*, 2002), 2.0% in Trinidad and Tobago (Nkogwe *et al.*, 2011), 0% prevalence for *Salmonella* spp. in Trinidad (Gopee *et al.*, 2000). The differences from the results of this study might be due to the differences in analytical methods, number of samples tested, and the different regional/geographical situations.

From this study, it can be concluded that rats provide a good chance for environment–rat–poultry interaction through ingestion of pathogens such as *Salmonella* in rodent fecal droppings. These *Salmonella* infected feces may be a source of very important *Salmonella* serotypes known to cause diseases in livestock and humans. Isolates such as: *S. newport*, *S.* typhimurium, *S. heidelberg, S. enteritidis, S. bongori, S. enteric* serovar paratyphi B, *S. tennessee* and *S. pullorum* that were found in this study are known pathogens responsible for serious outbreaks in humans and livestock globally. Therefore, the role of rats in the *Salmonella* transmission cycle in poultry farms and human environment should not underestimated.

4.5 REFERENCES

Ateba, C. & Mochaiwa, B. 2014. Use of *inv*A gene specific PCR analysis for the detection of virulent *Salmonella* species in beef products in the North West Province, South Africa. *Journal of Food and Nutrition Research*, 2(6):294-300.

Clapperton, B.K. 2006. A review of the current knowledge of rodent behaviour in relation to control devices. Vol. 263: Science & Technical Pub., Department of Conservation. Retrieved from https://www.doc.govt.nz/documents/science-and-technical/sfc263.pdf [2018-06-12].

de Freitas Neto, O., Penha Filho, R., Barrow, P. & Berchieri Junior, A. 2010. Sources of human non-typhoid salmonellosis: a review. *Revista Brasileira de Ciência Avícola*, 12(1):01-11.

Desenclos, J.-C., Bouvet, P., Benz-Lemoine, E., Grimont, F., Desqueyroux, H., Rebiere, I. & Grimont, P.A. 1996. Large outbreak of *Salmonella enterica* serotype p*aratyphi B* infection caused by a goats' milk cheese, France, 1993: a case finding and epidemiological study. *BMJ*, 312(7023):91-94.

Gopee, N.V., Adesiyun, A.A. & Caesar, K. 2000. Retrospective and longitudinal study of *Salmonellosis* in captive wildlife in Trinidad. *Journal of Wildlife Diseases*, 36(2):284-293.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. (*In*. Nucleic acids symposium series organised by: [London]: Information Retrieval Ltd., c1979-c2000. p. 95-98).

Harris, J.R., Bergmire-Sweat, D., Schlegel, J.H., Winpisinger, K.A., Klos, R.F., Perry, C., Tauxe, R.V. & Sotir, M.J. 2009. Multistate outbreak of *Salmonella* infections associated with small turtle exposure, 2007–2008. *Pediatrics*, 124(5):1388-1394.

Hendrickson, H., Slechta, E.S., Bergthorsson, U., Andersson, D.I. & Roth, J.R. 2002. Amplification–mutagenesis: evidence that "directed" adaptive mutation and general hypermutability result from growth with a selected gene amplification. *Proceedings of the National Academy of Sciences*, 99(4):2164-2169.

Henzler, D. & Opitz, H. 1992. The role of mice in the epizootiology of *Salmonella enteritidis* infection on chicken layer farms. *Avian Diseases*, 36(3):625-631.

Hilton, A.C., Willis, R.J. & Hickie, S.J. 2002. Isolation of *Salmonella* from urban wild brown rats (*Rattus norvegicus*) in the West Midlands, UK. *International Journal of Environmental Health Research*, 12(2):163-168.

Himsworth, C.G., Zabek, E., Desruisseau, A., Parmley, E.J., Reid-Smith, R., Jardine, C.M., Tang, P. & Patrick, D.M. 2015. Prevalence and characteristics of *Escherichia coli* and *Salmonella* spp. in the feces of wild urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) from an inner-city neighborhood of Vancouver, Canada. *Journal of Wildlife Diseases*, 51(3):589-600.

Hoffmann, M., Zhao, S., Pettengill, J., Luo, Y., Monday, S.R., Abbott, J., Ayers, S.L., Cinar, H.N., Muruvanda, T. & Li, C. 2014. Comparative genomic analysis and virulence differences in closely related *Salmonella enterica* serotype *Heidelberg* isolates from humans, retail meats, and animals. *Genome Biology and Evolution*, 6(5):1046-1068.

Jambalang, A.R., Buys, E.M. & Botha, F.S. 2017. Bacterial species from retailed poultry eggs in Tshwane, South Africa: Implication for consumers. *South African Journal of Science*, 113(11-12):1-7.

Johannes, N.S. 2015. Identification and characterisation of potential virulence genes of *Salmonella* from rooibos tea. Doctoral dissertation, University of the Western Cape. Retrieved from http://etd.uwc.ac.za/xmlui/handle/11394/4954 [2018-05-18].

Judy, L.a.W., R. . 2010. Animal damage management. *Controlling rodents in commercial poultry facilities*:1-2.

Jukes, T.H. & Cantor, C.R. 1969. Evolution of protein molecules. *Mammalian Protein Metabolism*, 3(21):121-132.

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(1):37-44.

Kretsinger, K., Noviello, S. & Moll, M. 2003. Tomatoes sicken hundreds: multistate outbreak of *Salmonella newport*. (*In*. 52nd Annual Epidemic Intelligence Service Conference. Atlanta, Georgia.

Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7):1870-1874.

Lapuz, R., Tani, H., Sasai, K., Shirota, K., Katoh, H. & Baba, E. 2008. The role of roof rats (*Rattus rattus*) in the spread of *Salmonella enteritidis* and *S. infantis* contamination in layer farms in Eastern, Japan. *Epidemiology and Infection*, 136(09):1235-1243.

Lapuz, R.R.S.P., Umali, D.V., Suzuki, T., Shirota, K. & Katoh, H. 2012. Comparison of the prevalence of *Salmonella* infection in layer hens from commercial layer farms with high and low rodent densities. *Avian Diseases*, 56(1):29-34.

Levings, R.S., Lightfoot, D., Hall, R.M. & Djordjevic, S.P. 2006. Aquariums as reservoirs for multidrug-resistant *Salmonella Paratyphi B*. *Emerging Infectious Diseases*, 12(3):507.

Liu, C.-C., Yeung, C.-Y., Chen, P.-H., Yeh, M.-K. & Hou, S.-Y. 2013. *Salmonella* detection using 16S ribosomal DNA/RNA probe-gold nanoparticles and lateral flow immunoassay. *Food Chemistry*, 141(3):2526-2532.

Meerburg, B.G. & Kijlstra, A. 2007. Role of rodents in transmission of *Salmonella* and *Campylobacter*. *Journal of the Science of Food and Agriculture*, 87(15):2774-2781.

Miko, A., Guerra, B., Schroeter, A., Dorn, C. & Helmuth, R. 2002. Molecular characterization of multiresistant d-tartrate-positive *Salmonella enterica* serovar *Paratyphi B* isolates. *Journal of Clinical Microbiology*, 40(9):3184-3191.

Mushtaq-ul-Hassan, M., Hussain, I., Shehzadi, B., Shaheen, M., Mahmood, M., Rafique, A. & Mahmood-ul-Hassan, M. 2008. Occurrence of some zoonotic microorganisms in faecal matter of house rat (*Rattus rattus*) and house mouse (*Musmusculus*) trapped from various structures. *Pakistan Veterinary Journal*, 28(4): 171-174.

Mushtaq, M., Kayani, A.R., Nadeem, M.S. & Beg, M.A. 2014. Distribution pattern of commensal rodents in shops of urban Rawalpindi, Pakistan. *Pakistan Journal of Zoology*, 46(6): 1585-1589.

Ngoma, L., Esau, B. & Babalola, O.O. 2013. Isolation and characterization of beneficial indigenous endophytic bacteria for plant growth promoting activity in Molelwane Farm, Mafikeng, South Africa. *African Journal of Biotechnology*, 12(26):4105-4114.

Nkogwe, C., Raletobana, J., Stewart-Johnson, A., Suepaul, S. & Adesiyun, A. 2011. Frequency of detection of *Escherichia coli*, *Salmonella* spp., and *Campylobacter* spp. in the faeces of wild rats (*Rattus* spp.) in Trinidad and Tobago. *Veterinary Medicine International*, 2011: 1-7.

Nwiyi, P. & Erumaka, G. 2012. Frequency of detection of *Salmonella* spp. and *Esherichia coli* in the faeces of house rat in Abia State, Nigeria. *Research Opinions in Animal & Veterinary Sciences*, 2(10): 521-524.

Oboegbulem, S.I. & Okoronkwo, I. 1990. *Salmonellae* in the African great cane rat (Thryonomys swinderianus). *Journal of Wildlife Diseases*, 26(1):119-121.

Olobatoke, R.Y. & Mulugeta, S.D. 2015. Incidence of non-typhoidal *Salmonella* in poultry products in the North West Province, South Africa. *South African Journal of Science*, 111(11-12):1-7.

Özbey, G., Seven, P.T., Muz, A., Ertaş, H. & ÇERÇI, H. 2008. Isolation of *Salmonella* spp. from faecal samples of cracked egg fed hens and polymerase chain reaction (PCR) confirmation. *Bulgarian Journal of Veterinary Medicine*, 11(2):103-112.

Parvej, M.S. 2013. Isolation, identification and molecular characterization of poultry *salmonella.* Doctoral dissertation, University of Mymensingh. Retrieved from researchgate.net/publication/ 297563780 Isolation Identification_and Molecular Characterization of Poultry *Salmonella* [2018-02-08].

Psifidi, A., Dovas, C.I., Bramis, G., Lazou, T., Russel, C.L., Arsenos, G. & Banos, G. 2015. Comparison of eleven methods for genomic DNA extraction suitable for large-scale whole-genome genotyping and long-term DNA banking using blood samples. *PloS one*, 10(1):e0115960.

Rocha-e-Silva, R., Cardoso, W., Albuquerque, Á., Horn, R., Lopes, E., Gomes Filho, V., Almeida, C., Santos, I., Machado, D. & Lima, S. 2014. Isolation of *Salmonella enterica* subsp. *enterica* (O: 4, 5: i) and *Salmonella enterica* subsp. typhimurium from free-living domestic pigeons (Columba livia). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 66(5):1435-1438.

Rodriguez, J., Rondón, I. & Verjan, N. 2015. Serotypes of *Salmonella* in Broiler Carcasses Marketed at Ibague, Colombia. *Revista Brasileira de Ciência Avícola*, 17(4):545-552.

Roomaney, R., Ehrlich, R. & Rother, H.-A. 2012. The acceptability of rat trap use over pesticides for rodent control in two poor urban communities in South Africa. *Environmental Health*, 11(1):32.

Seguin, B., Boucaud-Maitre, Y., Quenin, P. & Lorgue, G. 1986. Epidemiologic evaluation of a sample of 91 rats (*Rattus norvegicus*) captured in the sewers of Lyon. *Zentralblatt fur Bakteriologie, Mikrobiologie, und Hygiene. Series A, Medical Microbiology, Infectious Diseases, Virology, parasitology*, 261(4):539-546.

Shimi, A., Keyhani, M. & Hedayati, K. 1979. Studies on salmonellosis in the house mouse, *Mus musculus*. *Laboratory Animals*, 13(1):33-34.

Smith, A.M., Ismail, H., Henton, M.M., Keddy, K.H. & Network, G.-S.S. 2014. Similarities between *Salmonella enteritidis* isolated from humans and captive wild animals in South Africa. *The Journal of Infection in Developing Countries*, 8(12):1615-1619.

Stratton, J., Stefaniw, L., Grimsrud, K., Werker, D., Ellis, A., Ashton, E., Chui, L., Blewett, E., Ahmed, R. & Clark, C. 2001. Outbreak of *Salmonella paratyphi B* var java due to contaminated alfalfa sprouts in Alberta, British Columbia and Saskatchewan. *Canada communicable disease report= Releve des maladies transmissibles au Canada*, 27(16):133-137.

Taylor, S., Wakem, M., Dijkman, G., Alsarraj, M. & Nguyen, M. 2010. A practical approach to RT-qPCR—publishing data that conform to the MIQE guidelines. *Methods,* 50(4):S1-S5.

Timme, R.E., Pettengill, J.B., Allard, M.W., Strain, E., Barrangou, R., Wehnes, C., Van Kessel, J.S., Karns, J.S., Musser, S.M. & Brown, E.W. 2013. Phylogenetic diversity of the enteric pathogen *Salmonella* enterica subsp. enterica inferred from genome-wide reference-free SNP characters. *Genome Biology and Evolution*, 5(11):2109-2123.

Trkov, M. & Avguštin, G. 2003. An improved 16S rRNA based PCR method for the specific detection of *Salmonella* enterica. *International Journal of Food Microbiology*, 80(1):67-75.

Umali, D.V., Lapuz, R.R.S.P., Suzuki, T., Shirota, K. & Katoh, H. 2012. Transmission and shedding patterns of *Salmonella* in naturally infected captive wild roof rats (*Rattus rattus*) from a *Salmonella*-contaminated layer farm. *Avian diseases*, 56(2):288-294.

Wakawa, A., Mohammed, F. & Mamman, H. 2015. Isolation and antibiotic susceptibility of *Escherichia coli* and *Salmonella gallinarum* isolated from rats in commercial poultry farms with recurrent Colibacillosis and Fowl typhoid cases in Zaria, Nigeria. *Journal of Veterinaru Advance*, 5(11):1147-1152.

CHAPTER 5− THE VIRULENCE OF *SALMONELLA* ISOLATES FROM RATS USING DOCUMENTED VIRULENT GENE MARKERS OF THE *SALMONELLA* STRAINS

ABSTRACT

The ability to cause disease relies on several virulence determinants. *Salmonella* has a number of physiological and structural virulence factors that enable it to cause diseases. The objective of this study was to establish the virulence of the *Salmonella* isolated from *Rattus* species found in poultry houses using eleven virulence genes namely; *inv*A, *Sdf I*, *Spy*, *Spv*C, *hil*A, *spi*C, *mis*L, *orf*L, *Ppb23*, *fli*B and *fli*C. The genes were detected by PCR using specific primers.

Out of 68 *inv*A positive *Salmonella* isolates, 12 (17%), 26 (38%), 14 (20%), 28 (41%), 43 (63%), 31 (45%), 38 (55%) were positive for *SdfI*, *Spy*, *Spv*C, *hil*A, *mis*L, *Orf*L *spi*C genes, respectively. Some of the *Salmonella* serotypes were carrying multi-virulence genes i.e. *S.* typhimurium, *S. newport*, *S. heidelberg*, *S. enteritidis*, *S. bongori*, and *S. pullorum*, harbored 7 (10.3%), 6 (8.8%), 2 (2.9%), 3 (4.4%), 2 (2.9%) and 3 (4.4%), respectively. The occurrence of several virulence genes indicates the high pathogenic potential of these isolates and can be considered as an important health concern.

Keywords: *Salmonella*, virulence genes, PCR, *Rattus* spp.

5.1 INTRODUCTION

The capacity of *Salmonella* species to cause disease relies on several virulence determinants (Kaur & Jain, 2012; Li *et al.*, 2018; Wu *et al.*, 2017). Some of these genes may be regarded as virulence determinants in a broad sense. *Salmonella* species acquire a number of physiological and structural virulence factors, enabling them to cause chronic and acute diseases (Berkes *et al.*, 2003). Some of these structural and physiological virulence factors and some virulence genes are encoded on SPI within the species. There are about twenty one SPIs ranging from SPI1 to SPI21 and they code for a type T3SS (López *et al.*, 2012). Approximately 60 virulence genes are associated with SPIs (Zishiri *et al.*, 2016).

The most important characteristics responsible for the virulence of *Salmonella* spp., such as intracellular survival (Pathmanathan *et al.*, 2003), production of Vi antigens capsule (Hughes *et al.*, 2008) and cell invasion (Peixoto *et al.*, 2017) are all encoded by SPIs (Zishiri *et al.*, 2016). SPI-1 is required for bacterial invasion into intestinal epithelial cells, while systemic infections and intracellular accumulation of *Salmonella* spp. are dependent on the function of SPI-2 (Valle & Guiney, 2005). These SPIs are absent in non-pathogenic *Salmonella* strains (Leung *et al.*, 2011).

Some of the SPI encoded genes are invasive, *inv*A which is necessary for invasion of epithelial cells (Rahn *et al.*, 1992), *hil*A required for modulation of transcriptional regulator of the invasion genes (Darwin & Miller, 2000) and also for expression of SPI-1 invasion (Schechter & Lee, 2000), and *orf*L gene for auto-transporter survival in macrophages/ or colonization (Hughes *et al.*, 2008). Genes like *sip*C is a translocase mediating bacteria entry into epithelial cell (Hayward & Koronakis, 1999).

Infection with *Salmonella* is commonly inadequate to a localised intestinal event. However, the occurrence of virulence plasmids has been linked with non-typhoidal *Salmonella* spp. surviving in phagocytes and spreading from the small intestine to the spleen and liver (Dauda, 2010). The majority of *Salmonella* strains produce heat labile cytotoxins which causes damage to the intestinal mucosal surface and lead to general enteric signs and inflammation (Berkes *et al.*, 2003).

The virulence genes of *Salmonella* isolated from rats is not widely studied especially for the rats that are found in poultry houses without visible direct link to human diseases or surroundings. Studies have shown that *Salmonella* serotypes commonly isolated from rats are similar to those that have been implicated in major *Salmonella* outbreaks (Nkogwe *et al.*, 2011; Umali *et al.*, 2012). Earlier investigation on the same has also confirmed that similar serotypes to those known in many disease outbreaks are also found in rats collected in poultry farms around Mafikeng (Olobatoke & Mulugeta, 2015). The aim of this part of the study was, therefore, to assess the virulence of these *Salmonella* isolates from these rats using known virulence indicator genes in order to ascertain their potential pathogenicity.

5.2 MATERIAL AND METHODS

5.2.1. Study site, sampling, bacterial isolation, identification and DNA extraction

Study site, sampling, sample preparation, isolation and *Salmonella* identification was done as described in details under section 3.2.1, 3.2.2, 4.2.4−4.2.13, respectively.

5.2.2 Detection of virulence genes by PCR

Eleven known virulence genes namely: *inv*A, *SdfI, Spy*, *Spv*C, *hil*A, *spi*C, *mis*L, *orf*L, *Ppb23, fli*B and *fli*C were targeted for detection using published primers for PCR. Individual PCRs, for each virulent gene were set up in a 25 µL which consisted of 12.5 µL PCR Master Mix [AmpliTaq Gold® DNA Polymerase 0.05 units/µL, Gold buffer 930 mM Tris/HCl pH 8.05, 100 mM KCl0, 400 mM of each dNTP and 5 mM $MgCl_2$] (Applied Biosystems, California, USA). Then 2.5 of each primer, 2 µL of template DNA and ddH_2O was added to make the final volume. Test DNA was replaced with 5 µL of nuclease-free water as negative control. Cycling conditions for Polymerase Chain Reactions (PCR), as well as the virulence gene size, amplified fragments and sequences are shown in Table 5.1.

Amplified fragments of DNA were fractionated on a 1% w/v agarose gel at a constant voltage of 80 V in 0.5×TAE (Tris-Acetat EDTA). A 100-bp and 1-kb reference marker (Sigma, D7058) was used to allow standardization. Following staining with ethidium bromide (0.1µg/mL), the gel was visualized using Syngene Ingenius Bioimager (UK) under UV light to confirm the expected size of the product (Ekwanzala *et al.*, 2017; Zishiri *et al.*, 2016).

Table 5. 1: List of *Salmonella* virulence genes and PCR conditions used for amplification

Target gene	Sequence (5' - 3')	Amplicon size (bp)	Conditions	Reference
*inv*A	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	280	94°C for 5 min, 94°C for 45 sec, 58°C for 45 sec, 72°C for 70 min, 72°C for 7 min, 30 cycles	(Ekwanzala *et al.*, 2017)
SdfI	TGTGTTTTATCTGATGCAAGAGG TGAACTACGTTCGTTCTTCTGG	303	95°C for 2 min, 95°C for 1 min, 57°C for 1 min, 72°C for 2 min; 72°C for 5 min, 30 cycles	(Mohd Afendy & Son, 2015)
Spy	TTGTTCACTTTTTACCCCTGAA CCCTGACAGCCGTTAGATATT	401	95°C for 2 min, 95°C for 30 s, 57°C for 30 s, 72°C for 30 s, 72°C for 4 min, 30 cycles	(Alvarez *et al.*, 2004)
SPAB_01124	ACATAATGCTTTTCGTGCTCCTC GGCATAAATATCTTTCTCCCCTCC	384	95°C for 5 min, 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s, 35 cycles	(Zhai *et al.*, 2014)
fljB	ATCAACGGTAACTTCATATTTG GGCAACCCGACAGTAACTGGCGATC	135	95°C for 5 min, 95°C for 1 min, 60°C for 30 s, 72°C for 30 s, 72°C for 7 min, 30 cycles	(Perera & Murray, 2008)
fliC	CACTGGTCTTAATGATGCAGCTC CCTGTCACTTTCGTGGTTAT	222	95°C for 5 min, 95°C for 1 min, 60°C for 30 s, 72°C for 30 s, 72°C for 7 min, 30 cycles	(Ateba & Mochaiwa, 2014)
*Spv*C	GGGGCGGAAATACCATCTACA GCGCCCAGGCTAACACG	392	94°C for 90 sec, 94°C for 45 sec, 60°C for 45 sec, 72°C for 90 sec, 72°C for 3 min, 30 cycles	(Olobatoke & Mulugeta, 2015)
*hil*A	CGGAACGTTATTTGCGCCATGCTGAGGTAG GCATGGATCCCCGCCGGCGAGATTGTG	784	94°C, 5 min, 94°C for 1 min, at 65°C for 1 min, 72°C for 1 min, 72°C for 10 min, 30 cycles	(Modarressi & Thong, 2010)
misL	GTCGGCGAATGCCGCGAATA GCGCTGTTAACGCTAATAGT	400	94°C for 3 min, 1 min at 94°C, 1 min at 58°C, 1 min at 72°C, 5 min at 72° C35 cycles	(Zishiri *et al.*, 2016)
orfL	GGAGTATCGATAAAGATGTT GCGCGTAACGTCAGAATCAA	550	94°C for 3 min, 1 min at 94°C, 1 min at 58°C, 1 min at 72°C, 5 min at 72°C 35, cycles	(Zishiri *et al.*, 2016)
spiC	CCTGGATAATGACTATTGAT AGTTTATGGTGATTGCGTAT	309	94°C for 3 min, 1 min at 94°C, 60 min at 55°C, 60 min at 72°C and 5 min at 72°C, 30 cycles	(Zishiri *et al.*, 2016)

5.3 RESULTS

All the *Salmonella* isolates which were *inv*A positive (n = 68) had a 284 bp band (Figure 5.1) and were thus confirmed as *Salmonella* spp. They were then subjected to further virulence determination using the other virulent genes. Of these, *spy* gene was detected in 26 (38%) of *Salmonella* isolates and showed fragments of 401 bp (Figure 5.2); 12 (18%) of the isolates had 303 bp fragments of *SdfI* (Figure 5.3); 14 (20%) isolates had *Spv*C fragments of 392 bp (Figure 5.4); 28 (41%) isolates had *hilA* fragment of 784 bp (Figure 5.5); *mis*L was detected from 43 (63%) isolates with good fragments of 400 bp (Figure 5.6) and *Orf*L was detected from 31 (46%) showing fragments of 550 bp (Figure 5.7). Lastly, *spi*C was detected from 38 (56%) as represented by fragments of 309 bp (Figure 5.8). The virulent genes: *Ppb23*, *fli*B and *fli*C were not detected from any of the isolates from this study.

