Microbiol. Biotechnol. Lett. (2020), 48(2), 230–235
http://dx.doi.org/10.4014/mbl.1911.11008
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

Simplex PCR Assay for Detection of *bla*$_{TEM}$ and *gyrA* Genes, Antimicrobial Susceptibility Pattern and Plasmid Profile of *Salmonella* spp. Isolated from Stool and Raw Meat Samples in Niger State, Nigeria

Dickson A. Musa[1], Kolawole H. Aremu[2]*, Abraham Ajayi[3], and Stella I. Smith[4]

[1]*Department of Biochemistry, IBB University, Lapai, Niger State, Nigeria*
[2]*Department of Biochemistry, Osun State University, Osogbo, Osun State, Nigeria*
[3]*Department of Microbiology, University of Lagos, Lagos, Nigeria*
[4]*Molecular Biology and Biotechnology Department, Nigerian Institute of Medical Research Yaba, Lagos, Nigeria*

Received: November 19, 2019 / Revised: January 21, 2020 / Accepted: January 28, 2020

The global evolution of antibiotic resistance has threatened the efficacy of available treatment options with ravaging impacts observed in developing countries. As a result, investigations into the prevalence of antibiotic resistance and the role of plasmids are crucial. In this study, we investigated the presence and distribution of *bla*$_{TEM}$ and *gyrA* genes, plasmid profiles, and the antimicrobial susceptibility pattern of *Salmonella* strains isolated from raw meat and stool sources across Niger State, Nigeria. Ninety-eight samples, comprising 72 raw meat and 26 stool samples, were screened for *Salmonella* spp. The antimicrobial susceptibility of *Salmonella* isolates to 10 commonly used antimicrobial agents was determined using the Kirby-Bauer disc diffusion method. Isolates were further analyzed for plasmids, in addition to PCR amplification of beta-lactamase (*bla*$_{TEM}$) and *gyrA* genes. A total of 31 *Salmonella* spp. were isolated, with 22 from raw meat (70.97%) and 9 from stool (29.03%). *Salmonella* spp. with multiple resistance patterns to ceftazidime, cefuroxime, ceftriaxone, erythromycin, ampicillin, cloxacillin, and gentamicin were detected. Ofloxacin and ciprofloxacin were found to be the most effective among the antibiotics tested, with 67.7% and 93.5% susceptible isolates, respectively. Nine (29.03%) isolates harbored plasmids with molecular sizes ranging between 6557 bp and 23137 bp. PCR amplification of *gyrA* was detected in 1 (3.23%) of the 31 isolates while 28 isolates (90.32%) were positive for *bla*$_{TEM}$. This study shows the incidence of antibiotic resistance in *Salmonella* isolates and the possible role of plasmids; it also highlights the prevalence of ampicillin resistance in this local population.

Keywords: ***Salmonella*** **spp. Antibiotic resistance, polymerase chain reaction, plasmid, resistance genes, Niger State**

Introduction

Salmonella spp. causes salmonellosis, among other diseases and are also predominant cause of human food-borne outbreaks and diseases in tropical and sub-tropical countries leading to public health threats [1]. In order to facilitate treatment, recommended therapeutic regimen for *Salmonella* infections were based on the use of ampicillin, chloramphenicol and trimethoprim/sulfamethoxazole [2], however there had been the emergence of resistance resulting in great impairment of the efficacy of these antibiotics treatment options [3].

The global trend of resistance to antibacterial agents is alarming and poses grave threat. Consequently, this has prompted investigation into the incidence of antibiotics resistance in bacteria and underlying mechanisms

***Corresponding author**
Tel: +2348035592157
E-mail: harunaremu@gmail.com

involved in proliferation of resistance in various environments [4] in addition to the role of plasmid. Numerous ecological studies have shown that increased use of antibiotic in agriculture and treatment of human infections [5] contribute to the emergence of antibiotic resistance in various bacterial genera [6].

