Paper ISSN 0189-0514
Online ISSN 1427-4410

National Animal Production Research Institute
Ahmadu Bello University
P.M.B 1096, Shika-Zaria,
Kaduna State, Nigeria.
Email: japr@napri.gov.ng Url: journal.napri.gov.ng

*J. Anim. Prod. Res. (2021) 33(2):87-100*

# RESISTANCE OF NON-TYPHOIDAL *SALMONELLA* STRAINS ISOLATED FROM BROILER PRODUCTS TO ANTIBIOTICS

**[*1]Olobatoke, R. Y., [2]Ruzvidzo, O. and [2]Mulugeta, S.**

*[1]College of Agriculture, Division of Agricultural Colleges, Ahmadu Bello University, Kabba, Nigeria*
*[2]School of Agricultural Science, North West University, Mafikeng Campus, Private Bag X2046, Mmabatho, 2735, South Africa.*
*[*]Corresponding Author: yemisirose205@yahoo.com*

## ABSTRACT

This study was conducted to evaluate the resistance of non-typhoidal *Salmonella* strains (NTS) isolated from broiler products marketed in North-West Province, South Africa, to various antibiotics. A total of sixty (60) NTS isolates recovered from raw broiler products and confirmed by PCR were evaluated for antimicrobial resistance by disk diffusion method, using a panel of ten antibiotics. The NTS isolates that were identified and used in this study include *S.typhimurium* (n = 20), *S. enteritidis* (n = 20) and *S. newport* (n = 20). The antibiotics used were ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), trimethoprim-sulphamethoxasole (25 µg), tetracycline (30 µg) cefotaxime (30 µg), meropenem (10 µg), gentamicin (10 µg) and erythromycin (15 µg). All the strains tested were resistant to two or more antibiotics. However, all isolates were susceptible to cefotaxime, meropenem, gentamycin and amikacin.Whereas, all were resistant to tetracycline, resistance to trimethoprim-sulphamethoxasole, ciprofloxacin and chloramphenicol was low, being 8.3, 13.3 and 20% respectively. Multi-drug resistance was discovered only in nine *S.typhimurium* strains, representing 15% of the tested isolates. Analysis of multiple antibiotic resistance (MAR), revealed seven phenotypes, with resistance to ampicillin, tetracycline and erythromycin as the most common pattern. The observations of this study indicate that NTS strains isolated from broiler products are resistant to multiple antibiotics, and may pose significant health risks to the consumers.

**Key words:** Non-typhoidal *Salmonella*, antibiotics, broiler, disk diffusion, resistance genes

## INTRODUCTION

In recent years, occurrence of antimicrobial resistance among *S. enterica* serovars has been reported in many countries (Lee *et al*., 2009; Fashae *et al*., 2010; Wannaprasat *et al*., 2011). Antimicrobial resistance is a major global health problem in both human and veterinary medicine, with serious socioeconomic consequences. The problem is however more pressing in developing countries where disease burden is high and use of newer antimicrobials is constrained by cost (Okeke *et al*., 2005). β-lactam antibiotics such as ampicillin, tetracycline, chloramphenicol and trimethoprim-sulphamethoxasole are readily available antimicrobial agents for the treatment of invasive salmonellosis. Resistance to these drugs has however, been reported with increasing frequency in many countries (Ruiz *et al*., 1999; Kariuki *et al*., 2005). For instance, resistance of *Salmonella* isolates, from foods of animal origin, to ampicillin, tetracycline, sulphonamides and chloramphenicol was high in Malaysia (22 – 49%), Thailand (41 – 92%) and Vietnam (17 – 68%) (Benacer *et al*., 2010; Vo *et al*., 2010; Wannaprasat *et al*., 2011). Zewdu and Cornelius (2009) similarly reported high antimicrobial resistance (62.1%) in *Salmonella* isolates from chicken carcasses in Ethiopia.

Concurrently, resistance of human NTS isolates to the commonly used antibiotics (ampicillin, tetracycline, etc.) is high in the developing countries, suggesting the extensive use/abuse of

the antimicrobials in veterinary practice/human therapy. Sequel to this development, the fluoroquinolones became drugs of first choice in the treatment of invasive and systemic salmonellosis in both humans and animals (Yates and Amyes, 2005; Giraud *et al*., 2006). However, *Salmonella* strains with decreased susceptibility to fluorinated quinolones have been reported in many countries and are now considered widespread. Fashae *et al*. (2010) reported a high frequency (up to 87%) of reduced susceptibility to ciprofloxacin in NTS isolates from chickens in Nigeria. Similarly, Ellerbroek *et al*. (2010) observed that more than half of NTS spp, isolated from pigs, were resistant to ciprofloxacin. Reduced susceptibility of clinical *Salmonella* isolates to ciprofloxacin has also been reported in Philippines (15%) and Singapore (25%). Whereas the production of β-lactamase is the primary cause of resistance to β-lactam antibiotics in *Enterobacteriaceae*, resistance to quinolones is believed to result from chromosomal mutations in genes that encode target enzymes or affect drug accumulation (Arlet *et al*., 2006; Strahilevitz *et al*., 2009). TEM and SHV penicillinases seem to be the most widely spread extended-spectrum beta lactamases (ESBLs), and nosocomial strains of *K. pneumonieae* and *E.coli* are believed to be main reservoirs of ESBL genes (Miriagou *et al*., 2004). However, hospital NTS isolates producing TEM, SHV, CMY and CTX-type ESBLs have been reported in South Africa (Kruger *et al*., 2004; Usha *et al*., 2008; Keddy *et al*., 2009). Similarly, the first plasmid-mediated quinolone resistance in *Salmonella* from South Africa was observed in *S. typhimurium* isolated from the blood of HIV-seropositive patient (Govender *et al*., 2009). Poultry products have been incriminated in many outbreaks of human salmonellosis in South Africa (Smith et al., 2007), the antimicrobial resistance of NTS isolates from contaminated poultry products has not been elucidated. This study therefore aims at investigating the resistance of NTS species isolated from broiler products in the North West Province of South Africa to some common antimicrobials and to screen the isolates for the presence of antibiotic resistance genes.

