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ORIGINAL ARTICLE

Phenotypic Antimicrobial Resistance and Resistance Genes in *Salmonella* isolates from poultry farms in Umuahia, Abia State, Nigeria

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ABSTRACT

The spread of *Salmonella* species resistant to multiple antibiotics along the poultry supply chain, has become a threat to global public health. This study investigated the antimicrobial resistance profiles and resistance genes of *Salmonella* spp. isolated from poultry farms in Umuahia, Abia State, Nigeria. A total of 200 specimens consisting of 100 cloacal swabs and 100 faecal droppings were collected into sterile containers. The specimens were pre-enriched and inoculated into appropriate media. Antimicrobial susceptibility testing and extended spectrum Beta Lactamase (ESBL) production was assessed accordingly using standard methods. Detection of antibiotic resistance genes (*aada1, aac3-iv, bla-TEM, bla-CMY,* and *qnrA*) was done with Polymerase Chain Reaction. A total of 26 *Salmonella* isolates were obtained from the 200 specimens, representing an isolation rate of 13%. The rates of resistance to the antimicrobial drugs were as follows: Ofloxacin, 30.8% (8/26), Ciprofloxacin, 53.8% (14/26), Chloramphenicol, 69.2% (18/26), Ceftriaxone, 76.9% (20/26) and Augmentin and Cefuroxime, 100%, respectively. Nine (34.6%) isolates were extended spectrum beta lactamase (ESBL) producers. Multiple antimicrobial resistance index values ranged from 0.2 to 0.6 with 21(80.8%) exhibiting resistance to >3 antimicrobial agents tested. Out of the 9 most resistant isolates screened for antibiotic resistance genes, *bla-TEM* was detected in 2(22.2%) isolates and *aac-iv* was detected in 1(11.1%). None of the isolates was positive for *qnrA, bla-CMY* and *aada1*. This study suggests the need for surveillance of the emerging antimicrobial resistance in *Salmonella* species in poultry farms in Umuahia and to control the use of antibiotics in poultry production.

Keywords: Antimicrobial resistance; *Salmonella*; Poultry; Poultry farms; Antimicrobial resistance genes.

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1.0 INTRODUCTION

Salmonella species are major pathogens that cause severe economic damage in the poultry industry (Abdi et al., 2017). *Salmonella* is also a major zoonotic pathogen that can be transmitted from chickens to humans either directly or indirectly through consumption of poultry meat and eggs (Parvin et al., 2020). Globally, zoonotic *Salmonella* infections are estimated to cause over 200 million to 1 billion cases of human illnesses with over 93 million cases of gastroenteritis and over 155,000 deaths each year (Lamichhane et al., 2024). Another disturbing dimension is the capability of *Salmonella* to easily acquire antimicrobial resistance (AMR) either through mutation or through horizontal transfer of resistance genes (Alenazy, 2022). The emergence and spread of such resistant strains among food animals constitutes a global public health concern, as they are often difficult to treat with currently available antimicrobials. Resistant bacteria and resistance genes could be transmitted to humans either by direct contact between animals and humans or indirectly through the food production chain (Kumar et al., 2020). The increasing problem of spread of antibiotic resistant *Salmonella* spp. along the poultry supply chain is a growing global public health concern around the world (Castello et al., 2023). Additionally, a number of studies have demonstrated that *Salmonella* isolated from poultry meat can display resistance to a broad spectrum of antibiotic molecules (Hassena et al., 2019; Gambino et al., 2022). The emergence, selection, and dissemination of antimicrobial resistance (AMR) in *Salmonella* spp. within the poultry population are largely caused by the overuse or misuse of antimicrobials in poultry production (Kumar et al., 2020). In poultry farming, antibiotics are widely used to treat and prevent infections in the birds as well as to enhance growth performance; however, this may lead to the spread of multidrug-resistant *Salmonella* spp (Habiba et al., 2023), thus posing significant health concerns for humans because of the increased mortality/morbidity of infected individuals (Sin et al., 2020). Over the last ten years, there has been an increasing public health concern due to the emergence of multidrug-resistant (MDR) *Salmonella*. MDR strains isolated from poultry meat have been found to harbor AMR genes for β-lactams, aminoglycosides, tetracyclines, and sulfanamides, posing a significant threat to public health (Hindermann et al., 2017). In recent years, *Salmonella* strains from humans and other animals that show reduced susceptibility to ciprofloxacin and multidrug-resistance profiles have been rapidly emerging, with significantly high reported prevalence from Asia, Europe, and Africa (Pham et al., 2015; Abdi et al., 2017; Ekundayo and Enya, 2018).