Several literatures have reported the roles of plasmid and resistance genes in antibiotics resistance [7, 8]. Bacterial antibiotics resistance can be intrinsic or acquired via horizontal gene transfer of genetic mobile elements associated with resistance and mutations causing changes in genetic composition [4]. From earlier studies, resistance to ampicillin and ciprofloxacin has been attributed different genetic determinant including bla_{TEM} [9] and mutations in *gyrA* genes [10] respectively. In developing countries, partly due to easy accessibility and economic burden, there is wide spread of antibiotics resistance genes especially to low cost antibiotics in the region. Hence, using molecular technique, it is important to study the distribution of resistance genes for relatively high and low cost antibiotics namely ciprofloxacin and ampicillin respectively. In this study, we aim to investigate the antimicrobial susceptibility pattern, plasmid profile and presence of resistance gene in *Salmonella* strains isolated from raw meat and stool sources across Niger State, Nigeria.

Materials and Methods

This study was conducted with approval of ethics committee of Niger State Hospitals Management Board of Ministry of Health, Niger State (NSHMB No. 2018-08-009).

Study area and sample collection

This study was conducted across Niger State, Nigeria. Stool samples were collected from General Hospitals including Lapai, Bida, Minna, Suleja, Kontagora and Wushishi and raw meat samples were collected from sellers across the state. A total of ninety-eight (98) samples consisting of 26 stool samples collected from suspected typhoid fever patients and 72 raw meat samples from raw meat sellers. Table 1 shows distribution of samples across the state. Samples were aseptically collected in sterile sample bottles and Ziploc bags and transported to the laboratory at 4℃ for analysis.

Isolation and identification of isolates

Meat (25 g) and stool (5 g) samples were enriched and placed in sterile selenite broth and incubated for 24 h at 37℃. The aliquots were cultured in *Salmonella-Sigella* agar and incubated overnight. Colourless transparent colonies with black centre dot on *Salmonella-Sigella* agar were further confirmed as *Salmonella* spp. using biochemical tests as described by Chessbrough, [11] and Olutiola *et al.* [12].

Antimicrobial susceptibility of *Salmonella* isolates

Antimicrobial susceptibility of *Salmonella* isolates was determined by the Kirby-Bauer disc diffusion method. *Salmonella* isolates were screened for their susceptibility to erythromycin (5 μg), gentamicin (10 μg), cefuroxime (30 μg), ceftazidime (30 μg), ciprofloxacin (5 μg), ceftriaxone (30 μg), cloxacillin (5 μg), ampicillin (10 μg), ofloxacin (5 μg) and augmentin (30 μg). Two to three bacteria colonies were emulsified in sterile physiological saline to form a suspension and adjusted to 0.5 McFarland turbidity standard. Using sterile swab sticks bacteria suspensions were applied to the surface of Muller-Hinton agar plates. Afterwards, discs were carefully layered on the agar and incubated at 37℃ for 24 h. Results of each isolates were expressed as resistant, intermediate or susceptible to different antibiotics based on recommendation by CLSI [13].

Plasmid extraction of *Salmonella* isolates

Extraction of plasmid was performed on isolates using TENS-Mini Prep as described by Liu [14]. A 1% agarose

Table 1. Distribution of Samples across Niger State.

Sample	Bida	Lapai	Minna	Suleja	Kontangora	Wushishi	Total
Stool	5(2)	5(2)	5(1)	5(2)	1(0)	5(2)	26(09)
Raw meat	12(6)	12(5)	12(3)	12(3)	12(4)	12(1)	72(22)

*Values in "( )" indicate no. of *Salmonella* spp. identified in each location.

Table 2. Primer sequence and Amplicon size of Target gene.

