

Genomic Insights into Possible Zoonotic, Anthroponotic, and Environmental Transmission of Escherichia coli in Ekiti and Ondo States, Nigeria

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Abstract

Background

Escherichia coli can be pathogenic or non-pathogenic, depending on its genetic makeup, which characterises its virulence potential. The transmission dynamics of *E. coli* are complex due to its dual nature and its ability to be transmitted among humans, animals, and the environment in both directions. The objective of this study was to gain insight into the resistome, virulome, and microbiome of *E. coli* from matched samples using the One-Health Approach in Ekiti and Ondo States, Nigeria.

Methods

Whole-genome sequencing (WGS) was used to characterise the genomic features of 8 *E. coli* isolates from humans (n = 3), animals (n = 2), and the environment (n = 3). Antimicrobial resistance genes (ARGs), virulence factors, and mobile genetic elements (MGEs) were identified, followed by multi-locus sequence typing (MLST), core genome MLST, serotyping, phylogrouping, and local and global phylogenomic analysis.

Results

The 8 *E. coli* recovered in this study showed variation in the presence of ARGs, virulome, and mobilome, with total ARGs > 30 genes and virulome > 40 genes. There was an identical genetic makeup in three pairs (NGEK1 vs. NGEK1, NGEK13 vs. NGEK17, and NGEK21 vs. NGEK22A) recovered from humans, animals and environments. Some significant resistance genes (blaCTX-M-15, blaCMY-2, blaTEM-1B, blaOXA-1, blaOXA-181 and qnrS1) were found in the isolates. The MGEs analysis found two groups: plasmid-mediated and non-plasmid-mediated, with both carrying resistance (blaTEM, blaCTX-M-15, qnrS1, qnrB19, and blaOXA181) and virulence genes (hlyE, hha, senB). The STs found in the study were ST 2, 38, 181, 693, and 998. Clonality was observed among some *E. coli* isolates from the same household or farms, and these isolates were also phylogenetically associated with certain global *E. coli* isolates.

Conclusion

Our study emphasised that the *E. coli* isolates analysed possessed ARGs and virulence factors that drive pathogenicity and presented evidence for possible zoonotic and anthroponotic modes of transmission.

Introduction

The ubiquitous nature of *Escherichia coli* is due to its duality [1]. The duality of *E. coli* is its ability to act as a commensal in the intestinal tract of humans and other vertebrates, which is necessary for the maintenance of their microbiome, and it's also able to cause significant infection, ranging from diarrhoea, a self-limiting infection, urinary tract infection, to sepsis [2, 3]. This duality makes it a vital bacterium of public health concern. This ability to adapt to different environments is encoded in its genome [4, 5]. *E. coli* possesses an open pangenome with a core set of housekeeping genes, which provides the evolutionary backbone for phylogenomic insight and a vast variable accessory genome that is either borne on the chromosome or an extra-chromosomal element [6–8]. These accessory genomic materials are exchanged between strains, which equip them with antimicrobial resistance and virulence potentials [9, 10]. The genomic component of any *E. coli* determines its behaviour and virulent potential [4]. These genomic components include the resistome, virulome and mobilome, which are crucial to the pathogenicity and the extent to which it can cause an infection [11–13]. Therefore,

understanding the resistome, virulome, and mobilome of an *E. coli* isolate is fundamental to deciphering the epidemiology of any pathogenic *E. coli*, its transmission to humans and animals, and its contamination of their shared environment.

The line between commensalism and pathogenicity is not fixed but is a dynamic continuum shaped by genetic evolution. A study by Burgaya et al. [14] investigated and unravelled the genetic determinants that predispose an *E. coli* strain to cause invasive disease. In that study, a comparison of 912 *E. coli* strains from bloodstream infections and 370 strains from healthy individuals revealed that pathogenicity is a highly heritable trait, with a striking 69% of the variance explained between the two datasets. Some of these variances could be attributed to *E. coli* adaptation through the acquisition of MGEs, as reported by Byarugaba et al. [15] and Saini et al. [4]. Saini et al. particularly found that *E. coli* isolates from urban environments evolved by gaining fitness advantages, such as AMR and virulence factors, through horizontal gene transfer (HGT)- mediated acquisition of MGEs.

