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Antibiotic resistance and resistance genes in Escherichia coli from poultry farms, southwest Nigeria

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Abstract
Introduction: This study investigated the mechanisms of resistance in 36 E. coli isolated from waste, litter, soil and water samples collected from poultry farms in Southwestern Nigeria.
Methodology: Minimum inhibitory concentration (MIC) distributions of the isolates were determined using the methods of the Clinical and Laboratory Standard Institute and resistance genes detected by PCR.
Results: A total of 30 isolates (94%) showed resistance to more than one antimicrobial. Percentage resistance was: tetracycline 81%, sulphamethoxazole 67%, streptomycin 56%, trimethoprim 47%, ciprofloxacin 42%, ampicillin 36%, spectinomycin 28%, nalidixic acid 25%, chloramphenicol 22%, neomycin 14%, gentamicin 8%, amoxicillin-clavulanate, ceftiofur, ceftoxime, colistin, florfenicol and apramycin 0%. Resistance genes found among the isolates include bla-TEM (85%), sul2 (67%), sul3 (17%), aadA (65%), strA (70%), strB (61%), catA1 (25%), cmlA1 (13%), tetA (21%) and tetB (17%). Class 1 and 2 integrons were found in five (14%) and six (17%) isolates, respectively, while one isolate was positive for both classes of integrons. Seven out of eight isolates with resistance to ciprofloxacin and MIC ≤ 32 mg/L to nalidixic acid contained qnrS genes.
Conclusions: Our findings provided additional evidence that the poultry production environment in Nigeria represents an important reservoir of antibiotic resistance genes such as qnrS that may spread from livestock production farms to human populations via manure and water.

Key words: Farm waste; livestock production environment; qnrS.

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Introduction
Antimicrobial drug use in livestock production has been implicated as a risk factor in the development and dissemination of drug resistance from livestock production farms [1,2]. Food animals and their production environments are reservoirs of both resistant bacteria and resistance genes that could be transferred to humans either by direct contact between animals and humans or indirectly via the food production chain [3,4]; or as a result of the spread of animal waste on land [5,2]. In Nigeria, antimicrobial agents are routinely used in livestock production especially as additives to feed and water [6]. This may result in a selective advantage and a consequent increase in the abundance of resistant bacteria in animals, their wastes and surrounding environment [5]. Antibiotic resistant bacteria have been detected in poultry waste [7,8,6,9], commercial poultry production environments [10,11] and poultry products [12,13].

Most food animal production farms in Nigeria have no waste treatment facility. Wastes generated on these farms are either dumped in heaps on farmlands or at remote locations, often inside or close to water bodies. Wastes are also used as organic fertilizer or as feed supplements, especially in fish ponds. This raises the fear that human beings resident in the vicinity of the farm and/or animal waste dump may be exposed to antibiotic resistant bacteria in the waste. In addition, close interaction between human and animal ecosystems is a common occurrence in Nigeria with livestock kept in close proximity to or inside human residences. This practice has been shown to favour high rates of transmission of zoonotic E. coli between livestock and humans in rural Uganda [14]. Although E. coli is a normal inhabitant of the intestinal tract of
warm blooded animals, commensal *E. coli* from humans and animals can cause extra intestinal infections and are a potential reservoir of antimicrobial resistance genes [15]. These bacterial strains, especially when they carry mobile genetic elements such as plasmids, transposons and integrons, can play important roles in the dissemination of transmissible resistance genes [16].