Target gene	Primer	Oligonucleotide sequence (5′-3′)	Amplicon size (bp)	Reference
bla_{TEM}	ASNTF	GCTGGATCTCAACAGCGGTAAG	311	[15]
	ASNTR	CTGACAACGATCGGAGGACC		
gyrA	ASNGF	TGGGCAATTTTCGCCAGACGG	234	[16]
	ASNGR	ACTAGGCAATGACTGG		

gel was used for plasmid horizontal electrophoresis. Hind III digest of Lambda phage was used as a standard molecular marker.

DNA extraction

DNA extraction was carried out using the Qiagen QIAmp mini DNA kit (Germany) according to the manufacturers' specification.

PCR amplification of resistance genes for bla_{TEM} and *gyrA*

Targeting primers shown in Table 2, a 20 µl PCR reaction was carried out. The reaction mixture contained a buffer of 1x hot FirePol Master Mix (Solis BioDyne; Estonia), dNTPs (200 µM), each primer (20 pmol), hot DNA polymerase (2 unit), magnesium chloride (2 mM), proofreading enzyme and sterile distilled water (final volume 20 µl). Amplification reactions were performed under the following conditions: bla_{TEM} gene, initial denaturation of 15 min at 95℃, followed by 35 cycles of 30 sec at 95℃, 30 sec at 61℃ and 1 min at 72℃, and one cycle at 72℃ for 10 min. The *gyrA* gene: initial denaturation of 15 min at 95℃, followed by 35 cycles of 1 min at 95℃, 1 min at 61℃ and 1 min at 72℃, and one cycle 10 min at 72℃ for. The PCR products were separated on a 1.5% agarose gel at 100 Volts and 100 bp DNA ladder (Solis Biodyne) served as standard molecular weight marker.

Results

Among the 98 samples (72 meat and 26 stool), *Salmonella* spp. were detected in 31 samples (31.6%). The prevalence of *Salmonella* spp. in meat and stool samples are 30.6% (n = 22/72) and 34.6% (n = 9/26) respectively. As observed in Table 1, the prevalence rate of *Salmonella* spp. across the different sample locations are Bida 47.1% (n = 8/17); Lapai 41.2% (n = 7/17); Minna 23.5% (n = 4/17), Suleja 29.4% (n = 5/17); Kontangora 30.8% (n = 4/13); Wushishi 17.6% (n = 3/17).

As shown in Table 3, *Salmonella* isolates showed high resistance to augmentin (100%), ceftazidime (100%), ceftriaxone (100%), ampicillin (100%), cloxacillin (96.8%), cefuroxime (90.4%), erythromycin (77.4%), and gentamicin (67.8%). Isolates were highly susceptible to ofloxacin (67.7%) and ciprofloxacin (93.5%).

Table 3. Antibiotic susceptibility pattern of bacteria isolated from stool and raw meat samples (n = 31).

Antibiotic	Resistant (n)	Intermediate (n)	Susceptibility (n)
Ceftazidime	31	-	-
Cefuroxime	28	1	2
Ciprofloxacin	1	1	29
Gentamicin	21	2	8
Ceftriaxone	31	-	-
Erythromycin	24	5	2
Ampicillin	31	-	-
Cloxacillin	30	-	1
Ofloxacin	9	1	21

The PCR detection of resistance genes was carried out as observed in Fig. 1, 2. Of the 31 isolates screened for *gyrA* and bla_{TEM}, 1 isolate (3.23%) (Fig. 1; lane 15) showed presence of *gyrA* while 28 isolates (90.32%) were positive for bla_{TEM} (Figs. 2A and B; lane 1–11, 13–14, 17–31). In addition, plasmids were detected in 9 (29.03%) of the 28 *Salmonella* isolates (Fig. 3; lanes 5–6,

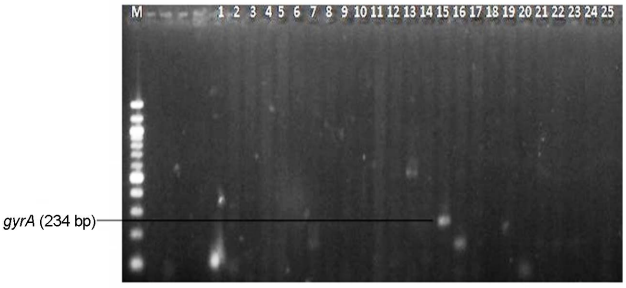

Fig. 1. Agarose gel band of *gyrA* gene. Lanes M: 100 bp ladder; lane 15: *gyrA* (234 bp) Positive.

