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Antibiotic resistance genes, mobile elements, virulence genes, and phages in cultivated ESBL-producing *Escherichia coli* of poultry origin in Kwara State, North Central Nigeria

Ahmad Ibrahim Al-Mustapha [a,b,c,*], Ibrahim Adisa Raufu [d], Oluwaseun Adeolu Ogundijo [c], Ismail Ayoade Odetokun [e], Ananda Tiwari [a], Michael S.M. Brouwer [f], Victoria Adetunji [b], Annamari Heikinheimo [a,g]

[a] Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Finland
[b] Department of Veterinary Services, Kwara State Ministry of Agriculture and Rural Development, Ilorin, Kwara State, Nigeria
[c] Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary medicine, University of Ibadan, Oyo State, Nigeria
[d] Department of Veterinary Microbiology, Faculty of Veterinary medicine, University of Ilorin, Kwara State, Nigeria
[e] Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary medicine, University of Ilorin, Kwara State, Nigeria
[f] Department of Bacteriology, Host-Pathogen Interaction and Diagnostics development, Wageningen University and Research, Lelystad, the Netherlands
[g] Finnish Food Authority, Laboratory and Research Division, Microbiology Unit, Helsinki, Finland

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ABSTRACT

The paucity of information on the genomic diversity of drug-resistant bacteria in most food-producing animals, including poultry in Nigeria, has led to poor hazard characterization and the lack of critical control points to safeguard public health. Hence, this study used whole genome sequencing (WGS) to assess the presence and the diversity of antibiotic resistance genes, mobile genetic elements, virulence genes, and phages in Extended Spectrum Beta Lactamase producing *Escherichia coli* (ESBL – *E. coli*) isolates obtained from poultry via the EURL guideline of 2017 in Ilorin, Nigeria. The prevalence of ESBL – *E. coli* in poultry was 10.5 % (n = 37/354). The phenotypic antibiotic susceptibility testing showed that all the ESBL— *E. coli* isolates were multi-drug resistant (MDR). The in-silico analysis of the WGS raw-read data from 11 purposively selected isolates showed that the isolates had a wide array of ARGs that conferred resistance to beta-lactam antibiotics, and 8 other classes of antibiotics (fluoroquinolones, foliate pathway antagonists, aminoglycoside, phenicol, tetracycline, epoxide, macrolides, and rifamycin). All the ARGs were in the bacterial chromosome except in two isolates where plasmid-mediated quinolone resistance (PMQR) was detected. Two isolates carried the *gyr*Ap.S83L mutation which confers resistance to certain fluoroquinolones. The mobilome consisted of several Col-plasmids and the predominant IncF plasmids belonged to the IncF64:A-:B27 sequence type. The virulome consisted of genes that function as adhesins, iron acquisition genes, toxins, and protectins. Intact phages were found in 8 of the 11 isolates and the phageome consisted of representatives of four families of viruses: *Myoviridae* (62.5 %, n = 5/8), *Siphoviridae* (37.5 %, n = 3/8), *Inoviridae* (12.5 %, n = 1), and *Podoviridae* (12.5 %, n = 1/8). ESBL - *E. coli* isolates harboured 1–5 intact phages and no ARGs were identified on any of the phages. Although five of the isolates belonged to phylogroup A, the isolates were diverse as they belonged to different serotype and sequence types. Our findings demonstrate the high genomic diversity of ESBL - *E. coli* of poultry origin in Ilorin, Nigeria. These diverse isolates harbor clinically relevant ARGs, mobile elements, virulence genes, and phages that may have detrimental zoonotic potentials on human health.

1. Introduction

The in-depth genomic surveillance of zoonotic antimicrobial resistance pathogens is critical in controlling the global silent antimicrobial resistance (AMR) pandemic. These AMR pathogens pose public health threats of transmission to humans as well as severe economic

* Corresponding author at: Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Finland.
E-mail address: ahmad.al-mustapha@helsinki.fi (A.I. Al-Mustapha).

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threats to the livelihoods of farmers (WHO, 2022a). To tackle AMR at the global level, the tripartite organization (World Health Organization, WHO; World Organisation for Animal Health, OIE, and the Food and Agriculture Organization of the United Nations, FAO) agreed to use the trends of the - Extended Spectrum Beta Lactamases (ESBL) producing *Escherichia coli* (ESBL - *E. coli*) - as a global indicator to monitor the success in curbing the emergence and spread of antimicrobial resistant bacteria (Hashim et al., 2022; WHO, 2022b). This organism was selected because of its increasing zoonotic potential, its potential to transfer resistance to other gut bacteria, and its ease of culture especially in resource-poor settings in Africa and Asia (Falgenhauer et al., 2019).

Several context-specific factors could affect the emergence and spread of multidrug-resistant (MDR) bacteria or extensively drug-resistant (XDR or superbugs) in poultry. These include social factors such as antibiotic usage, good management practices including optimal biosecurity, the impact of vaccinations, as well as bacterial intrinsic factors such as point mutations, and other factors such as acquired antibiotic resistance genes (ARG) which are transferred within and between bacterial species by Mobile Genetic Elements (MGE) such as plasmids and insertion sequences (IS elements) (Jain et al., 2021; Reygaert, 2018).

In most low- and middle-income countries, genomic surveillance activities are scarce both in human and animal health sectors. This was made even more evident with the COVID-19 pandemic (Adepoju, 2021; Brito et al., 2022). The utility of whole genome sequencing (WGS) in understanding and predicting key genetic determinants in the ever-changing bacterial dynamics cannot be over-emphasized (Deter et al., 2021). Hence, the WGS of microorganisms has become a powerful approach and reliable tool for screening antibiotic resistance, understanding pathogen diversity, and predicting the infection parameters based on observed mutations (Cunningham et al., 2011). WGS also holds great promise in developing newer antibiotics, controlling antimicrobial resistance, and enhancing diagnostics and public health microbiology (Cunningham et al., 2011; Hasman et al., 2014; Jain et al., 2021).

To date, only a handful of studies have conducted genomic surveillance of important pathogens in food-producing animals in Nigeria (Awosile et al., 2022; Ayandiran et al., 2018; Ayeni et al., 2020). Specifically, only a few published studies have utilized WGS for the molecular characterization of ESBL - *E. coli* of poultry origin in Nigeria. These studies have revealed that depending on the sampling area, there is a variation in the resistome and plasmidome of ESBL – *E. coli* from poultry (Aworh et al., 2020; Aworh et al., 2021; Ayandiran et al., 2018; Ayeni et al., 2020). Hence, very little is known about the genetic diversity of ESBL - *E. coli* of poultry origin across Nigeria despite its huge economic and public health implications. This poor genomic surveillance capacity meant Nigeria has no reliable baseline genomic data to monitor trends and antibiotic susceptibility patterns for important economic and zoonotic bacteria of interest such as ESBL - *E. coli*. This has made it very difficult to make simple inferences such as: "What is the clonality or diversity of MDR bacteria?"