## MATERIALS AND METHODS

### Bacterial isolation and identification

A total of 120 raw broiler products (whole carcasses, drumsticks, wings) obtained from different towns in the North West Province of South Africa were screened for non-typhoidal *Salmonella* contamination. Samples were collected during the periods of March and October, 2020. Bacterial culture, isolation and biochemical screening techniques were carried out using the MFHPB-20 procedure. *Salmonella typhimurium* ATCC 14028 and *E. coli* ATCC 25922 were used as positive and negative controls respectively.

### Molecular identification of isolates

DNA extraction of isolates was carried out by alkaline lysis method (Sambrook et al., 1989). Extracted DNA was purified and subjected to PCR targeting 16S rRNA and *Salmonella*-specific *invA*gene (Table 1). All PCR reactions were prepared in 25 µl volumes consisting of 1 µg/µl of the template DNA, 50 pmol of each oligonucleotide primer set, 1X PCR master mix, 1U Taq DNA polymerase and nuclease free distilled water. Amplifications were performed using a Peltier Thermal Cycler (model-PTC-220 DYAD™ DNA ENGINE). A reaction blank containing all the components of the reaction mixture except the template DNA was included in each PCR procedure to check for contamination. Thereafter, 10 µl of the PCR amplicons was analysed by standard submarine gel electrophoresis using 1% (w/v) agarose at 60 V for 6 hours. Amplicons which showed clear bands for 16S rRNA and *invA* genes were purified and sequenced (Inqaba Biotech, SA) for strain identification. The search for amino acid homology of the sequence results was done using the NCBI Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST).

**Antimicrobial susceptibility testing of isolates**

A total of sixty (60) identified NTS isolates (*S.typhimurium* = 20; *S. enteritidis* = 20; *S. newport* = 20) were subjected to antibiotic susceptibility testing according to Kirby-Bauer disc-diffusion method as previously described (Ndip *et al*., 2005). This method conforms to the recommended standard of the Clinical and Laboratory Standards Institute (CLSI). Briefly, a small inoculum of each pure *Salmonella* isolate, taken from a fresh culture on nutrient agar,was emulsified in 3 mL of sterile normal saline in Bijou bottles.The density of the emulsion was compared with a barium chloride standard (0.5 McFarland) to ensure appropriate turbidity. Mueller-Hinton agar plates (Mercks, South Africa) were then inoculated with the bacterial suspensions and the plates were allowed to dry. Antibiotic discs (Davies diagnostics, SA) with the following drug contents: ampicillin (10µg), chloramphenicol (30µg), ciprofloxacin (5µg), amikacin (30µg), trimethoprim-sulphamethoxasole (25µg), tetracycline (30µg) cefotaxime (30µg), meropenem (10µg), gentamicin (10µg) and erythromycin (15µg) were placed on the agar plates. Plates were incubated at 37$^{o}$C for 24 hours. Results were recorded as susceptible, intermediate or resistant. Multiple antibiotic resistance (MAR) phenotypes were generated for all isolates that showed resistance to two or more antibiotics. Based on antibiotic resistance patterns of the isolates, a dendrogram was constructed to show the relationship among the NTS isolates using the rmsd coefficient, Unweighted Pair Group Method with arithmetic mean (UPGMA) (Saitou and Nei, 1987).

**Molecular characterization of resistant isolates**

Overnight cultures of the isolates that showed resistance to ampicillin, tetracycline and chloramphenicol were prepared in 10 mL of nutrient broth, from which the DNA of the isolates were extracted. The extracted DNA was subjected to PCR amplification, targeting blaTEM, *tet* and *cat* genes (Türkyilmaz *et al*., 2009). The details of oligonucleotide sequences used and the PCR cycling conditions are shown in Table 1.