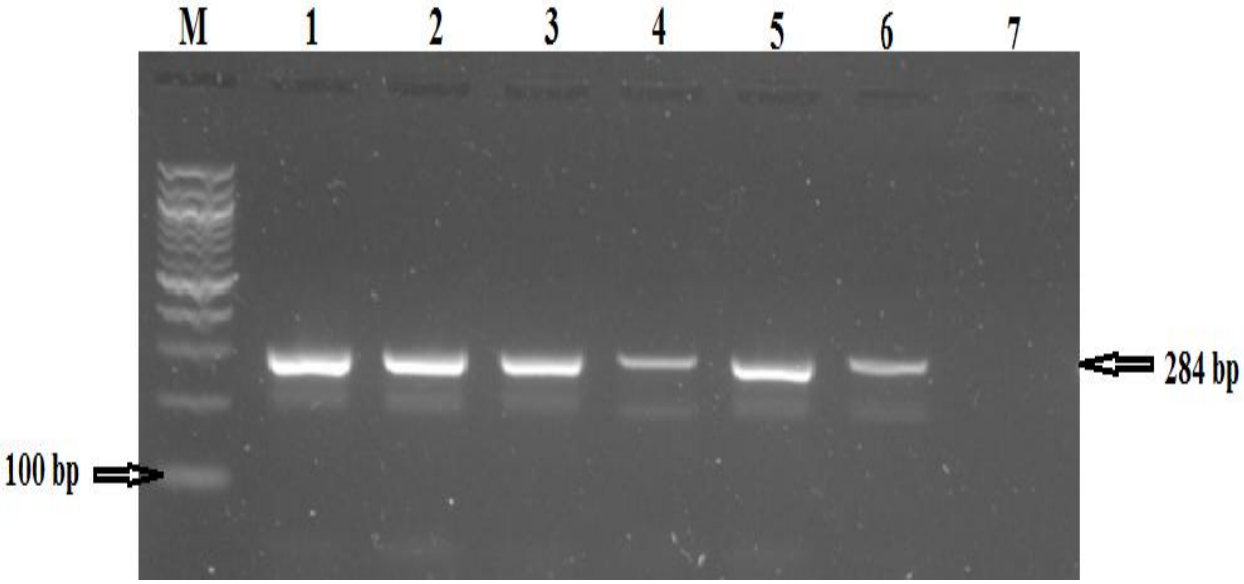


Figure 5. 1: The 284 bp *inv*A gene fragments from six representative isolates by agarose gel electrophoresis. Lane M 100 bp marker; lane 7 negative control; lane 1–6 test samples

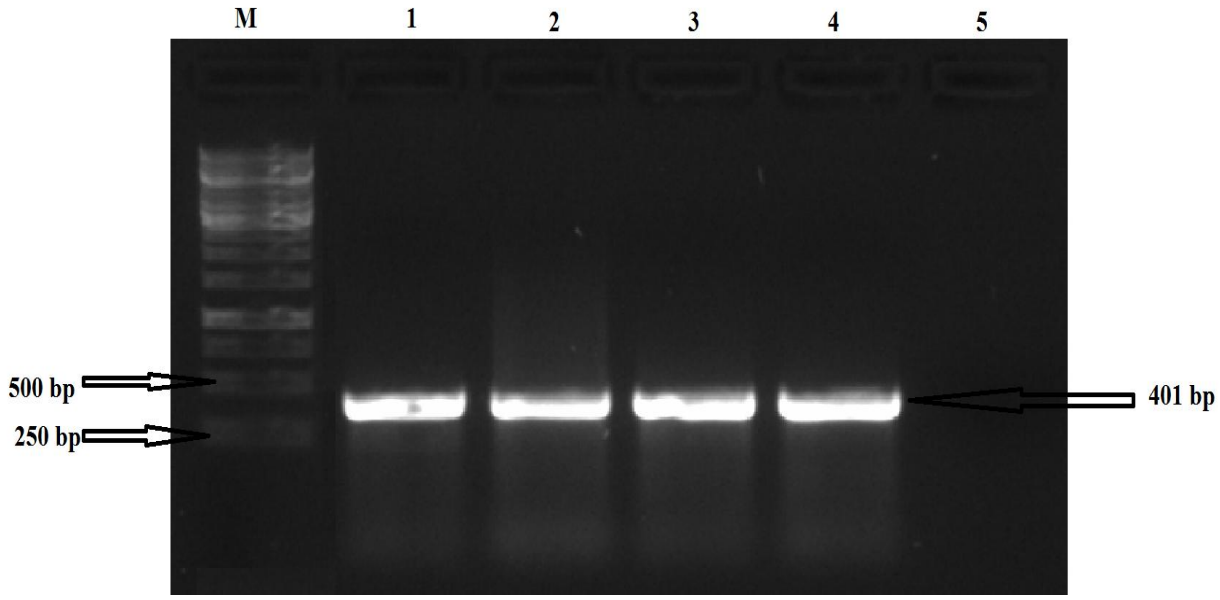


Figure 5. 2: The 401, bp *spy* gene fragments from four representative *Salmonella* isolates by agarose gel electrophoresis. Lane M 1kb marker, Lane 1–4 test samples; lane 5 negative control

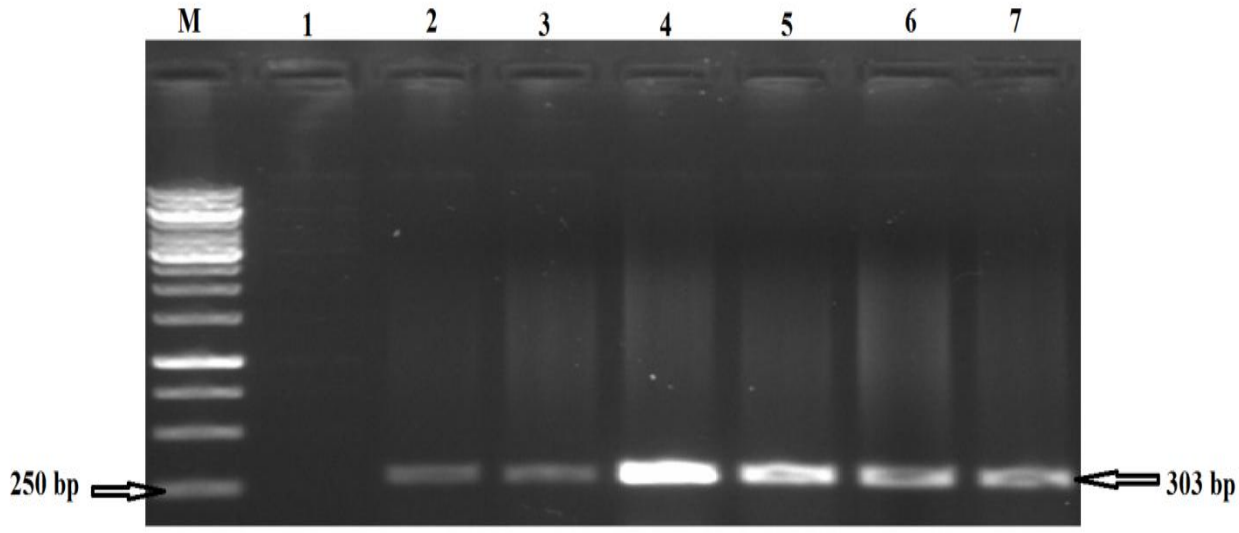


Figure 5. 3: The 303 bp *SdfI* gene fragments from six representative isolates by agarose gel electrophoresis. Lane 1 negative control; lane 2–7 test samples; Lane M 1kb marker

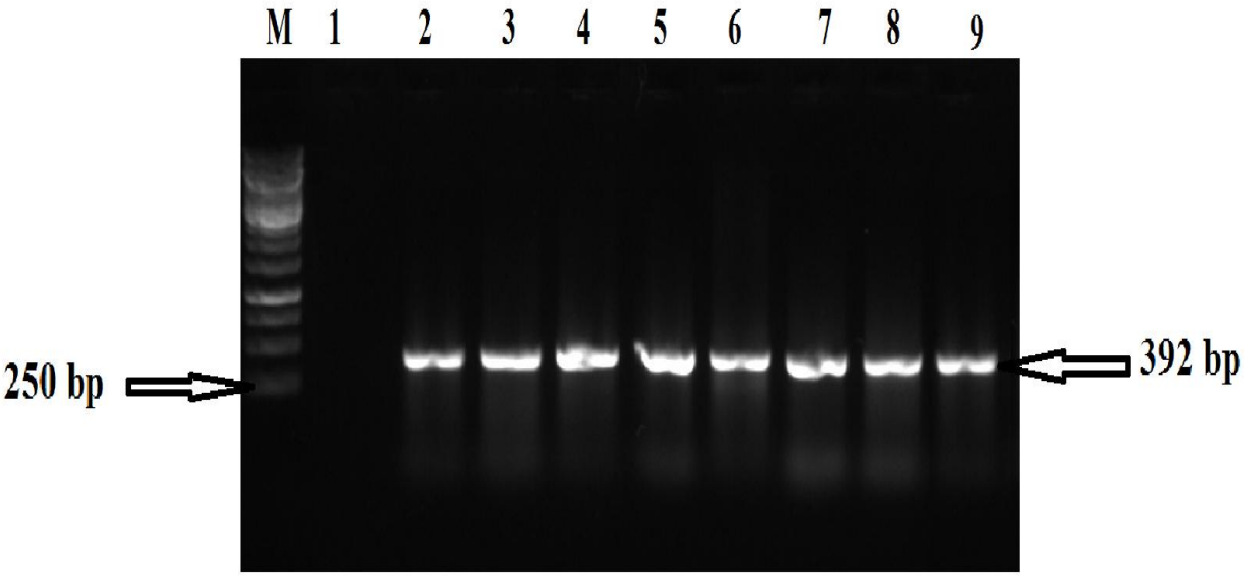


Figure 5. 4: The 392 bp *Spv*C gene fragments from eight representative *S* isolates by agarose gel electrophoresis. Lane M 1kb marker; Lane 1 negative control; lane 2–9 test samples

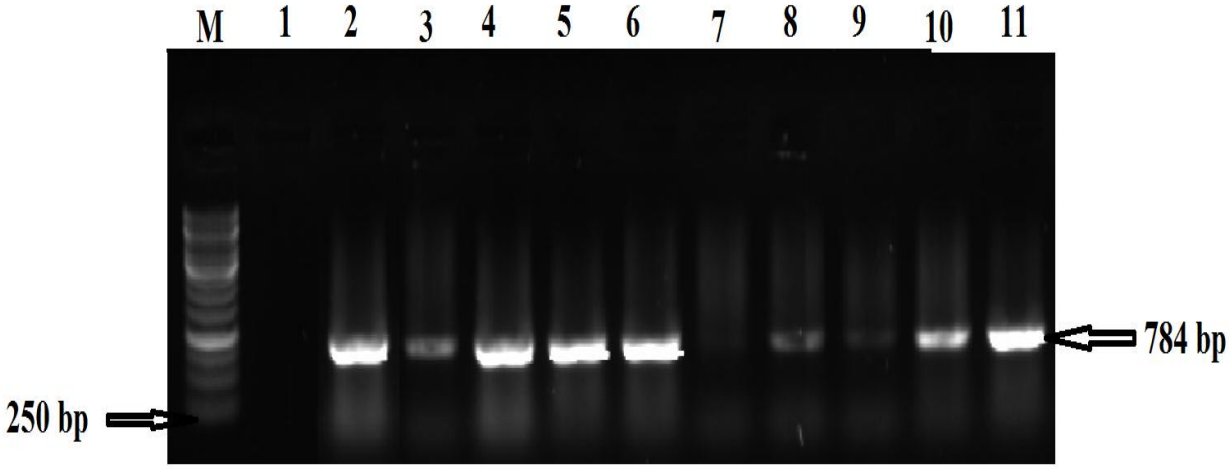


Figure 5. 5: The 784 bp *hil*A gene fragments from ten representative isolates by agarose gel electrophoresis. Lane M 1kb marker; Lane 1 negative control; lane 2–11 test samples

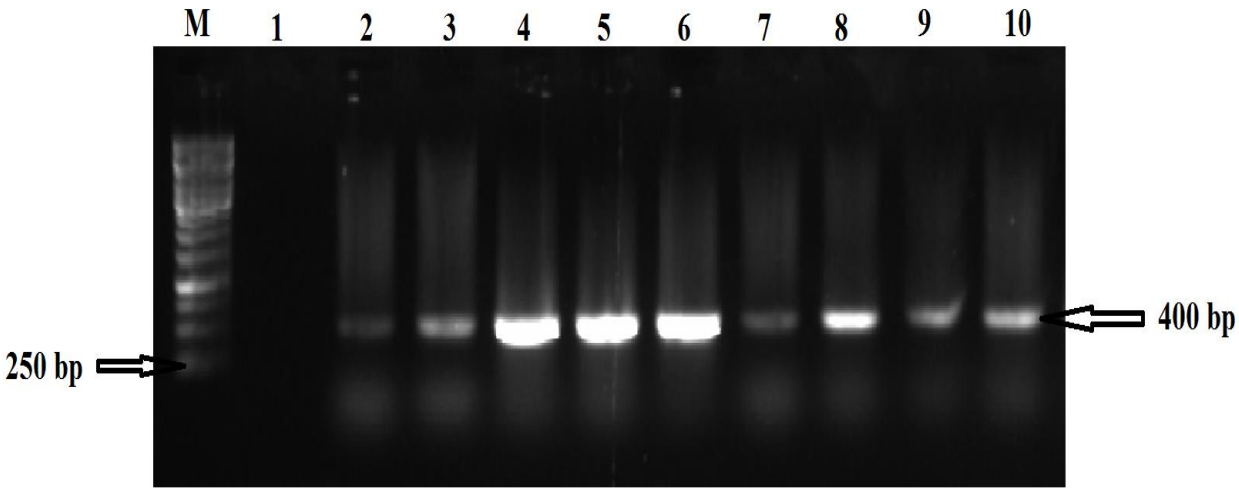


Figure 5. 6: The 400 bp *mis*L gene fragments from nine representative isolates by agarose gel electrophoresis. Lane M 1kb marker; Lane 1 negative control; lane 2–10 test samples

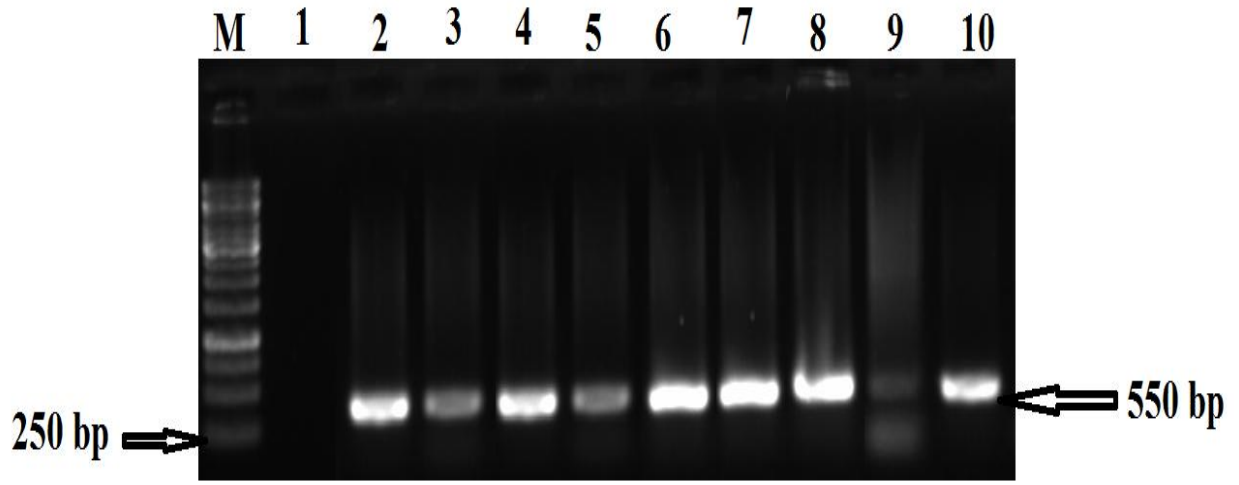


Figure 5. 7: The 550 bp *Orf*L gene fragments from nine representative *Salmonella* isolates by agarose gel electrophoresis. Lane M 1kb marker; Lane 1 negative control; lane 2–10 test samples

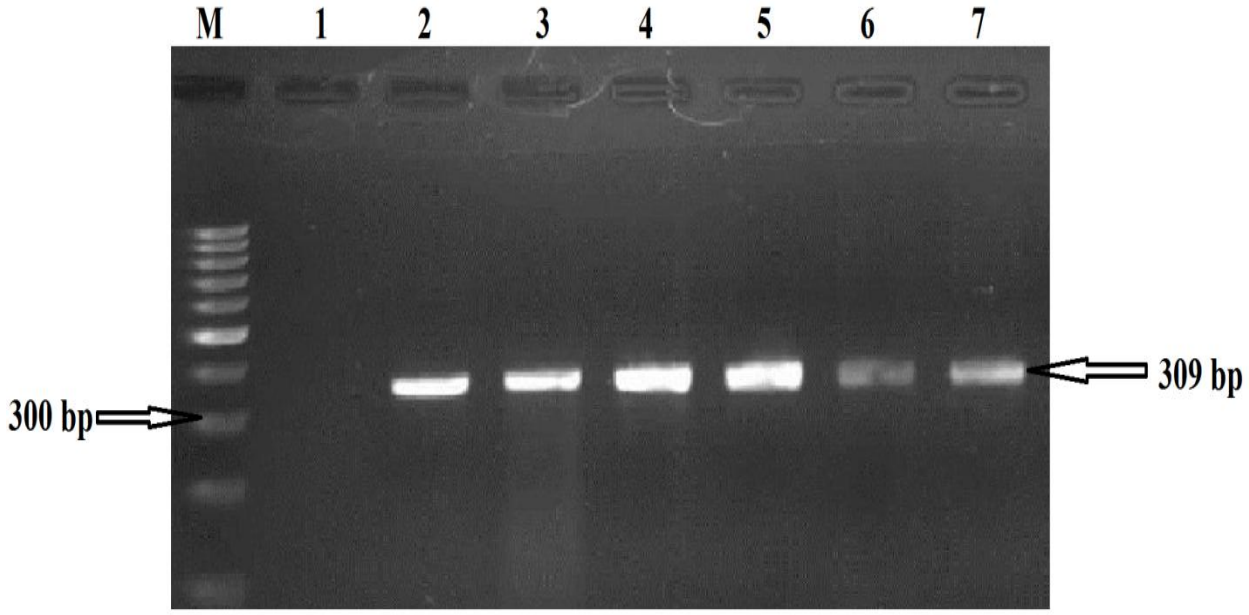


Figure 5. 8: The 309 bp *spi*C gene fragments from eight representative *Salmonella* isolates by agarose gel electrophoresis. Lane M 1kb marker; Lane 1, negative control; lane 2–7, test samples

Table 5.2 below shows which *Salmonella* isolates had virulent genes. Furthermore, the prevalence and distribution of these genes among the six farms was also assessed and is shown in Table 5.3. The bulk of the isolates at each farm were harboring *inv*A, *mis*L, *hil*A and *spi*C genes. The other genes were detected in varying percentages. Unlike *pPB23, fli*C *and fli*B genes which were not amplified from any of the isolates, *Spv*C gene were only absent from all the isolates from farm A and C.

Table 5. 2: Summary of virulence genes determined by PCR

***Salmonella* Isolates**	***inv*A**	***Sdf I***	***hil*A**	***pPB23***	***mis*L**	***fli*C**	***Spy***	***orf*L**	***fli*B**	***spi*C**	***Spv*C**
S. typhimurium	26	–	20(77%)	–	17(65%)	–	26(100%)	13(50%)	–	16(61%)	5(19%)
S. enteritidis	12	12(100%)	–	–	10(82%)	–	–	8(66%)	–	9(75%)	3(25%)
S. newport	8	–	–	–	3(37%)	–	–	2(25%)	–	4(50%)	2(25%)
S. heidelberg	7	–	3(42%)	–	7(100%)	–	–	3(42%)	–	3(42%)	1(14%)
S. bongori	6	–	3(50%)	–	2(33%)	–	–	3(50%)	–	3(50%)	1(14%)
S. enteric Paratyphi B	4	–	–	–	1(25%)	–	–	–	–	2(50%)	–
S. pullorum	2	–	2(100%)	–	–	–	–	2(100%)	–	1(50%)	–
S. tennessee	3	–	–	–	3(100%)	–	–	–	–	–	2(66%)
Total	**68**	**12(18%)**	**28(41%)**	**–**	**43(63%)**	**–**	**26(38%)**	**31(46%)**	**–**	**38(56%)**	**14(20%)**

Table 5. 3: Farm level distribution of *Salmonella* virulence genes among poultry farms

Farms	Isolates	*inv*A	*Sdf I*	*hil*A	*pPB23*	*mis*L	*fli*C	*Spy*	*orf*L	*fli*B	*spi*C	*Spv*C
A	*S.* typhimurium	4	–	4	–	4	–	4	2	–	4	–
	S. enteritidis	3	3	–	–	1	–	–	3	–	2	–
	S. newport	2	–	–	–	–	–	–	–	–	–	–
	S. heidelberg	3	–	1	–	3	–	–	2	–	2	–
	S. bongori	1	–	–	–	–	–	–	1	–	–	–
B	*S.* typhimurium	2	–	1	–	1	–	2	2	–	–	–
	S. enteritidis	1	1	–	–	1	–	–	–	–	1	1
	S. bongori	2	–	2	–	1	–	–	2	–	2	–
C	*S.* typhimurium	2	–	2	–	2	–	2	2	–	2	–
	S. enteritidis	1	1	–	–	1	–	–	1	–	1	–
	S. heidelberg	1	–	1	–	1	–	–	1	–	1	–
	S. enteric Paratyphi B	1	–	–	–	–	–	–	–	–	–	–
D	*S.* typhimurium	1	–	1	–	–	–	1	–	–	–	–
	S. enteritidis	2	2	–	–	2	–	–	–	–	–	2
	S. newport	3	–	–	–	3	–	–	2	–	2	–
E	*S.* typhimurium	16	–	12	–	10	–	16	6	–	10	5

	S. enteritidis	5	5	–	–	5	–	–	5	–	5	–
	S. newport	3	–	–	–	–	–	–	–	–	2	2
	S. heidelberg	3	–	1	–	3	–	–	–	–	–	1
	S. bongori	1	–	1	–	–	–	–	1	–	1	–
	S. enteric Paratyphi B	3	–	–	–	1	–	–	–	–	2	–
	S. pullorum	1	–	1	–	–	–	–	1	–	1	–
	S. tennessee	2	–	–	–	2	–	–	–	–	–	2
H	*S.* typhimurium	1	–	–	–	–	–	1	–	–	–	–
	S. bongori	2	–	–	–	1	–	–	–	–	1	1
	S. pullorum	1	–	1	–	–	–	–	–	–	–	–
	S. tennessee	1	–	–	–	1	–	–	–	–	–	–
	TOTAL	**68**	**12**	**28**	**–**	**43**	**–**	**26**	**31**	**–**	**38**	**14**

Table 5.4 shows the 24 (35.3%) *Salmonella* serotypes carrying more than two virulence genes. Among all the isolates; *S.* typhimurium 8 (11.8%), *S. enteritidis* 6 (8.8%), *S. newport* 2 (2.9%), *S. heidelberg* 3 (4.4%), *S. bongori* 2 (2.9%), *S. tennessee* 2 (2.9%) and *S. pullorum* 3 (4.4%) had more than two virulence genes. Two (2.9%) *S.* typhimurium isolates demonstrated high numbers of up to seven (*inv*A, *hil*A, *mis*L, *Spy*, *orf*L, *spi*C, *Spv*C) virulence genes, followed by 5 (7.4%) *S.* typhimurium isolates which were harboring six genes; *inv*A, *hil*A, *mis*L, *Spy*, *orf*L, *spi*C. On the other hand, 2 (2.9%) *S. enteritidis* each had six (*inv*A, *Sdf I*, *mis*L, *orf*L, *spi*C, *Spv*C) virulence genes. The other 4 isolates, *S. newport* and *S. heidelberg* showed five genes i.e. 1 (1.5%) S. *newport* (*inv*A, *mis*L, *orf*L, *spi*C, *Spv*C), 2 (2.9%) *S. heidelberg* isolates (*invA, hil*A, *mis*L, *Spy*, *orf*L) and 1 (1.5%) *S. heidelberg* isolate (*inv*A, *hil*A, *mis*L, *spi*C, *Spv*C). On the other hand, 2 (2.9%) of *S. bongori* and *S. pullorum* carrying three virulent genes (*inv*A, *orf*L, *spi*C) while none of *S. enteric* paratyphi B isolates was detected with multi-virulent genes.

Table 5. 4: *Salmonella* isolates carrying more than two virulence genes

Genes	Isolates							
	***S.* typhimurium**	***S. enteritidis***	***S. newport***	***S. heidelberg***	***S. bongori***	***S. paratyphi*B**	***S. pullorum***	**S, *tennessee***
*inv*A, *hil*A, *misL, Spy, orfL, spi*C, *Spv*C	2	–	–	–	–	–	–	–
invA, Sdf I, misL, orfL, spiC, SpvC	–	2	–	–	–	–	–	–
invA, hilA, misL, Spy, orfL, spiC	5	–	–	–	–	–	–	–
invA, Sdf I, misL, orfL, spiC	–	4	–	–	–	–	–	–
invA, hilA, misL, Spy, spiC	–	–	–	–	–	–	–	–
invA, hilA, misL, spiC, SpvC	–	–	–	1	–	–	–	–
invA, misL, orfL, spiC, SpvC	–	–	1	–	–	–	–	–
invA, hilA, misL, Spy, orfL	–	–	–	2	–	–	–	–
invA, misL, spiC, SpvC	–	–	1	–	–	–	–	–
invA, hilA, orfL, spiC	–	–	–	–	–	–	1	–
invA, orfL, spiC	–	–	–	–	2	–	2	–
invA, misL, spvC	1	–	–	–	–	–	–	2

5.4 DISCUSSION

The genes *inv*A, *Sdf I*, *Spy*, *Spv*C, *hil*A, *spi*C, *mis*L and *orf*L detected by this study are located in *Salmonella* pathogenicity island-1 and *Salmonella* pathogenicity island-2 and they encode for factors responsible for intestinal and systemic infections (Wu *et al.*, 2017). They are, therefore, indicative of the pathogenic potential of these isolates.

All the isolates had the invasion factor A (*inv*A) gene thus suggesting that they all have the ability to invade and cause gastroenteritis (Goodman *et al.*, 2017; Lan *et al.*, 2018; Li *et al.*, 2018; Sunar *et al.*, 2014). The *inv*A detection is a rapid, specific and sensitive method for *Salmonella*, especially for clinical samples (Ateba & Mochaiwa, 2014; Goodman *et al.*, 2017; Li *et al.*, 2018; Sunar *et al.*, 2014). This, therefore, means all the isolates having the *inv*A can potentially invade the epithelial cells of the animal and this can lead to development of a disease (Hu *et al.*, 2008; Ochman & Groisman, 1996). The *inv*A gene is located in the Pathogenicity island-1 and it is a type 3 secretion system apparatus, which secretes invasion effectors like invasion factor A (Sabbagh *et al.*, 2010).

The *spv*C gene was detected from only 14 (20%) of isolates. *Spv* genes are located on virulence plasmids located in the subspecies 1 lineage of *Salmonella* serovars such as *S. enteric* (Guiney & Fierer, 2011). It is translocated into the host cell cytoplasm by the SPI-2 TTSS (Browne *et al.*, 2008; Valle & Guiney, 2005). The *spv*C is required for survival within the host cell (Chaudhary *et al.*, 2015; Haneda *et al.*, 2012). *Spv*C gene influences the cellular pathways engaged in the pathogenesis of intracellular *Salmonella* infection (Browne *et al.*, 2008). Furthermore, *Spv*C gene is required for systemic infection (Ammar *et al.*, 2016; Chaudhary *et al.*, 2015; Gulig *et al.*, 1993; Haneda *et al.*, 2012; Hur *et al.*, 2011). The *Spv* genes have been shown to be responsible for intra-macrophages survival (Guiney & Fierer, 2011). They are situated within an extremely

homologous region enclosed on virulence plasmids (Guiney & Fierer, 2011; Kaur & Jain, 2012). Some serovars such as *S. enteritidis*, *S. choleraesuis*, *S.* typhimurium, *S. arizona* and *S. dublin* have been identified that carried the *Spv* gene from human disease (Amini *et al.*, 2010; Guiney & Fierer, 2011). A study has shown that five *Salmonella* serovars carry a virulence plasmid that has the *Spv* gene namely: *S. choleraesuis, S. enteritidis, S.* typhimurium, *S. dublin* and *S. gallinarum-S. pullorum* (Gulig *et al.*, 1993). The current study only found the gene in *S. newport, S.* typhimurium, *S. heidelberg, S. tennessee, S. bongori* and *S. enteritidis*.