Controlling AMR requires multisectoral collaborations using the one health concept (UNEP, 2022), promoting public awareness, operationalizing one health-sustainable integrated national AMR surveillance program, establishing antimicrobial stewardship programs (AMS), and generating genomic evidence-based data on AMR (WHO, n.d.). Hence, this study determined the prevalence of ESBL - *E. coli* in poultry and assessed their antibiotic susceptibility patterns. Finally, we used whole-genome Illumina sequencing to type isolates and to reveal the diversity of resistance genes (resistome), mobile genetic elements (mobilome), virulence factors (virulome), and coliphages (phageome) in selected isolates.

2. Materials and methods

2.1. Study settings and sampling

The samples used in this study were collected in Ilorin, Kwara State, Nigeria from June to July 2019 as part of a broader study evaluating the prevalence and epidemiology of AMR pathogens in food-producing animals in the state. A total of 354 caecal samples were collected from poultry (304 broilers and 50 spent layers). We collected 40–60 caecal samples weekly for six weeks from live birds slaughtered within Ilorin metropolis. The 50 samples from spent layers (old hens usually at two years of age) were obtained by randomly sampling approximately 1000 hens that were disposed off by their owners after noticing that they became economically inefficient as they have passed their prime production. All caecal samples were collected aseptically from nip incisions of the caecum in the freshly dressed carcasses.

2.2. Isolation of ESBL – Escherichia coli

One gram of caecal samples from freshly slaughtered poultry (broiler and spent layers) was aseptically collected into 9 mL of buffered peptone water (BPW, Oxoid, Basingstoke, UK) and incubated for 18–24 h at 37 °C. A loopful (10 μL) of the overnight medium was streaked onto MacConkey Agar (Oxoid, Basingstoke, UK) supplemented with 1 mg/L cefotaxime and incubated overnight at 37 °C. Presumptive *E. coli* colonies were purified on nutrient agar (Oxoid, Basingstoke, UK) and stored at −20 °C. After shipping the isolates to the University of Helsinki, we resuscitated the culture on fresh blood agar and confirmed the species using the Matrix-Assisted Laser Desorption Ionization Time of Flight - Mass Spectrometry (MALDI-ToF) (Bruker, Bremen, Germany) following the Biotyper protocol as previously described by Richter et al. (2020). The best match score value of >2.300 was used as the cut-off for reliable species differentiation. Score values between 2.000 and 2.3000 were considered reliable for the identification of bacterial genera.

2.3. Double disk synergy test and antibiotic susceptibility testing

The double-disk synergy test (DDST) was used to test for the production of extended-spectrum beta-lactamases in all confirmed *E. coli* isolates ($n = 37$) as previously described by Kurittu et al. (2021). Summarily, an isolate was regarded as an ESBL producer if it showed resistance towards third-generation cephalosporins [cefotaxime (CTX) or Ceftazidime (CAZ)] and the difference in the zone of inhibition between either the CTX and CTX-CLA (Cefotaxime- clavulanate) or the CAZ and CAZ-CLA (Ceftazidime – Clavulanate) was >5 mm. All the antibiotic disks (Neo-Sensitabs) were sourced from Rosco Diagnostics, Denmark. All ESBL - *E. coli* were screened for their antibiotic susceptibility patterns using the broth microdilution assay (BMD) to determine their minimum inhibitory concentration (MIC). The EUVSEC Sensititre (Thermo Fisher, Vantaa, Finland) plates were used for the BMD assay, and it consisted of a panel of 14 antibiotics (Table 1). All measurements and interpretations (DDST and BMD) were based on the epidemiological cut-off (ECOFF) values established by the European Committee on Antimicrobial Susceptibility Testing guidelines (EUCAST, 2022). The reference strain, *E. coli* ATCC 25922 was used as the negative control strain during all screening tests.

2.4. Genotyping of ESBL - E. coli

All the ESBL - *E. coli* isolates ($n = 37$) were genotyped using multiplex polymerase chain reaction (mPCR) to identify genes (*blaTEM*, *blaSHV*, *blaOXA-1*, *blaCTX-M-1* group, and *blaCTX-M-2* group) that confer resistance to beta-lactam antibiotics. Based on the phenotypic resistance profile, approximately one-third of the isolates ($n = 11$) were selected for WGS to have a holistic view of other resistance genes, virulence-associated factors, mobile genetic elements, phages, and to assess the

Table 1
Antibiotic susceptibility testing of ESBL-producing *Escherichia coli* isolates (n = 37).

Class of antibiotic	Antibiotic on EUVSEC (mg/L)	ECOFF (mg/L)	Resistance (%)
Folate pathway antagonist	Sulfamethoxazole (8–1024)	64	37 (100)
	Trimethoprim (0.25–32)	2	37,100)
Fluoroquinolones	Ciprofloxacin (0.0015–8)	0.064	35 (94.6)
	Nalidixic acid (4–128)	8	35 (94.6)
Tetracyclines	Tetracycline (2–64)	8	37 (100)
	Tigecycline (0.25–8)	0.5	6 (16.2)
Beta-lactam (third-generation cephalosporin)	Cefotaxime (0.25–4)	0.25	37 (100)
	Ceftazidime (0.5–8)	0.5	36 (97.3)
Beta-lactam (aminopenicillin)	Ampicillin (1–64)	8	37 (100)
Phenicol	Chloramphenicol (8–128)	16	14 (37.8)
Aminoglycoside	Gentamicin (0.5–32)	2	28 (75.7)
Polymyxin E	Colistin (1–16)	2	2 (5.4)
Macrolides	Azithromycin (2–64)	16	2 (5.4)
Carbapenems	Meropenem (0.03–16)	2	0 (0)
Carbapenems	Meropenem (0.03–16)	2	0 (0)

ECOFF – epidemiological cut-off; EUCAST is yet to set an ECOFF for Azithromycin. However, we used the tentative 16 mg/L.

genetic diversity of the isolates.