**Table 1: Oligonucleotide sequences and PCR conditions used for amplification**

| Primer | Sequence (5′ - 3′) | Target gene | Amplicon size (bp) | PCR conditions |
|---|---|---|---|---|
| TEM1 F | ATGAGTATTCAACATTTCCGTG | *bla*TEM | 793 | 95$^{o}$C, 3 mins; 30 cyc. of |
| TEM2 R | TTACCAATGCTTAATCAGTGAG | | | 95$^{o}$C, 1 min; 55$^{o}$C, 1min; 72$^{o}$C, 1min; ext. at |
| | | | | 72$^{o}$C, 5 mins |
| TET F | GCACTTGTCTCCTGTTTACTCCCC | *tet* | 659 | 94$^{o}$C, 2 mins; 35 cyc. of |
| TET R | CCTTGTGGTTATGTTTTGGTTCCG | | | 94$^{o}$C, 20 s; 53$^{o}$C, 10 s; 65$^{o}$C, 45 s; ext. at 65$^{o}$C, 4 mins |
| CAT F | TCCCAATGGCATCGTAAAGAAC | *cat* | 310 | 95$^{o}$C, 5 mins; 35cyc. of 94$^{o}$C, 30 s; 55$^{o}$C, 30 s; 72$^{o}$C, 30 s; ext. at 72$^{o}$C, 10 mins |
| CAT R | TCGTGGTATTCACTCGAGAGCG | | | |
| 27F | AGAGTTTGATCCTGGCTCAG | 16S ribosomal gene | 1450 | |
| 1492R | GGTTACCTTGTTACGACTT | | | 95$^{o}$C, 5 min;35 cyc of 94$^{o}$C, 30 s; 61$^{o}$C, 30 s; 72$^{o}$C, 1 min; ext. at 72$^{o}$C, 5 mins |
| | | *invA* | | |
| InvF | CGCGGCCCGATTTTCTCTGGA | | 321 | |
| invR | AATGCGGGGATCTGGGCGACAAG | | | 94$^{o}$C, 90 s;30 cyc of 94$^{o}$C, 45 s; 60$^{o}$C, 45 s; 72$^{o}$C, 90 s; ext. at 72$^{o}$C, 3 mins |

Cyc = cycle; ext = extension

## RESULTS AND DISCUSSION

### Antimicrobial susceptibility profiles

Although all the strains tested were resistant to two or more antibiotics, multi-drug resistance was discovered only in nine *S. typhimurium* isolates, representing 15% of all the tested isolates. The *S. typhimurium* isolates were resistant to ampicillin, tetracycline, chloramphenicol, ciprofloxacin, trimethoprim-sulphamethoxasole and erythromycin. Dogru *et al*. (2010) and Maripandi and Al-Salamah (2010) also reported resistance to multiple antimicrobials in *Salmonella* isolated from chicken carcasses. However, all isolates were susceptible to cefotaxime, meropenem, gentamycin and amikacin, but resistant to tetracycline. High resistance of isolates to ampicillin and tetracycline observed in the current study is in agreement with previous research findings (Antunes *et al*., 2003; Cheong *et al*., 2007; Fashae *et al*., 2010). Antunes *et al*. (2003) further reported tetracycline as one of the antibiotics most commonly resisted by

*Salmonella*. A low frequency of resistance was found towards trimethoprim-sulphamethoxasole (8.3%), ciprofloxacin (13.3%) and chloramphenicol (20%). *S. typhimurium* showed the highest resistance to both chloramphenicol and ampicillin (35% and 100% respectively) and this is in agreement with the report of Soler *et al*. (2006). Tetracycline and the β-lactams such as ampicillin are among the antibiotics used frequently in poultry production (Moussa *et al*., 2007). Thus, uncontrolled use of these antimicrobials in the live birds may have contributed to the observed resistance patterns. Reduced susceptibility of NTS isolates to ciprofloxacin noted in this study is in accordance with the observation of Fashae *et al*. (2010) in NTS isolates from Nigeria. In contrary, Salam *et al*. (2003) and Zaidi *et al*. (2006) reported 100% susceptibility of NTS isolates to ciprofloxacin in Bangladesh and Mexico respectively. Thus, the source of isolates with respect to geographical location may possibly be responsible for the disparity in observations noticed in this case. However, resistance to ciprofloxacin (a fluoroquinolone) noticed in the current study was found mainly in *S. Typhimurium*. This may be a cause for public health concern, particularly in the treatment of invasive salmonellosis. Figure 1 and Table 2 indicate the frequency of resistance to individual drugs and distribution of antimicrobial resistance in isolates respectively.

MAR analysis revealed eight resistance phenotypes, with resistance to ampicillin, tetracycline and erythromycin being the most common pattern (Table 3). The highest frequency of this resistance pattern was found in *S. typhimurium*. In addition, four other resistance patternswere shown by *S. typhimurium*, including resistance to ciprofloxacin, ampicillin, chloramphenicol and tetracycline. This observation agrees with the report of Soler *et al*. (2006) who noted resistance to ampicillin, chloramphenicol, sulphonamide and tetracycline as the most frequent resistant pattern of *S. typhimurium*, particularly DT104 strain. *S. newport* showed three resistance patterns including resistance to ampicillin, trimethoprim-sulphamethoxasole and tetracycline, whereas *S. enteritidis* showed only two resistance phenotypes.

**Cluster analysis**

Cluster analysis of all isolates based on antimicrobial resistance patterns revealed six major clusters (Fig.2). However, *S. typhimurium* strains belonged to four distinct clusters whereas *S.enteritidis* and *S. newport* strains were grouped into three and two clusters respectively. Most of the NTS isolates tested showed multiple antibiotic resistance, irrespective of their sources. Therefore the clonal diversity observed among the strains in the current study, is an indication of possible circulation of antimicrobial resistance genes in *Salmonella* strains within the environment. This may significantly add to the health care burden.

