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Confirmation of Antimicrobial Resistance by Using Resistance Genes of Isolated *Salmonella* spp. in Chicken Houses of North West, South Africa

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ABSTRACT

The widespread use of antibiotics for treatment of bacterial infections and growth promotion in the poultry industry has effectively increased antibiotic resistance around the world. Antibiotics resistance can be caused by different mechanisms and can be determined through phenotypic and molecular methods. The aim of the present study was to determine the occurrence of antibiotic resistance in *Salmonella* serovars isolated from layer chickens and rats in poultry houses. Phenotypic testing of antimicrobial resistance was performed using the Kirby-Bauer disc diffusion method. Furthermore, molecular evaluations and PCR assay were conducted for detecting resistance genes and class 1 integrons. A total of 144 *Salmonella* isolates (68 from rats and 46 from chickens) serovars were assessed. Evaluation of phenotypic resistance patterns demonstrated that *Salmonella* isolates have the highest antibiotic resistance for rifampicin (100%) followed by tetracycline (68%), ciprofloxacin (48%), sulphonamides (42%), chloramphenicol (39%), nalidixic acid (33%), ampicillin (28%), cephalothin (18%), streptomycin (18%), amoxicillin-clavulanic acid (6%), enrofloxacin (5%), and gentamicin (4%). Some *Salmonella* serovars revealed multi-drug resistance for up to four different antibiotics. According to PCR results, all the tested resistant gene markers (*tet*, *cat*, *bla*TEM, *sul*, *qnrA*, and *aadA*) were detected from the *Salmonella* isolates. The study further confirmed that 68% of *Salmonella* isolates were harboring class 1 integrons and the majority of the isolates (n=52) which were harboring these genes were recovered from the rats. The results of the present study provided that the *Salmonella* spp. isolated from chickens and rats in poultry houses, exhibited significant antibiotic resistance. Moreover, the current research ultimately highlights the importance of rats as carriers of antibiotic-resistant bacteria and the risk of transmission to chickens and humans.

Key words: Antibiotic resistance pattern, Class 1 integrons, Resistance genes, *Salmonella* serovars

INTRODUCTION

Several antimicrobial agents are used in animals and humans to treat certain infections (Raissy and Ansari, 2011). Despite the obvious benefits, improper use of antibiotics can lead to the development of bacterial resistance in infectious diseases (Hong et al., 2018). Approximately 95% of antibiotics administered to livestock are excreted unchanged, therefore, people who live in an environment close to animal waste are constantly exposed to antibiotics and this condition may develop resistance (Choi, 2007).

Antibiotic resistance can be conferred by intrinsic or acquired mechanisms (Schroeder et al., 2017). Mechanisms of acquired resistance can be through Horizontal Gene Transfer (HGT) or elevated mutation rates (Schroeder et al., 2017). In general, mechanisms of antibiotic resistance are classified into three categories including modification of cell permeability, replacement or modification of the antibiotic targets and inactivation of the antibiotics via enzymatic destruction or modification (Frye and Jackson, 2013; Schroeder et al., 2017).

Recent studies have demonstrated that *Salmonella* serotypes such as *S.* heidelberg, *S.* typhimurium, *S.* infantis, *S.* uganda, *S.* newport, *S.* typhi, *S.* paratyphi, *S.* agona and *S.* hadar exhibit antibiotic resistance (Mathole et al., 2017; Zhao et al., 2017; Odoch et al., 2018; Thung et al., 2018). Furthermore, the increasing frequency of resistance of *Salmonella* spp. to antibiotics such as chloramphenicol, tetracycline, and ampicillin has been reported in many countries (Olobatoke and Mulugeta, 2015; Odoch et al., 2018; Thung et al., 2018). Antimicrobial resistance, especially in *Salmonella* serovars, has also been implicated to play a role in their virulence (Mathole et al., 2017).

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It has now been established that *Salmonella* spp. contain different Antibiotic Resistance Genes (ARGs) (Abatcha et al., 2018). Most of the resistance genes are located on the plasmids, bacterial chromosome or transposons which can be transferred by mobile genetic elements. Apart from antibiotic resistance genes detected from *Salmonella* spp. and resistance integrons included class 1, 2 and 3 has also been identified from *Salmonella* spp. (Odoch et al., 2018). The majority of the ARGs have mostly located in class 1 integrons. So that, class 1 integron-mediated antimicrobial resistance among diverse *Salmonella* serovars (Thong and Modarressi, 2011).