2.5. DNA extraction

We compared two methods for genomic DNA extraction. These are the bacterial cell lysate method and the use of the Genomic DNA Kit. For the cell lysate, a single bacterial colony was swirled into 100 µl of TE buffer (10 mM Tris-HCl and 1 mM EDTA). This was boiled at 95 °C for 10 min. The lysate was centrifuged at 6,000 rpm for 5 min. The supernatant was further diluted in 100 µl of the Hyclone PCR grade water (Fischer Scientific, USA). For the kit extraction, the DNA of ESBL - *E. coli* isolates was also extracted using the commercially available PureLink Genomic DNA Kit (Invitrogen, Carlsbad, CA, USA). The DNA output of both DNA extraction methods was quantified with a Qubit Fluorometer 4.0 (Invitrogen, Singapore).

2.6. Genotypic detection of extended-spectrum and β lactamases genes

Each mPCR was conducted using a 50 µl reaction mix. The reaction mix was made up of 26.5 µl of the Hyclone PCR grade water (Fischer Scientific, USA), 10 µl of the 5× HF buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 1 µl of the dNTP mix (200 µM concentration), 1 U of the Phusion polymerase (Thermo Fisher Scientific, Leiden, Netherlands), 10 µl of oligonucleotides (Metabion, München, Germany), and 1 µl of the DNA template. The mPCR was performed in a Maxygene II thermocycler (Corning, USA) using the primer sequences and amplification parameters as previously described by Dallenne et al., 2010. The genetic targets were: Multiplex I (TEM, SHV, and OXA-1), Multiplex II (CTX-M group 1, group 2, and group 9), and CTX-M group 8/25. The mPCR amplicons were loaded onto 2 % Tris Acetate EDTA (TAE) gel and electrophoresis was performed at 100 V for 1 h. The separated bands were viewed on a gel imager (Alpha Innotech, California, USA). The *E. coli* ATCC 25922 was used as a negative control for all mPCR reactions. The positive controls used were: *E. coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603 (SHV), and *Enterobacter cloacae* NCTC 13464 for TEM-1, SHV, and CTX-M-2 group respectively.

2.7. Whole genome sequencing of ESBL - E. coli

We conducted whole genome sequencing of 11 of the 37 ESBL - *E. coli* isolates using the Illumina platform. The WGS was done at the Wageningen University and Research Center, Lelystad, The Netherlands. DNA samples were purified using a Qiagen Purification kit and the sequencing libraries were prepared using the Nextera Flex Kit according to the manufacturer's instructions. Pooled DNA samples from each isolate were sequenced on the Illumina HiSeq platform using the 2 × 200 paired-end read approach (Illumina Inc., San Diego, CA, USA). The raw sequencing read output had its adapters trimmed using Trimmomatic (Bolger et al., 2014), and the quality of the reads was assessed using the FastQC tool. The raw reads (Fastq) were assembled into contigs (Fasta) using Skesa (available on the Ridom SeqSphere+ bacterial analysis pipeline) and Assembler 1.2 (available on the Center for Genomic Epidemiology WGS bacterial analysis platform).

2.8. Analysis of whole genome sequence data

The sequences have been deposited at the European Nucleotide Archive (BioProject PRJEB58679 with the individual accession numbers). The assembled contigs were fed into two bacterial WGS analysis pipelines: the subscription-based RIDOM SEQSPHERE+ and the open-access CGE platform. The Ridom used the NCBI AMR Finder plus tool whereas CGE used its ResFinder 4.1 tool at default threshold ID (90 %) and minimum length (60 %) values to identify resistance genes (Florensa et al., 2022). All ARGs identified were designated as either chromosomal or plasmid-mediated based on their location in the genome using mlplasmids version 2.1.0. The CGE analytical pipeline were used for the identification of the sequence type (MLST 2.0), mobile genetic elements (Mobile Element Finder), plasmid replicons (PlasmidFinder), and replicon sequence typing (pMLST) using the recommended settings (Carattoli et al., 2014). Finally, we parsed the WGS data through the Serotype Finder 2.0 and Virulence Finder 2.0 web tools to determine the *E. coli* serotypes and the virulence-associated genes in the isolates respectively (Joensen et al., 2015; Malberg Tetzschner et al., 2020). The sequenced isolates were classified into phylogroups using the ClermonTyping tool (Beghain et al., 2018).

2.9. Detection of phages from WGS raw reads

To identify the phageome of our ESBL-*E. coli* isolates, we parsed WGS-assembled contigs into the PHASTER tool (Arndt et al., 2016). The tool enables rapid identification and annotation of prophage sequences within bacterial genomes and plasmids. The PHASTER tool classified the phageome into three classes (Intact, questionable, and incomplete) based on their completeness (phage score). This study only presents intact (fully identified) phages from the PHASTER tool. We classified phages into viral families by annotating identified intact phages against existing sequences in the NCBI Virus databases. Finally, we screened phages for ARGs using both the NCBI AMR Finder and RESFINDER as described above.

2.10. Data analysis

The qualitative information generated was presented as frequencies and proportions. We used the CHI-SQUARE analysis to test for association between qualitative variables in the Statistical Package for Social Sciences (SPSS) version 28.

3. Results

3.1. Prevalence and phenotypic antibiotic susceptibility profiles of ESBL-producing Escherichia coli

The prevalence of ESBL - *E. coli* in poultry (304 broilers and 50 layers) was 10.5 % (n = 37/354). The prevalence was 10.8 % in broilers (33/304 specimens) and 8 % in layers (4/50 specimens). The phenotypic ESBL production assay (double-disk synergy test) revealed that all 37

isolates produced extended-spectrum beta-lactamases. The BMD assay showed that most of the ESBL - *E. coli* isolates were MDR and conferred resistance to other classes of antibiotics.

All of the isolates showed resistance to folate pathway antagonists (Sulfamethoxazole and Trimethoprim), Tetracycline, and Ampicillin. This was closely followed by varying resistance to Fluoroquinolones (Ciprofloxacin and Nalidixic acid), Gentamicin, and chloramphenicol. Currently, no ECOFF is defined for the macrolide Azithromycin, however, using the tentative ECOFF of 16 mg/L, 2 isolates were determined to be resistant.

3.2. Prevalence of ESBL genes in E. coli isolates

The ESBL - *E. coli* isolates were genotypically screened for the ESBL/AmpC genes that confer resistance to beta-lactam antibiotics using mPCR. Our findings showed that the *blaTEM* gene was the most abundant narrow-spectrum B- lactamase as it was detected in 83.8 % ($n = 31/37$) of the isolates. The bla_{OXA-1}, another narrow-spectrum beta-lactamase gene, was detected in five isolates. The most abundant of the CTX-M group ESBLs was the CTX-M-1 group ($bla_{CTX-M-1}$, $bla_{CTX-M-3}$, and $bla_{CTX-M-15}$), which was detected in 91.8 % ($n = 34/37$) whereas $bla_{CTX-M-14}$ of the CTX-M-9 group was detected in three isolates (8.2 %). The co-carriage of both narrow and extended- spectrum beta-lactamase and ESBL genes was detected in 31 isolates (83.8 %).

3.3. Whole genome sequencing

The WGS provided useful in-depth genomic insight into the ESBL - *E. coli* isolates with special reference to the diversity of resistance genes, its MGE, and genetic relatedness to other veterinary or human *E. coli* isolates.

3.4. Resistome of ESBL-producing E. coli

There was a very high concordance (86 %) between the phenotypic AST (EUVSEC-2 plates) and the genotypic prediction (WGS raw reads). Similarly, there was 95 % concordance between the predicted resistance pattern generated by both Resfinder and the NCBI AMR Finder. Both search engines revealed that most of the ESBL *E. coli* harboured the $bla_{CTX-M-15}$ gene ($n = 6/11$). The $bla_{CTX-M-55}$ and $bla_{CTX-M-164}$ (both variants of $bla_{CTX-M-15}$) were also detected in three isolates whereas the genetically distinct $bla_{CTX-M-14}$ was detected in only two isolates. Of the narrow-spectrum beta-lactamase, the $blaTEM_{-1B}$ gene was the most detected variant and it was harboured by 7 of the 11 sequenced isolates (Table 2). Only one of the sequenced isolates contained the bla_{OXA-1} gene.

Our findings showed that most of the ESBL *E. coli* were MDR (and XDR) and conferred resistance to several classes of antibiotics (Table 2). The resistome of the ESBL - *E. coli* isolates consisted of a plethora of Aminoglycoside resistance genes [*aac*(3)-IId, *ant*(2″)-Ia, *aadA1*, *aph*(3′)-Ia, *aph*(3″)-Ib, *aph*(6)-Id, *aad*A5, and several variants such as (*aph*(3″)-Ib_AF321551), (*aac*(3)-IId_EU022314), etc.], Folate pathway antagonists [*sul*1, *sul*2, *sul*3, *dfr*A1, *dfr*A14, *dfr*A17, *dfr*A36], Tetracyclines [*tet*(A), *tet*(B), *tet*(M), and their mutants], and Fluoroquinolones [*gyr*A, *qnr*S1, *qnr*S2, *qnr*S11, *qnr*S13]. Table 2 shows the resistome and point mutations of the ESBL *E. coli* isolates. The resistome of these isolates also included genes that code for resistance to Fosfomycin, Chloramphenicol, and Rifamycin, as well as macrolide antibiotics such as erythromycin and Azithromycin. The multidrug efflux pump *mdf*(A) was detected in all of the isolates ($n = 11/11$).

Several mutations were detected in the ARGs. These include mutations in the *gyr*A, *gyr*B, *fol*P, *par*C, *pmr*B, *rpo*B, *amp*C, 16S_rrsB, and 16S_rrsC genes. For instance, the *gyr*A D87G or S83L (GAC to AAC) mutation confers resistance to nalidixic acid only but no other fluoroquinolone, if it occurs alone. Similarly, the *par*C mutation (AGC to ATC) produces an unknown resistance phenotype if the mutation occurs alone but can also be associated with *gyr*A mutations in which case it will result in resistance to Nalidixic acid and ciprofloxacin.

The findings of the WGS were in agreement with the result of the BMD assay as well as our mPCR molecular genotyping which showed that our isolates were MDR and that *blaTEM* and BlaCTX-M-1 groups were the most prevalent narrow- and extended-spectrum- beta-lactamase genes respectively in the isolates.

3.5. Mobilome of ESBL producing Escherichia coli

The mobilome of ESBL - *E. coli* was diverse as the isolates harboured diverse plasmids and insertion sequences. Most of the plasmids carried by these ESBL - *E. coli* isolates belonged to the incompatibility F (IncF) group. These include IncFIB(AP001918), IncFII, IncFII(pRSB107), IncFIC(FII), and IncFII(pCoo). Similarly, smaller plasmids of approximately 2 kb such as Col156, Col440I, ColRNAI, ColpVC, and Col (pHAD28) were also detected in most of the isolates (Table 2). As shown above, all of the isolates carried several plasmids and most of them harboured 2 or more plasmids except for Kw/B/04. The plasmid-mediated quinolone resistance (PMQR) was detected in only three isolates as they were carried on two ColpVC and a ColRNAI plasmid. In two other isolates, there was the co-carriage of a floR gene (977 bp) on a ColRNAI plasmid.

The replicon sequence typing (RST) for the IncF plasmids also revealed a high diversity of the IncF plasmids in the isolates. The IncF plasmids from two isolates (B21 and B48) belonged to the same subgroup IncF64:A-:B27 whereas the RST typed the IncF plasmids from three isolates (Kw/B/01, Kw/B/05, and Kw/B/07) as F18:A-:B-. Other IncF RST groups were: Kw/B/04 (F24:A-:B1) and Kw/B/09 (F16:A-:B37). The IncF plasmid in isolate B30 could not be typed as a novel allele in the IncFII plasmid was detected. Asides from plasmids, different families of autonomous transposable elements (IS elements) were detected in the isolates. The MITEEc1 (123 bp belonging to the IS630 family) was detected in all of the isolates. In addition to the ISEc9 (IS 1380 family), several other ISEc-type elements were detected (Table 2). These include ISEc78 (IS 66 family), ISEc10 (IS21 family), and ISEc38 (ISL3 family).

3.6. Virulome of ESBL - E. coli

Several virulence factors (VFs) have been identified in the ESBL - *E. coli* isolates. The most conserved VFs across most of the isolates were: *astA* (EAST-1 heat-stable toxin), *cea* (Colicin E1), *cel*b (Endonuclease colicin E2) *cva*C (Microcin C), *iss* (Increased serum survival), *sit*A (iron transport protein), *hyl*F (hemolysin F), *iuc*C (Aerobactin synthetase), and *tsh* (temperature-sensitive hemagglutinin). Several other VFs (Table 2) were seen in some of the isolates *tra*T (Outer membrane protein complement resistance), *ter*C (Tellurium ion resistance protein), *pap*C (Outer membrane usher P fimbriae), and *air* (Enteroaggregative immunoglobulin repeat protein).

3.7. Phageome of ESBL - E. coli

ESBL - *E. coli* isolates contained a wide array of phages. Our findings showed that 7 of the 11 sequenced ESBL *E. coli* isolates harboured at least 1 intact phage and the phages ranged in size from 5.8 kb to 94.8 kb. The coliphages contained open reading frames (ORFs) which could allow for the incorporation of other mobile genetic elements into the genome of the phages. The representatives of the family *Myoviridae* were the most abundant and it was isolated from 5 of the 8 *E. coli* isolates whereas members of the family *Siphoviridae* were detected in 3 isolates. Other detected phage families were: *Inoviridae* ($n = 1$) and *Podoviridae* sp. ($n = 1$). Several novel phages were detected and the phageome showed that ESBL - *E. coli* isolates harboured 1–5 intact phages (Table 3). No ARGs were identified on any of the phages from either the RESFINDER or NCBI databases.