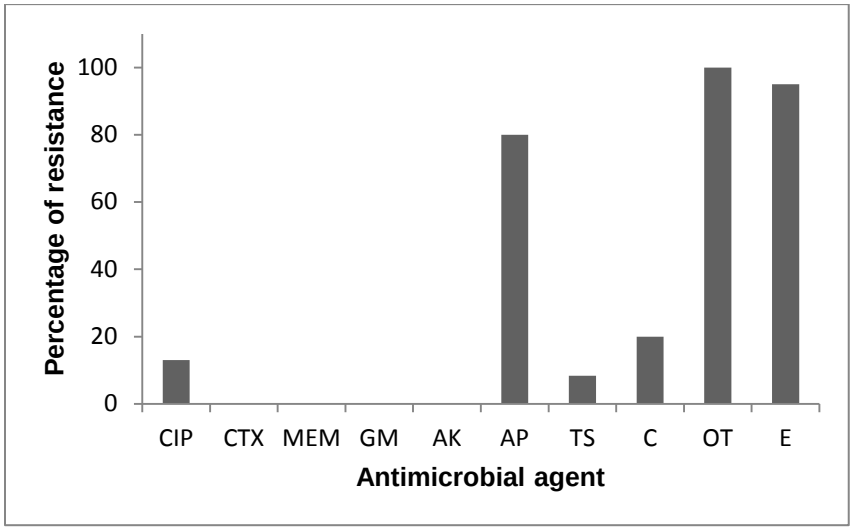



***Figure 1:*** *Frequency of resistance to individual antimicrobial agent. CIP = ciprofloxacin; CTX = cefotaxime; MEM = meropenem; GM = gentamycin; AK = amikacin; AP = ampicillin; C = chloramphenicol; OT = tetracycline; TS = trimethoprim-sulphamethoxasole; E = erythromycin*

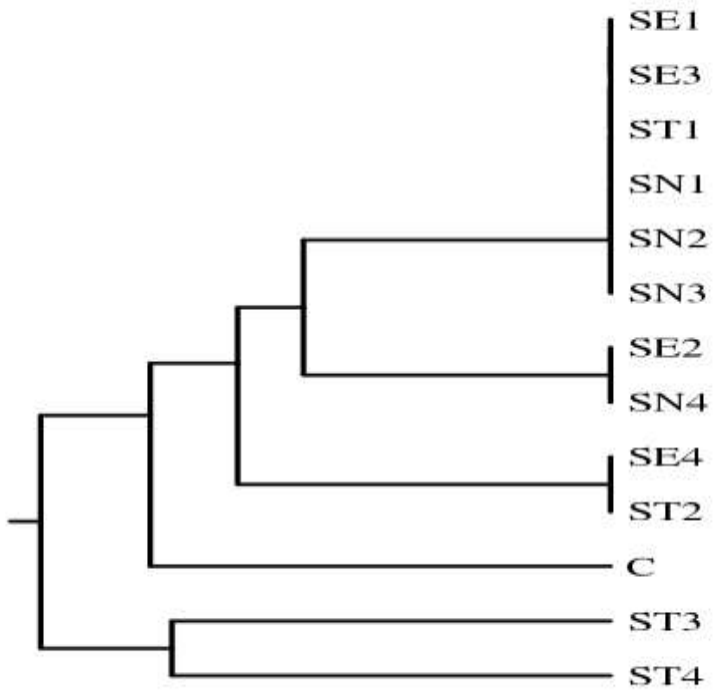



***Figure 2:*** *Dendrogram showing the relationship between NTS isolates obtained from raw broiler samples based on antibiotic resistance patterns. SE = Salmonella enteritidis; ST = Salmonella typhimurium; SN = Salmonella newport; C = control*

**Antimicrobial resistance genes**

Occurrence of resistance genes among antimicrobial-resistant isolates is shown in Table 4. Out of 48 ampicillin-resistant isolates that were screened, 15 (31.3%) carried the blaTEM gene (Fig. 5). This observation accords with the report of Hamidian*et al*. (2009) who investigated the presence of ESBLs in *Salmonella* species obtained from patients in Tehran hospitals. Turkyilmaz*et al*. (2009) similarly noted that three out of five ampicillin-resistant *Salmonella* species tested carried blaTEMgene. The presence of TEM-63, TEM-116 and TEM-131 in NTS isolates have also been reported in Durban of South Africa (Usha *et al*., 2008). The TEM type β-lactamases thus appear to be the most common gene that mediates resistance to ampicillin in *Salmonella* (Turkyilmaz*et al*., 2009). Similarly, 12 (20%) out of 60 tetracycline-resistant isolates were found to harbour the *tetB* gene (Fig. 6), which is in agreement with the observations of Roberts (2005) and Turkyilmaz*et al*. (2009). In addition, *cat3* (chloramphenicol resistance) gene was found in 5 (42%) out of twelve chloramphenicol-resistant NTS isolates whereas one isolate (8%) haboured both *cat1* and *cat3* genes (Fig. 7).