The resistance of *Salmonella* spp. to fluoroquinolones and quinolones is primarily mediated genetically by mutations in DNA gyrase and topoisomerase IV, which are the target structures of these antibiotics. Additionally, resistance can be conferred by modifications in the permeability of the external membrane, which is often transmitted by plasmids (plasmid-mediated quinolone resistance [PMQR]), and efflux pumps. This suggests that multiple biochemical mechanisms are involved in the resistance to quinolones and fluoroquinolones (Redgrave et al., 2014). Antimicrobial resistance monitoring in Nigeria is insufficient, especially with regards to food animals. As a result, there is a need for data on the current antimicrobial resistance profiles and resistance genes in poultry farms. Hence, the aim of the present study was to investigate the antimicrobial resistance profiles and resistance genes of *Salmonella* spp.

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isolated from cloacal swabs and faecal droppings of chickens from ten poultry farms in Umuahia, Abia State, South Eastern Nigeria.

2.0 MATERIALS AND METHODS

2.1 Sample collection and inoculation

Faecal droppings (n = 100) were collected in sterile universal bottles. Cloacal swabs (n = 100) were collected from the birds using swab sticks. The *Salmonella* species were isolated according to the methods outlined by the WHO (2010). The samples were initially inoculated into buffered peptone water, thereafter 1ml of the cultures was transferred into a 9 mL of Selenite F Broth and incubated at 37°C for 24 h (Al-Abadi & Al-Mayah, 2011). A loopful of the overnight Selenite F broth culture was streaked on *Salmonella Shigella* Agar (SSA) and the plates incubated overnight at 37°C for 24h (Menghistu et al., 2011). Upon establishment of growth, colonies typical of *Salmonella* were sub-cultured on nutrient agar plates and identified as *Salmonella* using standard biochemical characterization procedures.

2.2 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk agar diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) protocol (CLSI, 2020) on Mueller Hinton agar (Titan Biotech Ltd, India). Discrete colonies from a 24 h nutrient agar plates were suspended in sterile normal saline in a test tube to achieve a bacterial suspension equivalent to 0.5 McFarland Turbidity Standard**.** A cotton swab was dipped into the bacterial suspension and used to inoculate the entire surface of a Mueller Hinton agar plate, rotating the plate to ensure confluent growth of the bacterium. The antimicrobial susceptibility discs were placed on the surface of the inoculated plates with flame sterilized forceps. The plates were incubated 16– 18 h at 35-37°C. The diameter of the zone of inhibition produced by the antibiotic discs were measured to the nearest millimeter (mm) using a transparent ruler.

2.3 Test for ESBL

The isolates were selected for detection of ESBL in the double disc synergy test (DDST) as described by Kaur et al. (2013). A lawn culture of the resistant organisms was made on a Mueller-Hinton agar plate (Hardy Diagnostics, USA). Antibiotics discs (Oxoid, United Kingdom) containing amoxicillin-clavulanate (30 µg) was placed in the centre of the plate. Antibiotics discs of ceftriaxone (30 µg), cefotaxime (30µg) and ceftazidime (30 µg) were placed 15 mm and 20 mm apart respectively, centre to centre to that of the amoxicillin-clavulanate disc and the plates incubated at 37ºC for 18-24 h. A clear extension of the edge of the inhibition zone of any of the antibiotics towards the disc containing amoxicillin-clavulanate was considered as positive for ESBL production.

2.4 Genomic DNA extraction and gel electrophoresis

Genomic DNA was extracted from the isolates using the Zymo Research Bacterial/Fungal DNA Miniprep kit (Zymo Research Inc., California, USA) according to the manufacturer's instructions. Ten (10 µL) of extracted DNA mixed with 2 µL of loading dye was electrophoresed on 0.8% agarose gel in 1X TBE buffer containing 0.5 µg/mL ethidium bromide along with 1kb ladder (Fermentas) at 150V for 45 min. The gel was visualized using a UV transilluminator (Biobase, China) and photographed.