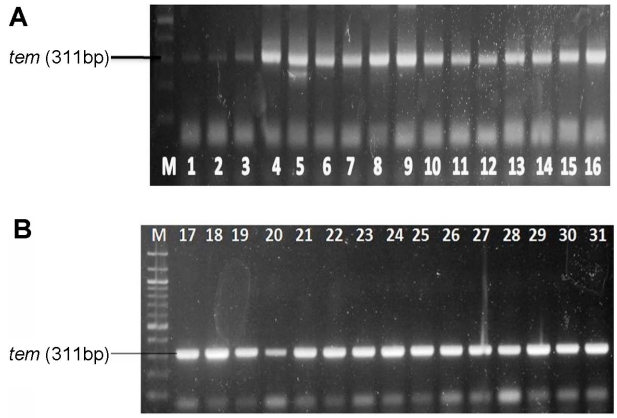

Fig. 2. (A) and (B): Agarose gel band of *bla*$_{TEM}$ genes. Lanes M: 100 bp ladder; lane 1-11, 13-14, 17-31 show presence of *bla*$_{TEM}$ (311 bp).

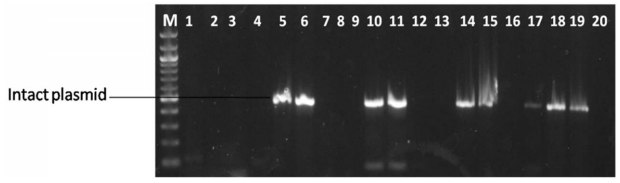

Fig. 3. Plasmid Profile of Isolates. Lanes 5-6, 10-11, 14-15, 17-19 show band of plamids.

10–11, 14–15, 17–19) that posses *bla*$_{TEM}$. Of the 9 *Salmonella* spp. that harboured plasmids, only one contained multiple size plasmids ranging between 6557 bp and 23130 bp (Fig. 3; lane 15).

Discussion

Salmonella spp. are predominant cause of human food-borne outbreaks worldwide [1]. In this study, *Salmonella* spp. was isolated from meat and stool samples. This agrees with reports of Kumar *et al.* [17] and Smith *et al.* [18] that reported the isolation of *Salmonella* spp. from stool and meat samples respectively. The development of antibiotics resistance to readily available treatment options remain a critical public health threat in tropical and subtropical countries [19]. Apart from notable intrinsic resistance, the development of antimicrobial resistance by microbes through gene transfer and mutation has also been described [20]. In our study, *Salmonella* strains showed high resistance to eight (8) antibiotics. Isolates were 100% resistant to augmentin, ceftazidime and cefriaxone, 96.8% resistant to cloxacillin, 93.5% resistant to ampicillin, 90.4% resistant to cefuroxime, 77.4% resistant to erythromycin and 67.8% resistant to gentamicin. This concurs with the findings of Adetunji *et al.* [21], Adabara *et al.* [22], Hemen *et al.* [23] and Omoya *et al.* [24] who had reported high resistance of *Salmonella* strains to these antibiotics. Also, similar to findings of Cardosso *et al.* [25] and Adetunji *et al.* [26], multiple antibiotics resistance was found in *Salmonella* strains.