The results obtained from this study showed that *spi*C gene was the second highest gene 38 (55%) detected. This gene it is a type III secretion system apparatus (Dione *et al.*, 2011), encoded within *Salmonella* pathogenicity island 2 protein C (Guiney & Fierer, 2011; Kaur & Jain, 2012; Uchiya & Nikai, 2008). It inhibits endosome-endosome fusion in vitro then affects the host cell by inhibiting fusion of SCV with lysosomes and endosomes thus interfering with normal trafficking of the transferring receptor (Marcus *et al.*, 2000). This gene helps the survival of *Salmonellae* inside the macrophage and their systemic spreading (Wu *et al.*, 2017).

Twenty eight (41%) isolates in this study were carrying the *hil*A gene. The *hil*A gene is a significant characteristic of *Salmonella* pathogenesis, as it is essential for bacterial colonization of the extracellular, luminal compartment of the host intestine (Pathmanathan *et al.*, 2003; Peixoto *et al.*, 2017). According to a study carried by Addwebi *et al.* (2014), it was established that *hil*A gene is also involved in the colonization of the spleen and liver. It is a transcriptional activator encoded in SPI1 and is required for expression and it's a regulator in the overall scheme of SPI1 genes (Pathmanathan *et al.*, 2003).

The *mis*L gene was detected from 63% of the isolates. It is located in the Pathogenicity island 3 (SPI-3) of *Salmonella* and it aids virulence by being involved in intra-macrophage survival by the pathogen (Hughes *et al.*, 2008).

Only 45% of the isolates in this study were harboring the *orf*L gene. *Salmonella* pathogenicity islands SP1-4 encode for the *orf*L genes (Zishiri *et al.*, 2016). It helps with adhesion and survival in macrophages (Niedergang *et al.*, 2000). It also has a secretion system that mediates the secretion of toxins (Hughes *et al.*, 2008; Odjadjare & Olaniran, 2015; Sánchez-Jiménez *et al.*, 2010) thus aiding in the pathogenicity of the *Salmonella* strain involved. It can survive in microphages (Dione *et al.*, 2011).

The *Spy* gene was found to be carried by all [26 (38%)] *S.* typhimurium isolates. *Spy* gene has been used by many types of research to identify *S.* typhimurium (Al *et al.*, 2016; Can *et al.*, 2014; de Freitas *et al.*, 2010; Olsen *et al.*, 1995). The *Spy* has been confirmed to act as an adenosine and Tri-phosphate (ATP) sovereign molecular chaperone (Wells, 2015). It assists protein breakdown in the periplasm by expression of *Spy* following its accumulation in a previously unstable protein (Quan *et al.*, 2011; Wells, 2015). ATP is required by chaperones at the sub-stoichiometric concentrations at which *Spy* functions (Powers & Balch, 2011; Wells, 2015). So *spy* does not have a well-known regulatory purpose and appears to exclusively act as a molecular chaperone (Wells, 2015).

The *SdfI* gene was detected in 12 (17%) of isolates in this study. This gene has been used for detection of *S. enteritidis* in previous studies (Al *et al.*, 2016; Ghazaey & Mirmomeni, 2012; Mirmomeni *et al.*, 2009; Mohd Afendy & Son, 2015; Shah *et al.*, 2011). An *SdfI* function is

encoded in *S. enteritidis* and is unique for identification of *S. enteritidis* (Agron *et al.*, 2001; de Freitas *et al.*, 2010; Mohd Afendy & Son, 2015; Trafny *et al.*, 2006).

The occurrence of several virulence genes indicates the high pathogenic potential of the isolates and this was seen especially for *S. enteritidis* and *S.* typhimurium isolates. These results agree with a previous study where S. *enteritidi*s and S. typhimurium strains were reported to be carrying high numbers of virulence genes (Bertelloni *et al.*, 2017). This is not surprising because, these two strains (*S.* typhimurium and *S. enteritidi*s) have been identified to be the main common causes of diseases in humans (Collard *et al.*, 2008; Fisher, 1999; Kaku *et al.*, 1995; Reddy *et al.*, 2010; Vaillant *et al.*, 2005; Varma *et al.*, 2005) and also in some animals (Barrow, 1990; Galán, 1996; Rodríguez *et al.*, 2018). Therefore, this study confirmed that these *Salmonellae* serovars from rats were also potentially pathogenic.

In conclusion, the findings from this study reveal the occurrence of virulence genes among *Salmonella* isolates from rats sampled at different farms in Mafikeng North West South Africa. These findings show that the rats are not only carrying *Salmonella* but that the *Salmonella* that they carry has the ability to cause diseases in both humans and livestock because they harbor virulent genes that are found in clinical cases of salmonellosis.

5.5 REFERENCES

Addwebi, T.M., Call, D.R. & Shah, D.H. 2014. Contribution of *Salmonella enteritidis* virulence factors to intestinal colonization and systemic dissemination in 1-day-old chickens. *Poultry Science*, 93(4):871-881.

Agron, P.G., Walker, R.L., Kinde, H., Sawyer, S.J., Hayes, D.C., Wollard, J. & Andersen, G.L. 2001. Identification by subtractive hybridization of sequences specific for *Salmonella* enterica serovar *enteritidis*. *Applied and Environmental Microbiology,* 67(11):4984-4991.

Al, S., Hizlisoy, H., Onmaz, N.E., Yildirim, Y. & Gönülalan, Z. 2016. Occurrence and antimicrobial resistance of *Salmonella* enterica subsp. enterica serovars typhimurium, *enteritidis*, and *typhi* isolated from chicken eggs and poultry products. *Turkish Journal of Veterinary and Animal Sciences,* 40(6):737-743.

Alvarez, J., Sota, M., Vivanco, A.B., Perales, I., Cisterna, R., Rementeria, A. & Garaizar, J. 2004. Development of a multiplex PCR technique for detection and epidemiological typing of *Salmonella* in human clinical samples. *Journal of Clinical Microbiology,* 42(4):1734-1738.

Amini, K., Salehi, T.Z., Nikbakht, G., Ranjbar, R., Amini, J. & Ashrafganjooei, S.B. 2010. Molecular detection of *inv*A and spv virulence genes in *Salmonella enteritidis* isolated from human and animals in Iran. *African Journal of Microbiology Research,* 4(21):2202-2210.

Ammar, A.M., Mohamed, A.A., El-Hamid, M.I.A. & El-Azzouny, M.M. 2016. Virulence genotypes of clinical *Salmonella* Serovars from broilers in Egypt. *The Journal of Infection in Developing Countries*, 10(04):337-346.

Ateba, C. & Mochaiwa, B. 2014. Use of invA gene specific PCR analysis for the detection of virulent *Salmonella* species in beef products in the north west province, South Africa. *Journal of Food and Nutrition Research,* 2(6):294-300.

Barrow, P. 1990. Immunity to experimental fowl typhoid in chickens induced by a virulence plasmid-cured derivative of *Salmonella gallinarum*. *Infection and Immunity*, 58(7):2283-2288.

Berkes, J., Viswanathan, V., Savkovic, S. & Hecht, G. 2003. Intestinal epithelial responses to enteric pathogens: effects on the tight junction barrier, ion transport, and inflammation. *Gut*, 52(3):439-451.

Bertelloni, F., Tosi, G., Massi, P., Fiorentini, L., Parigi, M., Cerri, D. & Ebani, V.V. 2017. Some pathogenic characters of paratyphoid *Salmonella* enterica strains isolated from poultry. *Asian Pacific Journal of Tropical Medicine*, 10(12):1161-1166.

Browne, S.H., Hasegawa, P., Okamoto, S., Fierer, J. & Guiney, D.G. 2008. Identification of *Salmonella* SPI-2 secretion system components required for *Spv*B-mediated cytotoxicity in macrophages and virulence in mice. *FEMS Immunology & Medical Microbiology*, 52(2):194-201.

Can, H.Y., Elmali, M., Karagöz, A. & Öner, S. 2014. Detection of *Salmonella* spp., *Salmonella enteritidis*, *Salmonella typhi* and *Salmonella* typhimurium in cream cakes by polymerase chain reaction (PCR). *Medycyna Weterynaryjna*, 70(11):689-692.

Chaudhary, J., Nayak, J., Brahmbhatt, M. & Makwana, P. 2015. Virulence genes detection of *Salmonella* serovars isolated from pork and slaughterhouse environment in Ahmedabad, Gujarat. *Veterinary World*, 8(1):121.

Chiu, C.-H. & Ou, J.T. 1996. Rapid identification of *Salmonella* serovars in feces by specific detection of virulence genes, *inv*A and *spv*C, by an enrichment broth culture-multiplex PCR combination assay. *Journal of Clinical Microbiology*, 34(10):2619-2622.

Clavijo, R.I., Loui, C., Andersen, G.L., Riley, L.W. & Lu, S. 2006. Identification of genes associated with survival of *Salmonella* enterica serovar *enteritidis* in chicken egg albumen. *Applied and Environmental Microbiology*, 72(2):1055-1064.

Collard, J., Bertrand, S., Dierick, K., Godard, C., Wildemauwe, C., Vermeersch, K., Duculot, J., Van Immerseel, F., Pasmans, F. & Imberechts, H. 2008. Drastic decrease of *Salmonella enteritidis* isolated from humans in Belgium in 2005, shift in phage types and influence on foodborne outbreaks. *Epidemiology & Infection*, 136(6):771-781.

Darwin, K.H. & Miller, V.L. 2000. The putative invasion protein chaperone *Sic*A acts together with *Inv*F to activate the expression of *Salmonella* typhimurium virulence genes. *Molecular Microbiology*, 35(4):949-960.

Dauda, M. 2010. Enterobacteria in drinking water: a public health hazard. *Reviews in Infection*, 1(5):224-230.

de Freitas, C.G., Santana, Â.P., da Silva, P.H.C., Gonçalves, V.S.P., Barros, M.d.A.F., Torres, F.A.G., Murata, L.S. & Perecmanis, S. 2010. PCR multiplex for detection of *Salmonella enteritidis*, *typhi* and typhimurium and occurrence in poultry meat. *International Journal of Food Microbiology*, 139(1-2):15-22.

Dione, M.M., Ikumapayi, U., Saha, D., Mohammed, N.I., Adegbola, R.A., Geerts, S., Ieven, M. & Antonio, M. 2011. Antimicrobial resistance and virulence genes of non-typhoidal** *Salmonella*** isolates in The Gambia and Senegal. *Journal of Infection in Developing Countries*, 5(11):765-775.

Ekwanzala, M.D., Abia, A.L.K., Keshri, J. & Momba, M.N.B. 2017. Genetic characterization of *Salmonella* and *Shigella* spp. isolates recovered from water and riverbed sediment of the Apies River, South Africa. *Water SA*, 43(3):387-397.

Fisher, I. 1999. *Salmonella enteritidis* in Western Europe 1995-98-a surveillance report from Enter-net. *Euro surveillance: bulletin Europeen sur les maladies transmissibles= European Communicable Disease Bulletin*, 4(5):56-56.

Galán, J.E. 1996. Molecular genetic bases of *Salmonella* entry into host cells. *Molecular Microbiology*, 20(2):263-271.

Ghazaey, S. & Mirmomeni, M.H. 2012. Microbial-resistant *Salmonella enteritidis* isolated from poultry samples. *Reports of Biochemistry & Molecular Biology*, 1(1):9.

Goodman, L.B., McDonough, P.L., Anderson, R.R., Franklin-Guild, R.J., Ryan, J.R., Perkins, G.A., Thachil, A.J., Glaser, A.L. & Thompson, B.S. 2017. Detection of *Salmonella* spp. in veterinary samples by combining selective enrichment and real-time PCR. *Journal of Veterinary Diagnostic Investigation*, 29(6):844-851.

Guiney, D.G. & Fierer, J. 2011. The role of the spv genes in *Salmonella* pathogenesis. *Frontiers in Microbiology*, 2(129):1-10.

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(6):825-830.

Haneda, T., Ishii, Y., Shimizu, H., Ohshima, K., Iida, N., Danbara, H. & Okada, N. 2012. *Salmonella* type III effector *Spv*C, a phosphothreonine lyase, contributes to reduction in inflammatory response during intestinal phase of infection. *Cellular Microbiology*, 14(4):485-499.

Hayward, R.D. & Koronakis, V. 1999. Direct nucleation and bundling of actin by the *Sip*C protein of invasive *Salmonella*. *The EMBO Journal*, 18(18):4926-4934.

Hu, Q., Coburn, B., Deng, W., Li, Y., Shi, X., Lan, Q., Wang, B., Coombes, B.K. & Finlay, B.B. 2008. *Salmonella* enterica serovar Senftenberg human clinical isolates lacking SPI-1. *Journal of Clinical Microbiology*, 46(4):1330-1336.

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. 2008. Characterisation of *Salmonella* enterica serotype typhimurium isolates from wild birds in northern England from 2005–2006. *BMC Veterinary Research*, 4(1):4.

Hur, J., Kim, J.H., Park, J.H., Lee, Y.-J. & Lee, J.H. 2011. Molecular and virulence characteristics of multi-drug resistant *Salmonella enteritidis* strains isolated from poultry. *The Veterinary Journal*, 189(3):306-311.

Kaku, M., Peresi, J., Tavechio, A., Fernandes, S., Batista, A., Castanheira, I., Garcia, G., Irino, K. & Gelli, D. 1995. Surto alimentar por *Salmonella enteritidis* no noroeste do estado de São Paulo, Brasil. *Revista de Saúde Pública*, 29:127-131.

Kaur, J. & Jain, S. 2012. Role of antigens and virulence factors of *Salmonella* enterica serovar *typhi* in its pathogenesis. *Microbiological Research*, 167(4):199-210.

Lan, T.T., Gaucher, M.-L., Nhan, N.T., Letellier, A. & Quessy, S. 2018. Distribution of virulence genes among *Salmonella* serotypes isolated from pigs in Southern Vietnam. *Journal of food protection*, 81(9):1459-1466.

Leung, K.Y., Siame, B.A., Snowball, H. & Mok, Y.-K. 2011. Type VI secretion regulation: crosstalk and intracellular communication. *Current Opinion in Microbiology*, 14(1):9-15.

Li, X., Liu, L., Li, Q., Xu, G. & Zheng, J. 2018. *Salmonella* contamination in layer farms in China: Detection and Genetic Analysis. *The Journal of Poultry Science*, 55(1):1-9.

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(2):842-851.

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

Mirmomeni, M., Naderi, S., Colagar, A.H. & Sisakhtnezhad, S. 2009. Isolation of *Salmonella enteritidis* using biochemical tests and diagnostic potential of SdfI amplified gene. *Research Journal of Biological Sciences*, 4(6):656-661.

Modarressi, S. & Thong, K.L. 2010. Isolation and molecular sub typing of *Salmonella* enterica from chicken, beef and street foods in Malaysia. *Scientific Research and Essays*, 5(18):2713-2720.

Mohd Afendy, A. & Son, R. 2015. Pre-enrichment effect on PCR Detection of *Salmonella enteritidis* in artificially-contaminated raw chicken meat. *International Food Research Journal*, 22(6):2571-2576.

Niedergang, F., Sirard, J.-C., Blanc, C.T. & Kraehenbuhl, J.-P. 2000. Entry and survival of *Salmonella* typhimurium in dendritic cells and presentation of recombinant antigens do not require macrophage-specific virulence factors. *Proceedings of the National Academy of Sciences*, 97(26):14650-14655.

Ochman, H. & Groisman, E.A. 1996. Distribution of pathogenicity islands in *Salmonella* spp. *Infection and Immunity*, 64(12):5410-5412.

Odjadjare, E.C. & Olaniran, A.O. 2015. Prevalence of antimicrobial resistant and virulent *Salmonella* spp. in treated effluent and receiving aquatic milieu of wastewater treatment plants in Durban, South Africa. *International Journal of Environmental Research and Public Health*, 12(8):9692-9713.

Olobatoke, R.Y. & Mulugeta, S.D. 2015. Incidence of non-typhoidal *Salmonella* in poultry products in the North West Province, South Africa. *South African Journal of Science*, 111(11-12):1-7.

Olsen, J., Aabo, S., Rasmussen, O. & Rossen, L. 1995. Oligonucleotide probes specific for the genus *Salmonella* and for *Salm.* typhimurium. *Letters in Applied Microbiology*, 20(3):160-163.

Parkhill, J., Dougan, G., James, K., Thomson, N., Pickard, D., Wain, J., Churcher, C., Mungall, K., Bentley, S. & Holden, M. 2001. Complete genome sequence of a multiple drug resistant *Salmonella* enterica serovar typhi CT18. *Nature*, 413(6858):848-852.

Pathmanathan, S., Cardona-Castro, N., Sanchez-Jimenez, M., Correa-Ochoa, M., Puthucheary, S. & Thong, K. 2003. Simple and rapid detection of *Salmonella* strains by direct PCR amplification of the *hil*A gene. *Journal of Medical Microbiology*, 52(9):773-776.

Peixoto, R.J., Alves, E.S., Wang, M., Ferreira, R.B., Granato, A., Han, J., Gill, H., Jacobson, K., Lobo, L.A. & Domingues, R.M. 2017. Repression of *Salmonella* host cell invasion by aromatic small molecules from the human fecal metabolome. *Applied and Environmental Microbiology*, 83(19):e01148-17.

Perera, K. & Murray, A. 2008. Development of a PCR assay for the identification of *Salmonella* enterica serovar *brandenburg*. *Journal of Medical Microbiology*, 57(10):1223-1227.

Powers, E.T. & Balch, W.E. 2011. Protein folding: protection from the outside. *Nature*, 471(7336):42-43.

Quan, S., Koldewey, P., Tapley, T., Kirsch, N., Ruane, K.M., Pfizenmaier, J., Shi, R., Hofmann, S., Foit, L. & Ren, G. 2011. Genetic selection designed to stabilize proteins uncovers a chaperone called *Spy*. *Nature Structural and Molecular Biology*, 18(3):262-269.

Rahn, K., De Grandis, S., Clarke, R., McEwen, S., Galan, J., Ginocchio, C., Curtiss Iii, R. & Gyles, C. 1992. Amplification of an *inv*A gene sequence of *Salmonella* typhimurium by polymerase chain reaction as a specific method of detection of *Salmonella*. *Molecular and Cellular Probes*, 6(4):271-279.

Reddy, E.A., Shaw, A.V. & Crump, J.A. 2010. Community-acquired blood stream infections in Africa: a systematic review and meta-analysis. *The Lancet infectious Diseases*, 10(6):417-432.

Rodríguez, F.I., Procura, F. & Bueno, D.J. 2018. Comparison of 7 culture methods for *Salmonella* serovar *enteritidis* and *Salmonella* serovar typhimurium isolation in poultry feces. *Poultry Science*. 97(11): 3826-3836.

Sabbagh, S.C., Forest, C.G., Lepage, C., Leclerc, J.M. & 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):1-13.

Sánchez-Jiménez, M.M., Cardona-Castro, N.M., Canu, N., Uzzau, S. & Rubino, S. 2010. Distribution of pathogenicity islands among Colombian isolates of *Salmonella*. *The Journal of Infection in Developing Countries*, 4(09):555-559.

Schechter, L.M. & Lee, C.A. 2000. *Salmonella* invasion of non-phagocytic cells. Bacterial Invasion Into Eukaryotic Cells. Springer. p. 289-320.

Shah, D.H., Zhou, X., Addwebi, T., Davis, M.A. & Call, D.R. 2011. In vitro and in vivo pathogenicity of S*almonella enteritidis* clinical strains isolated from North America. *Archives of Microbiology*, 193(11):811-821.

Sunar, N., Stentiford, E., Stewart, D. & Fletcher, L. 2014. Molecular techniques to characterize the *inv*A genes of *Salmonella* spp. for pathogen inactivation study in composting. *arXiv preprint arXiv:1404.5208*.

Trafny, E., Kozłowska, K. & Szpakowska, M. 2006. A novel multiplex PCR assay for the detection of *Salmonella* enterica serovar *enteritidis* in human faeces. *Letters in Applied Microbiology*, 43(6):673-679.

Uchiya, K.-i. & Nikai, T. 2008. *Salmonella* virulence factor *Spi*C is involved in expression of flagellin protein and mediates activation of the signal transduction pathways in macrophages. *Microbiology*, 154(11):3491-3502.

Vaillant, V., Valk, H.D., Baron, E., Ancelle, T., Colin, P., Delmas, M.-C., Dufour, B., Pouillot, R., Strat, Y.L. & Weinbreck, P. 2005. Foodborne infections in France. *Foodbourne Pathogens & Disease*, 2(3):221-232.

Valle, E. & Guiney, D.G. 2005. Characterization of *Salmonella*-induced cell death in human macrophage-like THP-1 cells. *Infection and immunity*, 73(5):2835-2840.

Varma, J.K., Greene, K.D., Ovitt, J., Barrett, T.J., Medalla, F. & Angulo, F.J. 2005. Hospitalization and antimicrobial resistance in *Salmonella* outbreaks, 1984–2002. *Emerging infectious diseases*, 11(6):943-946.

Wells, H. 2015. Further characterisation of the envelope stress responses of *Salmonella* Typhimurium. Doctoral dissertation, University of East Anglia. https://ueaeprints.uea.ac.uk/59224/1/H.C.WELLS_(4006879)_-_PhD_Thesis_Sept_2015.pdf [2018-05-18].

Wu, S.-y., Wang, L.-d., Xu, G.-m., Deng, Q.-f., Li, Y.-y. & Huang, R. 2017. spv locus aggravates *Salmonella* infection of zebrafish adult by inducing Th1/Th2 shift to Th2 polarization. *Fish & shellfish immunology*, 67:684-691.

Zhai, L., Yu, Q., Bie, X., Lu, Z., Lv, F., Zhang, C., Kong, X. & Zhao, H. 2014. Development of a PCR test system for specific detection of *Salmonella* paratyphi *B* in foods. *FEMS microbiology letters*, 355(1):83-89.

Zishiri, O.T., Mkhize, N. & Mukaratirwa, S. 2016. Prevalence of virulence and antimicrobial resistance genes in *Salmonella* spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. *Onderstepoort Journal of Veterinary Research*, 83(1):1-11.

CHAPTER 6− ANTIMICROBIAL RESISTANCE OF *SALMONELLA* ISOLATES FROM RATS USING DISK DIFFUSION AS WELL AS THROUGH RESISTANCE GENES KNOWN FOR EACH OF THE ANTIBIOTICS

ABSTRACT

The widespread use of antibiotics for the treatment of bacterial infections and growth promotion in the poultry industry has seen an enormous increase in antibiotic resistance worldwide. Antibiotics confer resistance in a number of ways and this resistance can be determined by many methods including phenotypic means and the use of known resistance genes. Rats are usually exposed to both bacteria in poultry houses as well as antibiotics used on that farm so any resistance patterns in them will generally reflect what is pertaining in both the poultry and human environments. Hence, the aim of this study was to determine the antimicrobial resistance of *Salmonella* isolated from rats using disk diffusion as well as known resistance genes. On disk diffusion, most of the *Salmonella* isolates showed resistance to Rifampicin 68 (100%), Tetracycline 32 (47.1%), Ciprofloxacin 21 (30.9%), Sulphonamides 12 (17.6%), Cephalothin 12 (17.6%), Chloramphenicol 9 (13.2%), Streptomycin 8 (11.8%), Enrofloxacin 6 (8.8%), Ampicillin 6 (8.8%), Amoxicillin/clavulanic acid 2 (2.9%) and Nalidixic acid 1 (1.5%). All *Salmonella* isolates were susceptible to Gentamicin. Several *Salmonella* serovars showed multiple drug resistance of up to four different antibiotics. Using molecular means, all the tested resistant genes (*tet, cat, bla*TEM, *sul, qnrA, aadA*) were detected in this study from some of *Salmonella* isolates. The findings in the present study confirmed seventy seven percent (n=52) isolates harbouring class 1 integrons at variable regions. We conclude that antibiotic resistance in *Salmonella* spp. rat's samples from Mafikeng is significant.

Keywords: resistance genes, antibiotic resistance pattern, class 1 integrons,

6.1. INTRODUCTION

Several antimicrobial agents are used in animals/human beings to prevent or treat certain diseases caused (Raissy & Ansari, 2011; Reyes Herrera, 2010). Despite the obvious benefits, improper use of antibiotics can lead to bacterial resistance against infectious diseases (Hong *et al.*, 2018; Singh *et al.*, 2018; Zeina *et al.*, 2013). Approximately 95% of antibiotics given to livestock are excreted unchanged therefore bacteria living on people who have usual contact with animal waste or in an environment close to animal waste are constantly exposed to antibiotics and may build up resistance (Choi, 2007; Fedorka-Cray *et al.*, 2002).

Antibiotics resistance can be conferred by either innate or acquired means (Schroeder *et al.*, 2017). It can be by Horizontal Gene Transfer (HGT) or Elevated Mutation Rates (Schroeder *et al.*, 2017). The other form is adaptive resistance; this includes enzymatic driven antibiotic inactivation, efflux pump regulation drug resistance in biofilms, changes in the antibiotic target and also environmentally provoke genetic changes such as biofilms and persister development (Brooks *et al.*, 2013; Schroeder *et al.*, 2017). A regular mechanism of antibiotic resistance requires the enzymatic destruction or modification of the antibiotic (Wilson, 2014; Wright, 2005). It is always coupled with alteration of the antibiotic target (Schroeder *et al.*, 2017), changes in cell permeability and efflux (Schroeder *et al.*, 2017; Wright, 2005). In general, mechanisms of antibiotic resistance occurs into three categories such as efflux in transport or permeability of the antimicrobial, replacement or modification of the antimicrobial target and inactivation of the antimicrobial (Foley & Lynne, 2008; Frye & Jackson, 2013).