Little is known about the factors contributing to the problem of antibiotic resistance in Nigeria. This is partly because there is a dearth of information on the types, quantity and frequency of antibiotics used in farm animal production system in Nigeria. Very little is also known about the antibiotic resistance profile of bacteria (such as *E. coli*) commonly associated with animal production systems in Nigeria despite the growing global concern about the transmission of antibiotic resistant bacteria through the food animal production chain. The few studies that have investigated antibiotic resistance in *E. coli* from the poultry production system in Nigeria stopped at phenotypic level without a corresponding investigation of the molecular mechanisms of resistance [17]. Yet such studies are warranted because of the potentials of *E. coli* as reservoirs of transferable antibiotic resistance genes that could be disseminated into human populations as a result of the contact with animal wastes. This study therefore investigated antimicrobial susceptibility and molecular mechanisms of resistance in *E. coli* isolated from eight poultry

### Table 1. Description of study farms

| Farm/City: | Age (yrs): | Description: | Antimicrobial use: | Animal population: |
|-----------|------------|--------------|-------------------|-------------------|
| Ogbomosho: |            |              |                   |                   |
| OG1       | 15         | Teaching and research farm, poultry, swine and fish operation. Swine and pond not operating at time of study. | Furazolidone, nitrofurantoin, ampicillin, neomycin, streptomycin, sulphonamides, tetracyclines, chloramphenicol, erythromycin. | 2000 birds |
| OG2       | 6          | Research and demonstration farm, poultry operation. | Furazolidone, nitrofurantoin, ampicillin, neomycin, streptomycin, sulphonamides tetracyclines, chloramphenicol, erythromycin. | 750 birds |
| OG3       | 9          | Poultry operation in a medium density residential area. Farm separated from adjoining building by a brickwall. | Furazolidone, nitrofurantoin, ampicillin, neomycin, streptomycin, sulphonamides tetracyclines, chloramphenicol, erythromycin. | 2500 birds |
| OG4       | unknown    | Integrated poultry, swine and aquaculture operation in a rural agricultural community. Uses poultry waste as feed supplement in pond. Pond operation suspended about 5 months before sample collection. | Furazolidone, nitrofurantoin, ampicillin, streptomycin sulphonamides, doxycycline, chloramphenicol. | 1200 birds, 120 pigs |
| Ibadan:   |            |              |                   |                   |
| IB1       | >20        | Teaching and research farm, poultry operation | Furazolidone, nitrofurantoin, ampicillin, neomycin, streptomycin, sulphonamides tetracyclines, chloramphenicol, erythromycin. | 2500 birds |
| IB2       | 17         | Integrated poultry, swine and aquaculture operation in a rural agricultural community. Swine and fish pond not operating at time of study. This is the only farm in this study that mentioned using enrofloxacin and tylosin. | Furazolidone, nitrofurantoin, ampicillin, streptomycin sulphonamides tetracyclines, chloramphenicol, enrofloxacin, tylosin. | 2500 birds |
| Osogbo:   |            |              |                   |                   |
| OS1       | 5          | Poultry and swine operation, swine operation relocated due to protests from surrounding residents | Furaltadone, nitrofurantoin, streptomycin, sulphonamides, tetracyclines, chloramphenicol, erythromycin. | 1000 birds |
| OS2       | unknown    | Poultry operation in a rural agricultural community. People living around this farm use water from a well located in the farm for domestic purposes | Furazolidone, nitrofurantoin, ampicillin streptomycin, sulphonamides, tetracyclines doxycycline, chloramphenicol. | 750 birds |

-a brief profile, including types of antibiotics used in each of the farms sampled in this study. OG1- OG4, represent the four farms sampled in Ogbomosho, IB1 – IB2, represent the two farms sampled in Ibadan, OS1-OS2, represent the two farms sampled in Osogbo.
production farms in Ogbomoso, Ibadan and Osogbo in Southwestern Nigeria. Four of the farms combined swine and/or fish ponds with poultry operations. The farms with aquaculture operations use the wastes generated from the poultry pens as feed supplement in the ponds. Our primary aim is to assess the potential of the poultry production environment in Nigeria as a reservoir of antibiotic resistance genes that can be mobilized into human populations.

**Methodology**

**Bacterial strains**

Thirty six *E. coli* isolated from samples of faecal materials, litter, farm soil, water and fish pond sediment collected from the study farms in May and June 2008 were included in the study. The water samples were collected as previously described [18] while samples of soil; litter and faecal materials were collected in sterile polythene sample bags as described [6]. The eight farms included in the study were selected from Ogbomoso and Ibadan in Oyo State (8° 0’N 4° 0’E) and Osogbo in Osun State (7° 46’N 4° 34’E) of Southwestern Nigeria. The farms are those who voluntarily agreed to participate in the study and are described in Table 1. Among the farms, only one has a comprehensive record of drug usage, it was also the only farm that admitted using enrofloxacin. The remaining farms admitted using one or more of furazolidone, furaltadone, nitrofurantoin, ampicillin, neomycin, streptomycin, sulphonamides, tetracyclines, doxycycline, chloramphenicol and erythromycin regularly on the farms for animal health management. The drugs are usually part of formulations used as feed supplements, commonly referred to as “premix” which often contained mixtures of antimicrobials. The farm with combined aquaculture operation suspended aquaculture operations about 5 months before the period of sediment collection from the fish pond.

The organisms were isolated from the samples within 24 hours on MacConkey agar as previously described [6] and were identified at the Unit of Antimicrobial Resistance and Molecular Epidemiology, National Food Institute, DTU, Copenhagen, Denmark by streaking on Blood agar, MacConkey agar and CHROMagar orientation Medium (Becton Dickinson GmBH, Heigelberg, Germany). Thirty-six non duplicated isolates confirmed as *E. coli* were randomly selected from the samples and stored at -80°C prior to further analysis for Minimum inhibitory concentration (MIC) determination and molecular characterization.

**Susceptibility testing**

The susceptibility of the isolates to antimicrobial agents was tested at the National Food Institute, DTU, Denmark using commercial dehydrated Sensititre panels (Trek Diagnostic Ltd, East Grinstead, UK) containing the antibiotics listed below at the stated test concentrations in mg/L. Amoxicillin (2-32 mg/L) – clavulanate (1-16 mg/L), ampicillin (1-32 mg/L), apramycin (4-32 mg/L), cefotaxime (0.125-4 mg/L), ceftiofur (0.5-8 mg/L), chloramphenicol (2-64 mg/L), ciprofloxacin (0.015-4 mg/L), colistin (1-16 mg/L), florfenicol (2-64 mg/L), gentamicin (0.5-16 mg/L), nalidixic acid (4-64 mg/L), neomycin (2-32 mg/L), spectinomycin (16-256 mg/L), streptomycin (8-128 mg/L), sulphamethoxazole (64-1024 mg/L), tetracycline (2-32 mg/L) and trimethoprim (1-32 mg/L). Panels were incubated aerobically at 37°C for 18-20 hours. MIC distributions were determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [19]. The MIC results were interpreted using the European Committee for Antimicrobial Susceptibility Testing (EUCAST) epidemiological cut-off values and *E. coli* ATCC 25922 was used as a positive control strain.

**Molecular mechanisms of resistance**

Polymerase chain reaction (PCR) amplification of class 1 and 2 integrons and antimicrobial resistance genes were performed as described previously [20]. One to four colonies of the tested isolates on blood agar were suspended in 100μl of TE buffer and lysed as previously described [20]. The lysates were used as template in PCR to detect strA, strB and aadA in streptomycin resistant isolates, blaTEM in ampicillin resistant isolates, sul2 and sul3 in sulphamethoxazole resistant isolates, tet(A), tet(B), tet(C), tet(D), tet(E), tet(G) and tet(39) in tetracycline resistant isolates, cmlA, catA1 and floR (primers for floR: FloR, 5'-ATGGCAGGCGCATATTCAATTA-3'; floR2, 5'-AAACGGGTGTGCACGATCATC-3') in chloramphenicol resistant isolates, acc(3)-IIV in gentamicin resistant isolates, qnrA, qnrB, qnrC, qnrD, qnrS, aac(6')Ib and qepA in isolates resistant to ciprofloxacin (≥ 0.125mg/L) but sensitive to nalidixic acid with MIC ≤ 32 mg/L [20, 22-25]. The variable regions of Class 1 and Class 2 integrons were amplified and representatives of the different sized amplicons sequenced to characterise their gene contents as described by Peirano et al [21]. The following were used as positive control strains in PCR: *E. coli* (tet(A)), Salmonella Typhimurium DT104 (tet(G)), *E. coli* CSH50::Tn10 (tet(B)), *E. coli*
Results

Antimicrobial susceptibility

Tables 2 and 3 showed the distribution of MICs and phenotypic patterns of resistance among the isolates. Resistance to tetracycline (81%), sulphamethoxazole (67%), streptomycin (56%), trimethoprim (47%), ciprofloxacin (42%) and ampicillin (36%) was the most commonly detected among the isolates tested while phenotypic resistance to spectinomycin (28%), nalidixic acid (25%), chloramphenicol (22%), neomycin (14%) and gentamicin (8%) was less commonly detected. In contrast, all the isolates were sensitive to amoxicillin-clavulanate, ceftiofur, cefotaxime, colistin, florfenicol and apramycin. Among the 15 ciprofloxacin resistant isolates, eight isolates had MICs to nalidixic acid ≤ 32 mg/L. While this study was not designed to investigate the relationship between antibiotic use and resistance on the study farms due primarily to the non-availability of antibiotic-use information as mentioned earlier, it is important to report that tetracycline, streptomycin, sulphonamides, and ampicillin are among the antimicrobials used on all the study farms.

Among isolates of the present study, resistance to two or more antibiotics was detected in 30 of 36 (83%) isolates. When looking at isolates from poultry droppings/litter, swine waste, soil (including fish pond sediment) and well water, 93% (14/15), 63% (5/8), 89% (8/9) and 75% (3/4) of the isolates respectively showed resistance to two or more antimicrobials. Four isolates from pig manure (EC137, EC146), farm soil (EC162) and well water (EC116) were fully susceptible to all antimicrobials tested (Table 3).

Mechanisms of resistance

Table 3 showed the phenotypic patterns of resistance and resistance genes profiles of the isolates. Among the 36 isolates tested, six (17%) carried class 2 integrons, five (17%) of 24 sulphamethoxazole resistant isolates carried class 1 integrons, while only one isolate (3%) (EC3) carried both classes of integrons. The variable region of the class 1 integrons of one representative isolate (EC117) selected from among the most common sized amplicon (1500 bp found in four isolates) was partially sequenced (935 bp). The partial sequence showed 99% sequence identity with position 695 to 1629 containing aadA5 in E. coli GL1 (GenBank accession number FJ807902).

As expected the sulphonamide resistance gene sul1 was detected in all class 1 integron-positive isolates as part of the integron. The variable regions of the class 2 integrons detected among the int2 positive isolates showed three different sized PCR products. The product of one representative isolate (EC3, EC111 and EC113) for each type was sequenced. The partial sequences showed 88% identity with position 1057 to 436 of E. coli encoding a sat1 (GenBank accession number X56815) for isolate EC111, 100% identity with position 2073 to 2608 of E. coli encoding a dfrA1-sat2(partial) (GenBank accession number HM439239) for EC113 and 100% identity with position 1995 to 2668 of E. coli encoding a sat2 (GenBank accession number AY183453) for isolate EC3.

In addition to sul1 carried as part of int1, sul2 and sul3 were detected in 16 (67%) and 4 (17%) of 24 sulphamethoxazole – resistant isolates. All the isolates positive for sul3 were from poultry waste or farm soil from Ogbomoso. Three of the int1/sul1 positive isolates were also positive for sul2. However, sul2/sul3 and sul1/sul3 combinations could not be detected in any of the isolates. Of 23 strains with phenotypic resistance to streptomycin, spectinomycin, or a combination of both antibiotics, aadA, strA and strB encoding resistance to spectinomycin / streptomycin were detected in 15 (65%) (including two isolates, EC123 and EC226 with intermediate resistance to streptomycin and spectinomycin), 16 (70%) and 14 (61%) isolates respectively. Five of these isolates were positive for the aadA/strA/strB combination while strA was found to occur together with strB in all 14 isolates positive for strB. Further, 12 (86%) of 14 isolates carrying the strA/strB combination were also positive for sul2.

The only tested ampicillin resistance gene bla-TEM was found in 11 (85%) of 13 ampicillin-resistant isolates in the present study. The highest prevalence (7 of 8 ampicillin resistant isolates) of this gene was found among isolates from Ogbomoso. catA1 and cmlA1 was detected in 2 (25%) and 1 (13%) of eight chloramphenicol resistant isolates respectively.
Table 2. Distribution of MICs and occurrence of resistance among the *E. coli* isolates from each source

| Compound                        | % Resistance | Distribution of MICs |
|---------------------------------|--------------|----------------------|
|                                 |              | 0.015    0.03 0.06 0.125 0.25 0.5 1 2 4 8 16 32 64 128 256 512 1024 >1024 mg/L |
| Chloramphenicol                 | PO=27        | 5        6   | 2   2 |
|                                 | SW=0         | 3        4   | 1   1 1 |
|                                 | SO=33        | 1        4   | 1   1 1 |
|                                 | WA=25        | 2        1   | 2   |
| Florfenicol                     | PO=0         | 5        8   | 2   |
|                                 | SW=0         | 3        5   |     |
|                                 | SO=0         | 2        5   | 2   |
|                                 | WA=0         | 2        2   |     |
| Trimethoprim                    | PO=53        | 7        8   |     |
|                                 | SW=25        | 7        1   |     |
|                                 | SO=56        | 4        1   | 1   4 |
|                                 | WA=75        | 1        3   |     |
| Amoxicillin + Clavulanate       | PO=0         | 3        5   | 5   2 |
|                                 | SW=0         | 5        3   |     |
|                                 | SO=0         | 1        6   | 1   1 |
|                                 | WA=0         | 2        1   | 1   1 |
| Cefitofur                       | PO=0         | 14       1   |     |
|                                 | SW=0         | 8        |     |
|                                 | SO=0         | 9        |     |
|                                 | WA=0         | 4        |     |
| Nalidixic Acid                  | PO=33        | 5        2   | 3   1 4 |
|                                 | SW=13        | 7        | 1   |
|                                 | SO=11        | 5        2   | 1   1 |
|                                 | WA=50        | 2        1   | 1   |
| Neomycin                        | PO=13        | 12       1   | 1   1 |
|                                 | SW=0         | 8        |     |
|                                 | SO=22        | 5        2   | 2   |
|                                 | WA=25        | 3        |     |
| Spectinomycin                   | PO=47        | 8        | 2   3 2 |
|                                 | SW=13        | 6        2   |     |
|                                 | SO=44        | 2        2   | 1   3 |
|                                 | WA=25        | 2        1   | 1   |
Table 2. (continued) Distribution of MICs and occurrence of resistance among the *E. coli* isolates from each source

| Antibiotic     | PO=67 | 1 | 8 | 1 | 1 | 3 |
|----------------|-------|---|---|---|---|---|
| Streptomycin   |       |   |   |   |   |   |
| SW=50          | 3     | 1 | 2 | 1 |
| SO=44          | 4     | 1 | 1 | 3 |
| WA=50          | 1     | 1 |   |   |
| Tetracycline   |       |   |   | 13|   |   |
| PO=87          | 1     |   |   |   |   |   |
| SW=75          | 2     |   |   |   |   |   |
| SO=89          | 2     |   |   |   |   |   |
| WA=75          | 1     |   |   |   |   |   |
| Sulphamethoxazole |      |   |   |   | 11|   |
| PO=73          | 4     |   |   |   |   |   |
| SW=25          | 6     |   |   |   |   |   |
| SO=89          | 1     |   |   |   |   |   |
| WA=75          | 1     |   |   |   |   |   |
| Colistin       |       |   |   |   |   |   |
| PO=0           | 15    |   |   |   |   |   |
| SW=0           | 8     |   |   |   |   |   |
| SO=22          | 9     |   |   |   |   |   |
| WA=0           | 4     |   |   |   |   |   |
| Ciprofloxacin  |       |   |   |   |   |   |
| PO=60          | 6     | 3 | 2 | 2 | 2 |
| SW=13          | 7     | 1 |   |   |   |
| SO=33          | 5     | 1 | 2 | 1 |
| WA=50          | 1     | 1 | 1 |   |
| Ampicillin     |       |   |   |   |   |   |
| PO=40          | 4     | 4 | 1 |   | 6 |
| SW=25          | 1     | 2 | 3 | 2 |
| SO=22          | 2     | 4 | 1 | 2 |
| WA=75          | 1     |   | 3 |   |
| Gentamicin     |       |   |   |   |   |   |
| PO=7           | 14    | 1 |   |   |
| SW=0           | 7     | 1 |   |   |
| SO=22          | 4     | 2 | 1 | 2 |
| WA=0           | 4     |   |   |   |
| Cefotaxime     |       |   |   |   |   |   |
| PO=0           | 14    | 1 |   |   |
| SW=0           | 7     | 1 |   |   |
| SO=0           | 9     |   |   |   |
| WA=0           | 4     |   |   |   |
| Apramycin      |       |   |   |   |   |   |
| PO=0           | 11    | 4 |   |   |
| SW=0           | 7     | 1 |   |   |
| SO=0           | 7     | 1 | 1 | 1 |
| WA=0           | 4     |   |   |   |

*the frequency of resistance to each concentration of antibiotics tested in this study. Solid vertical lines indicate EUCAST epidemiological cut off values. Where only a solid line is shown, the cut off value and breakpoint are the same. Footnotes: a: PO = Poultry waste (n = 15), b: SW = Swine waste (n = 8), c: SO = Farm Soil and fish pond sediments (n = 9), d: WA = well water (n = 4).*
Table 3. Phenotypic pattern of resistance and resistance genes profiles of *E. coli* isolates included in this study

| Isolates | Farm | Source      | Phenotypic resistance | Resistance genes profile |
|----------|------|-------------|-----------------------|--------------------------|
| EC3      | IB2  | Poultry droppings | chl, tmp, nal, neo, spe, str, smx, cip, amp | int1/sul1*, int2, sul2, bla-TEM, aadA, strA, strB |
| EC40     | IB2  | Poultry droppings | nal, str, tet, smx, cip, amp | sul2, bla-TEM, strA, strB |
| EC42     | IB2  | Poultry droppings | chl, tet, cip | qnrS |
| EC54     | IB1  | Poultry droppings | tmp, str, tet, smx, cip, amp | qnrS, sul2, bla-TEM, strA, strB |
| EC70     | OG1  | Poultry litter | tmp, spe, str, tet, smx, cip, amp | qnrS, int2, sul2, bla-TEM, aadA, strA, strB, tet(A) |
| EC75     | OG1  | Poultry litter | tet | tet |
| EC87     | OG1  | Poultry litter | chl, tmp, nal, spe, str, tet, smx, cip, amp | sul2, sul2, blA-TEM, aadA |
| EC114    | OG2  | Poultry droppings | spe, str, tet | int2, aadA |
| EC117    | OG4  | Poultry droppings | tmp, neo, str, tet, smx, amp | sul2, tet(A) |
| EC118    | OG5  | Poultry droppings | tmp, spe, str, tet, smx | sul2, aadA, strA, strB, tet(A) |
| EC119    | OG4  | Poultry droppings | nal, str, tet, smx, cip | qnrS, sul2, strA |
| EC232    | OG1  | Poultry droppings | chl, tet, smx, gen | sul3, aadA, catA1 |
| EC146    | OG4  | Swine waste | Susceptible to all Antibiotics tested | sul2, blA-TEM, strA, strB |
| EC150    | OG1  | Swine waste | str, tet, smx, amp | strA, strB, tet(B) |
| EC96     | OG4  | Fish pond sediment | nal, str, tet, smx, cip | sul2, strA, strB, tet(A) |
| EC233    | OG4  | Fish pond sediment | str, tet, smx, cip | qnrS, sul2, strA, strB |
| EC69     | OG4  | Manured soil | chl, spe, tet, smx, gen | sul3, aadA |
| EC113    | OG2  | Farm soil | chl, tmp, neo, spe, str, tet, smx, cip, amp | qnrS, int2, sul2, blA-TEM, catA1, aadA, strA, strB |
| EC117    | OG3  | Farm soil | tmp, neo, str, tet, smx, amp | int1/sul1, sul2, aadA, strA, strB, tet(B) |
| EC121    | OG3  | Farm soil | tmp, spe, tet, smx | int2, sul2, aadA, tet(B) |
| EC162    | OG3  | Farm soil | Susceptible to all Antibiotics tested | sul2 |
| EC1801   | OG3  | Farm soil | tmp, smx | sul2 |
| EC122    | OG4  | Farm soil | chl, tet, smx, gen | sul3, aadA |
| EC116    | OG3  | Well water 2 | Susceptible to all Antibiotics tested | sul2, blA-TEM, strA, strB, tet(B) |
| EC142    | OG3  | Well water 2 | tmp, nal, str, tet, smx, cip, amp | sul2, blA-TEM, strA, strB, tet(B) |
| EC164    | OG3  | Well water 5 | chl, tmp, nal, neo, str, tet, smx, cip, amp | int1/sul1, sul2, blA-TEM, catA1, strA, tet(B) |

chl: chloramphenicol, tmp: trimethoprim, nal: naldixic acid, neo: neomycin, str: streptomycin, spe: spectinomycin, smx: sulphamethoxazole, cip: ciprofloxacin, amp: ampicillin, gen: gentamicin, tet: tetracycline.

Footnote a: See Table 1 for farm codes.

Footnote b: *sul1* gene was detected as part of class 1 integrons in all *int1* positive isolates.
None of the eight chloramphenicol resistant isolates, including those that were negative for catA1 and cmlA, were positive for floR. Majority of the tetracycline resistant isolates did not give positive amplification signals for most of the tetracycline resistant genes we tested for.

Even though all positive controls gave positive amplification signals, tet(C), tet(D), tet(E), tet(G) or tet(39) could not be detected among the 29 tetracycline resistant isolates. However, tet(A) and tet(B) were found in six (21%) and five (17%) isolates respectively. Compared to the present study, other studies reported higher frequencies of tet(A) and tet(B). For example, in a study of 317 E. coli isolated from pigs, cattle and poultry in Germany, Guerra et al. [15] found tet(A) and tet(B) in 66% and 42% of the isolates respectively. Similarly, Enne et al [26] found 56% of 103 tetracycline resistant E. coli from farm animals at slaughter to contain tet(B). acc(3)-IV, the only gentamicin resistance gene tested for in this study was not detected in any of the four gentamicin resistant isolates.

Seven out of eight isolates that were resistant to ciprofloxacin and had MIC values to nalidixic acid of \( \leq 32 \text{ mg/L} \) contained qnrS. Of these isolates, the PCR products of three isolates (EC54, EC143, EC199) were sequenced and the sequences showed 100% identity to qnrS1 from E. coli (GenBank accession number GQ214053).

Discussion

Development of resistance to antimicrobial agents is a problem of global proportion which is increasingly frustrating efforts to treat infectious diseases. This has been attributed to human and agricultural use of antimicrobials. Thus, our study investigated the occurrence and molecular mechanisms of antibiotic resistance among commensal E. coli isolated from selected poultry farms in southwestern Nigeria, an area of the world where little is known on antibiotic use and its contribution to the development and dissemination of resistance. A serious limitation to this study was the difficulty encountered in sample collection which limited the number of isolates included in the study. It was difficult convincing farm owners to participate in the study and most of the volunteer farms restricted access to specified areas of the farms, and none allowed sample collection more than twice. Nonetheless, our findings demonstrated that the commensal E. coli included in this study were commonly resistant to antibiotics used in human and veterinary medicine. However, because our study did not include isolates from farms without antimicrobial use, it was difficult to draw a definite conclusion on the association between drug use on the study farms and the occurrence of resistance in the isolates.

The observed levels of resistance to ciprofloxacin (42%) and trimethoprim (47%) were however unexpected since only one of the eight farms admitted using the fluoroquinolone enrofloxacin and no farm used trimethoprim. Thus, it is quite possible that use of trimethoprim, a common component of poultry premixes used widely in the farms of the present study was underreported. More importantly, our observation about the levels of resistance to ciprofloxacin and trimethoprim suggested that factors other than antibiotic use may be contributing to the selection of resistance among the present isolates.

Similar to the observed pattern of phenotypic resistance, genes conferring resistance to streptomycin/spectinomycin, sulphamethoxazole and ampicillin were also found to be common among the isolates. However, it seemed that local and geographical factors played a role in the occurrence of some resistance genes; all isolates positive for sul3 and tetracycline resistance genes are from Ogbomoso. In a sharp contrast to this, other genes seem to have a widespread occurrence within the study farms. For example, isolates with qnrS originated from six different farms (including the farm admitting usage of fluoroquinolones) and from four different sources: poultry droppings, fish pond sediment and farm soil. The only isolate that did not contain any of the quinolone resistance genes screened for was from well water (EC142). The widespread occurrence of qnrS observed in this study may be a result of their co-selection and transfer with other resistance determinants on mobile genetic elements. qnr, a plasmid-mediated horizontally transferable gene encoding quinolone resistance was discovered for the first time in 1998 and has been in circulation for at least 20 years [27]. Qnr proteins are capable of protecting DNA gyrase from the action of quinolones and qnr are increasingly detected in isolates of clinical importance [28]. Our findings indicated that qnrS was widely spread among poultry and their production environment in Nigeria. qnrS1 was recently found among Salmonella and E. coli from humans and animal sources in Nigeria [29,13].

Our study provided additional evidence that food animals production environments may be important reservoirs of clinically important antibiotic resistance genes such as plasmid-mediated quinolone resistance (PMQR) genes in this part of the world. This is of
concern as fluoroquinolones were listed by the World Health Organization as critically important antimicrobials for human health [30]. Therefore, it seems very important to pay more attention to the spread of resistance through such production systems in the tropical climate, and regulation of antimicrobial usage in production systems with close interaction between human and animal ecosystems such as this, prevalent in the developing world, seems crucial.

Even though we tested for the resistance genes most commonly found in *E. coli*, resistance in a number of isolates appeared to be conferred by mechanisms other than those tested in this study. Thus resistance genes investigated in this study could not be detected in two ampicillin-resistant, one sulphamethoxazole-resistant, five chloramphenicol-resistant and eighteen tetracycline-resistant isolates. These isolates may contain known mechanisms not tested for in this study or unknown mechanisms. The large fraction of tetracycline resistant isolates without any of the tested tetracycline resistance genes is unexpected and may be due to geographical differences between this and previous studies on tetracycline resistant *E. coli*. The results of this study underscore the importance of routine screening for antibiotic resistance in commensal bacteria from food animal production farms using both phenotypic and genotypic methods. This is very crucial, especially in countries such as Nigeria with limited studies on occurrence of resistance genes in food animal production systems.

In conclusion, data from the present study suggested that commensal *E. coli* from the study farms may act as reservoirs of antimicrobial drug resistance genes such as *qnrS* which may be mobilized into human populations. To the best of our knowledge, this is the first comprehensive study of molecular mechanisms of resistance in commensal *E. coli* associated with livestock farms in Nigeria. Results should however be interpreted with caution as a limited number of isolates were investigated and a comprehensive history of antibiotic use necessary to make informed decision on the contribution of antibiotic use to the observed resistance is not available. It however pointed attention to the critical need for the regulation of antimicrobial drug usage in livestock production and continuous monitoring of antibiotic resistance in developing countries for public health safety.

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