Plasmids are major vectors in the global spread of antibiotic resistance genes especially in gram-negative bacteria [8]. In the study, plasmids were detected in 9 (29.03%) *Salmonella* spp. while multiple size plasmid ranging between 6557 bp and 23130 bp were found in one isolate. Isolates harbouring these plasmids were found to be resistant to ceftazidime, cefuroxime, ampicillin, gentamicin, ceftriaxone, erythromycin and cloxacillin. Conversely, isolates without detectable plasmid nonetheless exhibit resistance to some antibiotics. This observation infer that bacterial resistance can be attributed to several factors aside from being plasmid-mediated as suggested by Normark *et al.* [4]. Meanwhile, ofloxacin and ciprofloxacin were found to be most susceptible in our study. This is in agreement with the report of Soomro *et al.* [27] and Tadesse *et al.* [28] who described ofloxacin and ciprofloxacin respectively, as the drug of choice for the successful treatment of septicaemic salmonellosis in humans.

Furthermore, due to the rapid emergence of resistance to recommended therapeutic regimen (usually ampicillin and other third-generation cephalosporins or fluoroquinolones) for the treatment of *Salmonella* infections, the choice of antibiotics treatment options have been limited. *Salmonella* resistance to β-Lactam (ampicillin) has been related to the production of acquired β-Lactamases [29]. Among these, TEM-1 has been previously reported to mediate the resistance of *Salmonella* spp. to ampicillin [30]. In our study, *bla*$_{TEM}$ was detected in 28 (90.32%) *Salmonella* isolates recovered from stool and meat samples. The high prevalence of *bla*$_{TEM}$ observed among *Salmonella* spp. have been previously reported [31]. However, 100% resistance to ampicillin was recorded from the 31 *Salmonella* isolates. This therefore suggests that ampicillin resistance might be associated to other factors as reported by Michael *et al.* [30] who described that production of extended-spectrum β-lact-

amases of the TEM, SHV and CTX-M types mediate resistance of *Salmonella* isolates to ampicillin and other third-generation cephalosporins.

Similarly, fluoroquinolones resistance in *Salmonella* spp. have been attributed to target gene mutations [32]. Double *gyrA* mutations have been previously related to ciprofloxacin resistance [32]. In our study, we identified 1 (3.2%) isolate possessed *gyrA* which concurs with Kaichao *et al.* [33] who reported low occurrence rate of *gyrA* double mutations in *Salmonella* serovars. Notably, the data from antimicrobial susceptibility test in this study indicated more isolates were resistant to ciprofloxacin. Therefore, it suggests that resistant to ciprofloxacin is not only attributable to double *gyrA* mutation. Thus, other mechanisms like single *parC* mutation might be implicated as suggested by Yang *et al.* [32].

In conclusion, the development of antibiotics resistance continues to affect readily available treatment options which further burden the management of infectious disease in developing countries. Given the findings, we conclude there is an extensive spread of bla_{TEM} while *gyrA* is rare among *Salmonella* spp. in Niger State. According to Khatun *et al.* [34] and Akinyemi *et al.* [35] frequent overuse and misuse are among several factors that contribute to the resistance and spread of resistance determinant. Hence, there is an urgent need for concerted effort towards ensuring balance and coordination in the use and prescription of antibiotics agents in the environment.