Recent studies have revealed that *Salmonella* serotypes like *S. heidelberg*, *S.* typhimurium, *S. infantis, S. uganda, S. newport, S. typhi, S. paratyphi, S. agona* and *S. hadar* exhibit antibiotic resistance (Ghazaey & Mirmomeni, 2012; Kagambèga *et al.*, 2018; Mathole *et al.*, 2017; Odoch

et al., 2018; Olobatoke & Mulugeta, 2015; Özgen, 2007; Thung *et al.*, 2017; Zhao *et al.*, 2007; Zhao *et al.*, 2017). Some *Salmonella* species have been reported to be resistant to antibiotics such as Chloramphenicol, Tetracycline and Ampicillin, with escalating frequency in many countries (Kagambèga *et al.*, 2018; Odoch *et al.*, 2018; Olobatoke & Mulugeta, 2015; Thung *et al.*, 2017). Antimicrobial resistance, especially in *Salmonella* serovars, has been implicated to play a role in their virulence (Islam *et al.*, 2016; Mathole *et al.*, 2017).

Salmonella species have been established to contain different antibiotic resistance genes (Abatcha *et al.*, 2018; Thong & Modarressi, 2011). Most of these resistance genes are positioned on the plasmids, bacterial chromosome, transposons, clustered either resistance and transferred by mobile genetic elements (Wannaprasat *et al.*, 2011). Apart from the genes, resistance to integrons class 1, 2 and 3 has also been identified from *Salmonella* species (Odoch *et al.*, 2018; Povilonis *et al.*, 2010). The majority of the antibiotic resistance genes are mostly located in Class 1 integrons (Faldynova *et al.*, 2003). Integron Class 1 integron-mediated antimicrobial resistance is widespread among diverse *Salmonella* serovars (Khemtong & Chuanchuen, 2008; Srisanga *et al.*, 2017; Thong & Modarressi, 2011).

Although rodents are not treated with antibiotics, antibiotic-resistant bacteria occur in them because they may pick resistant bacteria from the environment and other sources. Rats can thus be used as good sentinels of monitoring antibiotic resistance in a given area. Transmission of antimicrobial resistance from rats to chickens and humans can be in the form of resistant pathogens carrying transferable resistance genes.

Currently, there is inadequate information about the antibiotic resistance pattern of *Salmonella* spp. in rats found in Mafikeng, North West, South Africa. This data is important since there are

plenty of rats in the poultry/human environment and it is well known that transfer of resistance genes can occur from rats to chickens/poultry products then human beings.

6.2. MATERIAL AND METHODS

6.2.1 Study site, sampling, bacterial isolation, identification and DNA extraction

Study site, sampling, sample preparation, isolation and *Salmonella* identification was done as described in details under section 3.2.1, 3.2.2, 4.2.4−4.2.13, respectively.

6.2.2 Phenotypic antimicrobial resistance of *Salmonella* isolates

The phenotypic antibiotic resistance test was performed by means of Kirby-Bauer disc diffusion method (Magiorakos *et al.*, 2012). Pure *Salmonella* isolates were sub-cultured on nutrient agar medium (Merck, Wadeville, South Africa), incubated at 37°C for up to 24 hours. Then fresh overnight cultures were used for antibiotic sensitivity tests. Aliquots of 100 µL from the suspensions were spread-plated on Mueller Hinton agar (MH) using a sterile cotton swab and plates were incubated for 24 hours at 37°C. Single disc diffusion method was used to assess the susceptibility of S*almonella* isolates to commonly used antimicrobial agents. A total of 12 antibiotic disc (Davies diagnostics, SA) were used in this study, containing; Gentamicin (CA 10 µg), Ciprofloxacin (CIP 5 µg), Rifampicin (RD 5 µg), Chloramphenicol (C 30 µg), Nalidixic acid (NA 30 µg), Ampicillin (AMP 10 µg), Enrofloxacin (ENR 5 µg), Tetracycline (TE 30 µg), Cephalothin (KF 30 µg), Sulphonamides (SSS 300 µg), Streptomycin (S 10 µg) and Amoxicillin/clavulanic Acid (AMC 30 µg). The antimicrobial profile of isolated *Salmonella* to

various antibiotics was determined following recommendations of the clinical laboratory institute standards interpreted as intermediate (I), sensitive (S), and resistant (R) (Watts *et al.*, 2008). *E. coli* ATCC 25922 was used as a reference strain for control (Bauer *et al.*, 1966).

Strains which showed resistance to at least 3 classes of antibiotics were considered as multi-drug-resistant (MDR) isolates (Abdel-Maksoud *et al.*, 2015; Zhao *et al.*, 2017). Table 6.1 shows how results were interpreted, it does not show.

Table 6. 1: Information on antibiotics used to investigate antimicrobial resistance obtained from clinical laboratory institute standards (Watts *et al.*, 2008).

Antibiotic	Abbreviation	Antibiotic concentrations disc(μg)	Inhibition zone (mm)		
			Resistant	Intermediate	Susceptible
Ampicillin	AMP	(10 μg)	≤13	14−16	≥17
Sulphonamides	SSS	(300 μg)	≤12	13−16	≥17
Cephalothin	KF	(30 μg)	≤14	15−17	≥18
Tetracycline	TE	(30 μg)	≤11	12−14	≥15
Ciprofloxacin	CIP	(5 μg)	≤20	21−30	≥31
Nalidixic acid	NA	(30 μg)	≤13	14−18	≥19
Chloramphenicol	C	(30 μg)	≤12	13−17	≥18
Gentamicin	CA	(10 μg)	≤12	13−14	≥15
Enrofloxacin	ENR	(5 μg)	≤16	17−20	≥21
Rifampicin	RD	(5 μg)	≤16	17−19	≥20
Streptomycin	S	(10 μg)	≤11	12−14	≥15
Amoxicillin/clavulanic Acid	AMC	(30 μg)	≤13	14−17	≥18

6.2.3. Genotypic antimicrobial resistant of *Salmonella* isolates

A total of six antimicrobial resistance genes (*bla*TEM, *tet*, *sul*, *cat*, *qnrA*, and *aadA*) were amplified by PCR using different primer sets targeting different antimicrobial resistance genes conferring resistance, including class 1 integrons. The details of oligonucleotide sequences, their base pairs (bp) including the PCR cycling conditions are shown in Table 6.2. Resistance to aminoglycoside is associated with carriage of *aadA* gene; resistance to quinolones is associated with carriage of *qnrA* gene; resistance to *β*-lactams is associated with carriage of a *bla*TEM (ESBL gene) gene; resistance to Chloramphenicol is associated with carriage of *cat* gene; resistance to sulfonamide is associated with carriage of *sul* genes; and resistance to tetracycline is associated to *tet* gene. Furthermore, *intI1* is associated with class 1 integrons.

Table 6. 2: Primer sequences specific to different antimicrobial resistant determinants in *Salmonella* spp.

Primers	Sequence (5'-3')	Amplicon size (bp)	Conditions	References
Sul3	F: TCAACATAACCTCGGACAGT	707	94°C, 5 mins; 30 cyc of 94°C, 30 S; 60°C for 40 s, 72°C for 30 S, ext. at 72°C, 5 mins	(Özgen, 2007)
Sul4	R: GATGAAGTCAGCTCCACCT			
tem1	F ATGAGTATTCAACATTTCCGTG	792	95°C, 3 min; 30 cyc. of 95°C, 1 mins, 55°C, l min, 72°C, 1 min, ext. at 72°C, 5 mins	(Olobatoke & Mulugeta, 2015),
tem2	R TTACCAATGCTTAATCAGTGAG			
tet	F: GCACTTGTCTCCTGTTTACTCCCC	659	94°C, 2 mins; 35 cyc. of 94°C, 20 S, 53°C, 10 S, 65°C, 45 s, ext. at 65°C, 4 mins	(Olobatoke & Mulugeta, 2015)
tet	R: CCTTGTGGTTATGTTTTGGTTCCG			
cat	F: TCCCAATGGCATCGTAAAGAAC	310	95°C, 5 mins; 35 cyc. of 94°C, 30 S, 55°C, 30 S, 72°C, 30 s, ext. at 72°C, 10 mins	(Olobatoke & Mulugeta, 2015)
cat	R: TCGTGGTATTCACTCGAGAGCG			
aadA	F: ATCCTTCGGCGCGATTTTG	282	94 °C, 3 mins; 30 cyc, 94 °C 30 s, 62 °C 30 s, 72 °C 1 mins, ext at 72 °C, 7 mins	(Aarestrup *et al.*, 2003)
aadA	R: GCAGCGCAATGACATTCTTG			
qnrA	F: TCAGCAAGAGGATTTCTCA	627	95°C, 5 mins, 30 cyc, 94C 40 s, 50°C 60 s, 72°C 90 s, ext at 72C, 10 mins	(Akiyama & Khan, 2011)
qnrA	R: GGCAGCACTATGACTCCCA			
intI1	F: ACATGTGATGGCGACGCACGA	568	95°C 3 mins; 30 cyc , at 95°C 30 s, 55°C 30 s, 72°C 30 s; ext 72°C for 10 mins	(Dunowska *et al.*, 2007)
intI1	R: ATTTCTGTCCTGGCTGGCGA			

Cyc= cycles, ext= extension, min= minutes, s= seconds

6.3 Data analysis

The antimicrobial resistance of a isolates was calculated as the percentage of isolates among the group that were resistant to a single antibiotic or a number of antibiotics (Johnson, Rajic & McMullen, 2005). Tables and graphs were used to display the relationships of various variables and also for comparative analysis of data on antimicrobial resistance pattern of *Salmonella* strains.

6.4 RESULTS

6.4.1 Phenotypic antimicrobial resistance of *Salmonella* spp.

The antibiotic resistances obtained for the isolates tested are shown in Table 6.3, and their inhibition zones are indicated in Appendix F2. The isolates demonstrated resistance to Rifampicin 68 (100%), Tetracycline 32 (47.1%), Ciprofloxacin 21 (30.9%), Sulphonamides 12 (17.6%), Cephalothin 12 (17.6%), Chloramphenicol 9 (13.2%), Streptomycin 8 (11.8%), Enrofloxacin 6 (8.8%), Ampicillin 3 (4.4%), Amoxicillin/clavulanic acid 2 (2.9%) and Nalidixic acid 1 (1.5%). All the isolates were susceptible to gentamicin. Among the *Salmonella* isolates, *Salmonella typhimurium* had the highest percentage of resistance followed by *Salmonella enteritidis* (Table 6.4).

Table 6. 3: Antimicrobial resistance of *Salmonella* isolates from rats

Antibiotic	Abbreviation	Breakpoint disc (µg)	Resistant	Intermediate
Ampicillin	AMP	(10 µg)	6	10
Sulphonamides	SSS	(300 µg)	12	–
Cephalothin	KF	(30 µg)	12	5
Tetracycline	TE	(30 µg)	32	–
Nalidixic Acid	NA	(30 µg)	1	18
Chloramphenicol	C	(30 µg)	9	8
Gentamicin	CA	(10 µg)	–	–
Enrofloxacin	ENR	(5 µg)	6	6
Rifampicin	RD	(5 µg)	68	–
Streptomycin	S	(10 µg)	8	12
Ciprofloxacin	CIP	(5 µg)	21	9
Amoxicillin/clavulanic Acid	AMC	(30 µg)	2	1

Table 6. 4: Percentage of antimicrobial resistance among *Salmonella* isolates from rats

Antimicrobial agents	*S.* typhimurium (n=26)	*S. enteritidis* (n=12)	*S. newport* (n=8)	*S. heidelberg* (n=7)	*S. bongori* (n=6)	*S. paratyphi* B (n=4)	*S. pullorum* (n=2)	*S. tennessee* (n=3)
AMP (10 µg)	2(7.7%)	–	1(12.5%)	2(28.6%)	1(16.7%)	–	–	–
SSS (300 µg)	6(23%)	4(33.3%)	–	1(14.3%)	1(16.7%)	–	–	–
KF (30 µg)	7(26.9)	2(16.7%)	–	1(14.3%)	2(33.3%)	–	–	–
TE (30 µg)	19(73%)	2(16.7%)	5(62.5%)	3(42.9%)	2(33.3%)	–	1(50%)	–
NA (30 µg)	–	1(8.3%)	–	–	–	–	–	–
C (30 µg)	5(19%)	2(16.7%)	–	1(14.3%)	–	1(25%)	–	–
CA (10 µg)	–	–	–	–	–	–	–	–
ENR (5 µg)	3(11.5%)	2(16.7%)	1(12.5%)	–	–	–	–	–
RD (5 µg)	26(100%)	12(100%)	8(100%)	7(100%)	6(100%)	4(100%)	2(100%)	3(100%)
S (10 µg)	4(15.4%)	–	3(37.5%)	1(14.3%)	–	–	–	–
CIP (5µg)	8(30.7%)	5(41.7%)	1(12.5%)	3(43.9%)	2(33.3%)	2(50%)	–	–
AMC (30 µg)	–	–	–	1(14.3%)	–	1(25%)	–	–

CA= Gentamicin, C= Chloramphenicol, CIP= Ciprofloxacin, NA= Nalidixic acid, RD= Rifampicin, AMP= Ampicillin, ENR= Enrofloxacin, TE= Tetracycline, KF= Cephalothin and SSS =Sulphonamides, S= Streptomycin, AMC= Amoxicillin/clavulanic Acid

Two isolates of *S. typhimurium* showed a multidrug resistance up to five different antibiotics (Rifampicin, Tetracycline, Enrofloxacin, Cephalothin, Ciprofloxacin, and Sulphonamides), one isolate of *S. enteritidis* and *S.* typhimurium to five (Rifampicin, Tetracycline, Enrofloxacin, Cephalothin and Ciprofloxacin) and one *S. enteritidis* to five (Rifampicin, Enrofloxacin, Cephalothin, Ciprofloxacin and Streptomycin). Furthermore, different isolates were resistance to three to four antibiotics as listed in Table 6.5.

Table 6. 5: *Salmonella* antimicrobial resistance and the prevalence of resistant strains in rats

MAR Phenotypes	Isolates							
	***S.* typhimurium**	***S. enteritidis***	***S. newport***	***S. heidelberg***	***S. bongori***	***S. paratyphi*B**	***S. pullorum***	S, *tennessee*
RD,TE,ENR,KF,CIP,SSS	2	–	–	–	–	–	–	–
RD,TE,ENR,KF,CIP	1	1	–	–	–	–	–	–
RD,ENR,KF,CIP,S	–	1	–	–	–	–	–	–
RD,C,TE,KF,CIP	–	1	–	–	1	–	–	–
RD,AMP,SSS,C	1	–	–	–	–	–	–	–
RD,AMP,TE,SSS	1	–	–	–	–	–	–	–
RD,C,TE,CIP	–	–	1	–	–	–	–	–
RD,C,TE,SSS	1	–	–	–	–	–	–	–
RD,TE,ENR,KF	1	–	–	–	–	–	–	–
RD,CA,TE,ENR	–	–	–	–	1	–	–	–
RD,TE,CIP,SSS	–	1	–	–		–	1	–
RD,AMP,KF,SSS	–	–	1	–	–	–	–	–
RD,TET,KF	–	–	–	1	–	–	–	–
RD,C,SSS	–	–	–	1	–	–	1	–
RD,AMP,CIP	–	–	1	–	–	–	–	–
RD,C,S	–	–	–	–	–	1	–	–
RD,TE,S	–	–	–	–	–	–	–	1

MAR= Multiple Antibiotic resistance

6.4.2 Genotypic antimicrobial resistant including class 1 integrons

The results of the analysis of *Salmonella* isolates for the occurrence of antibiotic resistance genes (ARGs) using conventional PCR are shown in Figures 6.1, 6.2, 6.3, 6.4, 6.5, 6.6 and 6.7.

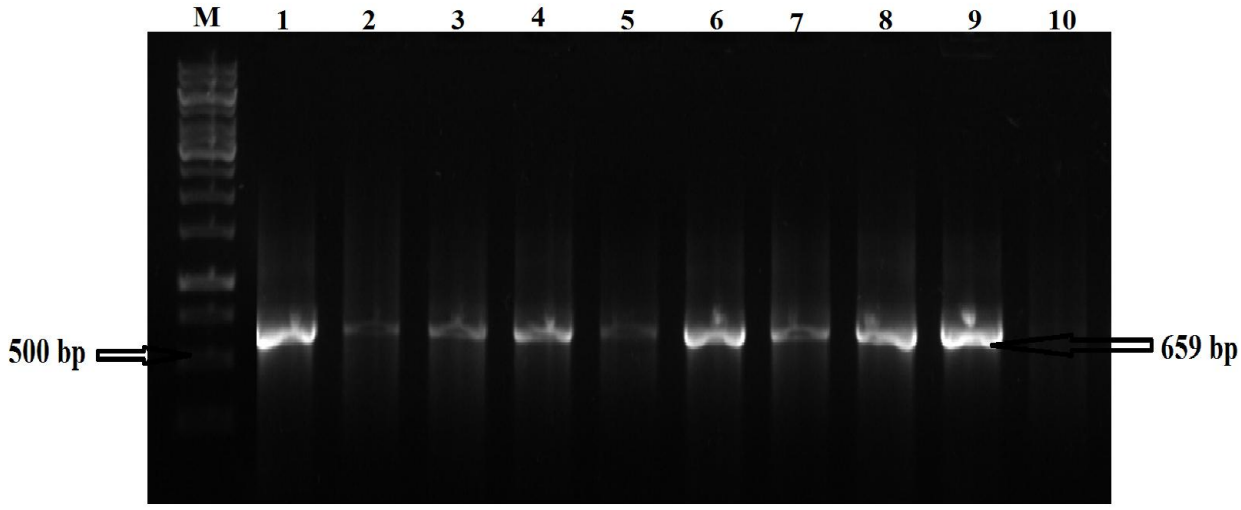


Figure 6. 1: Detection of the 659 bp *tet* gene fragments from nine representative isolates by agarose gel electrophoresis: Lane M 250 bp marker; Lane 1–9 test samples; Lane 10 negative control.

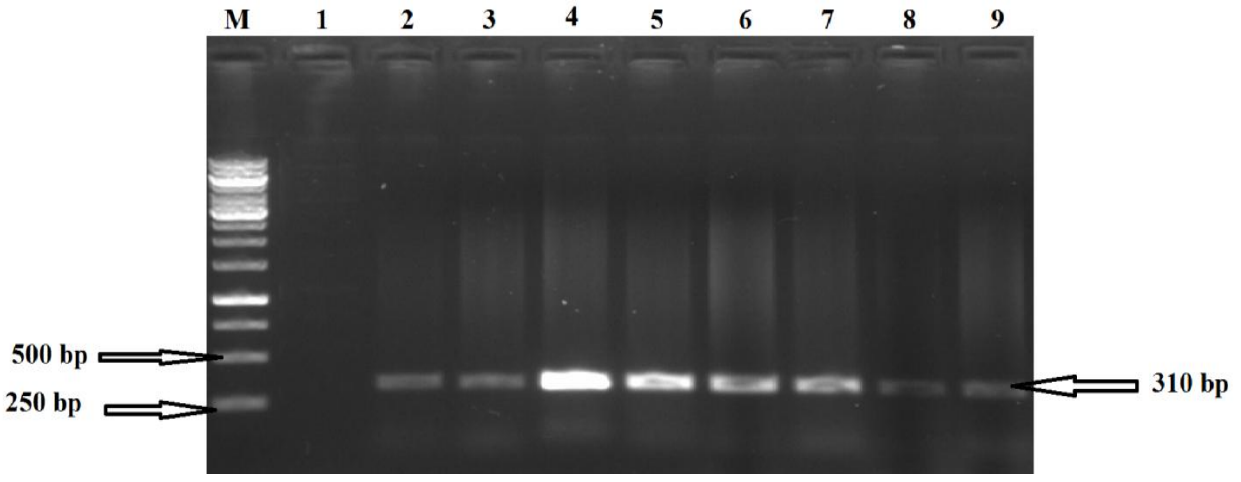


Figure 6. 2: Detection of the 310 bp *cat* gene fragments from eight representative isolates by agarose gel electrophoresis: Lane M 250 bp marker; Lane 1 negative control; Lane 2–9 test samples.

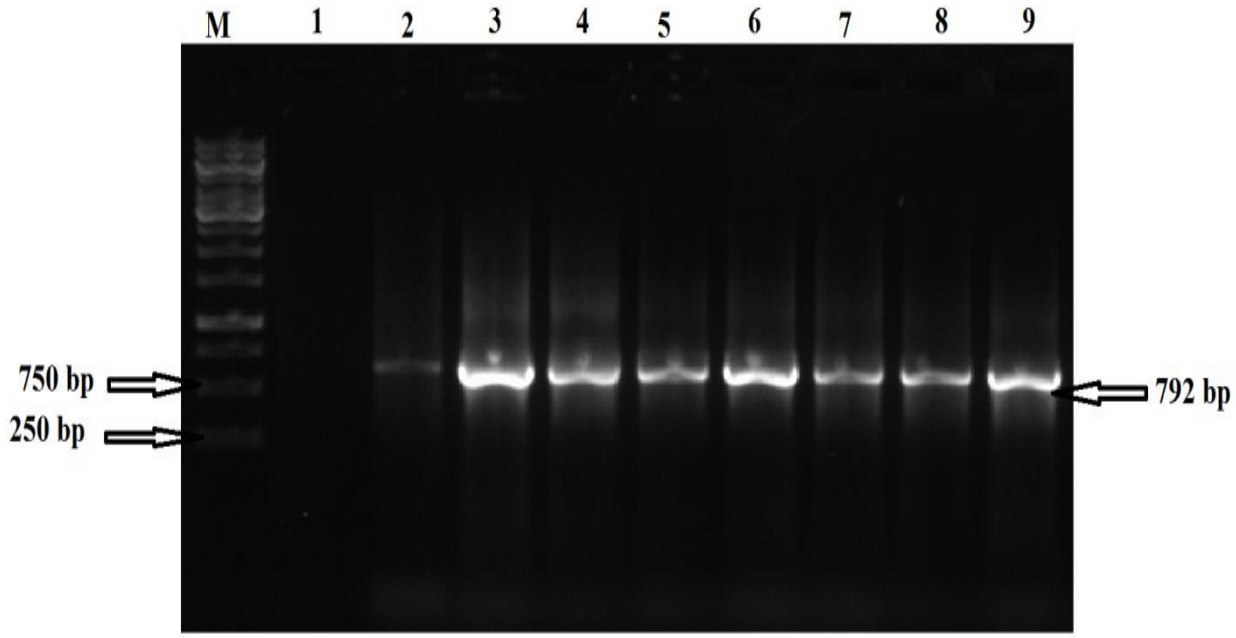


Figure 6. 3: Detection of the 792 bp *bla*TEM gene fragments from eight representative isolates by agarose gel electrophoresis: Lane M 250 bp marker; Lane 1 negative control; Lane 2–9 test samples.

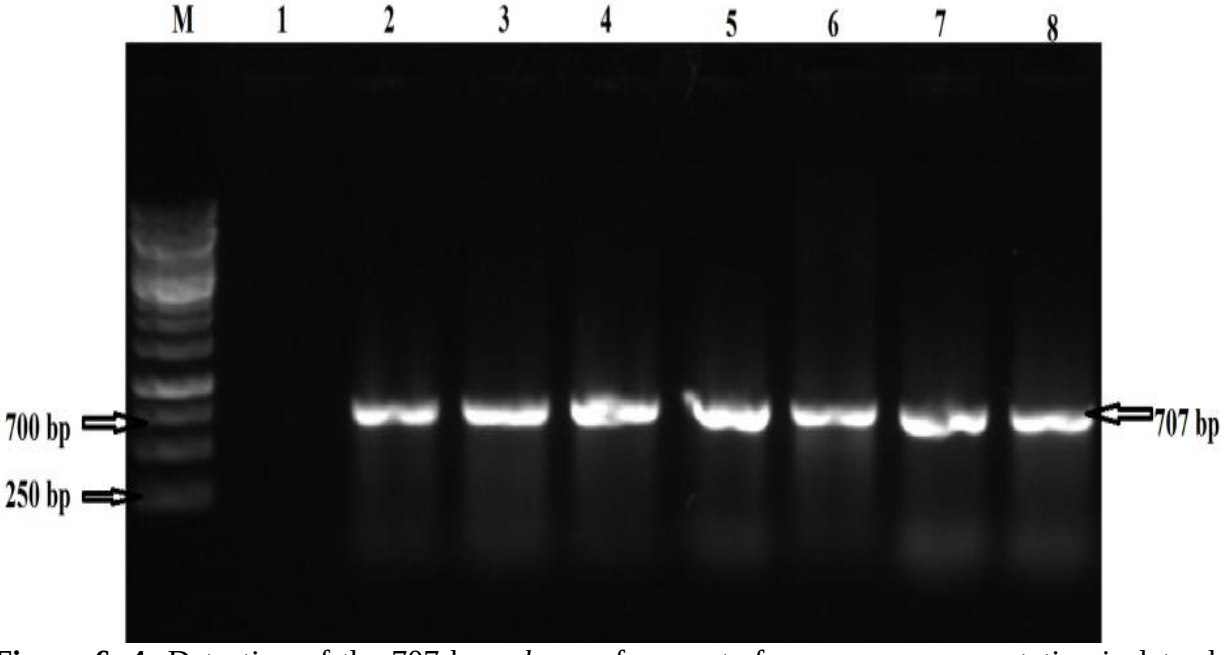


Figure 6. 4: Detection of the 707 bp *sul* gene fragments from seven representative isolates by agarose gel electrophoresis: Lane M 250 bp marker; Lane 1 negative control; Lane 2–8 test samples.