**Table 2: Occurrence of antimicrobial resistance among NTS isolates**

| Antimicrobial agent | *S.enteritidis* (n = 20 | *S.typhimurium* (n = 20) | *S.newport* (n = 20) |
|---|---|---|---|
| CIP(5) | 0(0) | 8(40) | 0(0) |
| CTX(30) | 0(0) | 0(0) | 0(0) |
| MEM(10) | 0(0) | 0(0) | 0(0) |
| GM(10) | 0(0) | 0(0) | 0(0) |
| AK(30) | 0(0) | 0(0) | 0(0) |
| AP(10) | 15(75) | 20(100) | 13(65) |
| TS(25) | 0(0) | 3(15) | 2(10) |
| C(30) | 5(25) | 7(35) | 0(0) |
| OT(30) | 20(100) | 20(100) | 20(100) |
| E(15) | 20(100) | 17(85) | 20(100) |

CIP = ciprofloxacin; CTX = cefotaxime; MEM = meropenem; GM = gentamycin; AK = amikacin; AP = ampicillin; C = chloramphenicol; OT = tetracycline; TS = trimethoprim-sulphamethoxasole; E = erythromycin

**Table 3: MAR patterns of obtained isolates**

| MAR phenotypes | *S. typhimurium* | *S. enteritidis* | *S. newport* | Total no. observed | MAR index |
|---|---|---|---|---|---|
| Ap-Ot-E | 15 | 5 | 12 | 32 | 9.6 |
| Cip-Ap-Ot-E | 3 | 0 | 0 | 3 | 1.2 |
| Cip-Ap-C-Ot-E | 3 | 0 | 0 | 3 | 1.5 |
| Ot-E | 0 | 3 | 10 | 13 | 0.8 |
| Ts-Ot-E | 0 | 0 | 3 | 3 | 0.9 |
| Ap-Ts-C-Ot-E | 3 | 0 | 0 | 3 | 1.5 |
| Ap-C-Ot | 3 | 0 | 0 | 3 | 0.9 |
| C-Ot-E | 0 | 5 | 0 | 5 | 1.5 |

Cip = ciprofloxacin; Ap = ampicillin; C = chloramphenicol; Ot = tetracycline; Ts = trimethoprim-sulphamethoxasole; E = erythromycin

**Table 4: Occurrence of resistance genes among antimicrobial-resistant NTS isolates**

| Antimicrobial resistance | No. of resistant isolates | Genes tested | No. of positive isolates (%) |
|---|---|---|---|
| Ampicillin | 48 | $bla_{TEM}$ | 15(31.3) |
| Tetracycline | 60 | *tet*B | 12(20) |
| Chloramphenicol | 12 | *cat* | 6(50) |

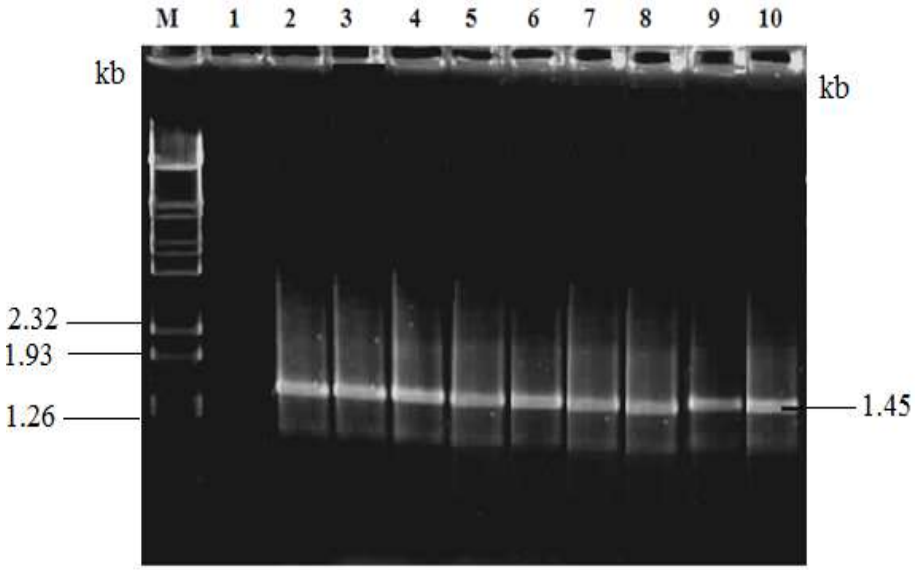



***Figure 3:*** *Agarose gel (1%) electrophoresis showing 16S rRNA gene fragments amplified from Salmonella spp. Lane 1 = No template (control); lane 2 = S. typhimurium ATCC 14028; lanes 3 - 10 = positive amplification of 16S rRNA gene fragments of Salmonella spp isolated from samples; lane M = DNA marker (Lambda).*

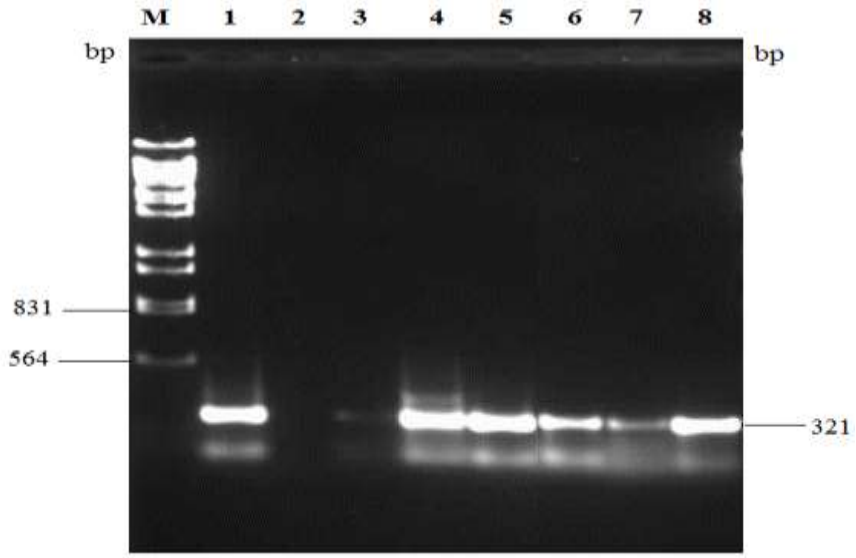



***Figure 4:*** *Agarose gel (1%) electrophoresis showing amplification of 321 bp fragments of invA genes of Salmonella. Lane 1 = S. typhimurium ATCC 14028; lane 2 = negative control; lanes 3 to 8 showing positive amplification of 321 bp fragments of Salmonella spp; lane M = DNA marker (Lambda).*