Although rodents are not normally treated with antibiotics, they have still been found to harbor antibiotic-resistant bacteria in the environment. Therefore, rats can be used as good indicators for the presence of antibiotic-resistant bacteria in a specific area. Currently, there is still inadequate information about the antibiotic resistance patterns of *Salmonella* spp. in chicken and rats in South Africa. Therefore, the present study sought to document the occurrence of antibiotic-resistant *Salmonella* serovars in chickens and rats found in chicken houses of North West Province, South Africa.

MATERIALS AND METHODS

Ethical Approval

Prior to the commencement of the study, the research proposal was approved by the Animal Research Ethics Committee (Ref No: NWU-00274-18-A5) following guidelines of North West University Research Ethics Regulatory Committee (NWU-RERC), North West, South Africa.

Sampling

A list of layer farms in Mafikeng, North West province of South Africa were randomly selected by using the records of Agriculture Department. A few farms in the north, south, east, and west were randomly selected. A total of 274 fecal samples were collected from chicken (n=120) and rats (n=154) in six commercial farms. Cleaning and disinfection of surfaces before sampling of rats were done with 70% ethanol to avoid cross-contamination. Dissection of the abdominal cavity was done using a surgical blade, a pair of forceps and the samples were harvested from cecum. The fecal samples of chickens were collected from three different floors in each poultry farm once a week. This sampling was done to have a good representation and distribution of the organisms. The collected samples were packed in properly labelled sterile polyethylene bags and transported under a complete aseptic condition in an icebox, then processed immediately upon arrival to the laboratory. All samples were labelled and prepared for analysis however the samples which did not analysed within 24 hours, immediately refrigerated at -4°C.

Bacteria isolates

Salmonella was isolated from feces following the International Organization for Standardization method (ISO-6579: 2002). The DNA of the isolates were amplified using Polymerase Chain Reaction (PCR) and then PCR products were sequenced. Generated sequences were aligned on the GenBank database using basic local alignment search tool from the National Center for Biotechnology Information to identify sequences with high similarity. The *16S rDNA* gene sequences of *Salmonella* isolates in the current study were deposited into the GenBank database and were given accession numbers ranging from MH352147 to MH352214 for rat and from MH356670 to MH356715 for chicken.

Phenotypic test for detecting antimicrobial resistance

The phenotypic antibiotic resistance test was performed using the Kirby-Bauer disc diffusion method (Magiorakos et al., 2012). Pure *Salmonella* isolates were sub-cultured on nutrient agar (Merck, Wadeville, South Africa) medium, incubated at 37°C for up to 24 h. Then fresh overnight cultures were used for antibiotic sensitivity tests. Aliquots of 100 µl from the suspensions were spread-plated on Mueller-Hinton agar (Biolab, supplied by Merck) using a sterile cotton swab. Single disc diffusion method was used to assess the susceptibility of S*almonella* isolates to commonly used antimicrobial agents. A total of 12 antibiotic discs (Davies diagnostics, SA) were used in this investigation including gentamicin (GM; 10 µg), ciprofloxacin (CIP; 5 µg), rifampicin (RIF; 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 (STR; 10 µg) and amoxicillin-clavulanic acid (AMC; 30 µg). The antimicrobial profile of isolated bacteria to different antibiotics was determined following recommendations of the clinical laboratory institute standards interpreted as intermediate (I), sensitive (S), and resistant (R). The *E. coli* (ATCC 25922) was used as quality control. Strains which showed resistance to at least three classes of antibiotics were considered as multi-drug-resistant (MDR) isolates (Zhao et al., 2017).

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Genotypic test for detecting antimicrobial resistant

A total of six antimicrobial resistance genes (*bla*TEM, *tet*, *sul*, *cat*, *qnrA*, and *aadA*) were amplified by PCR using respective primer sets targeting the different antimicrobial resistant genes including class 1 integrons. The details of oligonucleotide sequences, their base pairs (bp) including the PCR cycling conditions are shown in table 1. Resistance to aminoglycosides 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; 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 with *tet* gene. In addition, *intI1* is associated with class 1 integrons (Ozgen, 2007; Olobatoke and Mulugeta, 2015; Kim et al., 2016).