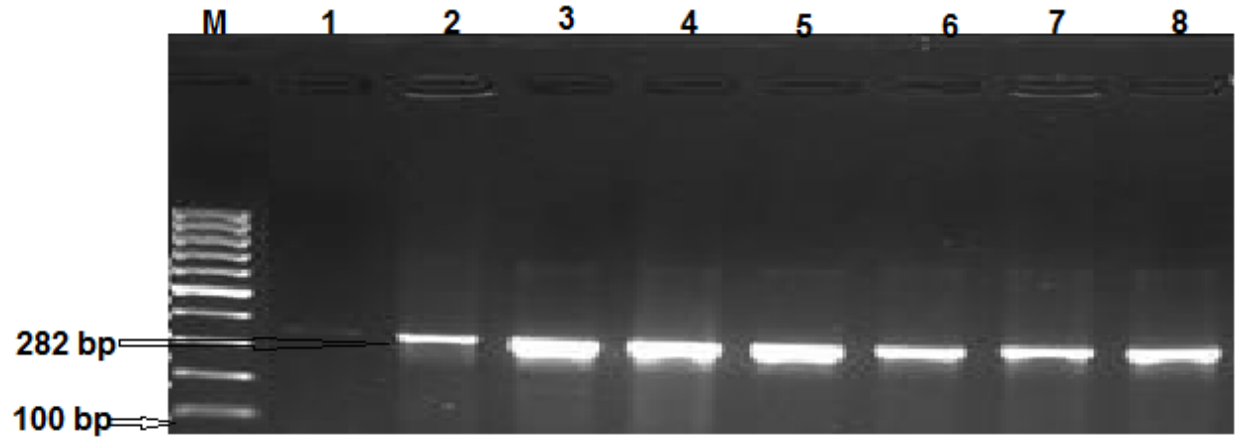


Figure 6. 5: Detection of the 282 bp *aadA* gene afragments from six representative isolates by agarose gel e electrophoresis: Lane M 100 bp marker; Lane 1 negative control; Lane 2–8 test samples.

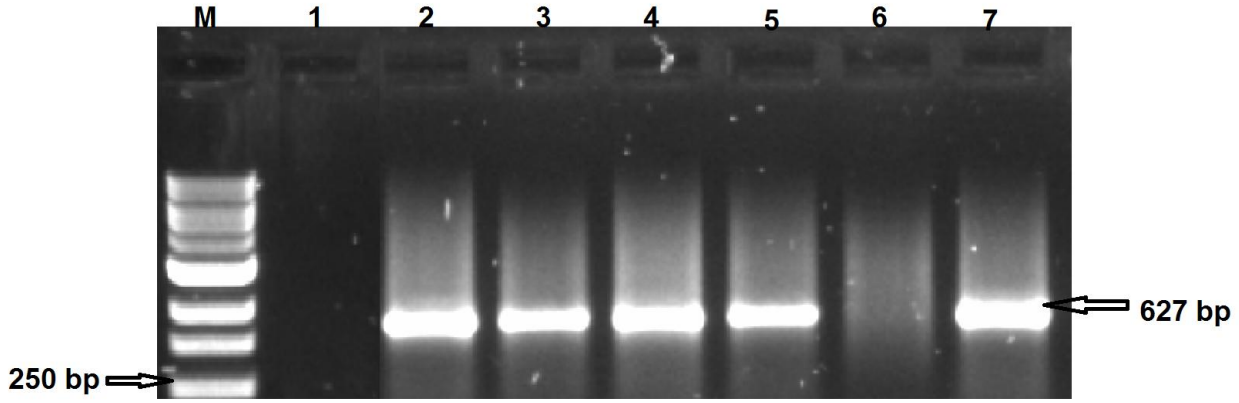


Figure 6. 6: Detection of the 627 bp *qnrA* gene amplicon from representative *Salmonella* isolates by agarose gel electrophoresis: Lane M, 250 bp marker; Lane 1, negative control; Lane 2–6 and 7 test samples

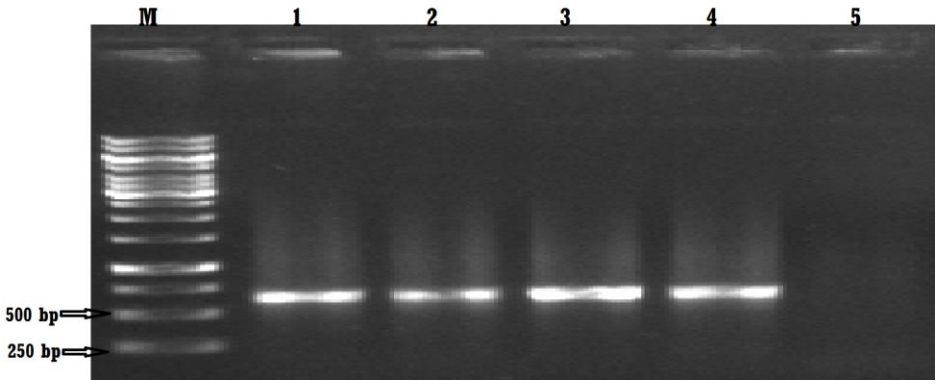


Figure 6. 7: Detection of the 568 bp *ntI*1 gene amplicon from seven representative isolates by agarose gel electrophoresis: Lane 5 negative control; Lane 1–4 test samples; Lane M 250 bp marker.

Table 6.6 shows 52 (77%) isolates encoding class 1 integrons detected from *Salmonella* spp. of which 26 (38%) were *S.* typhimurium followed by *S. enteritidis* 10 (15%), then *S. heidelberg* 4 (6%), *S. bongori* with 3 (4%), *S. newport* 4 (6%), *S. enteric serovar paratyphi* B 2 (3%), *S. tennessee* 2 (3%), then lastly *S. pullorum* 1 (2%) as shown in Table 6.7. One isolate (*S. typhimurium*) was carrying Class 1 Integrons gene however, it was not harbouring one of the resistance genes used in this study. *Salmonella* isolates harbouring antibiotic resistance genes are shown in Figure 6.8.

Table 6. 6: Antibiotic resistance genes among the different *Salmonella* isolates from rats collected from the poultry houses around Mafikeng

Antimicrobial agent	Class of antimicrobials	Genes tested	No. of isolates	Resistant phenotype (%)	Integrons and resistance genes (%)
Chloramphenicol	Phenicol	*cat*	68	9(13%)	9(13%)
Tetracycline	Tetracycline	*tet*	68	32(47%)	16(24%)
Ampicillin	Beta-lactam	*blaTEM*	68	3(4%)	1(2%)
Sulfonamide	Sulfonamide	*Sul*	68	12(18%)	6(9%)
Streptomycin	Aminoglycoside	*aadA*	68	8(12%)	4(6%)
Ciprofloxacin	Quinolones	*qnr-A*	68	21(31%)	15(22%)
Class 1 integrons	−	*intI1*	68	−	52(77%)

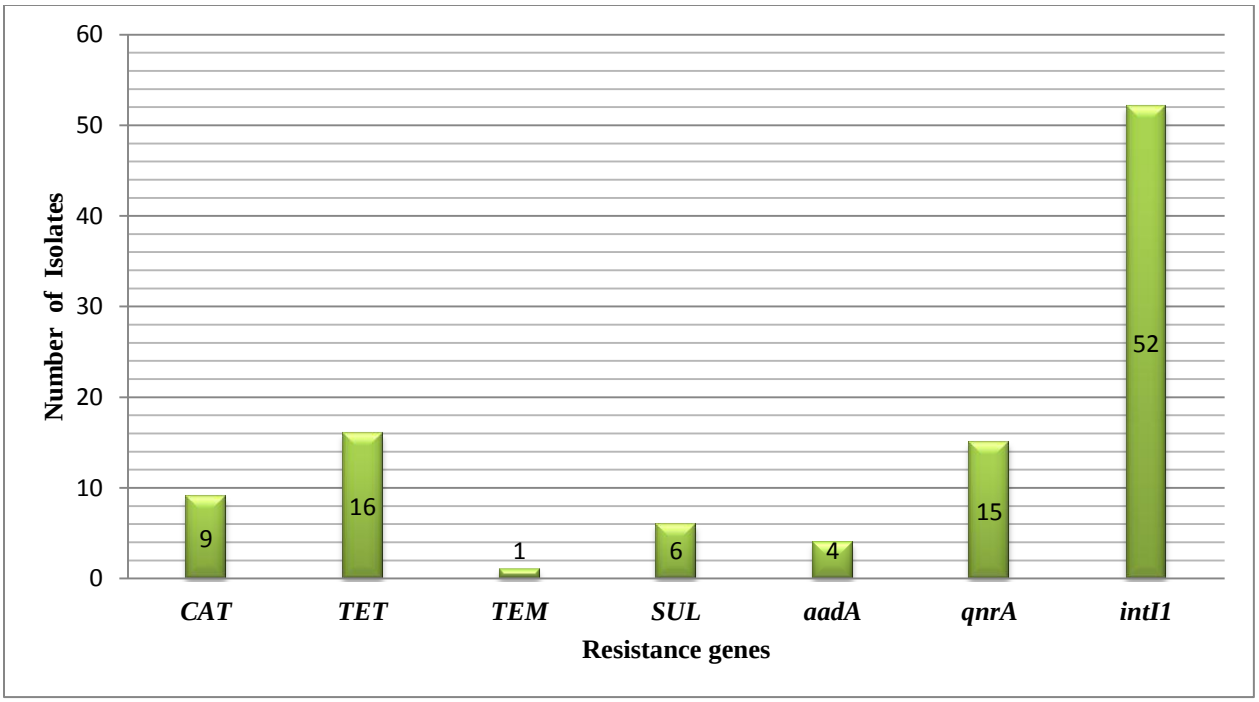


Figure 6. 8: The number of *Salmonella* isolates harbouring different antibiotic resistance genes

Table 6. 7: *Salmonella* isolates containing antimicrobial resistance genes and class 1 integrons

MAR Genotype	*S.* typhimurium	*S. enteritidis*	*S. newport*	*S. heidelberg*	*S. bongori*	*S. enteric serovar paratyphi* B	*S. pullorum*	S. *tennessee*	Total
CAT	3	1	1	–	1	–	1	2	9
TET	10	2	3	1	–	–	–	–	16
TEM	1	–	–	–	–	–	–	–	1
SUL	3	1	–	1	1	–	–	–	6
aadA	1	–	–	2	1	–	–	–	4
qnrA	7	6	–	–	–	2	–	–	15
intI1	26	10	4	4	3	2	1	2	52

aadA= Streptomycin, *bla*TEM= Ampicillin, *CAT*= Chloramphenicol, *Sul3, Sul4*= Sulfonamide, *qnrA*= Quinolones, *TET*= Tetracyclines, *ntI1*= Class 1 Integrons

6.4.2.2 Multidrug-resistant (MDR) genes

Two *S. typhimurium* serovars had resistance genes to more than two classes of antibiotics, therefore they were considered to be multi-drug-resistant (MDR) isolates. The first isolate was resistance to *tet*, *cat* and *qnr*A. However, the other isolate was resistant to *qnrA*, *tet*, *sul* and *aad*A. *S. enteritidis* was resistance to *tet, Sul and Cat* (Table 6.8).

Table 6. 8: Multi-resistance genes detected *Salmonella* isolates

MRG Genotypes	*S.* typhimurium	*S. enteritidis*	*S. newport*	*S. heidelberg*	*S. bongori*	*S. enteric paratyphi* B	*S. pullorum*	S. *tennessee*
Tet, Cat, qnrA	1	1	–	–	–	–	–	–
Tet, Sul, aadA, qnr-A	1	–	–	–	–	–	–	–

6.5 DISCUSSION

According to literature, this is the first study to demonstrate antimicrobial resistance in *Salmonella* spp. isolated from rats captured from chicken houses around Mafikeng. The twelve (n=12) antimicrobial drugs used are those with veterinary and human health significance and are commonly used in Mafikeng in particular and in North West Province as a whole. Finding resistance to these commonly used antimicrobials is very significant because rats are never themselves treated with antibiotics thus the resistance observed from them represents the contamination and circulation of resistant strains in the environment around Mafikeng, which ultimately affects both animals and humans.

The current study showed a very high prevalence (100%) of Rifampicin resistance by the *Salmonella* isolates on disc diffusion. This figure is similar to what was observed from studies

conducted in Egypt (Mahmoud *et al.*, 2018), in Sardinia, Italy (Piras *et al.*, 2011), in Shandong, China (Zhao *et al.*, 2017) and in United Arab Emirates (Khan *et al.*, 2010). Rifampicin in South Africa is used as the first-line drug for tuberculosis in humans (Dramowski *et al.*, 2012; McIntosh *et al.*, 2018; Narsing *et al.*, 2017; Sunpath *et al.*, 2014). North West Province is one of the provinces with high number of mines; therefore it has been confirmed as one of the provinces having high number of people suffering from tuberculosis (TB) due to silica dust exposure, particularly in gold mines, which is associated with silica dust-associated tuberculosis (Mathema *et al.*, 2015; Phillips *et al.*, 2014; Van Wyk *et al.*, 2013). Therefore, many people around this province have been taking TB treatment and the drug may be discharged in the environment where it is picked by rats and circulated, thus maintaining resistance. This finding is therefore a significant concern for public health and more research is necessary to assess the extent to which other bacteria species are affected.

Tetracycline is frequently used due to its low cost, compared to other antibiotics and thus is one of the extensively used antibiotics for therapy and prophylaxis of animal and human infections (Fritz & Zuo, 2007; Granados-Chinchilla & Rodríguez, 2017). It is also commonly used at sub-therapeutic levels for growth promotion (Chopra & Roberts, 2001). This encourages selection for resistance and results in higher percentages of bacteria in the environment that cannot respond to treatment. A high resistance rate of 32 isolates to Tetracycline was also noticed on isolates on disc diffusion. On the other hand, when the same isolates were assessed using molecular methods targeting the *tet* gene, only 16 of the isolates were positive. This may be enlightened by the fact that resistance to tetracycline can also be encoded by efflux pumps proteins like the *tet(K) and tet(L)* genes (Bilatu, 2012), hence other isolates were not having this *tet* gene. However, this is not surprising because previous studies have shown that the most frequently detected antibiotic

resistance gene is *tet* gene (Dahshan *et al.*, 2010; Keelara *et al.*, 2014; Roberts & Schwarz, 2016; Shekhar & Singh, 2015; Xu, 2017; Zishiri *et al.*, 2016), since the antibiotic is moderately cheaper on the market which is a big concern because of the limited access and high cost of other antibiotics (Bedada & Zewde, 2012). Tetracyclines are used for the prevention of diseases and also the promotion of growth in different animal production processes (Kim & ju Lee, 2017). Therefore, a high occurrence of *tet* genes is expected as Tetracycline is a widely used in veterinary and human medicine, for the reason that it is cheap and readily available (Odoch *et al.*, 2018).

Twelve (18%) isolates were resistant to Sulphonamides on disc diffusion compared to 6 (9%) of these isolates on molecular evaluation using the *Sul* gene. This is not surprising because sulphonamides are a common antibiotic in chickens flocks (Bertelloni *et al.*, 2017). Sulphonamides are used to treat some infectious diseases in chickens like Fowl typhoid, Coccidiosis coryza and Pullorum disease (Mehtabuddin *et al.*, 2012) and thus are a common contaminant in these poultry houses. The survey conducted by Eagar *et al.* (2012) in South Africa, showed that 95.4% of Sulphonamides sold are used for water medication. This, therefore, makes them common environmental contaminants as water spills and are picked by all organisms in that environment thus impacting on the selection pressure for resistance

Quinolones or Fluoroquinolones have been used as a treatment option for salmonellosis for over 40 years (Balasundaram *et al.*, 2017). The current study showed that 21 (31%) of the isolates were resistance to Ciprofloxacin on disc diffusion. However, when these isolates were subjected to molecular evaluation of resistance using the *qnr-A* gene, 15 (22%) out of 68 isolates were positive for *qnr-A* gene. The absence of this gene in isolates that were phenotypically positive could be that the target gene for this antibiotic is not always the same *qnr*ABSCD genes. The

high resistance percentage might be due to the reason that Fluoroquinolones are the common treatment for *Salmonella* infections (Zhao *et al.*, 2017). Fluoroquinolones have actually been considered one options for the treatment of *Salmonella* spp. (Abdel-Maksoud *et al.*, 2015). In a study conducted in South Africa, *Salmonella* isolates (*Salmonella enterica* serotype typhi) was isolated from a woman who was sick and this isolates was found to be resistant to Ciprofloxacin and also harbouring *qnr*A gene (Keddy *et al.*, 2010). So to find some of the isolates in the present study having this *qnr*A gene is concerning but not surprising.

A total of 8 (12%) isolates were found phenotypically resistance to Streptomycin, of which half of these isolates were harbouring *aad*A gene. These may be explained by the fact that different resistance genes can encode this class. One study has shown that poultry litter act as a reservoir of antibiotic-resistant microbes (Liljebjelke *et al.*, 2017). In a study conducted in Malaysia, 23 *Salmonella* isolates from poultry and environment were observed to be harboring the *aad*A genes (Chuah *et al.*, 2018). In another study conducted by Abatcha *et al.* (2018), 32 *Salmonella* isolates harboring *aad*A gene were isolated from the samples collected from related processing environments and chicken carcasses. Therefore, it is possible that these rats obtained this antibiotic-resistant *Salmonella* from poultry feces or even from the environment.

Since the discovery of Chloramphenicol, it was the main treatment used against *Salmonella* up to 1990 (Mirza *et al.*, 1995). The increasing resistance of *Salmonella* spp. to Chloramphenicol has been reported by Olobatoke & Mulugeta, 2015 from South Africa (North West Province) from chickens samples. The current study has isolated 9 (13%) *Salmonella* specimens which have appeared to be phenotypically resistant to Chloramphenicol and all these isolates were harbouring *cat* gene. This suggest that there is ongoing use of this antibiotic due to its broad spectrum activity (Mehdizadeh *et al.*, 2010), despite the awareness of its resistance.

The isolates were also subjected to disk diffusion to evaluate Ampicillin resistance, only 3 (4%) of the isolates were resistant. Furthermore, these isolates were subjected to molecular evaluation of resistance using the *blaTEM* gene and therefore one (2%) isolate was positive for *blaTEM* gene. The absence of this gene from isolates that were phenotypically positive indicates that the target gene for this antibiotic is not always the same; *SHV, CTX-M-9G, CTX-M-2G, CTX-M-64* and many more or the disk diffusion was more sensitivity and but not specificity to antibiotics resistance (Dickert et al., 1981). This is not surprising but more concerning because this antibiotic has been found to be resistant by the previous study around this area but from the chickens (Olobatoke & Mulugeta, 2015).

In this study, a high occurrence of the isolates has shown multi-drug resistance to Tetracycline, Ciprofloxacin and Sulphonamides, which are antimicrobial agents commonly used in veterinary medicine. This is worrying because some of these antibiotics are the recommended drugs for the treatment of salmonellosis (Bosco *et al.*, 2012; Walusansa, 2017). Studies elsewhere have also observed that *Salmonella* isolates can carry multidrug-resistance (MDR) (Kuan *et al.*, 2017; Thung *et al.*, 2017). Multi-drug resistance *Salmonella* isolates are considered to be highly virulent than non-multi-drug resistance (Dong *et al.*, 2014; Thung *et al.*, 2017) and this observation in this study is of great concern.

Seventy seven percent (n=52) of *Salmonella* isolates from rats in this study harbored class 1 integrons. Even though there are four classes of integrons linked with the resistance gene cassette, class 1 integrons have been more commonly observed than the other classes (Abatcha *et al.*, 2018; Kariaki *et al.*, 1996; Mahero *et al.*, 2013; Thong & Modarressi, 2011; Zhao *et al.*, 2007). According to literature, Class 1 integrons is the most common type of integron in MDR in *Salmonella* spp. and plays a significant role in assisting the transfer of the resistance genes

(Tankson *et al.*, 2005; Thong & Modarressi, 2011). It was observed in the current study that a high percentage of the isolates harboured class 1 integrons genes, which meant they contained one/more genes that encode antibiotic resistance.

Multi-drug resistant genes were encountered from *Salmonella* isolates harbouring more than two resistance genes. Furthermore, out of two isolates, one *S. typhimurium* showed multi-drug resistance for both disc diffusion and gene resistance. Multi-drug resistance found on *Salmonella* has been reported to cause illnesses in either humans or in animals from different countries including; the US and Denmark (Aarestrup *et al.*, 2007), in Italy (Graziani *et al.*, 2008), in Eastern China (Lu *et al.*, 2014), in Vietnam (Vo *et al.*, 2010). Therefore, multi-resistant genes must be put into consideration.

These are not the first study regarding *Salmonella* antibiotic resistance, several studies have been carried out in South Africa from different animals (Mathole *et al.*, 2017), including, pigs from commercial farms (Iwu *et al.*, 2016), chickens and human clinical isolates (Zishiri *et al.*, 2016), and from the aquatic environments (Suzuki *et al.*, 2015). However, this study has pioneered antibiotic resistance investigation on *Salmonella* isolates from rats inhabiting poultry farms.

In conclusion, the results of this study have revealed that both phenotype and genotype antibiotic resistance characteristics are well established in most of the *Salmonella* isolates infecting sampled rats. These findings provide a better understanding of the importance of rats in the transmission and maintenance of the antibiotic-resistant *Salmonella* in poultry premises which can potentially be transferred to humans via chicken products.

6.6 REFERENCES

Aarestrup, F.M., Hendriksen, R.S., Lockett, J., Gay, K., Teates, K., McDermott, P.F., White, D.G., Hasman, H., Sørensen, G. & Bangtrakulnonth, A. 2007. International spread of multidrug-resistant in food products. *Emerging Infectious Diseases*, 13(5):726-731.

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(4):715-718.

Abakpa, G., Umoh, V., Ameh, J., Yakubu, S., Kwaga, J. & Kamaruzaman, S. 2015. Diversity and antimicrobial resistance of *Salmonella* enterica isolated from fresh produce and environmental samples. *Environmental Nanotechnology, Monitoring & Management*, 3:38-46.

Abatcha, M.G., Effarizah, M.E. & Rusul, G. 2018. Prevalence, antimicrobial resistance, resistance genes and class 1 integrons of *Salmonella* serovars in leafy vegetables, chicken carcasses and related processing environments in Malaysian fresh food markets. *Food Control*, 91:170-180.

Abdel-Maksoud, M., Abdel-Khalek, R., El-Gendy, A., Gamal, R.F., Abdelhady, H.M. & House, B.L. 2015. Genetic characterisation of multidrug-resistant *Salmonella* enterica serotypes isolated from poultry in Cairo, Egypt. *African Journal of Laboratory Medicine*, 4(1):1-7.

Akiyama, T. & Khan, A.A. 2011. Molecular characterization of strains of fluoroquinolone-resistant *Salmonella enterica serovar schwarzengrund* carrying multidrug resistance isolated from imported foods. *Journal of Antimicrobial Chemotherapy*, 67(1):101-110.

Balasundaram, P., Veerappapillai, S. & Karuppasamy, R. 2017. Quinolones and Fluoroquinolones to Treat *Salmonella* typhimurium: A Review of Metabolism and Pharmacokinetics. *Current Drug Metabolism*, 18(12):1085-1094.

Bauer, A., Kirby, W., Sherris, J.C. & Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45(4):493.

Bedada, A.H. & Zewde, B. 2012. Tetracycline residue levels in slaughtered beef cattle from three slaughterhouses in central Ethiopia. *Global Veterinaria*, 8(6):546-554.

Bertelloni, F., Tosi, G., Massi, P., Fiorentini, L., Parigi, M., Cerri, D. & Ebani, V.V. 2017. Some pathogenic characters of paratyphoid *Salmonella* enterica strains isolated from poultry. *Asian Pacific Journal of Tropical Medicine*, 10(12):1161-1166.

Bilatu, A. 2012. Quantitative screening of antibiotics residues and identification of antibiotic resistant *Salmonella* from raw and ready to eat meat in Thailand. *International Journal of Advanced Live Science*, 5:51-64.

Bosco, K.J., Kaddu-Mulindwa, D.H. & Asiimwe, B.B. 2012. Antimicrobial drug resistance and plasmid profiles of *Salmonella* isolates from humans and foods of animal origin in Uganda. *Advances in Infectious Diseases*, 2(04):151-155.

Brooks, B.D., Brooks, A.E. & Grainger, D.W. 2013. Antimicrobial medical devices in preclinical development and clinical use. Biomaterials associated infection. Springer. p. 307-354.

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):1-7.

Choi, C.O. 2007. Pollution in solution-Drug-resistance dna as the latest freshwater threat: SCI American INC 415 Madison AVE, New York, NY 10017 USA.

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

Chuah, L.-O., Syuhada, A.-K.S., Suhaimi, I.M., Hanim, T.F. & Rusul, G. 2018. Genetic relatedness, antimicrobial resistance and biofilm formation of *Salmonella* isolated from naturally contaminated poultry and their processing environment in northern Malaysia. *Food Research International*, 105:743-751.

Dahshan, H., Shahada, F., Chuma, T., Moriki, H. & Okamoto, K. 2010. Genetic analysis of multidrug-resistant *Salmonella* enterica serovars *stanley* and typhimurium from cattle. *Veterinary Microbiology*, 145(1-2):76-83.

Dickert, H., Machka, K. & Braveny, I. 1981. The uses and limitations of disc diffusion in the antibiotic sensitivity testing of bacteria. *Infection*, 9(1):18-24.

Dong, P., Zhu, L., Mao, Y., Liang, R., Niu, L., Zhang, Y., Li, K. & Luo, X. 2014. Prevalence and profile of *Salmonella* from samples along the production line in Chinese beef processing plants. *Food Control*, 38:54-60.

Dramowski, A., Morsheimer, M., Jordaan, A., Victor, T., Donald, P. & Schaaf, H. 2012. Rifampicin-monoresistant *Mycobacterium tuberculosis* disease among children in Cape Town, South Africa. *The International Journal of Tuberculosis and Lung Disease*, 16(1):76-81.