2.5 PCR Amplification of antibiotics resistance genes

The Polymerase Chain Reaction (PCR) amplification reactions were performed in PX2 thermal cycler (Thermo Electron Corporation, USA) in a total volume of 50 µL as described by Jeamsripong et al. (2022) with slight modifications. The reaction mixture consisted of 25 µL of Taq 2X Master Mix (NEB, USA), 1 µL of each forward and reverse primer, 5 µL template DNA, and 18 µL of sterile nuclease free water in a PCR micro tube. The thermal cycling reaction was carried out as follows: initial denaturation at 95°C for 2 min, 35 cycles of denaturation at 95°C for 30s, annealing at 55 ºC for 45 s and extension at 68°C for 1min. Final extension was at 68°C for 5 mins and the reaction was held at 4°C. PCR products were separated by electrophoresis at 150V for 45 minutes on 1.5 % (w/v) agarose gel containing 0.5 µg/mL of ethidium bromide in 1X TBE buffer.
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Table **1**. Primer sequences used

Primer Name	Sequence(5'-3')	Annealing Temperature(^{0}C)	Reference
aada F	TATCCAGCTAAGCGCGAACT	58^{0}C	Ahmed et al., 2009
aada R	ATTTGCCGACTACCTTGGTC		
aac3-iv F	CTTCAGGATGGCAAGTTGGT	61^{0}C	Anjay et al., 2015
aac3-ivR	TCATCTCGTTCTCCGCTCAT		
bla-CMY F	TGGCCAGAACTGACAGGCAAA	58^{0}C	Bennett, 2008
bla-CMY R	TTTCTCCTGAACGTGGCTGGC		
qnra F	GGGTATGGATATTATTGATAAAG	53^{0}C	Canton, 2008
qnra R	CTAATCCGGCAGCACTATTTA		
bla-TEM F	CAGCGGTAAGATCCTTGAGA	55^{0}C	Maynard et al., 2003

3.0 RESULTS

A total of 26 *Salmonella* isolates (13.0%) were recovered from 200 poultry samples, with a higher isolation rate from cloacal samples (18.0%) compared to fecal samples (8.0%).

The disc agar diffusion assay showed that 18 (69.2%) isolates were resistant to chloramphenicol; 26 (100%) were resistant to cefotaxime and cefuroxime, respectively, 10 (38.5%) were resistant to nitrofurantoin; 20(76.9%) were resistant to ceftriaxone; and 8 (30.8%) were resistant to gentamicin (Figure 1). Greater than 20% of the isolates showed reduced sensitivity (intermediate category) to gentamicin and nitrofurantoin. Ofloxacin was the most active antibiotic as 69.2% of the isolates were susceptible to it.

The results as shown in Table 3 below describes the resistance patterns of the 26 *Salmonella* isolates to seven antibiotics. All isolates (100%) were resistant to two antibiotics (cefuroxime and augmentin). The incidence of multidrug resistance (MDR) phenotypes decreased as the number of antibiotics in the resistance pattern increased, from 80.8% for three antibiotics down to 37.5% for seven antibiotics. The Multiple Antibiotic Resistance Index (MARI) values ranged from 0.2 to 0.8.

Table **2.** Occurrence of *Salmonella* isolates from cloacal and fecal samples of poultry

Source of isolate	Number Examined	No (%) of Isolates
Cloacal	100	18 (18.0)
Fecal	100	8 (8.0)
Total	200	26 (13.0)

Table **3.** Multiple antimicrobial resistance profiles of *Salmonella* isolates

Number of antibiotics	Resistance pattern	Number of isolates (%)	Percentage of resistant isolates (%)	MARI (%)
2	CRX, AUG	26	100	0.2
3	CRO, AUG, GEN	21	80.8	0.3
4	CRO, AUG, NIT, GEN	18	69.2	0.4
5	CRO, AUG, NIT, GEN, CXM	16	61.5	0.6
6	CRO, AUG, NIT, GEN, CXM, CPR	13	50	0.7
7	CRO, AUG, NIT, GEN, CXM, CPR, OFX	6	37.5	0.8

Of the 26 *Salmonella* isolates screened, 9 (34.6%) tested positive for extended-spectrum Beta-Lactamase (ESBL) production (Table 4). ESBL production was more frequent among isolates from cloacal samples (38.9%) than from fecal samples (24.0%).