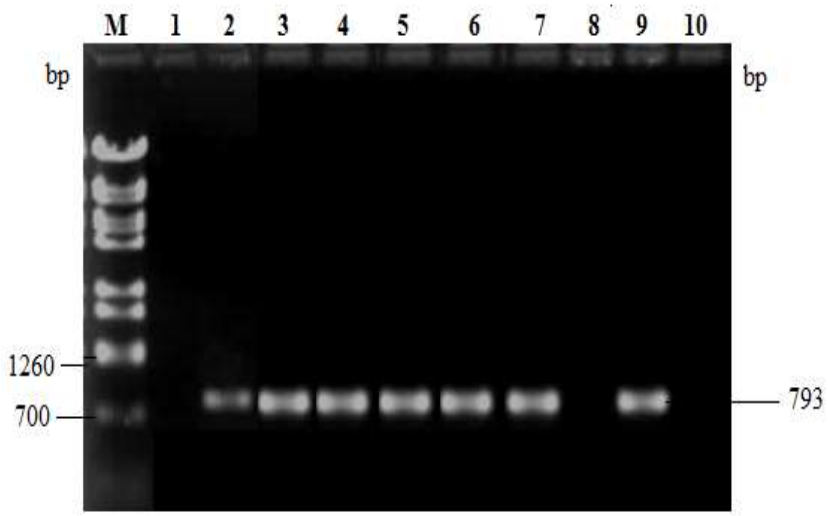



***Figure 5:*** *Agarose gel (1%) electrophoresis showing amplification of 793 bp fragments of $bla_{TEM}$ genes in ampicillin-resistant NTS isolates. Lane 1 = No template; lanes 2 to 7 and 9 = positive amplification of $bla_{TEM}$ gene; lanes 8 and 10 = negative; lane M = DNA marker (Lambda)*

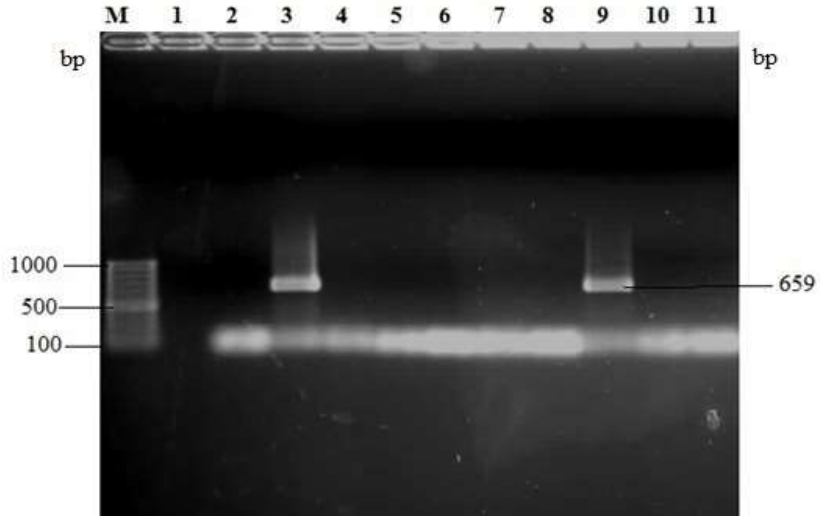



***Figure 6:*** *Agarose gel (1%) electrophoresis showing amplification of 659 bp fragments of tetB genes in tetracycline-resistant NTS isolates. Lane 1 = No template; lanes 3 and 9 = positive amplification of tetB fragment; lanes 2, 4, 5, 6, 7, 8, 10 amd 11 = negative; lane M = DNA marker (100 bp)*

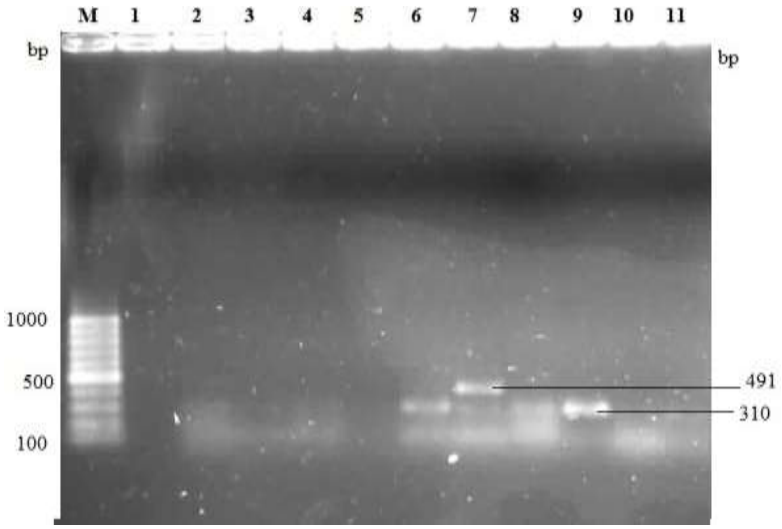



***Figure 7:*** *Agarose gel (1%) electrophoresis showing amplification of 310 bp and 491 bp fragments of cat3 and cat1 genes in chloramphenicol-resistant NTS isolates. Lane M = DNA marker (100 bp); lanes 2, 4, 6, 8 and 9 = positive amplification of cat3 fragment; lane 7 = positive amplification of cat1 and cat3 fragments*