Dunowska, M., Morley, P., Traub-Dargatz, J., Davis, M., Patterson, G., Frye, J., Hyatt, D. & Dargatz, D. 2007. Comparison of *Salmonella* enterica serotype Infantis isolates from a veterinary teaching hospital. *Journal of Applied Microbiology*, 102(6):1527-1536.

Eagar, H., Swan, G. & Van Vuuren, M. 2012. A survey of antimicrobial usage in animals in South Africa with specific reference to food animals. *Journal of the South African Veterinary Association*, 83(1):15-23.

Faldynova, M., Pravcova, M., Sisak, F., Havlickova, H., Kolackova, I., Cizek, A., Karpiskova, R. & Rychlik, I. 2003. Evolution of antibiotic resistance in *Salmonella* enterica serovar typhimurium strains isolated in the Czech Republic between 1984 and 2002. *Antimicrobial Agents and Chemotherapy*, 47(6):2002-2005.

Fedorka-Cray, P.J., Englen, M.D., Gray, J.T., Hudson, C. & Headrick, M.L. 2002. Programs for monitoring antimicrobial resistance. *Animal Biotechnology*, 13(1):43-54.

Foley, S. & Lynne, A. 2008. Food animal-associated *Salmonella* challenges: Pathogenicity and antimicrobial resistance 1. *Journal of Animal Science*, 86(14):E173-E187.

Fritz, J.W. & Zuo, Y. 2007. Simultaneous determination of tetracycline, oxytetracycline, and 4-epitetracycline in milk by high-performance liquid chromatography. *Food Chemistry*, 105(3):1297-1301.

Frye, J.G. & Fedorka-Cray, P.J. 2007. Prevalence, distribution and characterisation of ceftiofur resistance in *Salmonella* enterica isolated from animals in the USA from 1999 to 2003. *International Journal of Antimicrobial Agents*, 30(2):134-142.

Frye, J.G. & Jackson, C.R. 2013. Genetic mechanisms of antimicrobial resistance identified in *Salmonella enterica, Escherichia coli,* and *Enteroccocus* spp. isolated from US food animals. *Frontiers in Microbiology*, 4:135-166.

Ghazaey, S. & Mirmomeni, M.H. 2012. Microbial-resistant *Salmonella enteritidis* isolated from poultry samples. *Reports of Biochemistry & Molecular biology*, 1(1):9-13.

Granados-Chinchilla, F. & Rodríguez, C. 2017. Tetracyclines in food and feedingstuffs: From regulation to analytical methods, bacterial resistance, and environmental and health implications. *Journal of Analytical Methods in Chemistry*, 2017: 1315497.

Graziani, C., Busani, L., Dionisi, A., Lucarelli, C., Owczarek, S., Ricci, A., Mancin, M., Caprioli, A. & Luzzi, I. 2008. Antimicrobial resistance in *Salmonella* enterica serovar typhimurium from human and animal sources in Italy. *Veterinary Microbiology*, 128(3-4):414-418.

Guerra, B., Soto, S., Helmuth, R. & Mendoza, M.C. 2002. Characterization of a self-transferable plasmid from *Salmonella* enterica serotype typhimurium clinical isolates carrying two integron-borne gene cassettes together with virulence and drug resistance genes. *Antimicrobial Agents and Chemotherapy*, 46(9): 2977-2981.

Hong, Y.-P., Wang, Y.-W., Huang, I.-H., Liao, Y.-C., Kuo, H.-C., Liu, Y.-Y., Tu, Y.-H., Chen, B.-H., Liao, Y.-S. & Chiou, C.-S. 2018. Genetic Relationships among Multidrug-Resistant *Salmonella* enterica Serovar typhimurium Strains from Humans and Animals. *Antimicrobial Agents and Chemotherapy*:AAC. 62(5); 00213-00218.

Ishaleku, D., Umeh, E., Amali, O. & Gberikon, G. 2015. The Reemergence of Chloramphenicol Sensitive *Salmonella* species among Typhoid Fever Patients in the Southern Geographical Zone of Nasarawa State, Nigeria. *Journal of Infectious Diseases Therapy*, 3(3):1-3.

Islam, M.J., Mahbub-E-Elahi, A., Tanvir Ahmed, M. & Hasan, K. 2016. Isolation and identification of *Salmonella* spp. from broiler and their antibiogram study in Sylhet, Bangladesh. *Journal of Applied Biology & Biotechnology*, 4(03):046-051.

Iwu, C.J., Iweriebor, B.C., Obi, L.C., Basson, A.K. & Okoh, A.I. 2016. Multidrug-Resistant *Salmonella* Isolates from Swine in the Eastern Cape Province, South Africa. *Journal of Food Protection*, 79(7):1234-1239.

Johnson J.M., Rajic A. & McMullen, L.M. (2005). Antimicrobial resistance of selected *Salmonella* isolates from food animals and food in Alberta. *Canadian Veterinary Journal* 46, 141-146.

Kagambèga, A., Lienemann, T., Frye, J.G., Barro, N. & Haukka, K. 2018. Whole genome sequencing of multidrug-resistant *Salmonella* enterica serovar typhimurium isolated from humans and poultry in Burkina Faso. *Tropical Medicine and Health*, 46(1):4-27.

Kariaki, S., Gilks, C., Corkill, J., Kimari, J., Benea, A., Waiyaki, P. & Hart, C. 1996. Multi-drug resistant non-typhi *Salmonellae* in Kenya. *Journal of Antimicrobial Chemotherapy*, 38(3):425-434.

Karim, S.S.A., Churchyard, G.J., Karim, Q.A. & Lawn, S.D. 2009. HIV infection and tuberculosis in South Africa: an urgent need to escalate the public health response. *The Lancet*, 374 (9693):921-933.

Keddy, K.H., Smith, A.M., Sooka, A., Ismail, H. & Oliver, S. 2010. Fluoroquinolone-resistant typhoid, South Africa. *Emerging Infectious diseases*, 16(5):879-880.

Keelara, S., Scott, H.M., Morrow, W.M., Hartley, C.S., Griffin, D.L., Gebreyes, W.A. & Thakur, S. 2014. Comparative phenotypic and genotypic characterization of temporally related

nontyphoidal *Salmonella* isolated from human clinical cases, pigs, and the environment in North Carolina. *Foodborne Pathogens and Disease*, 11(2):156-164.

Khan, M., Suryanarayan, P., Ahmed, M.M., Vaswani, R.B. & Faheem, S.M. 2010. Antimicrobial susceptibility of *Salmonella* isolates from chicken meat samples in Dubai, United Arab Emirates. *International Journal of Food, Nutrition and Public Health*, 3(2):149-159.

Khemtong, S. & Chuanchuen, R. 2008. Class 1 integrons and *Salmonella* genomic island 1 among *Salmonella* enterica isolated from poultry and swine. *Microbial Drug Resistance*, 14(1):65-70.

Kim, J.E. & ju Lee, Y. 2017. Molecular characterization of antimicrobial resistant non-typhoidal *Salmonella* from poultry industries in Korea. *Irish Veterinary Journal*, 70(1):20-55.

Kuan, C., Rukayadi, Y., Ahmad, S., Radzi, W.C.M., Kuan, C., Yeo, S., Thung, T., New, C., Chang, W. & Loo, Y. 2017. Antimicrobial resistance of *Listeria monocytogenes* and *Salmonella enteritidis* isolated from vegetable farms and retail markets in Malaysia. *International Food Research Journal*, 24(4):1831-1839.

Liljebjelke, K.A., Hofacre, C.L., White, D.G., Ayers, S., Lee, M.D. & Maurer, J.J. 2017. Diversity of antimicrobial resistance Phenotypes in *Salmonella* isolated from commercial Poultry Farms. *Frontiers in Veterinary Science*, 4:96. doi: 10.3389/fvets.2017.00096.

Lu, Y., Zhao, H., Sun, J., Liu, Y., Zhou, X., Beier, R.C., Wu, G. & Hou, X. 2014. Characterization of multidrug-resistant *Salmonella* enterica serovars *indiana* and *enteritidis* from chickens in Eastern China. *PloS One*, 9(5):e96050. doi: 10.1371/journal.pone.0096050

Magiorakos, A.P., Srinivasan, A., Carey, R., Carmeli, Y., Falagas, M., Giske, C., Harbarth, S., Hindler, J., Kahlmeter, G. & Olsson-Liljequist, B. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, 18(3):268-281.

Mahero, M., Byarugaba, D., Doetkott, D., Olet, S. & Khaitsa, M. 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 Microbial* 2:128. doi: 10.4172/2327-5073.1000128

Mahmoud, M., Askora, A., Barakat, A.B., Rabie, O.E.-F. & Hassan, S.E. 2018. Isolation and characterization of polyvalent bacteriophages infecting multi drug resistant *Salmonella* serovars isolated from broilers in Egypt. *International Journal of Food Microbiology*, 266:8-13.

Mathema, B., Lewis, J.J., Connors, J., Chihota, V.N., Shashkina, E., Van Der Meulen, M., Graviss, E.A., Ha, N.P., Kreiswirth, B.N. & Grant, A.D. 2015. Molecular epidemiology of *Mycobacterium tuberculosis* among South African gold miners. *Annals of the American Thoracic Society*, 12(1):12-20.

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

McIntosh, A.I., Jenkins, H.E., White, L.F., Barnard, M., Thomson, D.R., Dolby, T., Simpson, J., Streicher, E.M., Kleinman, M.B. & Ragan, E.J. 2018. Using routinely collected laboratory data to identify high Rifampicin-resistant tuberculosis burden communities in

the Western Cape Province, South Africa: A retrospective spatiotemporal analysis. *PLoS Medicine*, 15(8):e1002638. https://doi.org/10.1371/journal.pmed.1002638

Mehdizadeh, S., Kazerani, H.R. & Jamshidi, A. 2010. Screening of chloramphenicol residues in broiler chickens slaughtered in an industrial poultry abattoir in Mashhad, Iran. *Iranian Journal of Veterinary Science and Technology*, 2(1):25-32.

Mehtabuddin, A., Ahmad, T., Nadeem, S., Tanveer, Z. & Arshad, J. 2012. Sulfonamide residues determination in commercial poultry meat and eggs. *Journal of Animal and Plant Science*, 22(2):473-478.

Mirza, S., Beeching, N. & Hart, C. 1995. The prevalence and clinical features of multi-drug resistant Salmonella typhi infections in Baluchistan, Pakistan. *Annals of Tropical Medicine & Parasitology*, 89(5):515-519.

Narsing, R., Karstaedt, A. & Sahid, F. 2017. The prevalence and management of rifampicin-resistant tuberculosis among adults at Chris Hani Baragwanath Academic Hospital, Johannesburg, South Africa. *African Journal of Thoracic and Critical Care Medicine*, 23(2):30-34.

Nisha, A. 2008. Antibiotic residues-a global health hazard. *Veterinary World*, 1(12):375-377.

Odoch, T., Sekse, C., L'Abee-Lund, T.M., Høgberg Hansen, H.C., Kankya, C. & Wasteson, Y. 2018. Diversity and Antimicrobial Resistance Genotypes in Non-Typhoidal *Salmonella* Isolates from Poultry Farms in Uganda. *International Journal of Environmental Research and Public Health*, 15(2):324. https://doi.org/10.3390/ijerph15020324

Olobatoke, R.Y. & Mulugeta, S.D. 2015. Incidence of non-typhoidal *Salmonella* in poultry products in the North West Province, South Africa. *South African Journal of Science*, 111(11-12):1-7.

Orrell, C., Cohen, K., Conradie, F., Zeinecker, J., Ive, P., Sanne, I. & Wood, R. 2011. Efavirenz and rifampicin in the South African context: is there a need to dose increase efavirenz with concurrent rifampicin therapy? *Antiviral Therapy*, 16(4):527-534.

Özgen, C. 2007. Isolation, molecular characterization of food-borne drug resistant *Salmonella* spp. and detection of class 1 integrons. Citeseer. (Doctoral dissertation, Middle East Technical University). Retrieved on the 25/04/2018.

Phillips, J., Vorajee, N., Davies, J., Nelson, G., Murray, J. & Ndlovu, N. 2014. Marikana autopsies highlight occupational diseases amongst platinum mine workers. *Occupational Health Southern Africa*, 20(4):6-12.

Piras, F., Brown, D.J., Meloni, D., Mureddu, A. & Mazzette, R. 2011. Investigation of *Salmonella enterica* in Sardinian slaughter pigs: prevalence, serotype and genotype characterization. *International Journal of Food Microbiology*, 151(2):201-209.

Povilonis, J., Šeputienė, V., Ružauskas, M., Šiugždinienė, R., Virgailis, M., Pavilonis, A. & Sužiedėlienė, E. 2010. Transferable class 1 and 2 integrons in *Escherichia coli* and *Salmonella enterica* isolates of human and animal origin in Lithuania. *Foodborne Pathogens and Disease*, 7(10):1185-1192.

Raissy, M. & Ansari, M. 2011. Antibiotic susceptibility of Lactococcus garvieae isolated from rainbow trout (*Oncorhynchus mykiss*) in Iran fish farms. *African Journal of Biotechnology*, 10(8):1473-1476.

Reyes Herrera, I. 2010. Pharmacokinetic Evaluation of the Blood: Tissue Relationship in Poultry: Screening for Antibiotic Residues in Chicken Muscle. (Doctoral dissertation, University of Arkansas, Fayetteville). Retrieved on the 11/06/2018.

Roberts, M.C. & Schwarz, S. 2016. Tetracycline and phenicol resistance genes and mechanisms: importance for agriculture, the environment, and humans. *Journal of Environmental Quality*, 45(2):576-592.

Schroeder, M., Brooks, B.D. & Brooks, A.E. 2017. The complex relationship between virulence and antibiotic resistance. *Genes*, 8(1):39.

Shekhar, C. & Singh, S. 2015. Multi-drug resistance in *Salmonella* serovars of zoonotic importance and detection of anti-microbial resistance genes. *The Indian Journal of Animal Sciences*, 85(2): 13-16.

Singh, P., Singh, R., Gupta, B., Tripathi, S.S., Tomar, K.S., Jain, S. & Sahni, Y. 2018. Prevalence study of *Salmonella* spp. in milk and milk products. *Asian Journal of Dairy & Food Research*, 37(1): 7-12.

Srisanga, S., Angkititrakul, S., Sringam, P., Le Ho, P.T., Vo, A.T. & Chuanchuen, R. 2017. Phenotypic and genotypic antimicrobial resistance and virulence genes of *Salmonella* enterica isolated from pet dogs and cats. *Journal of Veterinary Science*, 18(3):273-281.

Sunpath, H., Winternheimer, P., Cohen, S., Tennant, I., Chelin, N., Gandhi, R. & Murphy, R. 2014. Double-dose lopinavir-ritonavir in combination with rifampicin-based anti-tuberculosis treatment in South Africa. *The International Journal of Tuberculosis and Lung Disease*, 18(6):689-693.

Suzuki, S., Ogo, M., Koike, T., Takada, H. & Newman, B. 2015. Sulfonamide and tetracycline resistance genes in total-and culturable-bacterial assemblages in South African aquatic environments. *Frontiers in Microbiology*, 6:796. doi:10.3389/fmicb.2015.00796

Tankson, J., Fedorka-Cray, P., Jackson, C. & Headrick, M. 2005. Genetic relatedness of a rarely isolated *Salmonella*: *Salmonella* enterica serotype *niakhar* from NARMS animal isolates. *Journal of Antimicrobial Chemotherapy*, 57(2):190-198.

Thong, K.L. & Modarressi, S. 2011. Antimicrobial resistant genes associated with *Salmonella* from retail meats and street foods. *Food Research International*, 44(9):2641-2646.

Thung, T.Y., Radu, S., Mahyudin, N.A., Rukayadi, Y., Zakaria, Z., Mazlan, N., Tan, B.H., Lee, E., Yeoh, S.L. & Chin, Y.Z. 2017. Prevalence, virulence genes and antimicrobial resistance profiles of *Salmonella* serovars from retail beef in Selangor, Malaysia. *Frontiers in Microbiology*, 8:2697. doi:10.3389/fmicb.2017.02697

Van Wyk, D., Reyneke, S. & Cronje, F. 2013. Local communities and health disaster management in the mining sector. *Jàmbá: Journal of Disaster Risk Studies*, 5(2):1-12.

Vo, A.T., Van Duijkeren, E., Gaastra, W. & Fluit, A.C. 2010. Antimicrobial resistance, class 1 integrons, and genomic island 1 in *Salmonella* isolates from Vietnam. *PloS one*, 5(2):e9440.

Walusansa, A. 2017. Non-typhoidal *Salmonella* and Zoonotic *E. coli* as Potential Drivers of Antimicrobial Resistance in Pastoralist Communities in Kasese District, Uganda. Doctoral dissertation, Makerere University. Retrieved on the 2018-04-12.

Wannaprasat, W., Padungtod, P. & Chuanchuen, R. 2011. Class 1 integrons and virulence genes in Salmonella enterica isolates from pork and humans. *International Journal of Antimicrobial Agents*, 37(5):457-461.

Watts, J.L., Clinical & Institute, L.S. 2008. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard:

National Committee for Clinical Laboratory Standards-Third Edition https://www.dbt.univr.it/documenti/OccorrenzaIns/matdid/matdid485539.pdf.

Wilson, D.N. 2014. Ribosome-targeting antibiotics and mechanisms of bacterial resistance. *Nature Reviews Microbiology*, 12(1):35-48.

Wright, G.D. 2005. Bacterial resistance to antibiotics: enzymatic degradation and modification. *Advanced Drug Delivery Reviews*, 57(10):1451-1470.

Xu, Y. 2017. Isolation of *Salmonella* from flies on cattle farms and horizontal transfer of integron-mediated antibiotic resistance genes on microbiological media and selected farm samples. Doctoral dissertation, uga. Retrieved on the 2018-05-10.

Zeina, K., Pamela, A.K. & Fawwak, S. 2013. Quantification of antibiotic residues and determination of antimicrobial resistance profiles of microorganisms isolated from bovine milk in Lebanon. *Food and Nutrition Sciences*, 4(07):1-9.

Zhao, S., McDermott, P., White, D., Qaiyumi, S., Friedman, S., Abbott, J., Glenn, A., Ayers, S., Post, K. & Fales, W. 2007. Characterization of multidrug resistant *Salmonella* recovered from diseased animals. *Veterinary Microbiology*, 123(1-3):122-132.

Zhao, X., Ye, C., Chang, W. & Sun, S. 2017. Serotype distribution, antimicrobial resistance, and class 1 integrons profiles of *Salmonella* from animals in slaughterhouses in Shandong province, China. *Frontiers in Microbiology*, 8:1049. doi: 10.3389/fmicb.2017.01049

Zishiri, O.T., Mkhize, N. & Mukaratirwa, S. 2016. Prevalence of virulence and antimicrobial resistance genes in *Salmonella* spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. *Onderstepoort Journal of Veterinary Research*, 83(1):1-11.

CHAPTER 7-GENERAL CONCLUSION AND RECOMMENDATIONS

7.1 CONCLUSION

This study determined the occurrence of two types of rodents in poultry farms around Mafikeng using barcoding genes for species identification namely: *COI* and the *Cyt-b* genes. Two rodent pest species identified were; *R. tanezumi* and *R. rattus*. *R. rattus* (the black rat) was the most predominant species identified. Surprisingly, *R. tanezumi* (the Asian Rat/Asian House Rat) was the second and only other rodent species identified. This is surprising because although the rodent species have been reported in South Africa, especially in the coastal areas of KwaZulu-Natal, it is not an African but Asian species and its increasing numbers are an indication of its ability to proliferate in places outside its native environment. The study also found out that of the two mitochondrial genes used only *COI* gene identified all the samples while the *Cyt-b* gene identified only some of the samples and was not efficient enough. Therefore, *COI* gene should also serve as a reliable precise gene for rodent species identification.

The study managed to detect eight different *Salmonella* serovars (*S. tennessee*, *S. typhimurium*, *S. bongori*, *S. enteritidis*, *S. newport*, *S. heidelberg*, *S. enteric* serovar paratyphi B, and *S. pullorum*) from these rodent species using traditional culturing methods together with PCR. Two *Salmonella* serovars namely; *S. enteritidis* and *S.* typhimurium were the most abundantly detected serotypes. Most of these isolates were recovered from *R. rattus* as compared to *R. tanezumi*. This is not surprising because these species have been identified to carry these bacteria by a number of previous studies elsewhere (Himsworth *et al.*, 2015; Wakawa *et al.*, 2015). The results of this study, which have shown that *Rattus* spp. carry *Salmonella* serotypes which are known to be pathogens responsible for serious outbreaks globally, are very significant. This study, according to literature is the first one to indicate that *R. tanezumi* carries these *Salmonella*

species in South Africa and this is also a significant finding in as far as these rats' role in the epidemiology of *Salmonellosis* in South Africa and the region.

Virulence of *Salmonella* serovars isolated from rats in this study was determined using known virulence genes such as *inv*A, *Sdf I*, *Spy*, *Spv*C, *hil*A, *spi*C, *mis*L, *orf*L and they were encoding class 1 integrons. The strains had varying degrees and combinations of these virulent genes signifying that they are potentially highly pathogenic though at different scales. This finding is significant in that the *Salmonella* strains these rodents carry are not only present as commensal but are also capable of causing serious diseases in livestock and humans. Therefore, controlling these rats would indirectly also control the *Salmonellosis* outbreaks in both humans and livestock.

Finally, the response of these *Salmonella* isolates to antibiotics was evaluated and many strains were found to be resistant to numerous antibiotics due to the presence of genes such as; *tet, cat, bla*TEM, *sul, qnrA,* and *aadA*. Some of the serovars such as *S. typhimurium* and *S. enteritidis* were carrying more than three antibiotic resistant genes and were therefore classified as multiple antibiotic resistant strains. Furthermore, fifty-two (n=52) isolates were confirmed to harbor class 1 integrons, thus indicating that the isolates found in the study area contain one/more genes that encode antibiotic resistance. Class 1 integrons have been confirmed as a vital factor that distributes antibiotics resistant genes (Tankson *et al.*, 2005; Dunowska *et al.*, 2007; Gillings *et al.*, 2008; Gillings *et al.*, 2015; Hall & Stokes, 2004).

All the isolates, which were phenotypically resistant to antibiotics using antibiotic discs, were also found to contain resistance genes. This data is important for the treatment of the *Salmonella* as well as understanding how the rats are accountable for the selection of antibiotic-resistant

strains in the environment. Furthermore, this information will contribute in the formulation of strategies for future antibiotic use.

7.2 RECOMMENDATIONS

The observations made in this study have indicated that rats provide a good opportunity for the environment–rat–*Salmonella*-chicken which means there are possibilities that chickens could become infected with *Salmonella* via ingestion of *Salmonella*-positive feces and urine contaminated by infected rats. Therefore, rodent control should be considered as a vital measure for bio-security on poultry farms.

There is also a need for building effective barriers around poultry buildings, hygiene measures, and habitat management in order to limit rodent access to poultry houses as they are attracted by the availability of water and feed (Velkers *et al.*, 2017). Therefore, the containment of food sources is pretty more successful in reducing a rat population. Furthermore, this study suggests that the monitoring of *Salmonella* in rats around poultry buildings may be used as a supplementary useful tool in the evaluation of the *Salmonella* status of the chickens as well as infection of the environment.

Special emphasis also needs to be given for the rational use of drugs to avoid problems of resistance development among bacterial pathogens of poultry as well as in humans. This is because rats can pick up these resistant strains and maintain them as well as circulate them in the environment. Furthermore research will also be essential in order to identify some of the genes which were not included in this study such as class detection of class 2 and 3 integrons (*intI*2 and

*intI*3) which are reported to also contribute to antibiotic resistance development (Asgharpour *et al.*, 2018; Corrêa *et al.*, 2014; Mazel, 2006).

LIMITATIONS OF THE STUDY

1. The sampling should have been done seasonal in order to explain how seasonal variation could have had affected the capturing of rodents/rats as well as the final results i.e. the prevalence of *Salmonella* spp.
2. The chicken samples should have been collected together with the rat samples in order to compare the results of the isolates but this was not done due to a number of reasons.

7.3 REFERENCES

Asgharpour, F., Mahmoud, S., Marashi, A. & Moulana, Z. 2018. Molecular detection of class 1, 2 and 3 integrons and some antimicrobial resistance genes in *Salmonella infantis* isolates. *Iranian Journal of Microbiology*, 10(2):104-110.

Corrêa, F.E.L., da Silva Dantas, F.G., Grisolia, A.B., do Amaral Crispim, B. & Oliveira, K.M.P. 2014. Identification of class 1 and 2 integrons from clinical and environmental *Salmonella* isolates. *The Journal of Infection in Developing Countries*, 8(12):1518-1524.

Dunowska, M., Morley, P., Traub-Dargatz, J., Davis, M., Patterson, G., Frye, J., Hyatt, D. & Dargatz, D. 2007. Comparison of *Salmonella enterica* serotype *infantis* isolates from a veterinary teaching hospital. *Journal of Applied Microbiology*, 102(6):1527-1536.

Gillings, M., Boucher, Y., Labbate, M., Holmes, A., Krishnan, S., Holley, M. & Stokes, H.W. 2008. The evolution of class 1 integrons and the rise of antibiotic resistance. *Journal of Bacteriology*, 190(14):5095-5100.

Gillings, M.R., Gaze, W.H., Pruden, A., Smalla, K., Tiedje, J.M. & Zhu, Y.-G. 2015. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *The ISME Journal*, 9(6):1269-1279.

Hall, R.M. & Stokes, H. 2004. Integrons or super integrons? *Microbiology*, 150(1):3-4.

Mazel, D. 2006. Integrons: agents of bacterial evolution. *Nature Reviews Microbiology*, 4(8):608-620.

Tankson, J., Fedorka-Cray, P., Jackson, C. & Headrick, M. 2005. Genetic relatedness of a rarely isolated *Salmonella*: *Salmonella enterica* serotype *niakhar* from NARMS animal isolates. *Journal of Antimicrobial Chemotherapy*, 57(2):190-198.