Evidence in the molecular, genetic, and genome-wide analysis of *E. coli* obtained from a matched sample from different sampling cohorts, from companion animals and their owners, patients and their clinical environment, animals and their handlers and their environment has demonstrated transmission dynamics of pathogenic and MDR *E. coli* isolates. A global snapshot phylogenomic analysis by Mitra *et al.* [15], which used 158 *E. coli* isolates compared with isolates from India, suggests possible cross-transmission among humans, animals, and the environment, mainly via zoonotic transmission. Zoonosis is an established phenomenon in which an animal transmits an infection or pathogenic microorganism, including resistant bacteria, to humans [16–18]. Globally, this phenomenon, zoonosis, is well-established, as well as the transmission of microorganisms from contaminated environments to humans and animals [18, 19]. However, a phenomenon called anthroponosis, also known as reversed zoonosis, is the process by which an infected human host of microbial origin transmits pathogens to non-humans, whereas zooanthroponosis is exclusively a process by which humans transmit pathogens to animals; both phenomena are not well established in the literature [20, 21]. However, studies agreed that this is generally possible. Some examples of those types include that one by Ewers *et al.* [22], which found a specific *E. coli* clone that was initially restricted to humans; similarly, Rwego et al. [23] and Nicolas-Chanoine et al. [24] have independently asserted, based on data found in their studies, the possibilities of anthroponosis, significant efforts have been given to zoonosis, but if the chain of transmission is not fully understood, through concentrated attempt to unravel all possible means of transmission, the battle to turn the tide of infectious disease will yield limited result.. However, even since those earlier studies were done, little interest has been shown in this aspect of research. The gaps that limit this establishment of anthroponosis and zooanthroponosis lie in limitations in methods in sample collection, timing of collection, and pathogen recovery techniques. Also, such studies require large sample sizes, longitudinal designs, and epidemiological approaches. These types of studies require rigorous data analysis, including genome-wide analyses to determine the direction or origin of pathogenicity [25, 26]. Although, some subjects in the global north, has analysed large retrospective data set, which shows some evidence [4, 17], however, this is not so for global south, where hygiene, biosecurity in interaction with animals and agricultural and animal companion is minimal, as such the need for a study that can determine the direction of transmission of pathogenic micro-organism. This study provides a snapshot of pilot evidence and data that can be built on using genomic data to understand how pathogenic and MDR *E. coli* are transmitted within the One Health ecosystem. Therefore, this study reported a genomics-wide research on *E. coli* obtained from matched samples from human, animal, and environmental sources, limited to those with the same similarities in their phenotypic (AST susceptibility) and gene detection from Ekiti and Ondo states, Nigeria, a resource-limited setting and a low- and middle-income country.

Method

Bacterial Strains

The study obtained four hundred and thirty-five (435) *E. coli* isolates (unpublished). These isolates were cultured from matched samples from three cohorts: clinical settings, animal farms, and slaughterhouses. The clinical cohort includes

samples from consenting patients, animals, and environmental samples (water, tables, and bench-top swabs) collected from their homes. In the animal farm cohort, samples were collected from the animal handlers, their animals, and their environment (water samples, animal pens, and bench tops). Lastly, slaughterhouse cohorts included samples from slaughterhouse workers, slaughtered animals, and environmental samples (water, table tops, and bench-top swabs). The isolated *E. coli* were subjected to Phenotypic antimicrobial susceptibility testing (AST) in accordance with CLSI standards [27].

Eight *E. coli* isolates were selected for whole-genome sequencing based on similarities in antimicrobial susceptibility patterns from the same cohort and sample site. Table 1 presents the criteria used to select isolates for WGS. We selected eight *E. coli* isolates (humans, n = 3, animals, n = 2, and environmental samples, n = 3).

DNA Extraction and DNA Quantitation

DNA was extracted using the QIAamp DNA Mini Kit for genomic DNA. The modified protocol for Gram-negative bacteria DNA extraction was used, as provided by Yang [28]. The DNA was quantified using a Qubit fluorometer (Thermo Scientific, USA). The eluted DNA was pipetted into storage tubes and stored in a freezer at -80 degrees Celsius.

Whole Genome Sequencing

Library construction and the whole genome of the 8 *E. coli* isolates (NGEK1, NGK2, NGK7, NGK13, NGK16, NGK17, NGK21 and NGK22A) were sequenced using the Illumina NovaSeq PE150X (300bp) at the Beijing and Singapore Novogene Ltd. After the extracted DNA materials were shipped and passed the quality test. The Libraries were prepared according to the Novogene protocol. The Illumina NovaSeq PE150 was used to generate raw data (short reads). The clean data that was used for further analysis after the sequenced data were filtered and processed according to Cock et al [29].