Chuanchuen and Padungtod (2009) similarly reported the expression of $bla_{TEM}$, *tet* and *cmlA* genes in antibiotic-resistant NTS isolates that were obtained from live poultry. The isolates were found to be resistant to ampicillin, tetracycline and chloramphenicol which are drugs that are commonly used in poultry production (Moussa, *et al*., 2007). In conclusion, the observations of this study indicate that NTS strains isolated from broiler products in NWP of South Africa are resistant to multiple antibiotics, including quinolones. This may constitute significant public health problem and therefore calls for proper monitoring to reduce risk of exposure to the pathogens.

## ACKNOWLEGEMENTS

We wish to express our gratitude to the management of North West University, South Africa, for the funding of this research.

## REFERENCES

Antunes, P., Réu, C., Sousa, J. C., Peixe, L. and Pestana, N. (2003). Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. *International Journal of Food Microbiology*.82: 97 – 103.

Arlet, G., Barrett, T. J., Butaye, P., Cloeckaert, A., Mulvey, M. R. and White, D. G. (2006). *Salmonella* resistant to extended-spectrum cephalosporins: prevalence and epidemiology. *Microbesand Infection*,8: 1945–1954.

Benacer, D., Kwai lin, T., Haruo, W. and Savithri, D. P. (2010). Characterization of drug-resistant *Salmonella enterica* serotype Typhimurium by antibiograms, plasmids, integrons, resistance genes, and PFGE. *Journal of Microbiology and Biotechnology*, 20: 1042-1052.

Cheong, H. J., Lee, Y. J., Hwang, I. S., Kee, S. Y., Cheong, H. W., Song, J. Y., Kim, J. M., Park, Y. H., Jung, J. H. and Kim, W. J.

(2007). Characteristics of non-typhoidal *Salmonella* Isolates from human and broiler-chickens in Southwestern Seoul, Korea. *Journal of Korean Medical Science*, 22: 773-778.

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

Dogru, A.K., Ayaz, N.D. and Gencay, Y.E. (2010). Serotype identification and antimicrobial resistance profiles of *Salmonella* spp. isolated from chicken carcasses. *Tropical Animal Health and Production*,42: 893–897.

Ellerbroek, L., Narapati, D., Phu Tai, N., Poosaran, N., Pinthong, R., Sirimalaisuwan, A., Tshering, P., Fries,R., Zessin, K.-H., Baumann, M. and Schroeter, A.(2010). Antibiotic resistance in *Salmonella* isolates from imported chicken carcasses in Bhutan and from Pig Carcasses in Vietnam. *Journal of Food Protection*,73:376–379.

Fashae, K.., Ogunsola, F., Aarestrup, F.M., Rene, S. and Hendriksen, R.S. (2010). Antimicrobial susceptibility and serovars of *Salmonella* from chickens and humans in Ibadan, Nigeria. *Journal of Infection in Developing Countries*, 4: 484-494.

Giraud, E., Baucheron, S. and Cloeckaert, A. (2006). Resistance to fluoroquinolones in *Salmonella:* emerging mechanisms and resistance prevention strategies. *Microbes and Infection*,8: 1937–1944.

Govender, N., Smith, A.M., Karstaedt, A.S. and Keddy, K.H. (2009). Plasmid-mediated quinolone resistance in *Salmonella* from South Africa. *Journal of Medical Microbiology*,58: 1393-1394.

Hamidian, M., Tajbakhsh, M., Walther-Rasmussen, J. and Zali, M.R. (2009). Emergence of extended-spectrum beta-lactamases in clinical isolates of *Salmonella enterica* in Tehran, Iran. *Japanese Journal of Infectious Disease*, 62:368–371.

Kariuki, S., Revathi, G., Muyodi, J., Mwituria, J., Munyalo, A., Kagendo, D., Murungi, L.and Hart, C. A.(2005). Increasing prevalence of multidrug-resistant non-typhoidal salmonellae, Kenya, 1994–2003. *International Journal of Antimicrobial Agents*,25: 39–45.

Keddy, K.H., Dwarika, S., Crowther, P., Perovic, O., Wadula, J., Hoosen, A., Sooka, A., Crewe-Brown, H. H. and Smith, A. M. (2009). Genotypic and demographic characterization of invasive isolates of *Salmonella typhimurium* in HIV co-infected patients in South Africa. *Journal of Infectionin Developing Countries*,3: 585-592.

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

Lee, H.Y., Su, L.H., Tsai, M.H., Kim, S.W., Chang, H.H., Jung, S.I., Park, K. H., Perera, J., Carlos, C., Tan, B. H., Kumarasinghe, G., So, T., Chongthaleong, A., Hsueh, P. R., Liu, J. W., Song, J. H. and Chiu, C. H. (2009). High rate of reduced susceptibility to ciprofloxacin and ceftriaxone among nontyphoid *Salmonella* clinical isolates in Asia. *Antimicrobial Agents and Chemotherapy*, 53: 2696-2699.

