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Original Article

High resistance to tetracycline and ciprofloxacin in bacteria isolated from poultry farms in Ibadan, Nigeria

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Abstract

Introduction: Resistance to ciprofloxacin and tetracycline is increasing in the food chain especially in *E. coli* strains and more worrisome will be occurrence of extended-spectrum beta-lactamase (ESBL) producers among ciprofloxacin- and tetracycline-resistant isolates. This study was undertaken to investigate the occurrence and mechanism of ciprofloxacin-, tetracycline- and ESBL-resistant bacteria in poultry in Ibadan, Nigeria.

Methodology: Bacteria were isolated from poultry feces in two farms in Ibadan and identified by MALDI-TOF. Antibiotic susceptibility patterns of the isolates were determined by disc diffusion and Minimum Inhibitory Concentration (MIC) using Vitek-2 apparatus. Four tetracycline genes and six plasmids mediated quinolone resistance genes (PMQR) were investigated by PCR. Whole genome sequencing was done for strains that were ESBL producers.

Results: Bacterial strains ($\geq 10^5$ cfu/mL) were counted on ciprofloxacin and tetracycline supplemented plates. 106 bacteria from 14 different species were identified with high resistance to quinolones, tetracycline and trimethoprim. 49% of the strains were *E. coli* with 90% resistance for nalidixic acid, moxifloxacin (94%), ciprofloxacin (88%) levofloxacin (78%) and tetracycline (77%). The genes *tetA, tetB, qnrB, qnrS* and *qepA* were detected with 37%, 4%, 35%, 4% and 2% prevalence in *E. coli* respectively. Three ESBL-producing *E. coli* of the sequence type ST-6359 were found and harboured $bla_{\text{CTX-M-15}}$ located in the chromosome, at the same insertion site. All the ESBL producers harboured mutations in *gyrA* (S83L/D87N/D678E) and *parC* (S80I).

Conclusion: The observed high quinolones and tetracycline resistance with ESBL producers in this study calls for caution in the use of these antibiotics in poultry feeds.

Key words: Resistance; Poultry; Tetracycline; Ciprofloxacin.

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Introduction

Poultry products are a good source of protein for people living in developing countries; therefore, poultry farming is a thriving business in such locations. Many additives e.g. antibiotics are added to poultry feeds for prevention of disease, in therapeutic and sub-therapeutic doses. The quantities of antibiotics administered to livestock may be much more than human consumption [1] and this has been projected to increase by 2030 [2].

Beta-lactams, tetracyclines and enrofloxacin are common classes of antimicrobials frequently administered to poultry because of their affordability. Tetracyclines have a long history in the treatment of infectious diseases [3] while ciprofloxacin (major metabolite of enrofloxacin) is widely use in human and veterinary medicine [4]. As the use of these antibiotics increases, so do resistance to them. The application of beta-lactams is generally reducing because of the global problem of antibiotic resistance especially with the emergence of extended-spectrum beta-lactamase (ESBL) producers. Occurrence of ESBL-producing *E. coli* isolates in poultry has been reported [5-7]. Dissemination by transposons, mobile genetic elements, plasmids and bacteriophage enhances the spread of resistance in the community [8].

Antibiotics are used majorly among commercial poultry production in Nigeria. Nigerian government has regulated the importation of poultry meat thus leading to increase in poultry production in the country. However, our survey around Ibadan, Nigeria metropolis reveals that many poultry farms use unregulated

subtherapeutic doses of antibiotics in the poultry feeds. A further investigation reveals that tetracycline and enrofloxacin are the two commonest antibiotics usually included in the poultry feeds. They come under different brand names e.g. Reo oxyseyl, Neocerl plus and Enroveto 20 oral liquid. This unregulated antibiotics use in feeds will most probably select for bacteria highly resistant to ciprofloxacin and tetracycline in the poultry thus, serving as potential threat when transferred to human through the food chain. Ciprofloxacin and tetracycline resistant strains could also carry ESBL genes thus leading to multiple antibiotic resistance. Therefore, this study investigates the occurrence of ciprofloxacin- and tetracycline-resistant bacteria in poultry feces in two poultry farms in Ibadan metropolis, Nigeria with associated antibiotic resistance and ESBL genes.