The isolates were screened for the genes conferring resistance to the antibiotics to which the isolates exhibited a resistance phenotype. The most frequent genotype was the combination aac3-iv + Bla-TEM, which was found in 33.3% of the isolates (Table 5). The single Bla-TEM gene was present in 22.2% of isolates, while aac3-iv alone was found in 11.1% (Figures 1 and 2). The genes qnrA, aada1, and Bla-CMY were not detected in any of the isolates. These genes are known to confer resistance to β-lactams and aminoglycoside categories of antimicrobials. The *blaTEM-1*+*aac3-iv* resistance genes were detected in 3 of the isolates.

Table **4.** ESBL Profile of the *Salmonella* isolates

Source of isolate	No.(%) positive to ESBL Production
Cloacal sample (n= 18)	7 (38.9)
Fecal sample (n= 8)	2 (24.0)
Total (n= 26)	9 (34.6)

Table **5.** Frequency of multidrug resistance genotype patterns exhibited by the *Salmonella* isolates

Resistance Gene	No of Isolates	Number Positive (%)
Bla-TEM	9	2 (22.2)
qnrA	9	0 (0.0)
aac3-iv	9	1 (11.1)
aada1	9	0 (0.0)
***Bla*$_{-CMY}$**	9	0 (0.0)
aac3-iv + Bla-TEM	9	3 (33.3)

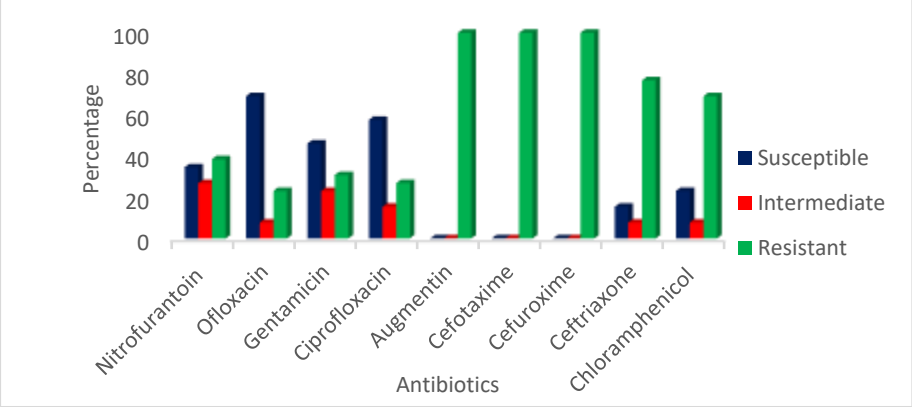


Figure **1.** Antimicrobial susceptibility of *Salmonella* isolates obtained from poultry samples

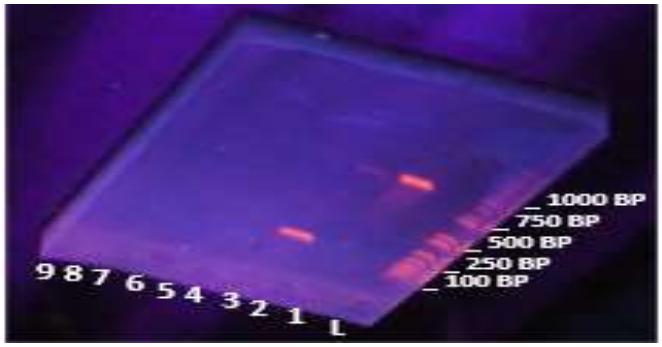


*Figure **2**. Gel Electrophoresis of PCR amplicon of bla $_{TEM}$ gene (618bp) in Salmonella isolates (Where: L = DNA Ladder, BP = Base pairs, 1 – 9: represents the isolates). Isolates 2 and 3 showed positive amplicons for bla $_{TEM}$ gene.*



*Figure **3**. Gel Electrophoresis of PCR amplicon of aac(3)-IV gene (491bp) in Salmonella isolates (Where: L = DNA Ladder, BP = Base pairs, 1 – 9: represents the isolates). Isolate 8 showed positive amplicons for aac(3)-IV gene.*