Velkers, F.C., Blokhuis, S.J., Veldhuis Kroeze, E.J. & Burt, S.A. 2017. The role of rodents in avian influenza outbreaks in poultry farms: a review. *Veterinary Quarterly*, 37(1):182-194.

APPENDIX

WEB REFERENCES

http://www.cdc.gov/ncidod/dbmd/phlisdata/salmtab/2005/SalmonellaAnnualSummar

http://www.cdc.gov/pulsenet/protocols/ecoli_salmonella_shigella_protocols.pdf

http://www.chembio.uoguelph.ca/merrill/research/enzyme_mechanisms

http://www.hpa.org.uk/hpa/inter/enter-net/07q1summ.pdf

http://www.hpa.org.uk/hpa/inter/enter-net_menu.htm

http://www.ncbi.nlm.nih.gov/BLAST/

http://www.who.int/salmsurv/links/GSSProgressReport2005.pdf

http://www.poultryhub.org/production/husbandry-management/housing-environment/pest-management/rodent-control/

Appendix T1 Sample code, Rodent specie, and results obtained for *Salmonella*

Sample code	Rats species	Gender	*Salmonella* detection
A1	*Rattus tanezumi*	M	−
A 2	*Rattus rattus*	F	−
A 3	*Rattus rattus*	M	+
A 4	*Rattus rattus*	F	−
A 5	*Rattus rattus*	F	−
A 6	*Rattus rattus*	M	−
A 7	*Rattus tanezumi*	F	−
A 8	*Rattus rattus*	F	−
A 9	*Rattus rattus*	M	+
A 10	*Rattus rattus*	M	−
A 11	*Rattus rattus*	F	−
A 12	*Rattus rattus*	M	−
A 13	*Rattus rattus*	F	−
A 14	*Rattus tanezumi*	F	+
A 15	*Rattus tanezumi*	F	−
A 16	*Rattus rattus*	F	−
A 17	*Rattus tanezumi*	M	−
A 18	*Rattus rattus*	M	−

A 19	*Rattus rattus*	F	−
A 20	*Rattus tanezumi*	F	−
A 21	*Rattus rattus*	M	−
A 22	*Rattus rattus*	F	+
A 23	*Rattus rattus*	F	−
A 24	*Rattus rattus*	F	−
A 25	*Rattus rattus*	M	−
B 26	*Rattus rattus*	M	+
B 27	*Rattus rattus*	M	+
B 28	*Rattus rattus*	F	−
C 29	*Rattus rattus*	M	−
C 30	*Rattus rattus*	M	−
C 31	*Rattus rattus*	F	−
C 32	*Rattus tanezumi*	M	−
C 33	*Rattus tanezumi*	F	−
C 34	*Rattus rattus*	M	−
C 35	*Rattus tanezumi*	M	−
C 36	*Rattus tanezumi*	M	−
C 37	*Rattus rattus*	F	−

C 38	*Rattus rattus*	M	+
C 39	*Rattus rattus*	M	−
C 40	*Rattus tanezumi*	F	−
C 41	*Rattus rattus*	F	−
C 42	*Rattus rattus*	M	−
C 43	*Rattus rattus*	F	−
C 44	*Rattus rattus*	F	−
C 45	*Rattus tanezumi*	F	−
C 46	*Rattus rattus*	F	−
C 47	*Rattus rattus*	M	−
C 48	*Rattus rattus*	F	−
C 49	*Rattus rattus*	F	−
D 50	*Rattus tanezumi*	M	−
D 51	*Rattus tanezumi*	F	−
D 52	*Rattus tanezumi*	M	−
D 53	*Rattus rattus*	M	−
D 54	*Rattus tanezumi*	F	−
D 55	*Rattus rattus*	F	−
D 56	*Rattus rattus*	F	−

D 57	*Rattus rattus*	M	−
D 58	*Rattus rattus*	F	−
D 59	*Rattus tanezumi*	F	−
D 60	*Rattus tanezumi*	M	+
D 61	*Rattus rattus*	F	−
D 62	*Rattus rattus*	M	−
D 63	*Rattus tanezumi*	F	−
D 64	*Rattus rattus*	M	+
D 65	*Rattus rattus*	F	−
D 66	*Rattus rattus*	F	−
E 67	*Rattus rattus*	M	−
E 68	*Rattus rattus*	F	−
E 69	*Rattus rattus*	M	−
E 70	*Rattus rattus*	F	−
E 71	*Rattus tanezumi*	M	+
E 72	*Rattus rattus*	M	−
E 73	*Rattus rattus*	F	−
E 74	*Rattus rattus*	M	+
E 75	*Rattus rattus*	M	−

E 76	*Rattus rattus*	M	−
E 77	*Rattus rattus*	F	−
E 78	*Rattus rattus*	F	−
E 79	*Rattus rattus*	M	+
E 80	*Rattus tanezumi*	F	−
E 81	*Rattus rattus*	F	−
E 82	*Rattus rattus*	M	−
E 83	*Rattus rattus*	M	−
E 84	*Rattus tanezumi*	M	−
E 85	*Rattus rattus*	F	−
E 86	*Rattus tanezumi*	F	−
E 87	*Rattus tanezumi*	M	−
E 88	*Rattus rattus*	M	−
E 89	*Rattus tanezumi*	M	+
E 90	*Rattus rattus*	M	−
E 91	*Rattus tanezumi*	F	−
E 92	*Rattus rattus*	M	−
E 93	*Rattus rattus*	M	−
E 94	*Rattus rattus*	M	−

E 95	*Rattus tanezumi*	M	−
E 96	*Rattus rattus*	F	−
E 97	*Rattus rattus*	M	−
E 98	*Rattus tanezumi*	M	+
E 99	*Rattus rattus*	F	−
E 100	*Rattus rattus*	F	−
E 101	*Rattus rattus*	M	−
E 102	*Rattus tanezumi*	F	−
E 103	*Rattus rattus*	M	+
E 104	*Rattus rattus*	M	−
E 105	*Rattus tanezumi*	F	−
E 106	*Rattus rattus*	M	+
E 107	*Rattus tanezumi*	M	−
E 108	*Rattus rattus*	M	−
E 109	*Rattus rattus*	F	−
E 110	*Rattus rattus*	F	+
E 111	*Rattus rattus*	M	−
E 112	*Rattus tanezumi*	M	−
E 113	*Rattus tanezumi*	F	−

E 114	*Rattus tanezumi*	M	−
E 115	*Rattus rattus*	F	−
E 116	*Rattus tanezumi*	M	−
E 117	*Rattus rattus*	F	−
E 118	*Rattus tanezumi*	M	−
E 119	*Rattus rattus*	M	−
E 120	*Rattus rattus*	M	−
E 121	*Rattus rattus*	M	−
E 122	*Rattus rattus*	F	−
E 123	*Rattus tanezumi*	M	−
E 124	*Rattus rattus*	M	−
E 125	*Rattus tanezumi*	F	−
E 126	*Rattus rattus*	M	−
E 127	*Rattus tanezumi*	F	−
E 128	*Rattus rattus*	M	−
E 129	*Rattus tanezumi*	F	−
E 130	*Rattus rattus*	F	−
E 131	*Rattus rattus*	M	+
E 132	*Rattus rattus*	F	−

E 133	*Rattus rattus*	M	−
E 134	*Rattus tanezumi*	M	−
H 135	*Rattus tanezumi*	M	−
H 136	*Rattus tanezumi*	F	−
H 137	*Rattus tanezumi*	F	−
H 138	*Rattus rattus*	M	−
H 139	*Rattus tanezumi*	M	−
H 140	*Rattus tanezumi*	F	−
H 141	*Rattus tanezumi*	F	−
H 142	*Rattus rattus*	M	−
H 143	*Rattus tanezumi*	F	−
H 144	*Rattus tanezumi*	M	−
H 145	*Rattus rattus*	M	−
H 146	*Rattus rattus*	F	−
H 147	*Rattus tanezumi*	M	−
H 148	*Rattus tanezumi*	F	−
H 149	*Rattus rattus*	M	+
H 150	*Rattus tanezumi*	F	−
H 151	*Rattus tanezumi*	M	−

H 152	*Rattus rattus*	M	−
H 153	*Rattus tanezumi*	M	−
H 154	*Rattus tanezumi*	F	−

A, B, C, D, E and H represent farm's name

Appendix T2 Results for 114 isolates; API and 16S sequencing (PCR)

	RATS				
	Isolates ID	Sequence length	**16S rRNA results**	**API identification**	**Accession number**
1	B1 (4)	692	*S.* typhimurium	+	MH352147.1
2	A19 (6)	928	*S. enteritidis*	+	MH352148.1
3	E25(2)	397	*S.* typhimurium	+	MH352149.1
4	C4(4)	894	*S. enteritidis*	+	MH352150.1
5	E54(6)	962	*S. heidelberg*	*Salmonella* spp.	MH352151.1
6	A12	863	*S.* typhimurium	+	MH352152.1
7	E34(4)	860	*S. enteritidis*	+	MH352153.1
8	E28(1)	956	*S.* typhimurium	+	MH352154.1
9	A12(1)	934	*S. pullorum*	+	MH352155.1
10	D2(2)	912	*S.* typhimurium	+	MH352156.1
11	E30	1035	*S.* typhimurium	+	MH352157.1
12	H12(2)	922	*S.* typhimurium	+	MH352158.1
13	E23(16)	1029	*S. tennessee*	+	MH352159.1
14	C4(1)	938	*S. newport*	+	MH352160.1

15	B2	918	*S. heidelberg*	+	MH352161.1
16	E57(4)	959	*S. heidelberg*	+	MH352162.1
17	D2(3)	853	*S. tennessee*	+	MH352163.1
18	H12	799	*S. pullorum*	+	MH352164.1
19	A19(1)	1031	*S. bongori*	+	MH352165.1
20	C4(6)	1014	*S. tennessee*	+	MH352166.1
21	E54(5)	503	*S. bongori*	+	MH352167.1
22	E25(3)	908	*S.* typhimurium	+	MH352168.1
23	E37(1)	1016	*S. heidelberg*	+	MH352169.1
24	E57(1)	999	*S. bongori*	+	MH352170.1
25	E30(1)	999	*S.* typhimurium	+	MH352171.1
26	A19(4)	1019	*S. heidelberg*	+	MH352172.1
27	E34(2)	898	*S. enteritidis*	+	MH352173.1
28	E23	941	*S.* typhimurium	+	MH352174.1
29	E25(13)	843	*S.* typhimurium	+	MH352175.1
30	H12(1)	1019	*S.* typhimurium	+	MH352176.1

31	E23(6)	999	*S. paratyphi* B	*Salmonella* spp.	MH352177.1
32	E32(20)	1070	*S. heidelberg*	+	MH352178.1
33	D4(3)	1049	*S. newport*	+	MH352179.1
34	E37(9)	688	*S. bongori*	+	MH352180.1
35	H1(6)	1016	*S. paratyphi* B	+	MH352181.1
36	E25(5)	898	*S.* typhimurium	+	MH352182.1
37	E32(3)	941	*S. enteritidis*	+	MH352183.1
38	C4(10)	937	*S. newport*	+	MH352184.1
39	H1(1)	941	*S.* typhimurium	+	MH352185.1
40	D4(X)	1100	*S. bongori*	*Salmonella* spp.	MH352186.1
41	E57(6)	688	*S. heidelberg*	+	MH352187.1
42	E54(1)	841	*S. bongori*	+	MH352188.1
43	A12(2)	926	*S.* typhimurium	+	MH352189.1
44	B2(2)	1019	*S. newport*	+	MH352190.1
45	E28(2)	1078	*S.* typhimurium	+	MH352191.1
46	H12(3)	909	*S.* typhimurium	+	MH352192.1

47	E34(8)	1070	*S. newport*	+	MH352193.1
48	B1(2)	1060	*S.* typhimurium	+	MH352194.1
49	D4	924	*S.* typhimurium	+	MH352195.1
50	F1(2)	1042	*S. enteritidis*	+	MH352196.1
51	E37(2)	1076	*S.* typhimurium	+	MH352197.1
52	B2(1)	957	*S. newport*	+	MH352198.1
53	E28	1020	*S.* typhimurium	+	MH352199.1
54	E23(X)	1031	*S* typhimurium	+	MH352200.1
55	E32(2)	1044	*S. newport*	+	MH352201.1
56	A12(3)	976	*S.* typhimurium	+	MH352202.1
57	A19(5)	999	*S. enteritidis*	+	MH352203.1
58	E34(1)	1058	*S. enteritidis*	+	MH352204.1
59	D4(1)	1059	*S. enteritidis*	+	MH352205.1
60	E25(1)	1089	*S.* typhimurium	+	MH352206.1
61	A19(X)	961	*S. newport*	+	MH352207.1
62	A32(19)	1061	*S. enteritidis*	+	MH352208.1

63	C4(2)	852	*S. Paratyphi* B	*Salmonella* spp.	MH352209.1
64	E25(12)	708	*S. enteritidis*	+	MH352210.1
65	D2	956	*S.* typhimurium	+	MH352211.1
66	E23(11)	1051	*S. paratyphi* B	*Salmonella* spp.	MH352212.1
67	C4(3)	1048	*S.* typhimurium	+	MH352213.1
68	E32(16)	1019	*S. enteritidis*	+	MH352214.1

Appendix T3 Resistance, susceptible and intermediate resistance patterns of *Salmonella* isolates from rats

S−ID	Isolates	CA_{10}	C_{30}	TE_{30}	AMC_{30}	AMP_{10}	NA_{30}	RD_5	ENR_5	KF_{30}	CIP_5	SSS3	S
1	B1 (4)	15	22	9	21	17	19	10	22	18	32	12	18
2	A19 (6)	22	31	18	26	22	24	10	32	24	19	28	11
3	E25(2)	24	26	10	31	28	21	12	19	22	32	22	17
4	C4(4)	27	10	26	34	13	23	10	36	21	38	11	26
5	E54(6)	21	25	21	19	14	19	13	25	15	36	26	14
6	A12	20	18	11	12	15	14	13	18	10	32	24	19
7	E34(4)	18	14	10	20	22	18	14	12	14	16	26	13
8	E28(1)	18	14	12	22	22	18	14	12	12	12	28	26
9	A12(1)	26	31	24	32	25	24	12	31	24	35	30	31
10	D2(2)	18	22	9	21	18	19	10	22	18	33	24	17

11	E30	18	21	20	10	15	17	13	20	12	33	26	13
12	H12(2)	26	11	10	20	24	15	14	24	22	19	28	15
13	E23(16)	20	18	8	18	15	14	14	26	16	17	26	10
14	C4(1)	25	20	17	26	12	20	12	29	19	20	36	23
15	B2	22	20	9	22	17	19	10	22	18	28	28	26
16	E57(4)	25	26	17	27	26	18	12	34	19	38	12	13
17	D2(3)	26	31	24	32	25	24	10	31	24	20	26	18
18	H12	18	20	14	18	18	19	10	22	18	24	28	23
19	A19(1)	22	14	22	28	26	24	14	16	14	16	28	11
20	C4(6)	26	10	11	24	22	20	10	17	18	32	10	26
21	E54(5)	16	19	18	21	22	20	12	22	14	24	28	14

22	E25(3)	24	25	19	30	25	24	13	30	25	20	28	19
23	E37(1)	15	22	16	21	17	19	12	22	18	28	26	14
24	E57(1)	16	29	18	22	15	21	12	32	18	34	30	26
25	E30(1)	18	16	10	20	22	18	14	12	10	31	28	31
26	A19(4)	24	22	16	26	22	18	10	22	20	16	32	17
27	E34(2)	22	31	11	22	24	20	12	29	24	35	10	12
28	E23	21	29	9	18	15	19	10	24	15	28	16	15
29	E25(13)	24	24	11	21	16	18	14	24	20	33	28	26
30	H12(1)	26	11	24	26	25	24	10	31	24	35	26	11
31	E23(6)	16	12	9	24	15	26	10	20	10	18	32	26
32	E32(20)	18	22	18	21	15	19	14	25	18	29	11	18

33	D4(3)	28	31	10	30	26	24	12	31	24	35	26	13
34	E37(9)	20	20	18	22	18	22	14	22	18	35	28	23
35	H1(6)	26	24	9	21	17	18	10	22	22	32	24	11
36	E25(5)	21	19	10	18	24	21	12	27	21	32	19	26
37	E32(3)	18	22	15	22	17	16	13	20	19	18	28	14
38	C4(10)	16	20	16	21	15	19	14	22	18	28	26	19
39	H1(1)	20	14	12	24	22	14	14	14	13	18	12	14
40	D4(X)	24	22	20	21	17	12	12	24	19	32	30	26
41	E57(6)	22	12	12	26	21	14	12	16	23	34	24	23
42	E54(1)	15	22	15	21	17	19	10	22	18	28	28	26
43	A12(2)	23	25	19	30	24	24	10	30	25	36	26	31

44	B2(2)	18	21	14	22	18	22	10	21	19	32	28	23
45	E28(2)	18	15	12	22	24	18	14	24	20	32	30	17
46	H12(3)	28	28	24	24	22	24	12	22	24	18	26	19
47	E34(8)	22	22	15	20	17	20	10	22	18	14	26	15
48	B1(2)	26	31	24	32	25	24	12	31	24	35	29	10
49	D4	28	24	22	28	20	18	10	28	20	20	28	31
50	F1(2)	16	22	11	22	17	19	12	22	18	32	32	23
51	E37(2)	21	28	22	18	15	18	10	24	14	32	30	13
52	B2(1)	22	22	10	21	18	19	14	22	18	17	11	26
53	E28	21	24	10	24	19	16	12	24	20	32	30	17
54	E23(X)	18	22	9	21	19	19	10	22	19	31	28	12

55	E32(2)	16	12	26	24	10	26	12	20	10	18	30	23
56	A12(3)	19	22	15	21	16	19	14	22	18	32	26	26
57	A19(5)	18	25	21	26	11	19	10	24	9	33	12	13
58	E34(1)	22	14	18	21	19	24	10	28	26	32	14	23
59	D4(1)	20	22	11	21	17	19	14	22	18	34	26	17
60	E25(1)	21	28	21	18	16	21	10	23	18	32	30	9
61	A19(X)	18	22	9	21	17	19	10	22	18	32	26	15
62	A32(19)	24	16	18	20	22	20	12	20	16	16	28	26
63	C4(2)	15	22	10	21	17	19	10	20	18	18	10	13
64	E25(12)	20	16	10	20	22	22	14	26	10	22	32	23
65	D2	26	11	24	32	25	24	10	31	24	35	11	17

66	E23(11)	22	27	11	18	15	19	10	24	16	31	28	26
67	C4(3)	26	31	11	32	25	24	10	31	24	35	32	10
68	E32(16)	20	10	9	20	20	17	16	24	16	14	12	25

Resistant-RED, Intermediate-GREEN, Susceptible-BLUE, Gentamicin CA_{10}, Chloramphenicol C_{30}, Ciprofloxacin CIP_5, Rifampicin RD_5, Nalidixic Acid NA_{30}, Ampicillin $AMP_{10,}$ Amoxicillin/clavulanic Acid AMC_{30}, Enrofloxacin ENR_5, Compound sulphonamides S_3 and Cephalothin K_{30}. Sulphonamides S_{300}

Appendix T4 Presence and absence of virulence genes from *Salmonella* isolates in rats captured from different farms

Farms	Isolates	*invA*	*Sdf I*	*hilA*	*pPB23*	*misL*	*fliC*	*Spy*	*orfL*	*fliB*	*spiC*	*SpvC*
A	*S.* typhimurium	4	–	4	–	4	–	4	2	–	4	–
	S. enteritidis	3	3	1	–	1	–	–	3	–	2	–
	S. newport	2	–	–	–	–	–	–	–	–	–	–
	S. heidelberg	3	–	1	–	3	–	–	2	–	2	–
	S. bongori	1	–	–	–	–	–	–	1	–	–	–
B	*S.* typhimurium	2	–	1	–	1	–	2	2	–	–	–
	S. enteritidis	1	1	–	–	1	–	–	–	–	1	1
	S. bongori	2	–	2	–	1	–	–	2	–	2	–
C	*S.* typhimurium	2	–	2	–	2	–	2	2	–	2	–
	S. enteritidis	1	1	1	–	1	–	–	1	–	1	–

	S. heidelberg	1	–	1	–	1	–	–	1	–	1	–
D	*S.* typhimurium	1	–	1	–	–	–	1	–	–	–	–
	S. enteritidis	2	2	–	–	2	–	–	–	–	–	2
	S. newport	3	–	–	–	3	–	–	2	–	2	–
E	*S.* typhimurium	16	–	12	–	10	–	16	7	–	10	5
	S. enteritidis	5	5	3	–	5	–	–	5	–	5	–
	S. newport	3	–	–	–	–	–	–	–	–	2	2
	S. heidelberg	3	–	1	–	3	–	–	–	–	–	1
	S. bongori	1	–	1	–	–	–	–	1	–	1	–
	S. enteric Paratyphi B	4	–	–	–	1	–	–	–	–	2	–
	S. pullorum	1	–	1	–	–	–	–	1	–	1	–

	S. tennessee	2	–	–	–	2	–	–	–	–	–	2
H	*S.* typhimurium	1	–	–	–	–	–	1	–	–	–	–
	S. bongori	2	–	–	–	1	–	–	–	–	1	1
	S. pullorum	1	–	1	–	–	–	–	–	–	–	–
	S. tennessee	1	–	–	–	1	–	–	–	–	–	–

Appendix T5 Antimicrobial disks and their contents used in the study (Davies diagnostics, SA).

Antibiotic	Symbol	Content (µg)
Gentamicin	CA	**10**
Chloramphenicol	**C**	**30**
Ciprofloxacin	CIP	**5**
Rifampicin	RD	**5**
Nalidixic Acid	NA	**30**
Ampicillin	AMP	**10**
Amoxicillin/clavulanic Acid	AMC	**30**
Enrofloxacin	ENR	**5**
Sulphonamides	**SSS**	**300**
Cephalothin	**KF**	**30**
Streptomycin	**S**	**10**