Methodology

Samples

Two medium sized poultry farms located in different vicinity of Ibadan, Oyo State, Nigeria (Akinyele and Ibadan North local government) were selected for this study. The two farms include enrofloxacin and oxytetracycline in their poultry feeds. A total of 10 faecal droppings from different poultry birds were sampled from each farm between January and April 2016. Aseptic conditions were ensured while collecting the sample in sterile collection bottle and transported to the laboratory within 30 minutes for analysis.

Isolation of bacterial strains

Following the British Society for Antimicrobial Chemotherapy manual guidelines [9], tetracycline and ciprofloxacin were supplemented in separate Nutrient Broth (NB) bottles to make a concentration of 5.0 mg/L and 2.0 mg/L respectively. 1 g of each faecal sample was inoculated into 9 ml of tetracycline and ciprofloxacin supplemented NB medium respectively for separate isolation of ciprofloxacin- and tetracycline-resistant bacteria. The mixture was vortexed and incubated at 37°C for 24 hours. They were serially diluted to obtain maximum recovery diluent (MRD). The total distribution of the tetracycline- and ciprofloxacin-resistant bacteria count in each incubated NB media were obtained by plating on different Nutrient Agar plate supplemented with 5 mg/liter and 2 mg/liter of tetracycline and ciprofloxacin respectively. The grown colonies were counted after incubation and distinct different colonies were isolated and stored for further characterization.

Identification of bacteria strains by MALDI-TOF mass spectrometry

All distinct bacterial colonies isolated were grown on Mueller Hinton agar (Oxoid, Cheshire, UK) for 24 h. A thin smear on MALDI target plate was made from the grown organisms. The smears were overlaid with 1 µL of matrix solution (saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid), and air dried at room temperature. Organisms were identified by comparing their mass spectra with reference spectra on the manufacturer (bioMérieux , Marcy l'Etoile, France) database. Data were interpreted as follows: scores of ≥ 2 were considered for species level identification. Identifications with scores below 1.7 were considered unreliable [10].

Antimicrobial Disc Susceptibility Test

The initial antimicrobial susceptibility to penicillin (10µg), nalidixic acid (30µg), ciprofloxacin (5µg), levofloxacin (5µg), tetracycline (30µg) and trimethoprim (5µg) were evaluated by disk diffusion [11] for all isolated strains. The plates were incubated aerobically at 37°C for 24 hours. The diameters of the respective zone of inhibitions were measured and interpreted following Clinical and Laboratory Standards Institute [11] guidelines.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of antibiotics for all strains were determined by AST N-214 antibiotics cards (bioMérieux, Marcy l'Etoile, France) using Vitek-2 apparatus (bioMérieux Marcy l'Etoile, France). The susceptible and resistant strains were determined according to manufacturer`s instructions [12].

Phenotypic Detection of Extended Spectrum Beta Lactamase Producers

All isolated strains were streaked on cefotaxime supplemented plates and incubated at 37ºC for 24 hours. All strains that grew on the plates were further tested for their MIC with cephalosporins by card AST-N248 (bioMérieux, Marcy l'Etoile, France) using the Vitek-2 apparatus (bioMérieux, Marcy l'Etoile, France). All isolates with observed resistance to third generation cephalosporins were selected for whole genomic sequencing (WGS) analysis.

PCR amplification

The most prevalent organism (*E. coli* strains) was selected for further molecular studies. All *E. coli* strains were selected for the presence of tetracycline and ciprofloxacin gene determinants. The genomic DNA was extracted by boiling method as earlier described by Mendonca *et al*. [13]. The primers used in this study were shown in Table 1 including the PCR condition, the primer sequence and their product sizes. Primers were synthesized by Inqaba Biotechnical Industries (Pty) Ltd, Hatfield, South Africa. All PCR amplifications contained 10 µL master mix (Inqaba, South Africa), primers 100 nM (1% of the final volume), molecular grade water and DNA template (1 µL) in a 20 µL reaction. (*tetA* and *tetB* was 25 µL reaction including 1 µL DNA template, 2 µL primers and 12.5 µL master mix).