Table 2
Genomic characteristics of ESBL - *E. coli* isolated from poultry ($n = 11$).

Isolate ID	Status	Class of antibiotics	ARGs	Point mutations in ARGs	Plasmids	Insertion Sequences (IS)	Virulence factors
Kw/B/01	MDR	1. Aminoglycoside 2. Beta-lactam 3. Fluoroquinolones 4. Phenicol 5. Tetracycline	1. *aac(3)-IId* 2. $bla_{CTX\text{-}M\text{-}15}$, $blaTEM_{\text{-}1B}$ 3. *qnrS13* 4. *floR* 5. *tet(A)*	No known mutations detected	Col(MG828), ColRANI, ColpVC, Col156, IncFIC(FII), col440I,	IS*102*, IS*421*, MITEEc*1*, IS*4*, ISEc*38*, ISEcl*1*, IS*26*, IS*100*	*lpfA*, *etsC*, *chuA*, *irp2*, *mchF*, *ompT*, *cea*, *traT*, *celb*, *ompT*, *hylF*, *astA*, *etsC*, *terC*, *ireA*, *fyuA*, *hylF*
Kw/B/02	XDR	1. Aminoglycoside 2. Beta-lactam 3. Folate pathway antagonist 4. Phenicol 5. Tetracycline 6. Fosfomycin 7. Macrolide	1. *ant(2″)-Ia*, *aadA2b*, *aadA1*, *aac(3)-IId* 2. $blaTEM_{\text{-}1B}$, $bla_{CTX\text{-}M\text{-}14}$ 3. *sul3*, *sul2*, *sul1*, *dfrA36* 4. *floR*, *cmlA1* 5. *tet(A)*, *tet(M)* 6. *fosA3* 7. *mef(B)*	parC, pmrB, ampC, 16S_rrsH	ColpVC, IncI2, Col156, IncHI2, IncFII.	ISUnCu*1*, ISKox*3*, IS*4*, ISEc*46*, ISVsa*3*, ISEc*31*, MITEEc*1*, ISKpn*8*, IS*630*, ISVsa*5*,	*iroN*, *terC*, *iucC*, *mchF*, *iss*, *fyuA*, *ireA*, *chuA*, *terC*, *hra*, *astA*, *traT*, *etsC*, *celb*, *tsh*, *ompT*, *irp2*, *air*, *kpsE*,
Kw/B/03	MDR	1. Beta-lactam 2. Fluoroquinolones 3. Folate pathway antagonist 4. Tetracycline	1. $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}55}$ 2. gyrAp.S83L 3. *sul2* 4. *tet(A)*	gyrA	Col440I, IncR,	IS*3*, ISEc*30*, ISEc*52*, MITEEc*1*, IS*26*, ISEsa*1*, IS*421*.	*ompT*, *hra*, *terC*.
Kw/B/04	MDR	1. Beta-lactam 2. Fluoroquinolone 3. Tetracycline 4. Phenicol	1. $blaTEM_{\text{-}1B}$, $bla_{CTX\text{-}M\text{-}15}$ 2. *qnrS11* 3. *tet(A)* 4. *floR*	gyrA, folP, ParC, pmrB, 16S_rrsB	IncFIB(AP001918), IncFII.	ISKpn*26*, IS*5*, IS*609*, IS*30*, IS*421*, MITEEc*1*, ISEc*38*, ISEc*78*, ISEc*31*, ISEc*32*, IS*3*, IS*26*.	*terC*, *cvaC*, *sma*, *gad*, *iss*, *traT*, *ompT*, *iroN*, *hylF*.
Kw/B/05	DR	1. Beta-lactam	1. $blaTEM_{\text{-}1B}$, $bla_{CTX\text{-}M\text{-}15}$	parC, pmrB, ampC.	IncFII,	ISEc*1*, ISSfl*8*, MITEEc*1*, ISEc*31*, IS*629*.	*ipfA*, *papC*, *hra*, *tratT*, *neuC*, *terC*
Kw/B/06	XDR	1. Aminoglycoside 2. Beta-lactam 3. Folate pathway antagonist 4. Phenicol 5. Tetracycline	1. *aac(3)-IIa*, *aadA1*. 2. $bla_{CTX\text{-}M\text{-}55}$ 3. *sul2*, *sul1*, *dfrA1* 4. *floR* 5. *tet(A)*	parC. gyrA, pmrB, ampC, 16S_rrsH	IncQ1, ColpVC, IncFIB (AP001918), IncFIC (FII)	ISEc*9*, ISVsa*5*, IS*26*, MITEEc*1*.	*papC*, *cma*, *iss*, *cea*, *celb*, *kpsE*, *terC*, *hra*, *iutA*, *papA_F19*, *ipfA*, *ompT*, *ireA*, *cvaC*, *traT*, *sitA*, *etsC*, *hylF*
Kw/B/07	XDR	1. Aminoglycoside 2. Beta-lactam 3. Folate pathway antagonist 4. Phenicol 5. Tetracycline	1. *ant(2″)-Ia*, *aph (6)-Id*, *aadA1*. 2. $bla_{CTX\text{-}M\text{-}15}$ 3.*sul1*, *sul2*, *dfrA36* 4. *floR*, *catA1* 5. *tet(B)*	parC. gyrA, pmrB, gyrB, rpoB, ampC, 16S_rrsB	ColRNAI, IncFIB (AP001918), IncFII, Col156, Col(MG828),	ISUnCu*1*, ISVsa*3*, IS*629*, ISEc*39*, MITEEc*1*, IS*100*	*papC*, *mchF*, *iss*, *iha*, *cea*,*celb*, *kpsE*, *terC*, *hra*, *iutA*, *papA_F20*, *gad*, *astA*, *ipfA*, *ompT*, *ireA*, *cia*, *iroN*, *eilA*, *cvaC*, *traT*, *sitA*, *etsC*, *hylF*
Kw/B/08	XDR	1. Aminoglycoside 2. Beta-lactam 3. Fluoroquinolones 4. Folate pathway antagonist 5. Phenicol 6. Tetracycline 7. Fosfomycin	1. *aph(6)-Id*, *aac (3)-IId* 2. $bla_{CTX\text{-}M\text{-}55}$, $bla_{CTX\text{-}M\text{-}164}$, $blaTEM_{-57}$ 3. *qnrS1* 4. *sul2*, *dfrA17* 5. *floR* 6. *tet(A)* 7. *fosA7*	parC, parC, gyrA, gyrB, pmrB, ampC, 16S-rrsB	Col156, Col(MG828), IncFIC(FII), Col 440I, ColRNAI	MITEEc*1*, IS*421*, IS*26*	*iucC*, *terC*, *traT*, *ompT*, *iutA*, *iss*, *etsC*, *hlyF*, *fyuA*, *Cea*, *Hra*, *etsC*, *lpfA*, *cvaC*, *traT*, *lpfA*, *gad*
Kw/B/09	XDR	1. Aminoglycoside 2. Beta-lactam 3. Fluoroquinolones 4. Folate pathway antagonist 5. Phenicol 6. Tetracycline 7. Rifamycin	1. *aac(6′)-Ib-cr*, *aac(3)-IId*, *aadA5*, *aph(3′)-Ia* 2. $bla_{OXA\text{-}1,}$ $blaTEM_{\text{-}1B}$, $bla_{CTX\text{-}M\text{-}15}$ 3. *aac(6′)-Ib-cr*, *qnrS1*, *qnrS2* 4. *sul2*, *dfrA17* 5. *catB3*, *catA2* 6. *tet(A)* 7. arr-3	parC, pmrB, rpoB, 16 s-rrsH	ColpVC, IncFII(pCoo), IncFIB(AP001918)	IS*26*, IS*3*, MITEEc*1*, ISKpn*8*, IS*421*	*traT*, *sitA*, *terC*, *cvaC*, *hylF*, *gad*
Kw/B/10	MDR	1. Beta-lactam 2. Fluoroquinolones 3. Phenicol 4. Tetracycline	1. $blaTEM_{\text{-}1B}$, $bla_{CTX\text{-}M\text{-}15}$ 2. *qnrS1* 3. *floR* 4. *tet(A)*	parC, folP, gyrA, pmrB, 16S-rrsH	p0111, IncI1, IncN, IncX1.	IS*30*, MITEEc*1*, IS*3*, IS*629*, ISKpn*8*, IS*421*, ISEc*38*.	*terC*, *gad*, *cia*, *iss*, *traT*.
Kw/B/11	DR	1.Beta-lactam 2. Fluoroquinolone	1. $bla_{CTX\text{-}M\text{-}15}$, $blaTEM_{\text{-}1B}$ 2. *qnrB19*	parC, gyrA, pmrB, ampC, gyrB, 16S-rrsB	Col440I, ColRNA1.	ISEc*9*, IS*4*, ISEc*10*, MITEEc*1*, ISKpn*8*,	*ipfA*, *terC*, *kpsE*, *gad*, *a fad*, *chuA*, *eilA*