Acknowledgments

The authors would like to acknowledge the valuable contributions of staff at the Molecular Biology and Biotechnology Department, Nigerian Institute of Medical Research, Yaba for their support during this research and AvH Foundation, Germany for the donation of photodocumentation system used in this study.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Amini K, Salehi TZ, Nikbakht G, Ranjbar R, Amini J, Ashrafganjooei SB. 2010. Molecular detection of *invA* and *spv* virulence genes in *Salmonella enteritidis* isolated from humans and animals in Iran. *Afr. J. Microbiol. Res.* **4**: 2202-2210.
2. Kariuki S, Gordon MA, Feasey N, Parry CM. 2015. Antimicrobial resistance and management of invasive *Salmonella* disease. *Vaccine* **33**: 21-29.
3. Butt F, Sultan F. 2011. In vitro activity of azithromycin in *Salmonella* isolates from Pakistan. *J. Infect. Dev. Ctries* **5**: 391-395.
4. Normark BH, Normark S. 2002. Evolution and spread of antibiotic resistance. *J. Int. Med.* **252**: 91-106.
5. Holmes AH, Moore LS, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, *et al.* 2016. Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet* **387**: 176-187.
6. NethMap. 2008. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. *Bilthoven*: RIVM.
7. Harrison E, Brockhurst MA. 2012. Plasmid-mediated horizontal gene transfer is a co-evolutionary process. *Trends Microbiol.* **20**: 262-267.
8. Zhang C-Z, Ding X-M, Lin X-L, Sun R-Y, Lu Y-W, Cai R-M, *et al.* 2019. The emergence of chromosomally located blaCTX-M-55 in *Salmonella* from foodborne animals in hina. *Front. Microbiol.* **10**: 1268.
9. Gebreyes WA, Altier C. 2002. Molecular characterization of multidrug-resistant *Salmonella* enteric subsp. enterica serovar Typhimurium isolates from swine. *J. Clin. Microbiol.* **40**: 2813-2822.
10. Jones ME, Boenink NM, Verhoef J, Köhrer K, Schmitz FJ. 2000. Multiple mutations conferring ciprofloxacin resistance in *Staphylococcus aureus* demonstrate long-term stability in an antibiotic-free environment. *J. Antimicrob. Chemother.* **45**: 353-356.
11. Cheesbrough M. 2006. District Laboratory Practice in Tropical Countries. 2nd Edn., Cambridge University Press, Cambridge, UK., ISBN-13: 9781139449298.
12. Olutiola PO, Famurewa O, Sonntag HG. 2000. Introduction to General Microbiology: A Practical Approach. 2nd Edition. *Bolabay Publications*, Ikeja, Nigeria.
13. Clinical and Laboratory Standards Institute (CLSI), 2016. Performance Standards for Antimicrobial Susceptibility Testing: Eighteenth Informational Supplement. CLSI Document M100-S27. Wayne: CLSL 28: 1.
14. Liu ST. 1981. Rapid procedure for detection and isolation of large and small plasmids. *J. Bacteriol.* **145**: 1365-1373.
15. Carlson SA, Bolton LF, Briggs CE, Hurd HS, Sharma VK, Fedorka-Cray PJ, *et al.* 1999. Detection of multi-resistant *Salmonella typhimurium* DT104 using multiplex and fluorogenic PCR. *Molecular Cell. Probes* **13**: 213-222.
16. Haque A, Haque A, Sarwar Y, Ali A, Bashir S, Tariq A, *et al.* 2005. Identification of drug resistance genes in clinical isolates of *Salmonella typhi* for development of diagnostic multiplex PCR. *Pakistan J. Med. Sci.* **4**: 402-407.
17. Kumar Y, Sharma A, Sehgal R, Kumar S. 2009. Distribution trends of *Salmonella* serovars in India (2001-2005). *Trans. Res. Soc. of Trop. Med. Hygiene* **103**: 90-94.
18. Smith SI, Fowora MN, Tiba A, Anejo-Okopi J, Fingesi T, Adamu ME, *et al.* 2015. Molecular detection of some virulence genes in *Salmonella* spp. isolated from food samples in Lagos, Nigeria.