Whole genome sequencing

The 3 ESBL-producing *E. coli* strains obtained were chosen for WGS analysis. DNA was extracted by Purelink Genome DNA Mini kit (Invitrogen, Darmstadt, Germany) according to the manufacturer's instructions. WGS was carried out using an Illumina Nextera XT library with 2x150bp paired-end reads on an Illumina NextSeq instrument (Illumina, San Diego, CA, USA). The raw data was assembled by SPAdes (version 3.0) [14]. The data of the sequenced isolates are available at the European Nucleotide Archive (ENA) under the project number PRJEB20802.

In silico analyses of resistance genes, MLST, plasmids and quinolone resistance-determining regions (QRDR) mutations

Sequences were analyzed for their plasmid replicon types, multi locus sequence types, antimicrobial resistance genes, and pMLST by MLST 1.8, ResFinder, Plasmidfinder and pMLST software from the Center for Genomic Epidemiology [15-17]. The genetic location of $bla_{\text{CTX-M-15}}$ was obtained by analyzing the contigs harboring $bla_{\text{CTX-M-15}}$ using *bla*stn. QRDR mutations were identified using *E. coli* MG1655 genes *parC* and *gyrA* as a reference [18].

Results

The number of bacteria enumerated ranged from ($3.4 \times 10^5 – 1.04 \times 10^6 \pm 0.10$) and ($3.4 \times 10^5 – 1.41 \times 10^6 \pm 0.95$) for ciprofloxacin and tetracycline supplemented plate respectively in poultry A to ($6.8 \times 10^5 – 1.44 \times 10^6 \pm 0.01$) and ($8.0 \times 10^5 – 1.84 \times 10^6 \pm 0.13$) for ciprofloxacin and tetracycline supplemented plates respectively in poultry B, (data not shown). 56 organisms grew on ciprofloxacin plates while 50 organisms grew on tetracycline supplemented plates, a total of 104 distinct organisms were identified from the two poultry farms (Table 2).

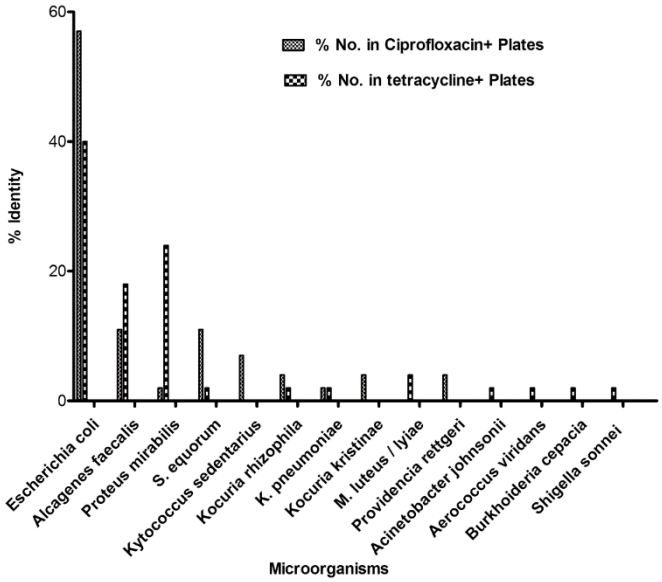
Table 1. Primer used for gene detection.