DR = resistance to one or two classes of antibiotics, MDR = resistance to at least 3 classes of antibiotics, and XDR = resistance to at least five classes of antibiotics.

Table 3
The phageome of ESBL producing *Escherichia coli* of poultry origin.

Isolate ID	No. of phages	Phage Score	No. of ORFs	Region length (kb)	Region position	Most common phage	GC content (%)	Family	Identity (%)	Accession No
Kw/B/02	1	90	16	11.3	1–11,326	PHAGE_Shigel_SfIV_NC_022749(7)	48.23	Myoviridae sp.	97	BK029289
Kw/B/04	1	133	8	5.8	2183–8058	PHAGE_Entero_I2_2_NC_001332(8)	41.37	Inoviridae sp.	87	BK029458
Kw/B/06	2	100	23	18	311–18,963	PHAGE_Pseudo_phiPSA1_NC_024365(7)	46.35	Siphoviridae sp.	100	BK027129
		140	16	8.1	1–8141	PHAGE_Entero_fiAA91_ss_NC_022750(13)	57.55	Myoviridae sp.	97	BK055845
Kw/B/07	2	115	34	31.3	171–31,506	PHAGE_Klebsi_4LV2017_NC_047818(21)	50.96	Myoviridae sp.	96	BK028253
		150	48	33.2	461–33,733	PHAGE_Entero_SfI_NC_027339(29)	51.49	Siphoviridae sp.	97	BK025314
Kw/B/08	4	150	47	37.7	504,112–541,839	PHAGE_Entero_fiAA91_ss_NC_022750(25)	49.79	Myoviridae sp.	97	BK055845
		150	52	41.1	500,415–541,579	PHAGE_Entero_P88_NC_026014(39)	51.18	Myoviridae sp.	98	BK024818
		110	54	47.1	71,860–119,035	PHAGE_Entero_HK630_NC_019723(21)	50.14	Podoviridae sp.	98	BK055190
		139	16	25.2	4011–29,270	PHAGE_Entero_P4_NC_001609(13)	50.44	ND	–	–
Kw/B/09	5	150	44	34.8	54,747–89,582	PHAGE_Escher_vB_EcoM_12474III_NC_049457(26)	50.62	ND	–	–
		150	22	15.4	1–15,443	PHAGE_Shigel_SfII_NC_021857(19)	53.96	Myoviridae sp.	97	BK026264
		110	23	29.8	14,495–44,350	PHAGE_Stx2_c_1717_NC_011357(3)	48.20	Siphoviridae sp.	100	BK048880
		150	20	15.9	59–16,014	PHAGE_Entero_cdtI_NC_009514(17)	53.34	Siphoviridae sp.	94	BK045453
		100	39	31	5848–36,884	PHAGE_Stx2_c_Stx2a_F451_NC_049924(3)	50.09	ND		
Kw/B/11	3	140	107	94.8	1–94,842	PHAGE_Salmon_SSU5_NC_018843(77)	48.34	ND		
		150	58	44.2	1–44,249	PHAGE_Pseudo_PMG1_NC_016765(10)	58.3	ND		
		150	50	40.9	47,972–88,951	PHAGE_Vibrio_VP882_NC_009016(13)	60.14	ND		

The score is the prediction of whether the region contains an intact (>90), questionable (identity score of 70–90), or incomplete (identity score of <70) alignment. Several other coliphages classified as questionable (identity score of 70–90) and incomplete (identity score of <70) were excluded from the list of phages from each isolate. The Region Position is the start and end positions of the region on the bacterial chromosome. The GC (%) is the percentage of GC nucleotides in the region. ND - Novel phages that could not be aligned to any known viral genome. kb- kilobase pair. ORF-open reading frames.