Quality Assessment and Assembly

Quality analysis was conducted using the Galaxy platform [30], which was accessed on February 3, 2024 (<https://usegalaxy.org/>). The quality assessment of raw data was performed using FastQC and MultiQC [31, 32]. The short-read sequences were assembled using SPAdes, and the assembly quality was assessed using Quast (Galaxy Version 5.0) [33]. An assembly was deemed unacceptable if it had more than 400 contigs, a N50 value lower than 40,000, or if it had between 300 and 400 contigs and an N50 value lower than 50,000.

Bioinformatic analysis of WGS data for the antimicrobial Resistance determinants of *E. coli* isolates

The identification and categorisation of genes that confer resistance were carried out using ResFinder 4.1, a bioinformatics tool developed by the Centre for Genomics Epidemiology (CGE) (<http://www.genomicepidemiology.org/>) (accessed on 2024/03/2). Each isolate's genes were compared to an annotated resistance gene using a threshold of 95–100% identity [34]. The plasmid replicon types of each *E. coli* isolate were identified using PlasmidFinder 2.1. As documented by Joensen et al. [33], the strain's O and H serotypes were determined by analysing the generated FASTA files using the Centre for Genomic Epidemiology (CGE) platform (<http://www.genomicepidemiology.org/> and (<https://cge.cbs.dtu.dk/services/CSIPhylogeny/>) accessed on 2024/03/2).

Multilocus sequence typing (MLST) of *E. coli* isolates

Eight *E. coli* isolates underwent *in silico* MLST analyses using schemes developed by Achtman [35, 36]. The MLST assignment for each *E. coli* isolate was determined from whole-genome sequence data, yielding complete correspondence with alleles in the MLST database. The MLST Finder 2.0, a bioinformatics tool developed by CGE, was

used to assign Sequence Types (STs) to the isolates. Isolates that showed a perfect 100% similarity with known MLST alleles were categorised accordingly. Nevertheless, isolates lacking a precise match were classified as unknown.

If an isolate's MLST alleles match an unknown ST in the MLST database, it is classified as a new type. The presence of virulence was determined using Virulence-Finder, a tool developed by Joensen et al [37]. The MLST, serotype, phylogroup, and fimH subtype were collectively referred to as phylogenetic characteristics.

3.17 Determination of *E. coli* Phylogroups, SNP Calling and Phylogeny

The phylogroups of *E. coli* genomes were determined using an *in-silico* Clermont typing method, as outlined by Beghain et al [38]. CATIBioMed (IAME UMR 1137) hosts the Clermont Typer web interface at <http://clermonttyping.iame-research.center/>. The phylogenetic trees were constructed to determine the phylogenetic similarity of the *E. coli* isolates using the Single Nucleotide Polymorphism (SNP) calling technique described by Kaas et al [39].

The *E. coli* reference strain was used to identify single-nucleotide polymorphisms (SNPs) and detect variations in the chromosomes of each isolate. A phylogenetic tree was then constructed using the online tool (<https://realphy.unibas.ch/realphy/>). The resulting phylogenetic tree was analysed and visualised using the interactive Tree of Life tool (iTOL) based on single-nucleotide polymorphisms (SNPs). The tool can be accessed at <http://itol.embl.de/itol.cgi>. The genomes were examined to determine the number of single-nucleotide polymorphisms (SNPs) between pairs of isolates from distinct sources, thereby evaluating their relationship. The 8 *E. coli* genomes compiled in this study have been submitted to the NCBI database, and a comparison with global *E. coli* isolates from different regions and sources was also made. Supplementary file 1 has its metadata and accession numbers.

Ethical Clearance

Ethical Clearance from the National Health Research Ethics Committee of Nigeria (NHREC/01/01/2007-21/03/2023), the Federal Teaching Hospital, Ido-Ekiti (ERC/2023/09/20/1034B), and the Ondo State Ministry of Agriculture (MNR/V.384/64). The study adhered to all approved guidelines for both human and animal research, as outlined by the relevant ethical bodies.