Table 1. The list of different primers used to detect antimicrobial resistance in *Salmonella* species

Primers	Sequence (5'-3')	Amplicon size (bp)	Conditions	References
Sul3 *Sul4*	F: TCAACATAACCTCGGACAGT R: GATGAAGTCAGCTCCACCT	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)
tem1 *tem2*	F ATGAGTATTCAACATTTCCGTG R TTACCAATGCTTAATCAGTGAG	792	95°C, 3 min; 30 cyc. of 95°C, 1 min, 55°C, l min, 72°C, 1 min, ext. at 72°C, 5 mins	(Olobatoke and Mulugeta, 2015),
tet *tet*	F: GCACTTGTCTCCTGTTTACTCCCC R: CCTTGTGGTTATGTTTTGGTTCCG	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 and Mulugeta, 2015)
cat *cat*	F: TCCCAATGGCATCGTAAAGAAC R: TCGTGGTATTCACTCGAGAGCG	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 and Mulugeta, 2015)
aadA *aadA*	F: ATCCTTCGGCGCGATTTTG R: GCAGCGCAATGACATTCTTG	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)
qnrA *qnrA*	F: TCAGCAAGAGGATTTCTCA R: GGCAGCACTATGACTCCCA	627	95°C, 5 mins, 30 cyc, 94C 40 s, 50°C 60 s, 72°C 90 s, ext at 72C, 10 mins	(Akiyama and Khan, 2011)
intI1 *intI1*	F: ACATGTGATGGCGACGCACGA R: ATTTCTGTCCTGGCTGGCGA	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	(Tankson et al., 2005)

RESULTS

Identification of *Salmonella* spp.

The *Salmonella* serovars detected from rats included *S.* typhimurium (n=26, 38.2%), *S.* enteritidis (n=12, 17.6%), *S.* newport (n=8, 11.8%), *S.* heidelberg (n=7, 10.3%), *S. bongori* (n=6, 8.8%), *S. enterica* serovar Paratyphi B (n=4, 5.9%), *S.* tennessee (n=3, 4.4%) and *S.* pullorum (n=2, 2.9%). The predominant *Salmonella* serovars isolated from chickens were *S.* typhimurium (n=18, 39.1%), *S.* heidelberg (n=9, 19.6%), *S. bongori* (n=7, 15.2%), *S.* enteritidis (n=6, 13.0%), *S.* paratyphi B (n=3, 6.5%) and *S.* newport (n=3, 6.5%).

Phenotypic antimicrobial resistance of *Salmonella* species

The *Salmonella* isolates revealed resistance to rifampicin (n=114, 100%), tetracycline (n=78, 68%), ciprofloxacin (n=55, 48%), sulphonamides (n=48, 42%), cephalothin (n=20, 18%), chloramphenicol (n=45, 39%), streptomycin (n=20, 18%), enrofloxacin (n=6, 5%), ampicillin (n=32, 28%), amoxicillin/clavulanic acid (n=7, 6%) and nalidixic acid 38 (33%), and gentamicin 5 (4%). All *Salmonella* isolated from rat were susceptible to gentamicin as shown in table 2.

Five *S.* typhimurium were MDR for up to six different antibiotics (rifampicin, tetracycline, enrofloxacin, cephalothin, ciprofloxacin, and sulphonamides). Some isolates like *S.* enteritidis and *S.* typhimurium exhibited resistance for up to five antibiotics (rifampicin, tetracycline, enrofloxacin, cephalothin, and ciprofloxacin) and one *S.* enteritidis for five (rifampicin, enrofloxacin, cephalothin, ciprofloxacin, and streptomycin). Furthermore, different isolates exhibited resistance for three to four antibiotics as listed in table 3.

Genotypic antimicrobial resistance of *Salmonella* isolates

The results of the analysis of *Salmonella* isolates for the occurrence of ARGs are shown in table 4. Seventy-eight (68%) isolates encoding class 1 integrons were detected from *Salmonella* spp. and most of the isolates which were harboring different resistance genes were also carrying class 1 integrons. In addition, the majority of the isolates which were harboring class 1 integrons were from the rats (n=52) while 26 isolates were from the chickens. Different resistance gene patterns were indicated by *S.* typhimurium, *S.* enteritidis, *S.* heidelberg, *S. bongori*, *S.* newport, *S. enterica* serovar Paratyphi B, *S.* tennessee and *S.* pullorum*;* respectively. The mentioned results are shown in table 5.