4.0 DISCUSSION

In this investigation, the isolation rate of *Salmonella* was 13.0%. The outcome is consistent with a prior similar study by Kassaye et al. (2011) in Ethiopia that found a 16.1% prevalence from faecal and cloacal swabs of birds in an intensive chicken farm. Similar outcomes have also been documented in Nepal (Maripandi & Al-Salamah, 2010) and Egypt (Ibrahim et al., 2013). In contrast however, a lower incidence (9.3%) was reported in East Showa, Ethiopia (Endris et al., 2013) and a greater prevalence (41.9%) in Jima (Alebachew & Mekonnen, 2013). A higher isolation rate of *Salmonella* species was detected in chickens' cloacal swabs than fecal sample. This difference implies that cloacal sample may provide higher diagnostic sensitivity and reflects active colonization, which has implications for surveillance procedures in the management of poultry health. Lankagbel et al. (2014) had earlier reported cloacal swab as the ideal sample for use in isolating *Salmonella* from poultry, this could explain the increased isolation rate of the organism from the cloaca rather than the fecal material in this study. Also, Mridha et al. (2020) in a related study recovered 59 (46.1%) *Salmonella* isolates from 128 cloacal swab samples collected. Our findings are consistent with those of other authors' research (Rahman et al., 2018). However, a cloacal swab study by Paul et al. (2017) revealed a very high prevalence (80%). High sensitivity levels of the isolates were seen for gentamicin (46.2%), ciprofloxacin (57.5%) and ofloxacin (69.2%). This finding is consistent with similar studies that reported a minimal level of resistance against the aforementioned antibiotics from local poultry *Salmonella* isolates (Khoo et al., 2015). However, high rates of resistance were demonstrated by the isolates to augmentin, (100%), cefuroxime (100%) as well as chloramphenicol (69.2%). Also, in the current study, 76.9% of *Salmonella* spp. isolated showed resistance to ceftriaxone, a finding, which was consistent to a previous observation by Mir et al. (2015) amongst poultry *Salmonella* isolates in India. Omoigberale et al. (2014) found that 80% of *Salmonella* isolates were susceptible to ciprofloxacin. Herrera-Sánchez et al. (2021) had also reported in an earlier study; strains that were resistant to ciprofloxacin (75%), levofloxacin (57.1%), and enrofloxacin (38.8%). These findings have led to widespread reports of increasing resistance to flouroquinolones in *Salmonella* spp. This observation is also in affirmation with previous finding in which the highest susceptibility was reported for Ofloxacin (85%) in *Salmonella enteritidis* (Asif et al., 2017) and contrary to an earlier study reporting the highest susceptibility for