Gene	Antimicrobial agent	Primers	Primer sequence	Annealing temperature	Product size	References
TetA	Tetracycline	*Tet(A)-F* *Tet(A)-R*	5-GGTTCACTCGAACGACGTCA-3 5'- CTGTCCGACAAGTTGCATGA -3'	57ºC	577bp	Randall ***et al.*** **[**36]
tetB	Tetracycline	*Tet(B)-F* *Tet(B)-R*	5'- CCTCAGCTTCTCAACGCGTG -3' 5'- GCACCTTGCTGATGACTCTT -3'	56ºC	639bp	Randall *et al.* [36]
tet(M)	Tetracycline	*Tet(M)-F* *Tet(M)-R*	5'-GTTAAATAGTGTTCTAACAA-3' 5'-CTAAGATATGGCTCTAACAA-3'	52ºC	406bp	Ng *et al*. [37]
tet(O)	Tetracycline	*Tet(O)-F* *Tet(O)-R*	5'-AAC TTA GGC ATT CTG GCT CAC-3' 5'-TCC CAC TGT TCC ATA TCG TCA-3	53ºC	515bp	Ng *et al*. [37]
qnrA	Ciprofloxacin	*Qnr(A)-F* *Qnr(A)-R*	5- ATTTCTCACGCCAGGATTTG-3 5 – GATCGGCAAAGGTTAGGTCA-3	53ºC	516bp	Wang *et al*. [38]
qnrB	Ciprofloxacin	*Qnr(B)-F* *Qnr(B)-R*	5 – GATCGTGAAAGCCAGAAAGG-3 5 – ACGATGCCTGGTAGTTGTCC-3	53ºC	469bp	Wang *et al*.[38]
qnrS	Ciprofloxacin	*Qnr(S)-F* *Qnr(S)-R*	5 – ACGACATTCGTCAACTGCAA-3 5 – TAAATTGGCACCCTGTAGGC-3	53ºC	417bp	Wang *et al*. [38]
qepA	Ciprofloxacin	*Qep(A)-F* *Qep(A)-R*	5 – CTTCTCTGGATCCTGGACAT-3 5- TGAAGATGTAGACGCCGAAC-3	53ºC	720bp	Şahinturk *et al.* [39]
oqxB	Ciprofloxacin	*Oqx(B)-F* *Oqx(B)-R*	5 – ATCGGTATCTTCCAGTCACC-3 5– ACTGTTTGTAGAACTGGCCG-3	56ºC	541bp	Şahinturk *et al*. [39]
aac(6')Ib – cr	Ciprofloxacin	*Aac(6') Ib-cr-F* *Aac(6') Ib-cr-R*	5 – TTGCGATGCTCTATGAGTGGCTA-3 5 – CTCGAATGCCTGGCGTGTTT-3	59ºC	482bp	Şahinturk *et al*. [39]

E. coli was the most prevalent organism on plates supplemented with ciprofloxacin in poultry A and B and tetracycline supplemented plates in poultry B. while *Proteus mirabilis* was the most prevalent organism on plates supplemented with tetracycline in poultry A (Table 2). Analysis of addition of all isolated bacteria from the 2 plates in the two poultry farms revealed that *E. coli* was the most abundant species (49%) followed by A*lcaligenes faecalis* (14%), *Proteus mirabilis* (12%), *Staphylococcus equorum* (7%), *Kytococcus sedentarius* (4%), *Kocuria rhizophila* (3%), *Klebsiella pneumoniae* (2%), *Kocuria kristinae* (2%), *M. luteus / lyiae* (2%), *Providencia rettgeri* (2%), *Acinetobacter johnsonii* (1%), *Aerococcus viridans* (1%), *Burkhoideria cepacia* (1%) and *Shigella sonnei* (1%).

Figure 1 shows that the most dominant organism on ciprofloxacin and tetracycline supplemented plates were *E. coli.* Growth of *Proteus mirabilis* (24%) and *Alcagenes faecalis* (18%) was more profuse on plates supplemented with tetracycline. *Kytococcus sedentarius*, *Kocuria kristinae* and *Providencia rettgeri* were specific to plates supplemented with ciprofloxacin while *Micrococcus luteus/lyiae*, *Acinetobacter johnsonii, Aerococcus viridans*, *Burkhoideria cepacia* and *Shigella sonnei* were specific for plates supplemented with tetracycline. The specific ciprofloxacin and tetracycline antibiotic general resistance pattern of all isolated bacteria isolated revealed that 77% were resistant to tetracycline, 78% were resistant to nalidixic acid, ciprofloxacin (73%), levofloxacin (67%) and 89% to moxifloxacin (data not shown). *Providencia rettgeri* were 100% resistant to all the antibiotics used. *Kytococcus sedentarius* and *Kocuria kristinae* were completely susceptible to penicillin G. Also, for all *E coli* strains, high resistance were observed for quinolones; (90% for nalidixic acid, moxifloxacin (94%), ciprofloxacin (88%) and levofloxacin (78%) while *Proteus* sp. were relatively susceptible to the tested antibiotics except tetracycline (Figure 2). Organisms isolated on ciprofloxacin plates were generally resistant to tetracycline and

Figure 1. Distribution of bacterial species on ciprofloxacin and tetracycline supplemented plates.