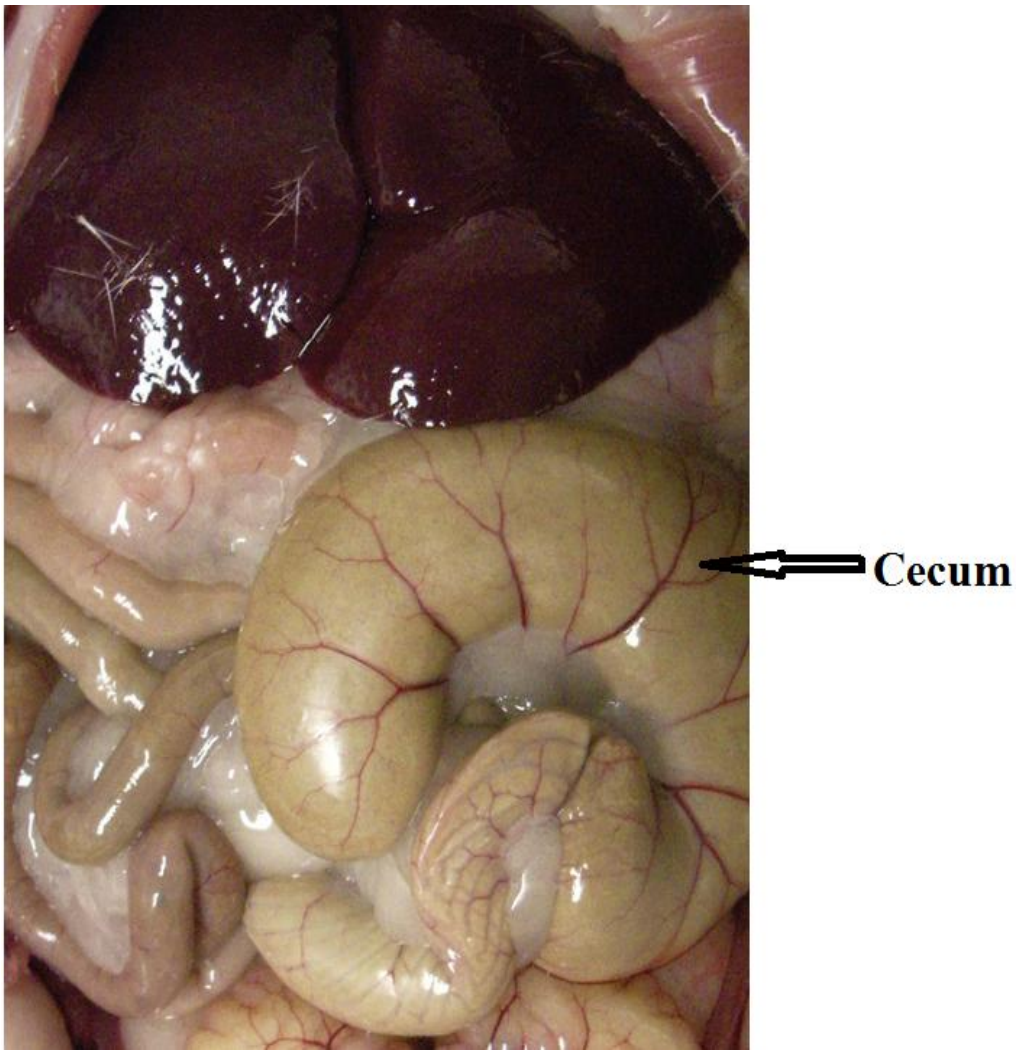
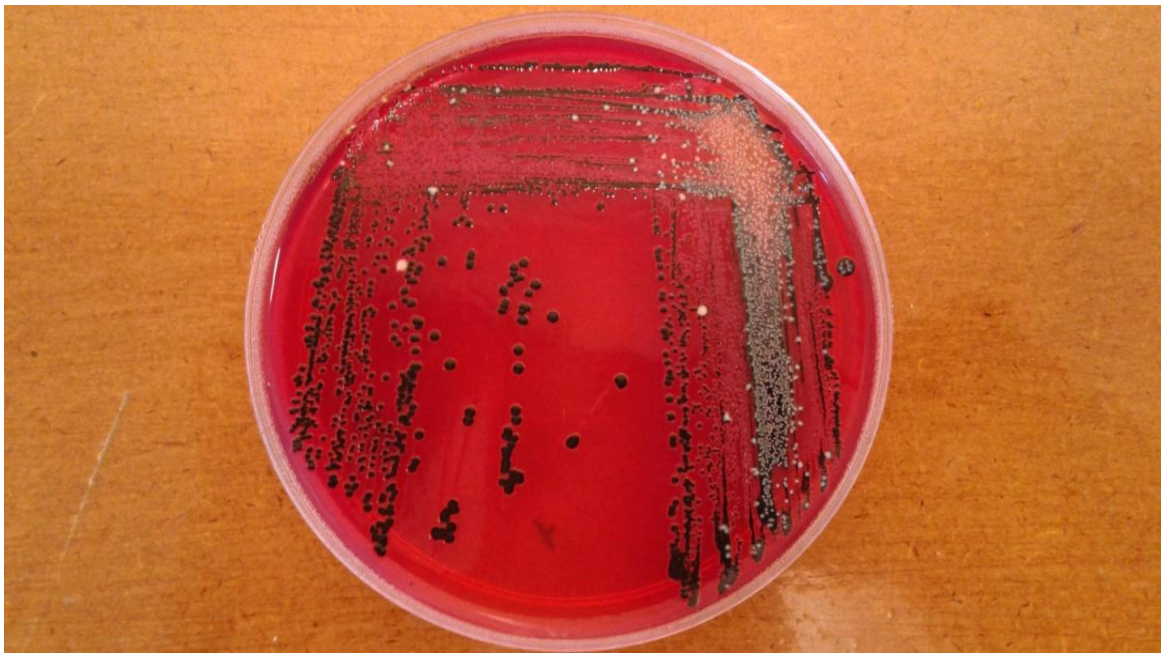
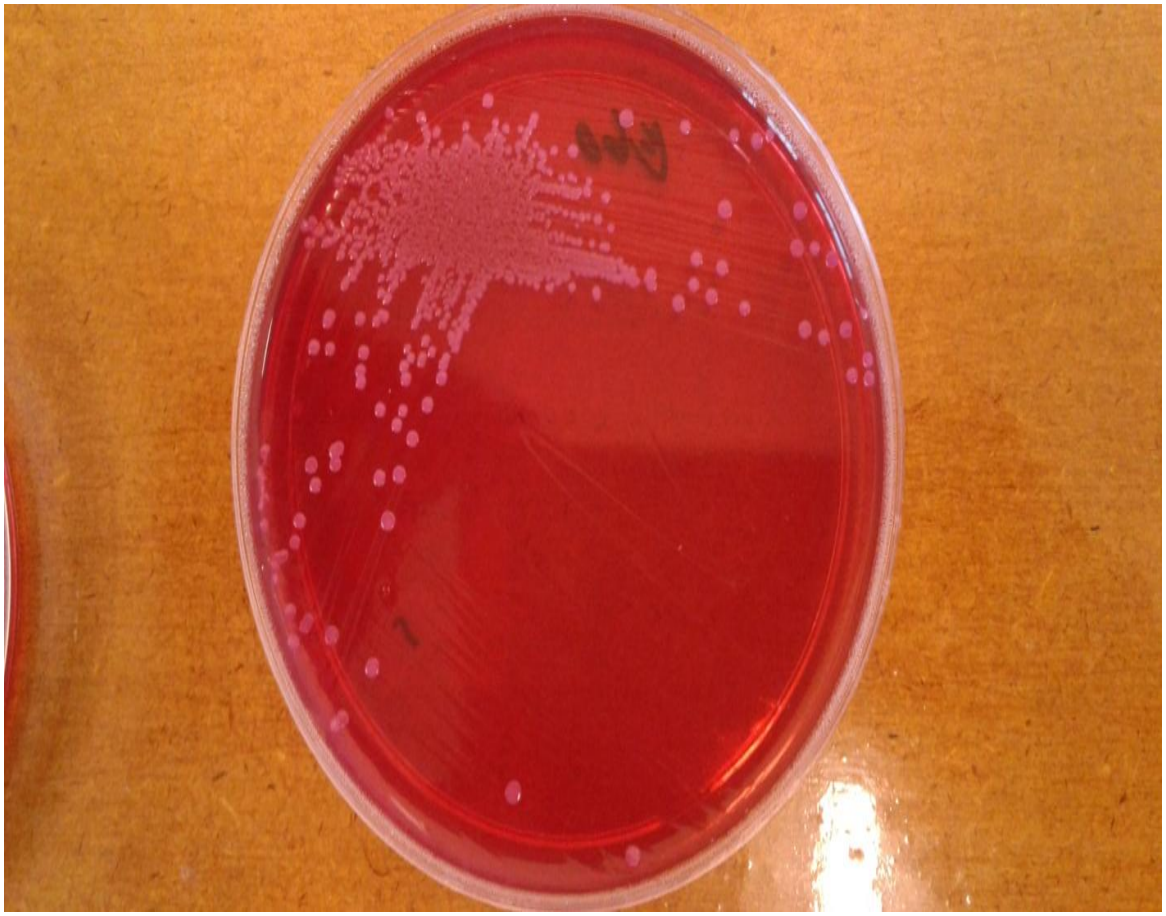


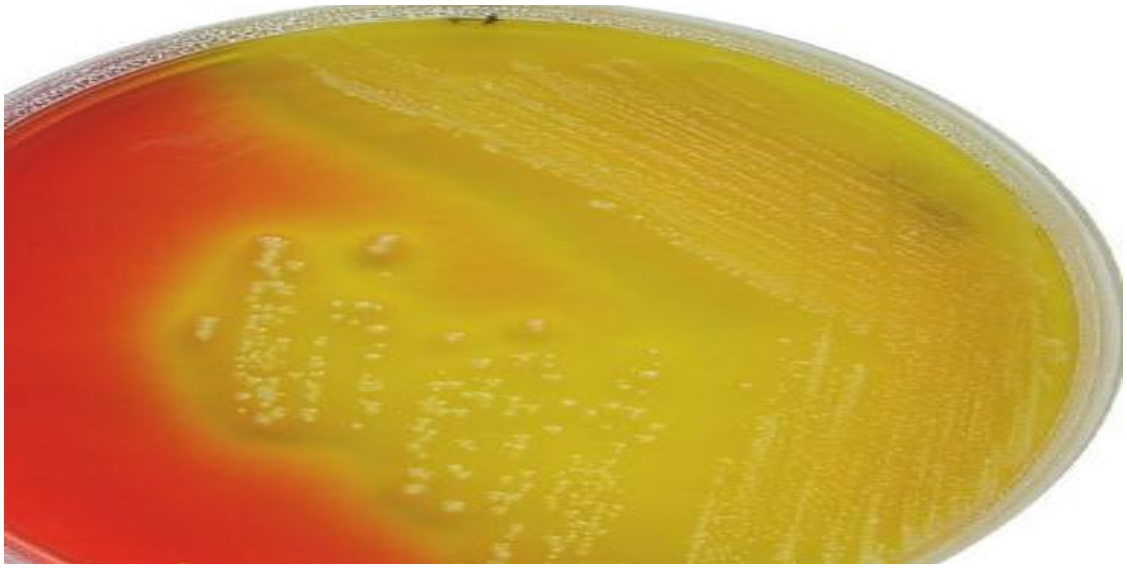
Figure F1: Indicate location of cecum where samples were obtained.



A

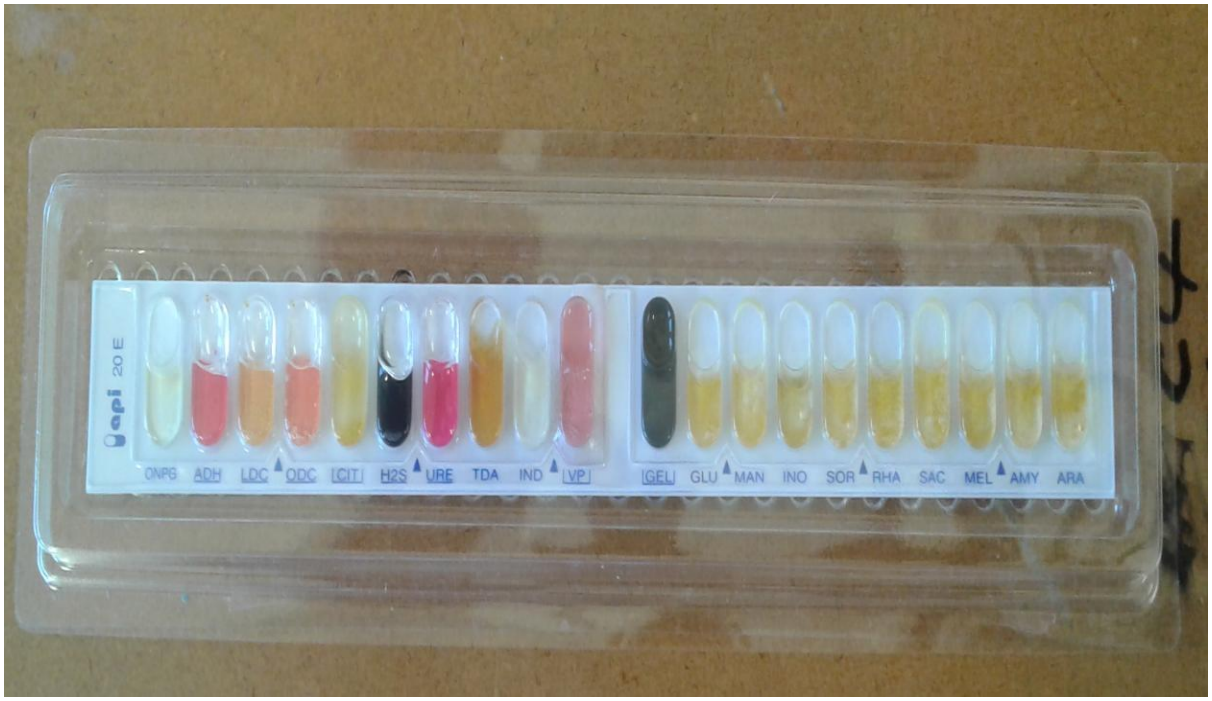


B

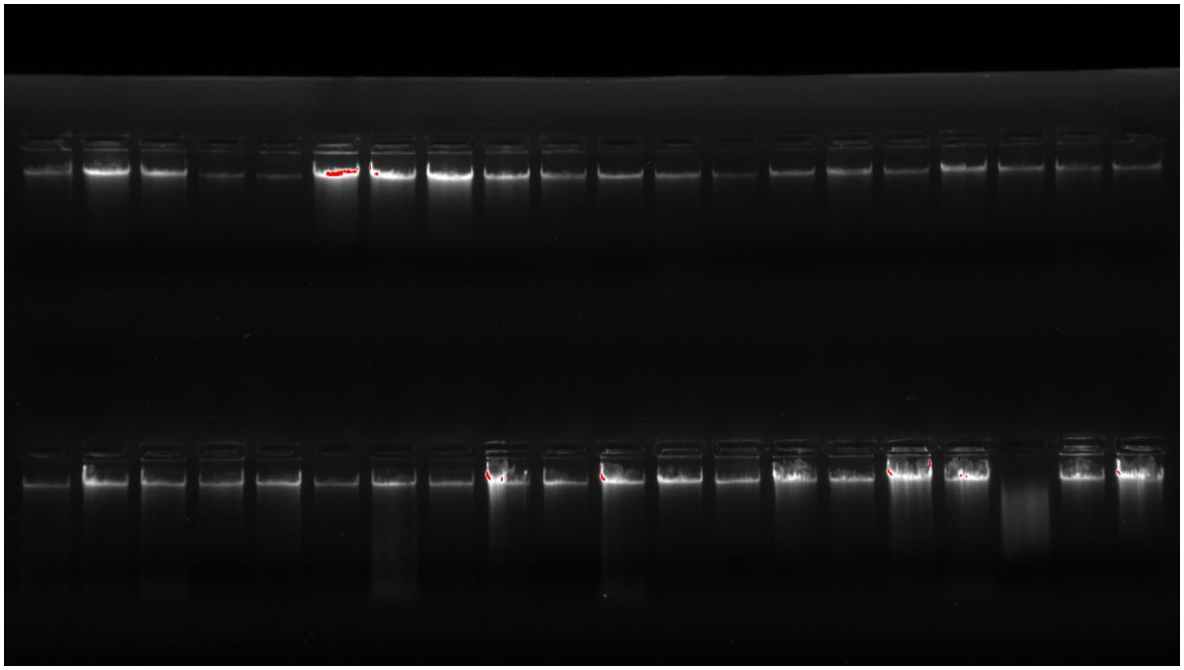


C

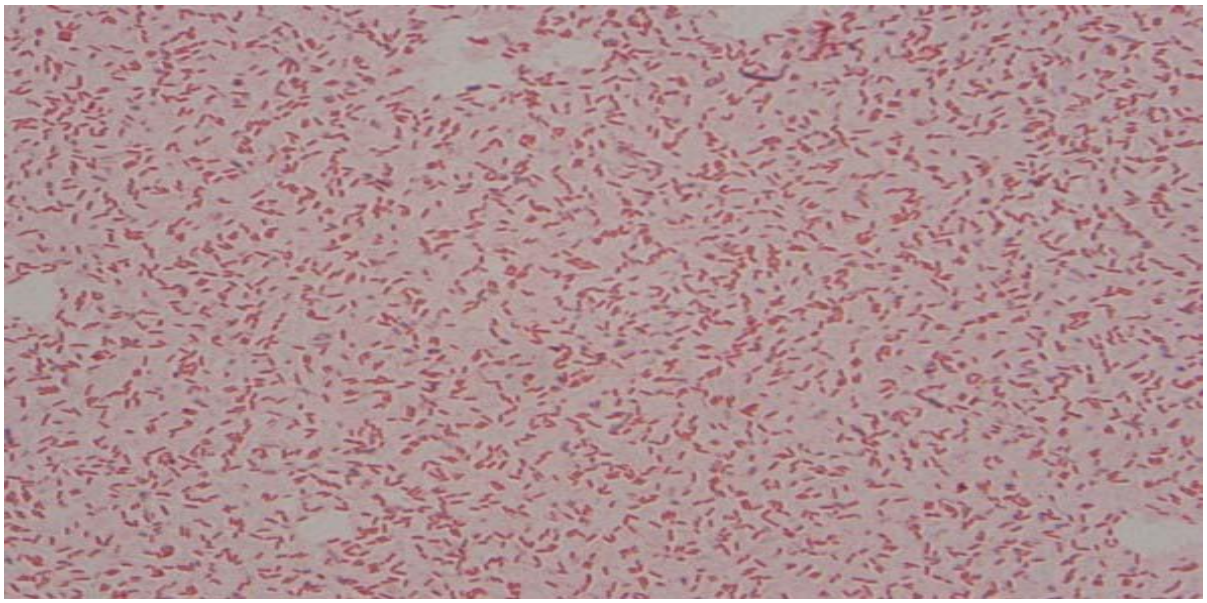
Appendix F2 Photographs of bacterial colonies on XLD media; [**A**] Colonies appear blackish; [**B**] Colonies appear pink on BGA agar, [**C**] yellow colonies of *E. coli* (Negative control) on XLD media following pre-enrichment.



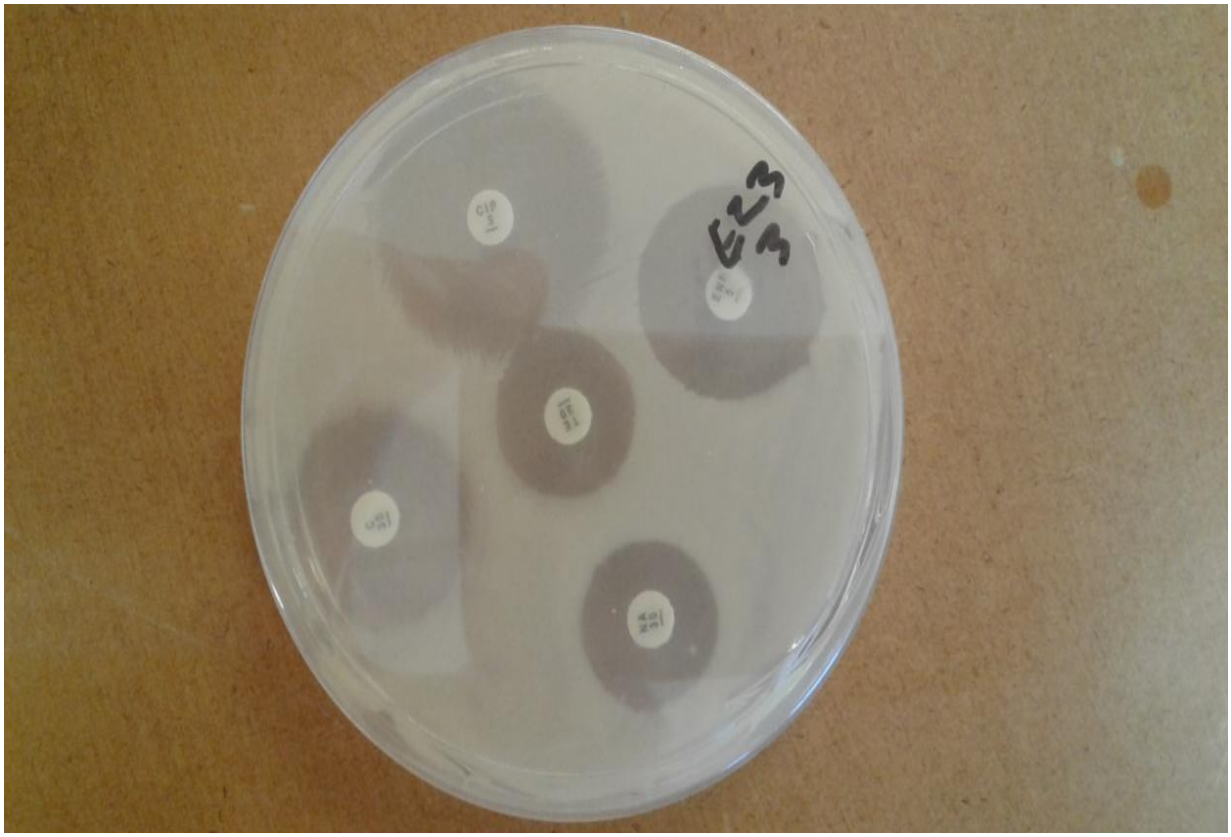
Appendix F3 The confirmatory test API 20E



Appendix F4 Agarose gel (1%) electrophoresis of extracted genomic DNA



Appendix F5 *Salmonella* serovars showing Gram-negative, small rods arranged singly or in pairs on Gram's staining



Appendix F6 Mueller Hinton Agar plate showing diffusion test for isolates against antibiotic discs

Sterilization procedures

Autoclaving

Culture media and discarded cultures were sterilized by autoclaving at 121ºC for 20 minutes while glassware with plastic covers was autoclaved at 121ºC for 15 minutes.

Disinfectants and antiseptics:

70% alcohol was used to disinfect the surfaces of benches before and after use.

Culture media

Buffered Peptone Water (BPW)

Formula	g/L
Enzymatic digest of casein	10,0
NaCl	5,0
Disodium hydrogen phosphate (Anhydrous)	3,5
Potassium dihydrogen phosphate	1,5

pH 7,0 ± 0,2 (before sterilization)

It was prepared by dissolving 15 grams of powder in 1 liter distilled water, the pH was adjusted to 7.4, then mixed well and distributed into test tubes 5 ml each and sterilized by autoclaving at 121º C for 15 minutes, then stored in the refrigerator at 4º C until used.

Rappaport Vassiliadis Broth (RVB)

Formula	g/L
Peptone from soymeal	4,5
Magnesium chloride hexahydrate	29,0
NaCl	8,0
Di-potassium hydrogen phosphate	0,4
Potassium di-hydrogen phosphate	0,6
Malachite-green	0,036

pH 5,2 ± 0,2 (before sterilization)

42,5 g of dehydrated media was suspended in 1 L distilled water. Then it was dispensed into 10 mL test tubes and sterilized in autoclave (115°C / 15 min).

Müller-Kauffmann Tetrathionate/Novobiocin Broth (MKTTn)

Formula	g/L
Meat extract	4,3
Enzymatic digest of casein	8,6
NaCl	2,6
Calcium carbonate	38,7
Sodium thiosulphate (anhydrous)	30,5
Ox bile	4,78
Brilliant green	0,0096

Novobiocin solution (0,8%) 5,0 mL Iodine-Iodide solution 20,0 mL pH 8,0 ± 0,2 (before sterilization) 89,5 g of dehydrated media was suspended in 1 L of sterile distilled water. The suspension mixed well and brought to boil with frequent agitation. When it was completely dissolved, iodine-iodide and novobiocin (Novobiocin Selective Supplement, Oxoid Ltd., UK) solutions were added and dispensed into 10 mL sterile tubes. This media was not sterilized in the autoclave.

Xylose-Lysin Desoxycholate (XLD) Agar

Formula	g/L
Yeast extract	3,0
L-Lysine HCl	5,0

Xylose	3,75
Lactose	7,5
Sucrose	7,5
Sodium desoxycholate	1,0
NaCl	5,0
Sodium thiosulphate	6,8
Ferric ammonium citrate	0,8
Fenol red	0,08
Agar	12,5

pH 7,4 ± 0,2 (before sterilization)

26,5 g of dehydrated media was suspended in 500 mL distilled water. The agar in the composition was melted in the boiling water bath by avoiding overheating. Subsequently, it was cooled down to 55^{o}C and transferred 15-20 mL in sterile Petri plates. This media was not sterilized in autoclave.

Müller-Hinton Agar

Formula	g/L
Meat Extract	300
Casein hydrolysate	17,5
Starch	1,5
Agar	17,0

This medium used for cultivation of Neisseria and antimicrobial susceptibility testing. pH 7,3 ± 0,1 (before sterilization) 38 gram dehydrated media was dissolved in 1000 mL distilled water

and sterilized at 121°C for 15 min in autoclave. After cooled down to 55°C, 10-15 mL was dispensed into sterile Petri dishes and stored at +4oC.

Nutrient Agar

Meat Extract	10g
Peptone	50g
Yeast Extract	2g
Sodium Chloride	8g
Agar	15g

28 grams of medium were added to 1 liter of distilled water and boiled to dissolve completely, the pH was adjusted to 7.4, and then the medium was sterilized by autoclaving at 121º C for 15 minutes and distributed aseptically in 15 ml amounts into sterile Petri dishes. Nutrient agar slops were also prepared and stored in refrigerator at 4ºC until used.

Eosin Methylene Blue (EMB) Agar

Formula	g/L
Peptone	10
Di-potassium hydrogen phosphate	2
Lactose	5
Sucrose	5
Eosin Y, yellowish	0,4

Methylene blue	0,07
Agar	13,5

pH 7,1 ± 0,2 (before sterilization)

36 g dehydrated media was dissolved in 1000 mL distilled water and sterilized at 121 °C for 15 min in autoclave. Then it was cooled down to 55^{o}C and poured into sterile Petri plates approximately 15-20 mL

Stock Solutions

50X Tris-Borat-EDTA (TBE) Stock Solution

Formula

(0.9 M Trizma-base, 0.9 M Boric acid, 0.02 M EDTA)

Tris (hydroxylmethyl) aminomethane	2M
0.5 M EDTA (pH 8.0)	50mM
Glacial acetic acid	57.1ml

About 600 ml of distilled water was placed in a 10 000ml beaker. While stirring, 2 M of Tris (hydroxylmethyl) aminomethane was added. Thereafter, 50 mM EDTA (pH 8.0) was added and the volume adjusted to 900 ml using water. The solution was stirred until Tri dissolved completely and then was adjusted to 1000 ml. A 1X TAE working solution was prepared and used as an electrophoresis running buffer.

Ethidium bromine

A stock solution of 10mg/ml was prepared by dissolving the powder in distilled water. A final concentration 0.1 ul/ml was used for staining extracted DNA and PCR product.

Biochemical median/reagents

Triple sugar Iron Agar medium (TSI) (Oxoid):

It contains (grams per liter) Lab-Lemco powder (Oxiod L29) 3g, yeast extract (Oxoid L20) 3 grams, peptone (Oxoid L37) 20 g, sodium chloride 5 g, lactose 10 g, sucrose 10 g, dextrose 1 g, ferric citrate 0.3, sodium thiosulfate 0.3, phenol red 0.025 g and agar No. 3 (Oxoid L13) 12 g. Triple sugar iron agar was prepared by adding 65 gram of powder to 1 liter of DW, the pH adjusted into 7.4, then boiled to dissolve completely, mixed well, distributed in 5 ml amount into McCarteny bottles and sterilized by autoclaving at 121º C for 15 min. The medium was allowed to set in a slope position about one inch butt and stored at 4º C.

Christensen's Urea Agar

The medium was composed of (grams per liter) peptone 1.0 g, dextrose 1.0 g, sodium chloride 5.0 g, disodium phosphate 1.2 g, potassium dihydrogen phosphate 0.8 g, phenol red 0.012 g and agar 15 g. According to the manufacturer instructions, 2.4 g of dehydrated medium were dissolved in 95 ml of distilled water by boiling, pH was adjusted to 6.8, sterilized by autoclaving at 115º C for 20 min, then cooled to 50º C and aseptically 5 ml of sterile 40% urea solution were added. The medium was poured into sterile screw-capped bottles 10 ml each and then allowed to set in the slope position.

Indole test

Inoculate the tryptophan broth with broth culture or emulsify isolated colony of the test organism in tryptophan broth. Then incubate at 37°C for 24-28 hours in ambient air, 0.5 ml of Kovac's reagent to the broth culture.

Catalase Test

A small amount of bacterial colony was transferred to a surface of clean, dry glass slide using a loop or sterile wooden stick then places a drop of 3% H_2O_2 on to the slide and mix. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling.