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Al-Mustapha, Ahmad Ibrahim

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Co-occurrence of antibiotic and disinfectant resistance genes in extensively drug-resistant *Escherichia coli* isolated from broilers in Ilorin, North Central Nigeria

Ahmad Ibrahim Al-Mustapha[a,b,c,*], Shafi Abdullah Alada[d], Ibrahim Adisa Raufu[e], Adedeji Nurudeen Lawal[e,f], Katarina Eskola[g], Michael SM Brouwer[h], Victoria Adetunji[b], Annamari Heikinheimo[c,i]

[a] *Department of Veterinary Services, Kwara State Ministry of Agriculture and Rural Development, Ilorin, Kwara State, Nigeria*
[b] *Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Oyo State, Nigeria*
[c] *Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Finland*
[d] *Veterinary Microbiology Laboratory, University of Ilorin Veterinary Teaching Hospital, Ilorin, Kwara State, Nigeria*
[e] *Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ilorin, Kwara State, Nigeria*
[f] *Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria*
[g] *Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Finland*
[h] *Department of Bacteriology and Host-Pathogen Reaction, Wageningen University and Research, Lelystad, The Netherlands*
[i] *Finnish Food Authority, Seinäjoki, Finland*

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ABSTRACT

Objectives: The occurrence of multidrug-resistant (MDR) bacteria in poultry poses the public health threat of zoonotic transmission to humans. Hence, this study assessed the occurrence of drug-resistant *Escherichia coli* in broilers in the largest live bird market in Kwara State, Nigeria in December 2020.
Methods: Presumptive *E. coli* isolates were isolated using the European Union Reference Laboratory guideline of 2017 and confirmed via matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). Broth microdilution was performed on confirmed *E. coli* isolates to determine the minimum inhibitory concentration. Five extensively drug-resistant (XDR) isolates were selected for Illumina whole genome sequencing to predict the resistome, phylotype, sequence type, serotype, and diversity of mobile genetic elements in these isolates.
Results: Of the 181 broiler caecal samples, 73 *E. coli* isolates were obtained, of which 67 (82.0%) and 37 (50.6%) were determined as MDR (resistant to at least three classes of antibiotics) and XDR (resistant to at least five classes of antibiotics), respectively. Whole genome sequencing revealed diverse sequence types, phylogroups, and serotypes (ST165/B1 - O80:H19, ST115/A - Unknown: H7, ST901/B1 - O109:H4, ST4087/F - O117:H42, and ST8324/A - O127:H42). The XDR *E. coli* isolates encoded resistance to fluoroquinolones, fosfomycin, sulfamethoxazole, ampicillin and cephalosporins, trimethoprim, aminoglycosides, chloramphenicol, tetracycline, and macrolides. Mutations in the *gyrA* gene conferring resistance to fluoroquinolones were also detected. There was a positive correlation between phenotypic resistance patterns and the antibiotic resistance genes that were detected in the sequenced isolates. The XDR isolates also harbored two disinfectant resistance genes (*qac*E and *sit*ABCD) that conferred resistance to hydrogen peroxide and quaternary ammonium compounds, respectively. The genome of the XDR isolates harbored several mobile genetic elements and virulence-associated genes, which were conserved in all sequenced XDR isolates.
Conclusions: This is the first report of co-carriage of antibiotic resistance genes and disinfectant resistance genes in *E. coli* isolated from broilers in Ilorin, Nigeria. Our findings suggest that poultry are potential carriers of clonally diverse, pathogenic, MDR/XDR *E. coli*, which may have detrimental zoonotic potentials on human health.

1. Introduction

Antimicrobial resistance (AMR) is a global health threat that requires a multisectoral One Health approach [1,2]. It encompasses

* Corresponding author. Mailing address:
E-mail address: ai.almustapha42@gmail.com (A.I. Al-Mustapha).

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resistance to antibiotics, anthelmintics, antivirals, and chemical agents such as disinfectants [1]. Globally, there has been an increase in the emergence and dissemination of multidrug-resistant (MDR) bacteria, especially in the family Enterobacteriaceae [3,4]. Several studies have reported empirical evidence on the effect of antimicrobial use in food-producing animals (FPAs) on human health [5,6]. Contact with livestock, particularly poultry, has been described as a risk factor for the emergence and spread of foodborne pathogens such as *Escherichia coli, Salmonella,* and *Campylobacter* [5]. The illicit use of antimicrobial agents in the food industry, especially FPAs (as growth promoters, for disease prevention, and disease treatment), enhanced the selection pressures on bacterial pathogens [7].

In Nigeria, most antimicrobials (antibiotics, antiprotozoal, anthelminthic, and disinfectants) are readily available to livestock farmers, hence their unregulated usage in humans, livestock, agriculture, and the environment. Most disinfectants have a broad spectrum of activity, multiple targets, and non-specific modes of action [8,9]. Studies have reported a direct link between exposure to disinfectants and the development of resistance [9–11]. However, it is unclear whether resistance to biocides in clinical isolates confers cross-resistance to antibiotics [8].

Despite the demonstrated importance of whole-genome sequencing (WGS) in understanding pathogen diversity and epidemiology, only a few studies in Nigeria have utilized WGS to study bacterial pathogens in FPAs. Recently, Aworh et al. [12] reported diverse antibiotic resistance genes (ARGs) in extended-spectrum beta-lactamase producing *E. coli* from poultry in Abuja, Nigeria. Similarly, Sharma et al. [13] reported resistance to clinically important reserve antibiotics such as colistin in *E. coli* that were isolated from poultry in Nigeria. In the same vein, Monarrez et al. [14] reported the occurrence of *E. coli* from Nigeria carrying two disinfectant resistance genes (*qac*E and *sit*ABCD) in a 125 Kb self-transmissible IncFII plasmid, pMB2, which also harbored the $bla_{\text{CTX-M-15}}$ gene and seven other functional resistance genes. The *qac*E genes confer the efflux-mediated resistance to QACs, whereas SitABCD is an operon of several genes that together build the ABC transporter conferring resistance to the bactericidal effects of hydrogen peroxide. This manganese and ferrous transport system was first described in *Salmonella enterica* [14]. The transport system is made up of four regions: *sit*A is a periplasmic binding protein; *sit*B functions as the ATP-binding component; *sit*C functions as a permease; and *sit*D is the inner membrane component of the system [14].

As alternatives to antibiotics, several studies have emphasized the importance of good management practices, biosecurity, and disinfection to limit the dissemination of these AMR bacteria in FPAs and to ensure wholesome food products from the farm to the fork [15,16]. However, it remains unclear whether illicit use of chemical disinfectants could result in resistance to chemical biocides and if these confer cross-resistance to antibiotics. Here, we present the first report of concurrent resistance to two classes of disinfectants and several classes of antibiotics in extensively drug-resistant (XDR) *E. coli* isolated from broilers in Ilorin, North Central Nigeria.

2. Materials and Methods

2.1. Sampling and isolation of Escherichia coli

A total of 181 caecal samples were collected from slaughtered broilers in December 2020 as part of a broader study on the epidemiology and surveillance of AMR in FPAs in Ilorin, Kwara State, Nigeria. The samples were obtained from the Obo Road live bird market (LBM), which is the biggest LBM in the state. This LBM was selected because it is a meeting point for small-scale holder poultry farmers and accommodates several hundred poultry farmers and marketers from across the state, especially during religious festivities.

The samples were aseptically collected into transport medium (buffered peptone water, Oxoid, Basingstoke, UK) and plated on MacConkey agar and Eosin Methylene Blue agar media after overnight incubation (18–24 hours) at 37°C. Single colonies with the typical colonial morphological characteristics and green metallic sheen on Eosin Methylene Blue agar (Oxoid, Basingstoke, UK), respectively, were picked and sub-cultured in blood agar (Oxoid, Basingstoke, UK) to obtain pure isolates. Isolates were stored at -20°C in nutrient broth supplemented with glycerol. Matrix-Assisted Laser Desorption Ionization Time of Flight - Mass Spectrometry (MALDI-TOF/MS) (Bruker, Bremen, Germany) was used following the Biotyper protocol to reliably confirm that the isolates were *E. coli*. The best match score value of greater than 2.300 was used as the cut-off for reliable species differentiation as previously described by Hou et al. [17].

2.2. Phenotypic antimicrobial susceptibility testing

The isolates were subjected to antibiotic susceptibility tests against 14 antibiotics belonging to 10 different classes contained in EUVSEC Sensititre plates using broth microdilution assay according to the manufacturer's instructions (Thermo Scientific, Vantaa, Finland). The antibiotics included in the EUVSEC Sensititre (and their concentrations) were as follows: sulfamethoxazole (8–1024 µg/L); trimethoprim (0.25–32 µg/L); ciprofloxacin (0.0015–8 µg/L); tetracycline (2–64µg); meropenem (0.03–6 µg/L); azithromycin (2–64 µg/L); nalidixic acid (4–128 µg/L); cefotaxime (0.25–4 µg/L); Chloramphenicol (8–128 µg/L); tigecycline (0.25–8 µg/L); ceftazidime (0.5–8 µg/L); colistin (1–16 µg/L); ampicillin (1–64 µg/L); and gentamicin (0.5–32 µg/L). The obtained results obtained were used to classify isolates as being resistant or susceptible using the epidemiological cut-off (ECOFF) values that were established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (https://mic.eucast.org/). *E. coli* strain ATCC 25922 was used as a reference. An isolate was designated as MDR if it conferred resistance to at least three classes of antibiotics and XDR if it conferred resistance to at least five different classes of antibiotics [18].

2.3. Genomic DNA extraction, genome sequencing, assembly, and annotation

Genomic DNA was extracted from overnight grown bacterial cultures using a PureLink Genomic DNA purification kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The purity and concentration of the DNA samples were quantified using a Qubit 4 Fluorometer (Invitrogen, Singapore). Libraries were prepared for the five XDR isolates using the Nextera Flex Kit (Illumina Inc., San Diego, CA) according to the manufacturer's instructions. Thereafter, these five isolates were sequenced using the Nextera Illumina sequencing platform (using a 2 × 300 paired-end approach (Illumina Inc., San Diego, CA). Adapters of the raw sequencing reads (fastq files) were quality filtered and trimmed using Trimmomatic (http://www.usadellab.org/cms/index.php?page=trimmomatic), and the FastQC tool was used to assess the quality of reads (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The sequence reads were assembled into contigs using Center for Genomic Epidemiology (CGE) Assembler (https://cge.food.dtu.dk/services/Assembler). Assembled contigs were annotated to determine the ARGs and multi-locus sequence types (MLSTs) using CGE's Resfinder (https://cge.food.dtu.dk/services/ResFinder/) and MLST finder (https://cge.food.dtu.dk/services/MLST), respectively, using the default settings [19,20]. The MLST was determined using the Achtman *E. coli* scheme 1,

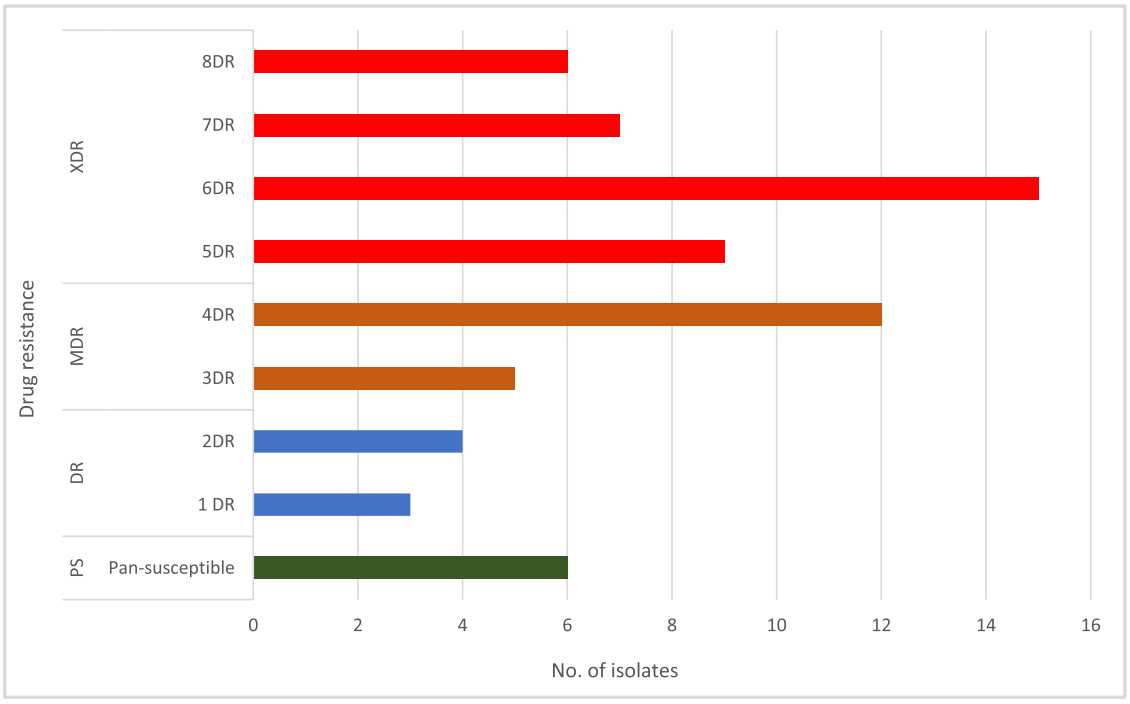

Fig. 1. Drug resistance profile of *Escherichia coli* isolated from broilers in the Obo Road live bird market in Kwara State, Nigeria.
DR, drug resistance, i.e. resistance to one or two classes of antibiotics; MDR, multidrug resistance, i.e. resistance to at least 3 classes of antibiotics; XDR, extensive drug resistance, i.e. resistance to at least five classes of antibiotics; PS, pan-susceptible.

which employed seven housekeeping *E. coli* genes (*adk*, *fum*C, *gyr*B, *icd*, *mdh*, *pur*A, *rec*A). To determine the serotype of these isolates, SerotypeFinder 2.0 from the CGE webserver was used (https://cge.food.dtu.dk/services/SerotypeFinder/). The plasmids and the insertion sequences were determined in these isolates using the PlasmidFinder 2.1 tool (https://cge.food.dtu.dk/services/PlasmidFinder/) [21] and the IS finder tool (https://isfinder.biotoul.fr/blast.php), respectively. Finally, the in-silico phylotyping was performed by ClermonTyping, which separates the isolates into phylotypes based on the presence or absence of five genes: *chu*A, *yja*A, *tsp*E4.C2, *arp*A, and *trp*A [22]. The sequence data were deposited in the NCBI database under the bio project PRJNA764473.

3. Results

3.1. Antibiogram of E. coli

A total of 73 presumptive *E. coli* isolates were retrieved from the 181 broiler caecal samples tested, resulting in a prevalence of 40.3%. MALDI-TOF/MS confirmed that all of the presumptive isolates were *E. coli*, with a minimum match score value of 2.34. Of the 73 *E. coli* isolates, 8.2% (n = 6) showed no resistance to the tested antibiotics. However, 56.2% (n = 41) of the isolates were MDR, and 35.6% (n = 26) of the isolates were extensively drug-resistant (XDR) (Fig. 1).

The isolates showed absolute (100%) resistance to sulfamethoxazole and ciprofloxacin. Similarly, very high resistance of 94.5% was detected for tetracycline, nalidixic acid, ampicillin, and chloramphenicol (Fig. 2). There was moderate resistance to cephalosporins, as 65.6% (n = 44) of the isolates showed resistance to cefotaxime and 55.2% (n = 37) of the isolates were resistant to ceftazidime. In general, the resistance rates were two- to four-fold higher than the recommended ECOFF values. For instance, the ECOFF of sulfamethoxazole was 64 μg/L. However, all isolates showed growth at 1024 μg/L. No isolate showed resistance to colistin or meropenem. Although there is currently no ECOFF or Clinical and Laboratory Standards Institute breakpoint (CLSI M100 performance standards) for azithromycin in *E. coli*, we recorded growth between 8–32 μg/L.

Table 1
Molecular features of XDR *Escherichia coli* isolated from broilers in Ilorin, Nigeria

Isolate ID	No. of ARG variants	DRG	Serotype	ST	Phylogroup
AL-D-16	7	1	O80:H19	165	B1
AL-D-24	9	2	H7	Novel	B1
AL-D-25	7	2	O109:H4	4087	F
AL-D-47	6	0	O117:42	8324	A
AL-D-91	8	2	O127:H42	115	A

ARG, antibiotic resistance gene; DRG, disinfectant resistance gene; ST, sequence type.

3.2. Genetic diversity of XDR E. coli

WGS analysis of five of the XDR isolates gave molecular insight into the resistome, the sequence type, the phylotype, the virulence-associated genes, and the genetic diversity in these isolates. Our findings further showed that the isolates were of diverse sequence types (Table 1). None of the isolates belonged to the globally dominant multidrug-resistant ST131 clone. The five isolates were of diverse serotypes: O80:H19; H7; O109:H4; O117:42; and O127:H42, and they were clonally diverse (Fig. S1). Based on the Clermont phylotypes, two isolates belonged to phylogroup A (n = 2), phylogroup B1 (n = 2), and one isolate was typed as phylogroup F.

3.3. Antimicrobial resistance genes

WGS revealed that several genes were present, conferring resistance to macrolides, tetracyclines, sulfonamide, trimethoprim, aminoglycosides, chloramphenicol, fluoroquinolones, fosfomycin, and cephalosporins. A total of five different bla_{AmpC} and bla_{ESBL} genes conferred resistance to beta-lactam antibiotics by inducing

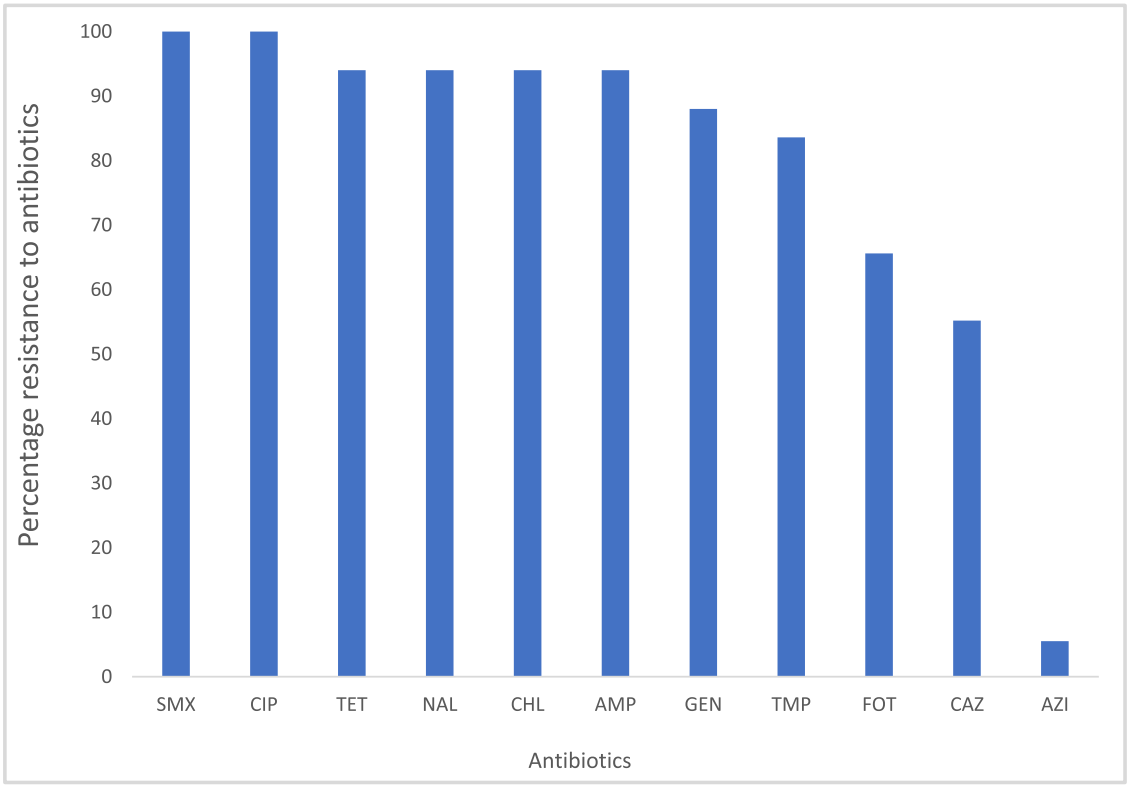

Fig. 2. Antibiotic resistance pattern of XDR *Escherichia coli* isolated from broilers in Ilorin, Nigeria (n = 67).
SMX, sulfamethoxazole; CIP, ciprofloxacin; TET, tetracycline; NAL, nalidixic acid; CHL, chloramphenicol; AMP, ampicillin; GEN, gentamicin; TMP, trimethoprim; FOT, cefotaxime; CAZ, ceftazidime; AZI, azithromycin (this was based on a tentative ECOFF of 16μg/L).

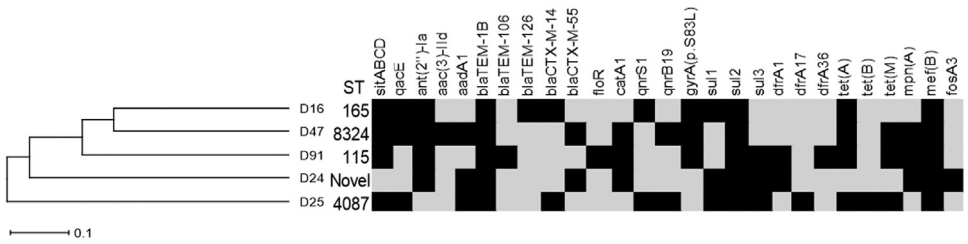

Fig. 3. SNP-based neighbor-joining phylogeny of extensively drug-resistant XDR *Escherichia coli* isolated from broilers in Ilorin, Nigeria.

the production of beta-lactamases and cephalosporinases in the isolates (Fig. 1). The narrow spectrum $bla_{TEM\text{-}1B}$ gene was detected in the five sequenced isolates. Similarly, two isolates also harbored extended-spectrum beta-lactamase (ESBL) genes, $bla_{TEM\text{-}106}$ and $bla_{TEM\text{-}126}$. In addition, two isolates contained $bla_{CTX\text{-}M\text{-}14}$, and an isolate harbored $bla_{CTX\text{-}M\text{-}55}$. Other clinically important ARGs detected were *fosA3,* the plasmid-mediated quinolone resistance genes (*qnrS1* and *qnrB19*), as well as *sul1, sul2,* and *sul3,* which conferred resistance to fosfomycin, fluoroquinolones, and sulfonamides, respectively. All other antimicrobial resistance genes are shown in Fig. 3. Several point mutations were identified in the quinolone-resistance gene, *gyr*A. The multidrug-resistant efflux pump gene, *mef(B)* was detected in all isolates. There was a positive correlation between phenotypic resistance patterns and the antibiotic resistance genes that were detected in the sequenced isolates.

Three of the XDR isolates harbored two DRGs, which conferred resistance to hydrogen peroxide (*sit*ABCD) and quaternary ammonium compounds (*qacE*), respectively (Fig. 3), Whereas isolate AL-D-16 only harbored the *qacE* gene, isolate AL-D-47 did not harbor any DRG. The DRGs were all chromosomally located and no mutations were detected in both DRGs.

3.4. Virulence-associated genes

Several virulence-associated genes (VAGs) harbored by these XDR isolates were detected. These were: *astA* (enteroaggregative heat-stable enterotoxin 1), *cea* (colicin E1), *cvaC* (microcin C), *iss* (increased serum survival), *hylF* (hemolysin F), *iucC* (aerobactin synthetase), and *tsh* (temperature-sensitive hemagglutinin). Several other VAGs detected in some of the isolates include: *sitA* (iron transport protein; 80%, n = 4), *traT* (outer membrane protein complement resistance, 40%, n = 2), *terC* (tellurium ion resistance protein, 20%, n = 1), *papC* (outer membrane usher P fimbriae; 40%, n = 2), and *air* (enteroaggregative immunoglobulin repeat protein;

Table 2
Diversity of plasmids and virulence-associated genes in XDR *Escherichia coli* isolated from broilers in Ilorin, Nigeria.

	Variable	Isolate ID AL-D-16	AL-D-24	AL-D-25	AL-D-47	AL-D-91
Plasmids	IncFII	+	+	+	+	+
	IncFII(pRSB107)	+	+	+	+	+
	IncFIB(AP001918)	+	+	+	+	+
	IncFIC(FII)	+	+	+	+	+
	IncFII(pCoo)	+	+	+	+	+
	IncHI2A	+	-	-	+	+
	p0111	+	+	+	+	+
	IncQ1	-	+	-	+	-
	IncR	+	-	-	-	+
	IncI1-I(Alpha)	+	+	-	-	-
	IncN	-	-	+	-	+
	IncX1	-	+	-	-	-
	IncFIC	-	-	+	+	+
	Col156	+	+	+	+	+
	Col440I	+	+	+	+	+
	ColRNAI	+	+	+	+	+
	ColpVC	+	+	+	+	+
	Col(pHAD28)	+	+	+	+	+
VAGs	sitA	+	-	+	+	+
	astA	+	+	+	+	+
	cea	-	+	+	-	-
	iss	+	+	+	-	-
	hylF	-	+	+	+	-
	iucC	+	-	-	+	+
	air	+	-	-	+	-
	tsh	+	+	-	+	+
	traT	+	-	-	-	-
	ompT	-	+	+	-	+
	chuA	-	-	-	-	+
	hra	-	-	-	-	-
	cvaC	+	+	-	+	+
	papC	-	+	-	-	+
	uphT	-	+	-	-	-

60%, n = 3). An average of seven VAGs were detected in the isolates (Table 2).

3.5. Mobile genetic elements

WGS revealed that two main categories of plasmid replicons were identified in the XDR *E. coli* isolates. Most of these plasmid replicons belonged to the incompatibility F (IncF) group and the others were of the Col plasmid group. A total of 18 plasmids replicon types were contained in the five XDR *E. coli* isolates. Of the IncF plasmids, IncFIB(AP001918), IncFII, IncFII(pRSB107), IncFIC(FII), p0111, IncHI2A, and IncFII(pCoo) were found in all the XDR isolates. Other Inc plasmids detected in the isolates were: IncI1-I(Alpha) (40%, n = 2), IncN (40%, n = 2), IncX1 (20%, n = 1), IncFIC (40%, n = 2), IncQ1 (40%, n = 2), and IncR (60%, n = 3). The Col plasmids (relatively small plasmids of approximately 2 kb) detected in all the XDR isolates were Col156, Col440I, ColRNAI, ColpVC, and Col(pHAD28).

The following AMR genes were carried on plasmid replicons: *tet(M)* (tetracycline resistance) on IncFIB(AP001918) plasmid (n = 5); *mef(B)* (macrolide efflux protein) on IncFIB plasmid (n = 5); *qnrS13* (fluoroquinolone resistance) on Col440I (n = 2); *catA1* on IS3; *sul2* (sulfonamide resistance) on an IncFII plasmid (n = 5); *aph(3)-Ib* (aminoglycoside resistance) on IncI1-I (Alpha) plasmid (n = 1); and two isolates that had bla_{TEM-1B} (beta-lactam antibiotics) on IncFIC(FII) plasmids. Similar to plasmids, the isolates also contained several insertion sequences (IS) and miniature inverted repeats. These include: MITEEc1 (100%, n = 5), ISEc9 (100%, n = 5), IS26 (100%, n = 5), IS3(80%, n = 4), ISKpn26 (80%, n = 4), ISKpn8 (60%, n = 3), IS6100 (60%, n = 3), IS629 (60%, n = 3), IS421(40%, n = 2), ISEc10 (20%, n = 1), IS4 (60%, n = 3), and ISEam1 (20%, n = 1).

4. Discussion

The detection of several ARGs and two DRGs in three of the five XDR *E. coli* isolates showed the in-depth information that could be obtained from WGS and its importance in ameliorating the global challenge of AMR, especially in the Low- and Middle- Income Countries (LMICs) of Africa and Asia [23]. This high AMR profile could be attributed to the multidrug combinations, poor drug quality, misuse of antimicrobials in humans and animals, self-medication, lack of antimicrobial stewardship programs, and inadequate genomic surveillance programs in most LMICs [24]. Hence, this study assessed the repertoire of resistance genes in phenotypically XDR *E. coli* that were isolated from broilers in Ilorin, North Central Nigeria.

Phenotypically, 82% of all *E. coli* isolates showed resistance to at least three classes of antibiotics. This high drug resistance pattern could be attributed to one or more of the above-listed factors, which are common in LMICs. This finding is similar to several other studies conducted across Nigeria that reported the occurrence of MDR/XDR *E. coli* in poultry [12,25–28] and other FPAs [29–32]. Specifically, the high resistance to gentamicin, fluoroquinolones, sulfamethoxazole, and tetracyclines could be attributed to the misuse of these antibiotics in poultry farms in Kwara State, Nigeria [33]. The increasing rate of MDR/XDR pathogens — especially *E. coli* — in FPAs poses a food safety concern and the possible emergence of the inter-host zoonotic spread between humans, animals, and the environment.

WGS showed that XDR *E. coli* isolates harbored a repertoire of resistance genes, showing that WGS can be used to positively predict phenotypic resistance patterns [34]. Resistance to aminoglycosides, conferred by *ant(2')-Ia, aac(3)-IId*, and *aadA1,* was prevalent in the sequenced isolates. Two chloramphenicol resistance genes (*floR* and *catA1*) were detected in the XDR isolates. This poses a great public health concern as usage of chloramphenicol was banned in food-producing animals because of its potential carcinogenic effect and the development of non-dose-related aplastic anemia in humans [35]. All XDR isolates harbored the multidrug efflux pump (encoded by the *mef*(B) gene), which confers resistance to macrolides. Similarly, the *mph*(A) gene, which confers resistance to macrolide antibiotics, was detected in three isolates. Previously, Sonda et al. [36] reported that high clonal diversity in human *E. coli* and 50% of their isolates harbored the *mph*(A) gene. As with other antibiotics, high exposures to erythromycin, tylosin, and azithromycin, especially in antibiotic combinations, could be a plausible reason for the emergence of resistance to macrolides.

The ESBL genes harbored by the isolates were similar to the findings of previous studies by Adefioye et al. [31], Borges et al. [37], and García-Béjar et al. [38] who reported that the bla_{TEM} was the most predominant $bla_{\text{AmpC/ESBL}}$ gene in poultry *E. coli* (avian pathogenic *E. coli*). Our findings were however contrary to the reports of Alonso et al. [39] and Aworh et al. [26] who reported that $bla_{\text{CTX-M}}$ were the most common ESBL genes in poultry in Africa and Nigeria respectively. The co-carriage of several beta-lactamases genes has been previously reported by Okpara et al. [40] who reported that detected that MDR *E. coli* from poultry and other FPAs harbored $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-55}}$ genes, and in few cases bla_{TEM}.

While we must preserve antibiotics through prudent usage and antimicrobial stewardship programs, the detection and co-carriage of two DRGs by three XDR *E. coli* isolates is worrying. This could be attributed to the incorrect or continuous (excessive) usage of disinfectants (in concentration and time) by farmers. Previously, reports by Randall et al. [9] as well as Berlanga and Vinas [10] detected increased phenotypic antibiotic resistance when bacteria were cultured in the presence of sub-inhibitory concentrations of disinfectants. Furthermore, the development of resistance to disinfectants may be associated with the development of resistance to antibiotics and heavy metals (usually a component of poultry feed) [41–43]. Thus, the excessive use of metals (especially Cu) as feed additives and disinfectants in poultry and other FPAs may have the potential to promote antibiotic resistance through co-selection. Alarmingly, this co-selection can maintain and promote antibiotic resistance even in the absence of antibiotic selection pressure [42,44,45]. In a study by Pal et al. [43], copper co-selected for resistance to last-resort antibiotics such as colistin. In yet another study, Wand et al. [46] reported that the cross-resistance to colistin following chlorhexidine adaptation in Klebsiella *pneumoniae* isolates was likely due to upregulation of the operon containing *pmr*K. Finally, Jin et al. [47] reported that chlorine disinfection naturally accelerated the genetic exchange in or across bacterial genera, resulting in the enrichment of ARGs in bacteria after chlorination.

In LMICs, the co-carriage of ARGs and DRGs poses a serious public health threat. This is particularly important because of the possibility of horizontal gene transfer with commensal bacteria. Furthermore, these organisms could contaminate water bodies, hence persisting in the environment. Because chemical disinfection is an essential component of the hazard analysis and critical control point protocol in the food industry, genomic surveillance of ARGs, DRGs, and heavy metal resistance genes in FPAs should be intensified to safeguard public health.

Several mobile genetic elements (conjugative plasmids and insertion sequences) have been implicated in the acquisition and transmission of ARGs, especially in the family Enterobacteriaceae [48]. Our XDR *E. coli* isolates harbored several plasmid replicons as well as insertion sequence elements that were responsible for the horizontal gene transfer of ARGs. The IncF family is the most commonly reported among the *E. coli* isolates from food-producing animals across Nigeria [13,49–51], as well as in Tanzania [52] and China [53]. Other conjugative plasmids that were detected in XDR *E. coli* isolates such as IncI1-I(Alpha), IncN, IncX1, IncQ1, IncR, Col156, Col440I, ColRNAI, ColpVC, and Col(pHAD28), had been previously reported in MDR *E. coli* in humans and FPAs [13,26,50]. Reid et al. [54] observed the ubiquitous carriage of ColV plasmids, especially among poultry and porcine isolates, and concluded that the acquisition of ColV plasmids contributed to the divergence of major ST58 sublineages. The occurrence of plasmids that have a high bearing cost has been a major concern, especially since it could increase the antimicrobial resistance trajectory and potentially circumvent efforts to contain AMR through restricted use of antimicrobials [14]. For instance, Monarrez et al. [14] and Huang et al. [55] reported plasmids that harbored several classes of ARGs.

The diversity of XDR *E. coli* isolated from this study was evident by the absence of the isolates belonging to the same sequence type or serotype. Moreover, none of the XDR *E. coli* isolates belonged to the pathogenic phylogroup B2. However, Reid et al. [54] reported an emerging globally disseminated uropathogenic *E. coli* ST58, which belonged to the environmental Phylogroup B1, and two of our isolates belonged to the same phylogroup. Similar to our finding, Adefioye et al. [31] and Aworh et al. [12] reported that XDR *E. coli* from FPAs belonged to diverse sequence types (STs), serotypes, and phylogroups, and most of their isolates belonged to the phylogroup B1. In addition, Aworh et al. [26] reported MDR *E. coli* isolates belonging to the same ST (ST-48, ST-155, ST-1638, and a novel ST) that were shared by humans, FPAs, and the environment, suggesting a possible clonal spread.

While this study provides useful genomic insights in MDR bacteria from FPAs, it has some limitations, especially that the number of sequenced isolates were few and there could be selection bias for the sequenced isolates. Hence, they might not be representative of the microbial diversity of MDR bacteria in FPAs in Nigeria.

5. Conclusion

The occurrence of drug-resistant pathogens in FPAs poses a threat of zoonotic transmission to humans and has severe economic implications for the livestock sector. Here, we document the occurrence of MDR/XDR *E. coli* isolates in broilers in Ilorin, Nigeria. The sequenced XDR isolates were of diverse ST, serotypes, and phylotypes, and harbored a plethora of mobile genetic elements and VAGs. This is the first report of co-carriage of ARGs and DRGs in *E. coli* isolated from broilers in Ilorin, Nigeria. This study emphasizes the need for active surveillance of DRGs and ARGs in FPAs and the need to establish critical control points in production and processing chain of animals meant for human consumption to safeguard public health.

Competing interests

The authors declare that they have no competing interests.

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Ethical approval

This study does not require ethical approval as caecal samples were only obtained from freshly slaughtered broilers.

Availability of data

The sequence raw reads are openly available at NCBI under the bio project PRJNA764473

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Supplementary materials

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