(M: *Micrococcus,* K: *Klebsiella,* S: *Staphylococcus)*.

Table 2. Number of grown bacteria on plates supplemented with antibiotics in two poultry farms in Ibadan.

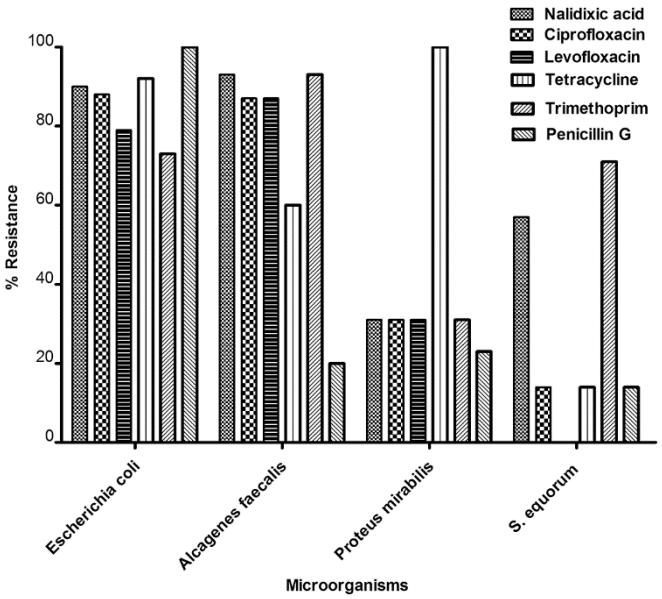
	Bacteria	Poultry A Number of bacteria on plate supplemented with antibiotics		Poultry B Number of bacteria on plate supplemented with antibiotics		Total
		Ciprofloxacin	Tetracycline	Ciprofloxacin	Tetracycline	
1	*Escherichia coli*	6	6	26	14	52
2	*Alcagenes faecalis*	5	4	1	5	15
3	*Proteus mirabilis*	0	10	1	3	13
4	*Staphylococcus equorum*	5	1	1	0	7
5	*Kytococcus sedentarius*	4	0	0	0	4
6	*Kocuria rhizophila*	2	1	0	0	3
7	*Klebsiella pneumoniae*	2	0	0	0	2
8	*Kocuria kristinae*	0	0	1	1	2
9	*Mytococcus luteus / lyiae*	0	0	0	2	2
10	*Providencia rettgeri*	0	0	2	0	2
11	*Acinetobacter johnsonii*	0	0	0	1	1
12	*Aerococcus viridans*	0	0	0	1	1
13	*Burkhoideria cepacia*	0	1	0	0	1
14	*Shigella sonnei*	0	1	0	0	1
	Total	**24**	**24**	**32**	**26**	**106**
	Percentage	**22.64%**	**22.64%**	**30.19%**	**24.53%**	**100%**

ciprofloxacin, (Figure 3) while organisms isolated on tetracycline plates were generally resistant to tetracycline, trimetroprim, ampicillin, ciprofloxacin and moxifloxacin (Figure 4).

Resistance genes were amplified from organisms grown on ciprofloxacin and tetracycline plates. There was coexistence of tetracycline genes on organisms from ciprofloxacin plates and vice versa. The only *qep* gene in this study was isolated on a ciprofloxacin plate. Interestingly, 12 *qnr*B were observed from organisms grown on ciprofloxacin plates and 6 from tetracycline plates. 12 and 7 *tetA* genes were from tetracycline and ciprofloxacin plates respectively while 1 *tetB* was isolated on ciprofloxacin plate and 1 on tetracycline plate. Overall, the most prevalent antibiotic resistant genes were *tetA* (37%) and *qnrB* (35%). The percentage of *tetB* observed was 4%, *qnrS* (4%) and *qepA* (2%) from the total *E. coli* studied. However, none of the *E. coli* had *tetM*, *tetO*, *qnrA*, *oqxB* and *aac(6')Ib – cr.*