3.8. Genomic diversity of ESBL producing Escherichia coli

Four *E. coli* phylogroups (A, B1, D, and F) were represented in our isolates. The most prevalent was phylogroup A (45.4 %, n = 5/11). Others were F (27.3 %, n = 3/11), B1 (18.1 %, n = 2/11) and D (9 %, n = 1/11). The isolates were genetically diverse as all the eleven sequenced isolates were of diverse sequence types (STs). Only two isolates (Kw/B/02 and Kw/B/11) that belonged to the avian pathogenic serogroups (O1 and O2) were detected in this study. There was no association between the antibiotic resistance profile of isolates and the number of phages contained in their genome. Isolate Kw/B/11 (A/ST132 - serotype 09:H11) harboured the most intact phages (n = 5) and was resistant to 6 classes of antibiotics (10 predicted antibiotics). On the contrary, we detected only one intact phage in isolate Kw/B/02 (D/ST45 - O2:H9) which had the highest resistance profile (8 antibiotic classes and 14 antibiotics) (Table 4).

Table 4
Genomic diversity of sequenced ESBL – *Escherichia coli* (n = 11).

Isolate ID	ST	ST cc	Serotype	Phylogroup
Kw/B/01	117	469	O143:H4	F
Kw/B/02	45	38	O2:H9	D
Kw/B/03	Novel	N/A	O[a]:H37	A
Kw/B/04	206	206	O185:H5	A
Kw/B/05	668	N/A	O171:H2	B1
Kw/B/06	162	469	O134:H19	B1
Kw/B/07	69	69	O17:H18	A
Kw/B/08	Novel	N/A	O143:H10	F
Kw/B/09	48	10	O9:H11	A
Kw/B/10	Novel	N/A	O[a]:H21	A
Kw/B/11	1088	N/A	O1:H25	F

[a] Unknown O (somatic) antigen.

4. Discussion

An in-depth understanding of the genetic diversity of an organism is essential to understanding its transmission dynamics and predicting its source and potential for zoonotic transmission (Ikhimiukor et al., 2022). Here, we demonstrate the importance of WGS as a vital genomic surveillance tool to detect the genetic backbone of Nigerian poultry ESBL - *E. coli* isolates.

The prevalence of ESBL - *E. coli* in poultry was 10.5 %. This was slightly higher in broilers than in spent layers. This prevalence rate is slightly higher than the 9.4 % reported by Chah and Oboegbulem (2007) in poultry from Enugu State and lower than the 12.6 % prevalence rate reported in Abuja, Nigeria (Aworh et al., 2021). Earlier, a study reported that the prevalence of ESBL - *E. coli* in the poultry value chain in South Western Nigeria was 1.0 % (Ojo et al., 2016). In yet another study, Ayandiran et al. (2018) also reported that the prevalence of ESBL - *E. coli* in poultry was 15 %. Several factors including antibiotic uses, farm management practices, and horizontal gene transfer) could be responsible for the emergence of ESBL-producing bacteria and other MDR bacteria in poultry (Barbosa and Levy, 2000). While several studies have noted antibiotic use and misuse as major forces associated with the

development of resistance in bacteria, a direct causal link has not been established (Schechner et al., 2013). For instance, despite no prophylactic antibiotic usage in the poultry industry in Finland, Päivärinta et al. (2020) reported the prevalence of ESBL/AmpC *E. coli* in broilers feces and meat samples were 18 % and 32 % respectively.

In Nigeria, several studies have reported excessive antibiotic usage and prescription in both humans and animals (Al-Mustapha et al., 2020; Ayandiran et al., 2018; Ojo et al., 2016). Primarily, the ESBL- *E. coli* specifically CTX-M enzymes in food animals were thought to be proliferated by the use of cephalosporins. To date, it remains unclear if this is the primary driver of resistance. In low- and middle-income countries (LMICs), other contextual factors such as poor diagnostic capacity, lack of antibiotic stewardship programs, poor animal disease surveillance system and biosecurity measures, symptom-based and endemicity-guided diagnosis, and the subsequent prescription of broad-spectrum antimicrobials for unconfirmed infections could contribute to the emergence and spread of MDR bacteria including ESBL - *E. coli* in humans and animals (Ayandiran et al., 2018; Ikhimiukor et al., 2022; Okeke, 2022).

The occurrence of ESBL in *E. coli* has always been associated with resistance to other classes of antibiotics especially fluoroquinolones (Dupouy et al., 2019). This study reports the occurrence of phenotypic resistance to 1–7 other antibiotic classes asides from the beta-lactam antibiotics. The most frequent resistance was against fluoroquinolones (especially ciprofloxacin, nalidixic acid, levofloxacin, moxifloxacin), tetracyclines (oxytetracycline and tigecycline), Aminoglycosides (gentamicin, streptomycin, kanamycin, tobramycin) as well as Phenicols (florfenicol and chloramphenicol). These findings are similar to other studies across Nigeria that reported multidrug resistance among ESBL - *E. coli* isolates of poultry origin (Al-Mustapha et al., 2022; Aworh et al., 2021; Ayandiran et al., 2018; Ayeni et al., 2020; Tama et al., 2021).

The resistome of ESBL - *E. coli* consists of a plethora of genes. Firstly, the occurrence of multidrug efflux pump *mdf*(A) which confers resistance to chemically unrelated, clinically important antibiotics such as chloramphenicol, erythromycin, and certain aminoglycosides and fluoroquinolones is worrisome (Edgar and Bibi, 1997; Lewinson et al., 2003). The $bla_{\text{CTX-M-15}}$ gene ($bla_{\text{CTX-M-1}}$ group) was the most prevalent ESBL gene, which is similar to other reports across Nigeria (Awosile et al., 2022). Furthermore, Alonso et al. (2017) reported that the $bla_{\text{CTX-M-15}}$ was the most prevalent ESBL gene in food-producing animals including poultry across Africa. The high diversity of the isolates and the co-carriage of several clinically significant ARGs is worrisome. The diversity of our isolates is evident in that we detected the three known variants of sulfamethoxazole ARGs (*sul*1, *sul*2 & *sul*3), the three known tetracycline ARGs (*tet*(A), *tet*(B), & *tet*(M), two fosfomycin ARGs (*fos*A3 & *fos*A7), and five of the phenicol ARGs (*flo*R, *cat*A1, *cat*A2, *cat*B3, & *cml*A1). Similarly, we detected a wide array of mutations in chromosomal genes which lead to several resistance patterns. These includes mutations in the *par*C, *gyr*A, *pmr*B, *amp*C, *gyr*B, *fol*P, 16S-*rrs*B genes. In addition, it is interesting that one isolate has an ampC promotor mutation and an ESBL. Further, we detected four variants of trimethoprim ARGs, as well as *mef*B (resistance to macrolide antibiotics such as Tylosin, erythromycin, and azithromycin), and the first report of *arr*-3 which confers resistance to rifamycin, a first-line anti-tuberculosis drug from FPAs in Nigeria (Table 2). The plethora of ARGs qualifies some of these ESBL - *E. coli* to be termed "superbugs" (Morris et al., 1998).