Results

Resistome and Virulome

Among the 8 *E. coli* isolates, there were 35 antimicrobial resistance genes (ARGs) with no co-occurrence of any ARGs in all the 8 *E. coli* isolates. Of the 35 ARGs found in all the *E. coli* isolates, the *bla*TEM gene was found in 5 of the eight *E. coli* isolates, the *bla*CTX-M-15, *qnr*S1, *aph*(3'')-Ib, *aph*(6)-Id and *sul*2 genes were found in 4 of the *E. coli* isolates. ARGs *aac*(3)-IIId, *mph*(A), *qep*A4 and *tet*(A) were found in 3 of the isolates, *aad*A2, *aac*(6')-Ib-cr, *bla*OXA-1, *cat*B3, *dfr*A17 and *qnr*B19 were found in 2 isolates, while *aad*A5, *bla*ADC-25, *sul*1, *dfr*A17, *aph*(3')-IIb, *aad*A10, *bla*PAO, *bla*OXA-488, *bla*CMY-2, *bla*OXA-181, *bla*OXA-1, *fos*A, *cat*B7, *flo*R, *cat*B3, *crp*P, *qnr*VC1, *tet*(B) and *dfr*B5 genes occurred only in one isolates. The occurrence of the ARGs is shown in Fig. 1.

A total of 58 virulence factors were identified in 8 *E. coli* isolates. Two of the isolates (NGEK1 and NGK2) had 15 virulence factors; isolates NGK13 and NGK17 had 16; isolates NGK21 and NGK22A had 31; and isolates NGK7 and NGK16 had 17 and 43, respectively. Virulence gene *csg*A, *fde*C, *gad*, *nl*pI, *ter*C, *yeh*A, B, C, and D were all found in all the 8 *E. coli* isolates, followed by *hly*E(7), *iss*(7), *fim*H (6), *hha* (6), *As*IA (5), *tra*T (5), *ygh*J(4), *h*ra (4), *chu*A (3), and *kps*MII_K5 (2). The occurrence of other virulence genes is shown in Figs. 2a and 2b, respectively.

Multi-locus Sequence Typing (MLST) and Core Genome Multi-locus Sequence Typing (cgMLST)

Multilocus sequence type (MLST) analysis identified five different STs among the *E. coli* isolates (ST 2, n = 2; ST 181, n = 2; ST 38, n = 2), while ST 693 and 998 were each identified. Equally, five cgMLSTs were identified in the cgMLST analysis, with 6 *E. coli* isolates, two each having cgST 147613, 195700, and 47723, respectively. The other 2 *E. coli* isolates had cgST values of 47720 and 137699, respectively. It was observed that isolates NGEK1 from human and NGEK2 from the environment had the same ST and cgMLST, also, isolates NGEK13 from chicken and NGEK17 from the environment had the same ST and cgMLST. Similarly, isolate NGEK21 from an animal handler (poultry worker) and isolate NGEK22A from chicken (poultry) had the same ST and cgMLST. However, *E. coli* isolate NGEK7 from humans and isolate NGEK16 had different ST and cgMLST, respectively. It was also observed that the cgMLST of NGEK21, 22A, and 7 were relatively close (cgST 77723 vs 47720) as shown in Fig. 3. The results suggest possible transmission of *E. coli* among humans, animals, and across different environments.

Serotypes and Phylogroups

Five serotypes (O101:H9, O55:H35, O76:H9, O2050:H6, and O101:H15) were identified among the 8 *E. coli* isolates, with similar serotypes observed in 3 matched pairs (Fig. 4). The phylogrouping of the 8 *E. coli* isolates shows that three were in phylogroup A, two each in phylogroups B2 and D, and one in phylogroup C. The results are depicted in Fig. 3.

Mobile Genetic Element

The MGEs analysis shows the presence of several plasmids and other MGEs. Table 2 shows the plasmids found in all *E. coli* isolates. Of the sixteen plasmids found among the 8 *E. coli* isolates, IncFIA(HI1) found in *E. coli* isolates NGEK1 and NGEK2 carried a virulence gene (clpK1). Plasmid Col440I was found to carry the qnrB19 gene in isolates NGEK13 and NGEK17 associated with phenotypic resistance to ciprofloxacin and a virulence factor (mrkA). Plasmid IncFII(pRSB107) was found in three isolates (NGEK7, NGEK21, AND NGEK22A). It was found to carry resistance gene *bla*TEM-1B in NGEK21 AND NGEK22A isolates and virulence genes *anr*, however, it was not associated with any resistance or virulence genes in NGEK7. Plasmid ColKP3 carried *qnrS1* and *bla*OXA-181 genes; similarly, plasmid IncQ1 carried *aph(6)-Id*, *aph(3'')-Ib*, and *sul2* genes, all found in *E. coli* isolate NGEK7. Plasmid IncFII(pRSB107) in *E. coli* isolate NGEK16 was found to carry resistance to tetA and virulence genes *anr* and *traT*, respectively. In the same *E. coli* isolates, plasmid Col156 was found to carry the virulence factor *senB*.