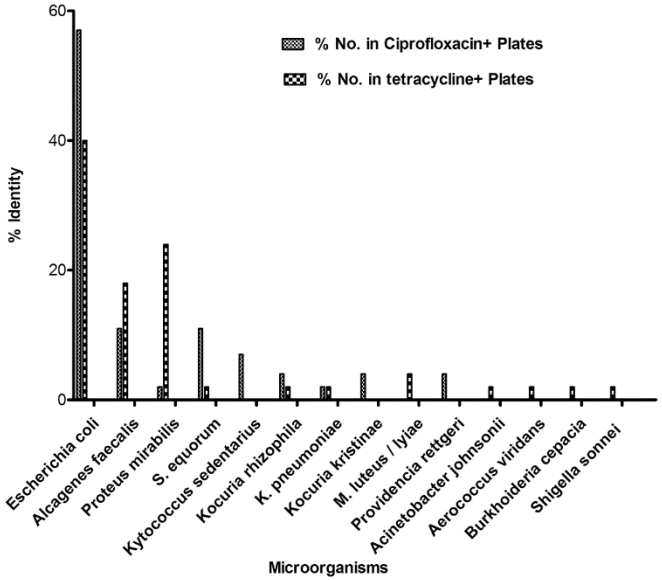
Three ESBL-producing *E. coli* were isolated from farm B (1 from ciprofloxacin plate and 2 from tetracycline plate). All are members of the sequence type ST-6359. They carried the $bla_{\text{CTX-M-15}}$ located in the chromosome at an identical insertion site. In addition, they harbored the beta-lactamase $bla_{\text{TEM-1B}}$, and in 2/3 cases *aac(3)-IId, strB, sul2,* and *dfrA17* antibiotic resistance genes. They all displayed mutations of the GyrA (positions S83L/D87N) and ParC proteins (S80I). Plasmids of the incompatibility group IncFIA were detected in most of the ESBL-producers (Table 3).

Figure 2. Distribution of antibiotic resistance among isolated bacterial species.



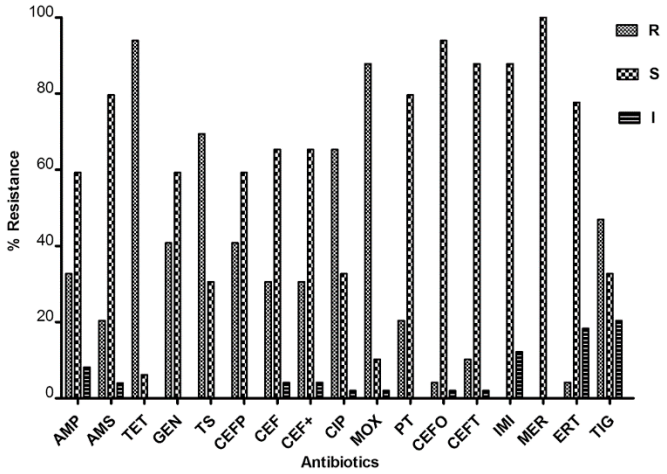
(S: *Staphylococcus)*.

Figure 3. Comparison of MIC of tested antibiotics for organisms isolated on ciprofloxacin supplemented plates



(S: Susceptible, R: Resistant, I: intermediate. AMP: Ampicillin, AMS: Ampicillin/Sulbactam, TET: Tetracycline, GEN: Gentamicin, TS: Trimethoprim/Sulphamethoxazole, CEFP: Cefpodoxime, CEF: Cefuroxime, CEF+: Cefuroxime (oral formulation), CIP: Ciprofloxacin, MOX: Moxifloxacin, PT. Piperacillin-Tazobactam, CEFO: Cefotaxime, CEFT: Ceftazidime, IMI: Imipenem, MEM: Meropenem, ERT: Ertapenem, TIG: Tigecycline).

Figure 4. Comparison of MIC of tested antibiotics for organisms isolated on tetracycline supplemented plates.