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Table 2. Antimicrobial resistance of *Salmonella* serovars isolated from rats (*Rattus* spp.) and layer chickens in poultry houses, North West, South Africa

Antibiotic	Abbreviation	Breakpoint disc (µg)	Chickens (n=46)	Rats (n=68)	Total (n=114)
Ampicillin	AMP	(10 µg)	29(63%)	3(4%)	32(28%)
Sulphonamides	SSS	(300 µg)	36(78%)	12(18%)	48(42%)
Cephalothin	KF	(30 µg)	8(17%)	12(18%)	20(18%)
Tetracycline	TE	(30 µg)	46(100%)	32(47%)	78(68%)
Ciprofloxacin	CIP	(30 µg)	34(74%)	21(31%)	55(48%)
Nalidixic acid	NA	(30 µg)	37(80%)	1(2%)	38(33%)
Chloramphenicol	CA	(10 µg)	36(78%)	9(13%)	45(39%)
Gentamicin	GM	(5 µg)	5(11%)	0(0%)	5(4%)
Enrofloxacin	ENR	(5 µg)	0(0.0%)	6(9%)	6(5%)
Rifampicin	RIF	(10 µg)	46(100%)	68(100%)	114(100%)
Streptomycin	STR	(5 µg)	12(25%)	8(12%)	20(18%)
Amoxicillin/clavulanic Acid	AMC	(30 µg)	5(11%)	2(3%)	7(6%)

Table 3. Antimicrobial resistance and the prevalence of resistant strains in *Salmonella* isolates from rats (*Rattus* spp.) and layer chickens in poultry houses, North West, South Africa

Isolates / MAR Phenotypes	*S.* typhimurium	*S.* enteritidis	*S.* newport	*S.* heidelberg	*S. bongori*	*S. enterica* paratyphi *B*	*S.* pullorum	*S.* tennessee
ENR, TE, GM, KF, STR, SSS	5	–	–	–	–	–	–	–
ENR, TE, GM, KF, STR	1	3	–	–	–	–	–	–
ENR, GM, KF, STR, RIF	1	1	–	1	–	–	–	–
ENR, NA, TE, KF, STR	–	1	–	–	1	–	–	–
ENR, AMP, SSS, NA	1	–	–	–	–	–	–	–
ENR, AMP, TE, SSS	1	–	–	–	–	–	–	–
ENR, NA, TE, STR	–	–	1	–	–	–	–	1
ENR, NA, TE, SSS	1	–	–	–	2	–	–	–
ENR, TE, GM, KF	3	–	–	–	–	–	–	–
ENR, CA, TE, GM	–	–	–	–	1	–	–	–
ENR, TE, STR, SSS	–	1	–	–		–	1	–
ENR, AMP, KF, SSS	–	–	1	–	–	–	–	–
ENR, TET, KF	–	–	–	1	–	–	–	–
ENR, NA, SSS	–	–	–	1	–	–	1	–
ENR, AMP, STR	1	–	1	–	–	–	–	–
ENR, NA, RIF	–	1	–	–	–	1	–	–
ENR, TE, RIF	–	–	–	–	–	–	–	1

MAR: Multiple Antibiotic Resistance, GM: Gentamicin, RIF: Rifampicin, NA: Nalidixic Acid, AMP: Ampicillin, ENR: Enrofloxacin, TE: Tetracycline, KF: Cephalothin, SSS: Sulphonamides, STR: Streptomycin; *S*: *Salmonella.*

Table 4. Antibiotic resistance genes among the different *Salmonella* spp. isolated from rats (*Rattus* spp). and layer chickens in poultry houses around North West, South Africa.

Antimicrobial agent	Class of antimicrobials	Genes tested	No. of isolates	Resistant phenotype (%)	Resistance genes and integrons (%)
Chloramphenicol	Phenicol	*cat*	114	45(39%)	11(10%)
Tetracycline	Tetracycline	*tet*	114	78(68%)	21(18%)
Ampicillin	Beta-lactam	*blaTEM*	114	32(28%)	4(4%)
Sulfonamide	Sulfonamide	*Sul*	114	48(42%)	16(14%)
Streptomycin	Aminoglycoside	*aadA*	114	20(18%)	6(5%)
Ciprofloxacin	Quinolones	*qnr-A*	114	55(48%)	20(18%)
Class 1 integrons	–	*intI1*	114	–	78(68%)

No: number,

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Table 5. The number of *Salmonella* isolates containing antimicrobial resistance genes and class 1 integrons recovered from rats (*Rattus* spp.) and layer chickens in poultry houses around North West, South Africa.