amoxicillin/clavulanic acid, gentamicin, tetracycline, and trimethoprim (Thung et al., 2016). Ofloxacin and ciprofloxacin are rarely used in veterinary practice in the study area. This may explain the reason why the *Salmonella* strains were not totally resistant to these antimicrobial agents. The high resistance rates recorded amongst the *Salmonella* isolates, points to an increased risk upon its eventual transmission to human. All 26 isolates (100%) exhibited resistance to at least two antibiotics (cefuroxime and augmentin). However, there was a progressive decline in the proportion of resistant isolates with increasing antibiotic resistance patterns from 80.8% resistance to three antibiotics down to 37.5% resistance to seven antibiotics. This suggests that while multi-drug resistance is common, higher-level resistance combinations are less frequent. The detection of extended-spectrum β-lactamase (ESBL) production in 34.6% of the *Salmonella* isolates is worrisome, as ESBLs confer resistance to critically important beta-lactam antibiotics, including third-generation cephalosporins. The higher ESBL production rate among cloacal isolates (38.9%) compared to fecal isolates (24.0%) further emphasizes the potential role of cloacal carriage in the dissemination of resistant strains. Among the beta-lactams associated resistance genes assayed in this study, blaTEM-1 was detected in 22.2% (2/9) of the *Salmonella* isolates, which is in agreement with a previous study where it was detected as the most prevalent (Deeshit et al., 2012). Amplification of the *blaTEM* antibiotic resistance gene in 2 (22.2%) of the screened isolates correlated with resistance to the Cephalosporin group of antibiotics used in this study (100% for augmentin and cefuroxime and 79.6% for ceftriaxone). Higher percentages of 93.3% and 90.5% have been reported by Lebdah et al. (2017) and Hur et al. (2011). Still in another related study, the percentage of *bla*TEM as reported by Ahmed et al. (2009) was 10%. The *blaCMY-2* antibiotics resistance genes conferring resistance to cephalosporins were absent in the screened isolates inferring newer methods of resistance to this class of antibiotics. The study of Gbotche et al. (2020) also indicated a high prevalence of blaCTX-MI (35.02%) and blaCTX-M15 (42.73%) among bacterial isolates from the Enterobacteriaceae family. the study reported that blaCTX-M15 was the most frequently found ESBL. Although, this current study did not screen for the gyrA gene, PCR amplification in the study of Usman *et al*. (2026) on similar organisms successfully detected the gyrA gene in all isolates, confirming the presence of the target gene. In our study, the *aac(3)-IV* gene was found only in one isolate (11.1% n=1/9). The aminoglycoside acetyltransferase gene *aac(3)-IV* have been detected amongst *S. enterica* isolates in a similar study by Aldred et al. (2014) further corroborating the results of this current investigation. Our result revealed the absence of neither *aadA1* nor *Qnra* genes in the nine phenotypically aminoglycoside and fluoroquinolone resistant *Salmonella* isolates, a finding in line with those of Velhner et al. (2014). Furthermore, Nwiyi et al. (2018) in a study on the identification of resistance genes in Salmonella obtained from chicken farms discovered that none of the ten isolates resistant to gentamicin carried aac(3)-IV genes. The existence of additional and more peculiar resistance mechanisms encoded by genes not included in this study may be the cause of the lack of association between genotypic resistance genes and the reported phenotypic resistance as observed.

The phenotypic MDR pattern of the *Salmonella* in this study to aminoglycosides and quinolones showed partial correlation with the genotypic analysis as only one of the *qnrA, aada1* and *aac3-iv* genes were expressed. Deekshit et al. (2012) also showed that the presence/absence of a resistance gene does not necessarily result in resistance to the antibiotic in question in a study where it described that some non-typhoidal *Salmonella* spp. strains can carry silent antibiotic-resistance genes.
The combination of high *Salmonella* prevalence, multidrug resistance, and ESBL development in poultry poses a triple threat to public health. These resistant strains may cause diseases that are resistant to conventional antibiotic if they get into the human food chain through contaminated chicken products, manure used as fertilizer, or direct contact with poultry workers (Ojo et al., 2009). The close interactions among humans, animals, and the environment pose a risk for the emergence of infectious diseases, and this risk is increasing (Anekpo et al., 2024). Consequently, it is crucial to focus on understanding the intricate dynamics between humans, animals, and the environment, as well as the various farming systems and their biosecurity levels.

Conclusion

The results of this study portrays the major contribution of poultry and its associated products (wastes) as a significant source/reservoir of *Salmonella*. This study revealed a significant prevalence of *Salmonella* in poultry, with an overall isolation rate of 13.0% from the 200 samples examined. The isolates demonstrated improved susceptibility to gentamicin (46.2%), ciprofloxacin (57.5%) and ofloxacin (69.2%), while displaying high levels of resistance isolates to Augmentin, (100%), cefuroxime (100%), chloramphenicol (69.2%) and ceftriaxone (76.9%). Extended-Spectrum Beta-Lactamase (ESBL) production was found in 34.6% of the *Salmonella* isolates. Genotypic analysis of resistance determinants revealed that the combination of aac3-iv (aminoglycoside resistance) and Bla-TEM (beta-lactam resistance) was the most prevalent genotype, occurring in 33.3% of the isolates tested. The single Bla-TEM gene was present in 22.2% of isolates, while aac3-iv alone was found in 11.1%. The findings demonstrate the rise of multi-drug resistant *Salmonella* strains in poultry, including isolates that produce ESBL and are resistant to several antibiotic. This underscores the need for strengthened improved biosecurity measures and regular surveillance of antimicrobial resistance.

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Competing interests

No conflicts of interests is declared by the authors.

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Author contributions

Chigozie Mgbemena, Enya Emmanuel and Ekundayo Emmanuel contributed in Material preparation, data collection, analysis and design and writing of the manuscript.

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