(S: Susceptible, R: Resistant, I: intermediate. AMP: Ampicillin, AMS: Ampicillin/Sulbactam, TET. Tetracycline, GEN: Gentamicin, TS: Trimethoprim/Sulphamethoxazole, CEFP. Cefpodoxime, CEF: Cefuroxime, CEF+. Cefuroxime (oral formulation), CIP: Ciprofloxacin, MOX: Moxifloxacin, PT: Piperacillin-Tazobactam, CEFO: Cefotaxime, CEFT: Ceftazidime, IMI: Imipenem, MEM. Meropenem, ERT: Ertapenem, TIG: Tigecycline).

Table 3. Resistant genes and plasmid groups in ESBL producers.

E. coli		Resistance Genes in each antibiotics					Plasmids	
Strain	Aminoglycoside	Q	S	T	Te	Bl	Plasmid incompatibility groups	IncF pMLST
fuy0103			*sul2*			$bla_{CTX-M-15}$,bla_{TEM-1B}	IncFIA	F-:A1*:B32*
fuy090	*aac(3)-IId,strA,strB*	*qepA*	*sul2*	*dfrA17*	*tet(B)*	$bla_{CTX-M-15}$,bla_{TEM-1B}	IncFIA,IncFIB(pB171),IncQ1	F-:A1*:B32*
fuy0054	*aac(3)-IId,strB*		*sul2*	*dfrA17*	*tet(B)*	$bla_{CTX-M-15}$,bla_{TEM-1B}	IncFIA,IncQ1	F-:A1*:B32*

Q = Quinolones; S = Sulphonamides; T = Trimethoprim; Te = Tetracycline; Bl = Beta lactam.

Discussion

Fourteen different bacterial species were identified in this study with the most abundant organism being *E. coli*. Gong *et al*. [19] reported that Gram-positive bacteria were the dominant organisms found in poultry intestines. The prevalence of Gram-negative organism here may be because ciprofloxacin and tetracycline had been supplemented in the media before sample inoculation and isolation. This eliminates the majority of susceptible bacteria and permits the growth of resistant strains that are Gram-negative bacteria such as *Escherichia coli*, *Alcagenes faecalis* and *Proteus mirabilis.*

A preliminary survey around Ibadan showed that antibiotics were used in poultry farms to control diseases. All the farmers visited employed multi-drug combinations and use antibiotics for therapeutic, prophylactic and as growth promoter. This finding is consistent with previous reports of use of antibiotics at sub-therapeutic levels by 86% of poultry farms in Ibadan. [20]. Some species of bacteria grew in media supplemented only with particular antibiotics. *Kytococcus sedentarius* and *Kocuria kristinae* were only found in medium supplemented with ciprofloxacin. Gram-positive rods are less susceptible to ciprofloxacin. Also, *Shigella sonnei* was isolated from medium supplemented with tetracycline. However, *Acinetobacter johnsonii* was susceptible to both antibiotics. A more diverse group of bacteria were detected on tetracycline-supplemented plates. This might be due to the variation in the strength of the two antibiotics and their mode of action. However, *E. coli* were the most abundant bacteria in medium supplemented with either of the antibiotics.

Most of species from the identified bacteria were resistant to moxifloxacin, tetracycline, ciprofloxacin and trimetroprim/ sulphamethoxazole. The resistance to these antibiotics may be due to the incessant use of antibiotics by most farmers in Nigeria. High usage of quinolones, tetracycline and other antibiotics at sub-therapeutic level has been reported in Ekiti state, Nigeria [21]. Also, Ogunleye *et al.* [22] reported sub therapeutic use of antibiotics in many poultries in Abeokuta, Nigeria where enrofloxacin and tetracycline are on the majority list.

Escherichia coli were isolated in this study as the most dominant and resistant of all the identified bacteria. This has been previously reported by Moon *et al.* [23]. The level of resistance in *E. coli* decreases across the family of quinolones, with nalidixic acid being the most resistant, followed by ciprofloxacin and levofloxacin. This is due to the presence of fluorine group on both ciprofloxacin and levofloxacin which is absent in nalidixic acid [24]. Although high resistance was still observed in levofloxacin, it was lower than that of other quinolones. Although the microorganisms used in this study were isolated from ciprofloxacin and tetracycline media respectively, the resistance pattern and number of resistance genes were quite high and interwoven so much that resistance were detected in organisms irrespective of the initial isolating media.