Genotype	*S.* typhimurium	*S.* enteritidis	*S.* newport	*S.* heidelberg	*S. bongori*	*S. enterica* paratyphi B	*S.* pullorum	*S.* tennessee
cat	17	6	6	7	7	2	1	2
tet	25	7	5	9	7	2	–	–
*bla*TEM	17	7	–	1	3	1	–	–
sul	21	6	–	7	3	3	–	–
aadA	1	1	–	5	2	1	–	–
qnrA	13	8	1	–	1	2	–	–
intI1	30	19	11	4	7	5	1	1

aadA gene mediates bacterial resistance to streptomycin, *bla*TEM mediate bacterial resistance to ampicillin, *cat* gene mediates bacterial resistance to chloramphenicol, *Sul3, Sul4* genes mediate bacterial resistance to sulfonamide, *qnrA* gene mediates bacterial resistance to quinolones, *tet* gene mediates bacterial resistance to tetracyclines, *ntI1* = Class 1 Integrons, *S.*: *Salmonella.*

DISCUSSION

This is the first study to demonstrate antimicrobial resistance in *Salmonella* spp. isolated from rats captured from chicken houses in North West province and to our knowledge in the whole of South Africa. The 12 antimicrobial drugs were tested in the present study, are commonly used for the treatment of bacterial infections of animals and human's health. Finding resistance to these commonly used antimicrobials is very significant because rats are never treated with antibiotics thus the antimicrobial resistance observed in *Salmonella* isolates from rats represents the contamination and circulation of resistant strains in the environment which ultimately affects both animals and humans.

The current study demonstrated a very high prevalence (100%) resistance of *Salmonella* isolates to rifampicin using disc diffusion test. This result is consistent with previous studies conducted in Egypt (Mahmoud et al., 2018), in Sardinia, Italy (Piras et al., 2011), in Shandong, China (Zhao et al., 2017) and in the United Arab Emirates (Khan et al., 2010). Rifampicin is used as the first-line drug for the treatment of humans Tuberculosis (TB) in South Africa (McIntosh et al., 2018). In South Africa due to the presence of gold mines, a high number of people suffer from TB because of silica dust exposure (Phillips et al., 2014). Therefore, many people around the North West province, a province which has minings, are taking TB treatment (Chirehwa et al., 2018) and this may explain the high possibility that the drug is being discharged into the environment and as a result, environmental bacteria and rats are constantly exposed to the drug. Therefore, this finding is a significant concern for public health and further research is necessary to evaluate its effect on other bacterial species.

A high resistance rate of 78 isolates to tetracycline was observed on disc diffusion. However, when the same isolates were assessed using molecular methods targeting the *tet* gene, only 55 of the isolates were positive. Tetracycline is a low-cost medication in comparison to other antibiotics (Odoch et al., 2018) and therefore is extensively used for therapy and prophylaxis of animal and human infections (Ammons and Copié, 2013; Granados-Chinchilla and Rodríguez, 2017; Almaaytah et al., 2018). It is also commonly used at sub-therapeutic levels for growth promotion (Chopra and Roberts, 2001). This encourages selection for resistance and results in the presence of higher percentages of bacteria in the environment that cannot respond to treatment. Thus the high resistance finding did not surprising because previous studies have also reported that the most frequently detected ARG is *tet* gene (Zishiri et al., 2016).

Forty-eight (42%) *Salmonella* isolates were resistant to sulphonamides on disc diffusion compared to 40 (35%) of these isolates on molecular evaluation using the *Sul* gene. This result 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 such as fowl typhoid, coccidiosis coryza and pullorum disease (Mehtabuddin et al., 2012) and thus are common in poultry houses. According to the results of a survey, 95.4% of sulphonamides are used as water medication in South Africa (Eagar et al., 2012). Thus, this condition makes these drugs common environmental contaminants in water spills that can be picked up by all organisms of environment, consequently exert selective pressure on ARGs.

The results of the present study indicated 55 (48%) of the *Salmonella* isolates were resistant to ciprofloxacin on disc diffusion. However, when these isolates were subjected to molecular evaluation of resistance using the *qnr-A* gene, 25 (22%) out of 114 isolates were positive for the *qnr-A* gene. Quinolones or fluoroquinolones have been used as a treatment option for salmonellosis for over 40 years (Balasundaram et al., 2017). Fluoroquinolones have actually been considered as one of the last options for the treatment of *Salmonella* spp. (Abdel-Maksoud et al., 2015). The absence of the *qnr-A* gene in isolates that had a positive phenotype indicated that the disc diffusion method may either be more sensitive and causing a few false positive. To explain this contradiction it can also be noted that the target gene for ciprofloxacin is not always the *qnr*ABSCD genes (Kim et al., 2016).