Maripandi, A. and Al-Salamah, A.A. (2010). Analysis of *Salmonella enteritidis* outer membrane proteins and lipopolysaccharide profiles with the detection of immune dominant proteins. *American Journal of Immunology*,6:1-6.

Miriagou, V., Tassios, P.T., Legakis, N.J. and Tzouvelekis, L.S. (2004). Expanded-

spectrum cephalosporin resistance in non-typhoid *Salmonella*. *International Journal of Antimicrobial Agents*,23: 547–555.

Moussa, I.M., Gassem, M.A., Al-Doss, A.A., Mahmoud,W.A.S. and Abdel-Mawgood, A.L. (2010). Using molecular techniques for rapid detection of *Salmonella* serovars in frozen chicken and chicken products collected from Riyadh, Saudi Arabia. *African Journal of Biotechnology*,9: 612-619.

Ndip, R.N., Dilonga, H.M., Ndip, L.M., Akoachere, J.F. K.and Nkuo-Akenji, T. (2005). *Pseudomonas aeruginosa* isolates recovered from clinical and environmental samples in Buea, Cameroon: current status on biotyping and antibiogram. *Tropical Medicine and International Health*,10:74 – 81.

Okeke, N.I., Laxminarayan, R., Bhutta, Z.A., Duse, A.G., Jenkins, P., O'Brien, T.F., O'Brien, T. F., Pablos-Mendez, A. and Klugman, K. P.(2005). Antimicrobial resistance in developing countries. Part I: recent trends and current status. *Lancet Infection and Disease*, 5:481–493.

Roberts, M.C. (2005). Update on acquired tetracycline resistance genes. *FEMS Microbiology Letters*,245: 195-203.

Ruiz, J., Capitano, L., Núñez, L., Castro, D., Sierra, J.M., Hatha, M., Borrego, J. J. andVila, J.(1999). Mechanisms of resistance to ampicillin, chloramphenicol and quinolones in multiresistant *Salmonella typhimurium* strains isolated from fish. *Journal of Antimicrobial Chemotherapy*,43:699-702.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*,4: 406-425.

Salam, M.A., Saha, D., Khan, W. A. and Bennish, M. L.(2003). Non-typhoidal *Salmonella* infection and their antimicrobial resistance in Bangladesh. In Interscience Conference on Antimicrobial Agents and Chemotherapy, Dhaka, Bangladesh.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular cloning 2nd ed. Edition. Cold Spring Harbour Laboratory Press.

Smith, A.M., Gouws, A., Hoyland, G., Sooka, A. and Keddy, K.H. (2007). Outbreaks of food-borne disease – a common occurrence but rarely reported. *South African Medical Journal*, 97:1272.

Soler, P., Gonzalez-Sanz, R., Bleda, M.J., Hernandez, G., Echeita, A. and Usera, M.A. (2006). Antimicrobial resistance in non-typhoidal *Salmonella* from human sources, Spain, 2001–2003. *Journal of Antimicrobial Chemotherapy*, 58: 310–314.

Strahilevitz, J., Jacoby, G.A., Hooper, D.C. and Robicsek, A. (2009). Plasmid-mediated quinolone resistance: a multifaceted threat. *Clinical Microbiology Review*, 22:664–689.

Türkyılmaz, S., Hazımoğlu, Ş. and Bozdoğan, B. (2009). Antimicrobial susceptibility and resistance genes in *Salmonella enterica* serovar Enteritidis isolated from turkeys. *Israel Journal of Veterinary Medicine*, 64: 72-77.

Usha, G., Chunderika, M., Prashini, M., Willem, S.A. and Yusuf, E.S. (2008). Characterization of extended-spectrum beta-lactamases in *Salmonella* spp. at a tertiary hospital in Durban, South Africa.*Diagnostic Microbiology and Infectious Diseases*, 62: 86-91.

Vo, A.T., van Duijkeren, E., Gaastra, W. and Fluit, A.C. (2010). Antimicrobial resistance, class 1 integrons, and genomic island 1 in *Salmonella* isolates from Vietnam. PLoS ONE5, e9440

Wannaprasat, W., Padungtod, P. and Chuanchuen, R. (2011). Class 1 integrons and virulence genes in *Salmonella enterica* isolates from pork and humans.

*International Journal of Antimicrobial Agents*, 37:457–461.

Yates, C. and Amyes, S. (2005). Extended-spectrum β-lactamases in non-typhoidal *Salmonella* spp. isolated in the UK are now a reality: why the late arrival? *Journal of Antimicrobial Chemotherapy*,56: 262−264.

Zaidi, M.B., McDermott, P.F., Fedorka-Cray, P., Leon, V., Canche, C., Hubert, S.K., Abbott, J., Leon, M., Zhao, S. and Headrick, M.(2006). Nontyphoidal *Salmonella* from human clinical cases, asymptomatic children, and raw retail meats in Yucatan, Mexico. *Clinical Infections and. Disease,* 42:21–28.

Zewdu, E. and Cornelius, P. (2009). Antimicrobial resistance pattern of *Salmonella* serotypes isolated from food items and personnel in Addis Ababa, Ethiopia. *Tropical Animal Health andProduction*, 41:241–249.