The observed presence of *qnrB, qnrS* and *qepA* in this study was consistent with work done in China by Bao *et al.* [25], who observed that 7.9% of their *E. coli* strains carried *qnrB*, *qepA* (3.6%) and *qnrS* (12.7 %) but no isolate was found to be positive for *qnrA*. Robicsek *et al*. [26] reported that PMQR determinants could confer low-level resistance to quinolones and enhance the selection of high-level resistant strains with mutations on the chromosome.

Although there is high tetracycline resistance in this study, the strains were generally susceptible to tigecycline. Tigecycline resistance mechanism involves evasion of basic tetracycline-resistance mechanisms (e.g. ribosomal protection proteins and efflux pumps) with a more efficient interaction with the ribosome [27]. This renders it to be more active than other first and second generation tetracycline. High number of *E. coli* strains was seen to be resistant to tetracycline. Farmers use it in their poultry indiscrimately, as it is one of the cheapest and easy to administer drug via the oral route. Tigecycline only route of administration is intravenously and it is very expensive as it is use as last resort drug.

Of the four tetracycline resistant genes investigated, only *tetA* and *tetB* were detected. Previous studies have

shown that the efflux *tet*A and *tet*B are the commonest tetracycline resistance genes generally observed in *E. coli* [28,29]. The *tet* genes occurrence are frequent on mobile genetic elements e.g. transposons, plasmids, and integrons [30]. In this study, higher number of *tetA* gene was found in the investigated *E. coli* than *tetB* which was in conformity with a report by Sengeløv *et al.* [31]. None of the *E. coli* in this study shows the presence of *tetM* and *tetO* determinants which was in agreement with what was reported by Bryan *et al*. [32]. However, Ayeni *et al*. [7] reported on the prevalence of *tet (M)* among poultry Enterococci strains in Ogun State, Nigeria.

Additional investigation on *E. coli* strains showed that there were three ESBL producers. ESBL producers occur in different regions and are detected in different *E. coli* strains from various sources. They also occur in other Enterobacteriaceae such as *Klebsiella spp*, *Proteus spp, Citrobacter spp*, *Enterobacter spp* and non-lactose fermenters like *Pseudomonas aeruginosa* [33]. Our previous study in Ibadan, Nigeria also reported some phenotypic ESBL-producing *E. coli* isolated from bird faeces in Ibadan, Nigeria [12]. The 3 ESBL producers in this study were from the same farm but have different resistant phenotype. They all harboured $bla_{\text{CTX-M-15}}$ located in the chromosome, at the same insertion sites with sequence type- ST-6359 indicating clonality. Recent studies involving isolates from animals and humans have shown the $bla_{\text{CTX-M-15}}$ allele to be predominant in humans, fish, and animals, suggesting a circulation of this gene among different settings [34,35].

Although a PMQR (*qepA*) was detected only in one ESBL strains, they all harbored mutations in GyrA (S83L/D87N) and ParC (S80I). Bagel *et al.* [18] have noted that the combination of these two mutations confers high resistance to fluoroquinolones.

Conclusion

The *E. coli* strains identified in this study were generally resistant to levofloxacin, the efficacious member of the quinolone family and tigecycline show lethal effect on *E. coli* as none was observed to be resistant to it. A high prevalence of *tetA* and *qnrB* with association of resistance to tetracyclines, quinolones and ESBL-producers in *E. coli* strains in this study could potentially lead to the transfer of the genes into the human food chain thereby lowering the efficacy of the respective antibiotics when used in for treatment of human illness. Our studies underline the need for regulation of antimicrobial drug usage in livestock farms in developing countries for better public health.

Limitation of the study

This was an explorative study and limited numbers of samples from two poultry farms were investigated. Future studies should cover a larger geographical location with more samples studied in more farms.

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Authors Contributions

TOA carried out laboratory work and wrote the manuscript, LF analysed the WGS and corrected the manuscript, JS corrected the manuscript, TC provided funding and corrected the manuscript. FAA conceived the study, carried out laboratory work and wrote the manuscript.

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