This diversity is equally evident in the phylogrouping as four phylogroups were detected. Our findings were similar to the report of Aworh et al. (2020) which demonstrated that ESBL - *E. coli* from poultry in Nigeria belonged to phylogroups A and B1. Across Africa, similar reports have been documented (Alonso et al., 2017; Seni et al., 2016). We detected three isolates that belonged to phylogroup F. These poultry-originated phylogroup F isolates, especially if they belong to dominant human ST types, might be recognized as a high-risk foodborne pathogen because of their ability to cause multiple diseases in animal models of avian colibacillosis and human infections (sepsis, meningitis, and urinary tract infections) (Zhuge et al., 2020).

The mobilome and virulome of the isolates were also very diverse. The most common plasmids were the col-type plasmids (seen in 8 of the 11 sequenced isolates). These small plasmids are believed to increase bacterial fitness and reduce susceptibility to several classes of antibiotics (Oladeinde et al., 2018). Surprisingly, only three ARGs were plasmid-mediated. These were: Fluoroquinolone (*qnr*S11 and *qnr*S19) and Phenicol (*flo*R). This could be due to the fact that only short-read sequencing was used and a combination of short- and long-read sequencing could detect more plasmid-mediated ARGs (Hickman et al., 2021; Solà-Ginés et al., 2015). The most common plasmids carrying cephalosporin resistance genes in *E. coli* isolated in poultry farms belong to the IncI1, IncFIB, and IncN families (Bortolaia et al., 2010). Of the Inc-type plasmids, the IncF was the most abundant (seen in 8 of the 11 isolates) and they belonged to 4 IncF STs. Previously, Aworh et al., *and* Ayandiran et al., had reported high numbers of IncF plasmids in ESBL - *E. coli* of poultry origin in Nigeria (Ayandiran et al., 2018; Aworh et al., 2021). Similarly, we had a few copies of IncI, IncN, IncX, IncR, and IncQ plasmids. The ESBL isolates contained virulence-associated genes such as adhesins, toxins, anti-host defense factors, iron acquisition systems, etc. These genes have been implicated in avian colibacillosis as well as in extraintestinal pathogenic *E. coli* (ExPEC) (Ewers et al., 2007; Nakazato et al., 2009). Hence, these suggest that some of our ESBL - *E. coli* of poultry origin may be zoonotic pathogens, could be a reservoir of virulence and resistance genes, and has the potential to cause human infections (Johnson et al., 2003; Mora et al., 2013; Ngbede et al., 2021).

The isolates belonged to varying ST and clonal complexes. Although Ayeni et al., reported ST206 as the most prevalent among Nigerian poultry chains, only one of our isolates (KW/BB24) belonged to ST 206 clonal complex (Ayeni et al., 2020). Similarly, MDR ST206, ST48, and ST117 *E. coli* have been reported in humans, poultry, and the environment in Abuja, Nigeria (Aworh et al., 2020). In another study, *E. coli* ST48 from drinking water carrying the $bla_{\text{CTX-M-1}}$ IncI1/ST3 plasmid similar to other $bla_{\text{CTX-M-1}}$ IncI1/ST3 plasmids found previously in animals and humans were detected in France (Madec et al., 2016). Other studies have reported *E. coli* isolates of poultry origin (ST117) that were indistinguishable from or closely related to *E. coli* from human urinary tract infections (Bergeron et al., 2012; Vincent et al., 2010).

To the best of our knowledge, this is the first study to report the diversity of phages in humans or animals in Nigeria as there were no previous reports on the phageome (coliphage or any other phages). Our findings detected representatives of four phage families: *Myoviridae*, *Siphoviridae*, *Inoviridae*, and *Podoviridae*. These and other families of phages have been previously reported in humans, animals, and the environment (Gogarten et al., 2021; Tisza and Buck, 2021; Wang et al., 2021). Also, Shousha et al. (2015) reported that phages conferred kanamycin, chloramphenicol, tetracycline, and ampicillin resistance when transduced into *Escherichia coli* ATCC 13706, hence, bearing a cost. Similarly, several other studies such as those of Gómez-Gómez et al. (2019) and Calero-Cáceres et al. (2019) reported that bacteriophages are vehicles for gene transmission in meat that should not be underestimated as a risk factor in the global crisis of antibiotic resistance. Contrary to these findings, we did not detect any ARG in the phageome of these Nigerian ESBL - *E. coli* isolates. The findings of Billaud et al. (2021) supported our research findings as they also reported in their analysis of porcine virome and microbiome that phages and prophages rarely carried antibiotic resistance genes. To better characterize and understand the diversity of phages (viral dark matter), their role in AMR, and their use as alternatives to antibiotics, it is essential to improve their detection accuracy (single-cell vs metagenomic approaches), use a combination of different analytical software to increase data output, and improve host-linkage of known and novel bacteriophages (Fitzgerald et al., 2021).

Although it is widely believed that illicit antimicrobial usage is the main driver of AMR, the relationship is complicated (Holmes et al., 2016). Studies have shown that several other determinants impact the

emergence and spread of AMR pathogens (Holmes et al., 2016; Silva et al., 2021). Generally, there is no single solution, and several, synergistic, overlapping, and complementing approaches will be needed, with a strong overarching shared goal to reduce the zoonotic transmission of MDR bacteria from FPAs and associated economic loss. Further, LMICs must invest heavily in genomic surveillance to provide evidence-based data and contribute to the understanding of the molecular dynamics of AMR.

5. Conclusions

This study demonstrated a high diversity of ESBL - *E. coli* in poultry that pose a significant public health threat to humans. WGS revealed that the resistome, mobilome, and virulome contained diverse ARGs, mobile genetic elements, and virulence factors that could increase the spread of AMR and increase their fitness and survival in humans. In addition, WGS revealed most of the cultured ESBL – *E. coli isolates* contained several coliphages belonging to four viral families. More genomic studies are needed to identify resistance mechanisms and the role of phages in the emergence and spread of MDR pathogens.

Ethics approval and consent to participate

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

Consent for publication

Not Applicable.

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Authors' contributions

AIA did the initial bacterial isolation and AST. AIA, IAR, OAO, IAO, VOA, and AH wrote the draft manuscript. BMSM did the WGS. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

The sequence raw reads are openly available at the ENA under the BioProject ID: PRJEB58679.

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