Ani. Vet. Sci. **1**: 22-27.
19. Aishwarya R, Mariappan S, Uma S. 2017. Detection of *blaCTX-M* extended spectrum beta-lactamase producing *Salmonella* enterica serotype Typhi in a tertiary care centre. *J. Clin. Diagn. Res*. **11**: DC21-DC24.
20. DubMandal M. 2005. Experiments on exploration of environmental bacteria degrading a pesticide used in agriculture. Thesis, University of Jadavpur, India.
21. Adetunji VO, Odetokun IA. 2012. Antibiogram profiles of *Escherichia coli*, *Salmonella* and *Listeria Species* isolated along the processing line of sale of frozen poultry foods. *Res. J. Microbiol.* **7**: 235-241.
22. Adabara NU, Ezugwu BU, Momojimoh A, Madzu A, Hashiimu Z, and Damisa D. 2012. The prevalence and antibiotic susceptibility pattern of *Salmonella typhi* among patients attending a military hospital in Minna, Nigeria. *Adv. Prev. Med*. **2012**: 875419.
23. Hemen JT, Johnson JT, Ambo EE, Ekam VS, Odey MO, Fila WA. 2012. Multi antibiotic resistance of some gram negative bacterial isolates from poultry litters of selected farms in Benue State. *Int. J. Sci. Technol*. **2**: 543-545.
24. Omoya FO, Ajayi KO. 2016. Antibiotic resistance pattern of pathogenic bacteria isolated from poultry droppings in Akure, Nigeria. *FUTA J. Res. Sci.* **12**: 219-227.
25. Cardosso MO, Ribeiro AR, dos Santos LR, de Moraes HLS, Salle CTP. 2006. Antibiotic resistance in *Salmonella enteritidis* isolated from broiler carcasses. *Brazil J. Microbiol.* **37**: 368-371.
26. Adetunji VO, Isola TO. 2011. Antibiotic resistance of *Escherichia coli*, *Listeria Salmonella* isolates from retail raw meat tables in Ibadan municipal abattoir, Nigeria. *Afr. J. Biotechnol.* **10**: 5795-5799.
27. Soomro AH, Khaskheli M, Bhutto MB, Shah G, Memon A, Dewani P. 2010. Prevalence and antimicrobial resistance of *Salmonella* serovars isolated from poultry raw meat in Hyderabad, Pakistan. *Turkey J. Vet. Ani. Sci.* **35**: 455-460.
28. Tadesse G, Habtamu M, Zelalem T, Dadi M. 2019. *Salmonella* and *Shigella* among asymptomatic street food vendors in the dire Dawa city, Eastern Ethiopia: Prevalence, antimicrobial susceptibility pattern, and associated factors. *Environ. Health Insights* **13**: 1-8.
29. Chen S, Cui S, McDermott PF, Zhao S, White DG, Paulsen I, *et al.* 2007. Contribution of target gene mutations and efflux to decreased susceptibility of *Salmonella* enteric serovar typhimurium to fluoroquinolones and other antimicrobials. *Antimicrob. Agents Chemother.* **51**: 535-542.
30. Michael GB, Butaye P, Cloeckaert A, Schwarz S. 2006. Genes and mutations conferring antimicrobial resistance in *Salmonella*: an update. *Microbes Infect.* **8**: 1898-1914.
31. Ammar AM, Abdeen EE, Abo-Shama UH, Fekry E, Kotb EE. 2019. Molecular characterization of virulence and antibiotic resistance genes among *Salmonella* serovars isolated from broilers in Egypt. *Lett. Appl. Microbiol.* **68**: 188-195.
32. Yang B, Qu D, Shem J, Xi M, Zhi S, Cui S, *et al.* 2010. Antimicrobial susceptibility and related genes of *Salmonella* serovars from retail food in Shaanxi province, *Wei Sheng We Xue Bao* **50**: 788-796.
33. Kaichao C, Ning D, Edward WC, Sheng C. 2019. Transmission of ciprofloxacin rersistance in *Salmonella* mediated by a novel type of conjugative helper plasmids. *Emerg. Microb. Infect.* **8**: 857-865.
34. Khatun F, Faruque ASG, Koeck JL, Olliaro P, Millet P, Paris N, *et al.* 2011. Changing species distribution and antimicrobial susceptibility pattern of *Shigella* over a 29-year period (1980-2008). *Epidemiol. Infect.* **139**: 446-452.
35. Akinyemi KO, Smith SI, Oyefolu AO, Fasure KA, Coker AO. 2007. Bioline international: trends of multiple drug resistance in *Salmonella Enterica* Serovar Typhi in Lagos, Nigeria. *East Central Afr. J. Surg.* **12**: 83-88.