A total of 20 (18%) isolates were found to be phenotypically resistant to streptomycin, of which half of these isolates were harboring *aadA* gene. This observation may be explained by a fact that this class can be encoded by different resistant genes. Chloramphenicol was used as the main treatment against *Salmonella*, since its discovery up to 1990 (Ishaleku et al., 2015). The increasing resistance of *Salmonella* spp. to chloramphenicol has previously been reported (Olobatoke and Mulugeta, 2015) from chicken samples in North West province, South Africa. The present evaluation isolated 45 (39%) *Salmonella* specimens which were phenotypically resistant to chloramphenicol and 44 (39%) of them were harboring *cat* gene. This suggests that there is an ongoing use of this antibiotic due to its broad-spectrum activity, despite awareness of resistance.

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Only 32 (28%) *Salmonella* isolates in this study were phenotypically resistant to ampicillin while in the molecular evaluation of the *blaTEM* gene, 29 (25%) isolates were resistant. The absence of mentioned gene from some isolates that were phenotypically positive may indicate that the target gene for this antibiotic is not always the same or the disk diffusion test has high sensitivity and but not specificity to antibiotics resistance (Dickert et al., 1981). This finding causes more concerns because antibiotic resistance for ampicillin has previously been detected from poultry products around this area (Olobatoke and Mulugeta, 2015). A high number of the isolates showed MDR to tetracycline, ciprofloxacin, and sulphonamides, which are antimicrobial agents commonly used in veterinary medicine. This is worrying because some antibiotics are recommended drugs for the treatment of salmonellosis (Hirose et al., 2001). MDR *Salmonella* has been observed in other countries such as Malaysia (Thung et al., 2018). MDR *Salmonella* isolates are considered to be highly virulent than non-MDR (Thung et al., 2018) and this finding was a major concern in the current study.

In the present research, seventy-eight (68%) *Salmonella* isolated from chickens and rats were harboring class 1 integrons and the majority of the isolates belonged to the rats (n=52). It was possible that these rats picked up *Salmonella* infections with resistance gene from the environment. Even though there were four classes of integrons associated with the resistance gene cassette, class 1 integrons had been more commonly observed than the other classes (Thong and Modarressi, 2011; Abatcha et al., 2018). According to literature, class 1 integrons are the most common integron types in MDR *Salmonella* spp. and plays a significant role in assisting the transfer of the resistance genes (Thong and Modarressi, 2011). The obtained results in the present study revealed that the *Salmonella* isolates had one or more genes that encode antibiotic resistance.

MDR genes were encountered from two *Salmonella* isolates harboring more than two resistance genes. Out of two the isolates, *S.* typhimurium was MDR regarding both disc diffusion test and gene resistance markers. MDR *Salmonella* isolates has been reported to cause illnesses in either humans and animals in different countries including; the USA and Denmark (Aarestrup et al., 2007), Italy (Graziani et al., 2008), Eastern China (Lu et al., 2014) and Vietnam (Vo et al., 2010). Moreover, the presence of MDR genes in isolated *Salmonella* spp. from rats must be taken seriously as these vertebrates can act as reservoirs and potentially can spread the bacteria to both human and animal surroundings.

CONCLUSION

The current research has pioneered antibiotic resistance investigation on *Salmonella* isolates from rats inhabiting chicken farms in North West, South Africa. The obtained results in the present study revealed that antibiotic resistance is well established in most of the *Salmonella* isolates infecting chickens and rats and the majority of isolates harbor more than two resistance genes. These findings provided a better understanding of the importance of rats in the transmission and maintenance of the antibiotic-resistant *Salmonella* spp. in poultry premises which can potentially be transferred to humans via chicken products.

DECLARATIONS

Author's contributions

Ramatla T. performed the experiments and wrote the first draft. Moeti OT and Thekisoe OMM provided the analysis tools and data analysis and reviewed the manuscript. Michelo S conceived and designed the experiments, provided reagents, materials and approved the final paper.

Consent to publish

All the authors agreed to publish the manuscript and declared that this work has not been previously published elsewhere.

Competing interests

The authors declare that they have no conflict of interest

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