Table 3 shows other types of MGEs found among the 8 *E. coli* strains; some were associated with antimicrobial resistance genes and virulence factors, whereas others were not associated with any function. Five types of non-plasmid MGEs were found in the 8 *E. coli*. They were: unit transposon, insertion sequence, miniature inverted repeat, integrative conjugative element, and composite transposon. Among the eight *E. coli* isolates, isolate NGEK16 had the highest number of non-plasmid MGEs carrying genes associated with antibiotic resistance and virulence factors. There were 7 them (Tn5403, ISEc9, IS6100, ICEEclHE3034-1, MITEEc1, ISEc17, and cn_31761_ISEc17). A Tn2, a unit transposon, was found in *E. coli* isolates NGEK1 and NGEK2, which carried *bla*TEM-1B. In the same isolates, ISKox3 and ISEc1, insertion sequences that carried virulence genes (*hlyE* and *fdeC*), were found. Insertion sequence ISEc38 was found in *E. coli* isolates NGK13 and NGEK17, which carried the *hha* gene, a virulence factor. *E. coli* isolates NGEK21 and NGEK22A carried ISKpn19, IS30, and MITEEc1, all non-plasmid MGEs; ISKpn19 carried three resistance genes (*tet*(A), *qnrS1*, and *bla*CTX-M-15). While the IS30 and MITEEc1 carried virulence factors. *E. coli* isolate NGEK7 had ISKpn19 and MITEEc1; the ISKpn19 carried *qnrS1* and *bla*OXA-181 antibiotic resistance genes, while MITEEc1 carried nine virulence genes (*fdeC*, *yehA*, B, C, D, *hra*, *papA_F13*, *papC*, and *nlpl*).

Phylogenetic Analysis and Comparative Genomics

Local Phylogenetic Analysis Results

The phylogenetic tree of the different 8 *E. coli* isolates is presented in Fig. 3. Figure 4 also shows the evolutionary history of the local *E. coli* isolates. Of the 8 *E. coli* strains, 7 showed a close relationship and were organised into three clades (clade1, clade2, and clade3). Clade 1, where isolates *E. coli* NGEK1 and NGEK2 were of the exact ancestry origin, as indicated by their distance and relationship, NGEK1 was from a human (blood). In contrast, isolate NGEK2 was isolated from the environment (home table), from which isolate NGEK1 was also isolated. These findings suggest possible transmission between humans and the environment, or vice versa. Similarly, NGEK13 isolated from poultry (chicken) and NGEK17 isolated from the environment (farm water) were also closely related, sharing the same ancestry level (clade 2). Isolate NGEK22A from an animal (chicken), and isolate NGEK21 from a human (rectal swab), which also showed similar ancestry roots but were closely related. Clade 3 showed four isolates from the same root, but only NGEK22A and NGEK21, which originated from the same ancestral root, and also NGEK16 from the environment (home water), seem to be related to some extent. Isolates *E. coli* NGEK1 was isolated from a human sample, while isolate NGEK2 was isolated from the bench, the house bench, and the home of that individual. Based on this phylogenetic analysis, they are likely from the same source. So, there is a possible cross-transmission between humans and the environment or from the environment to humans. The same explanation applies to isolate pairs NGEK13 and NGEK17 and NGEK22A and NGEK21. The whole-genome sequencing results for *E. coli* isolates NGEK7 and NGEK16 showed that they were significantly different and did not share a common ancestral root.

Global Phylogenetic Analysis Results

The analysis of the 8 *E. coli* isolates compared to global *E. coli* isolates phylogenetically showed similarity to *E. coli* isolates from Ghana, South Africa, Cuba, China, the United Kingdom, the Netherlands, Switzerland, Nigeria, and the United States of America, obtained from humans, animals, and wastewater, as shown in Fig. 4. Of the 8 *E. coli* isolates, one of the isolates (NGEK16-04-2023) from home water (Environment) was found to be of the same ancestry root as the *E. coli* isolate (GCA_002510125.1), in a human from Pretoria in South Africa. It was distantly related to the *E. coli* isolates (GCA_016767775.1 from a chicken in the USA, GCA_002002225.1 from a human in Sydney, Australia, and GCA_002416865.1 from a human in KwaZulu-Natal, South Africa).

E. coli isolate (NGEK7-04-2023) from a urine sample in a human was associated with *E. coli* isolate GCA_024585725.1 from poultry in Santa Cruz del Norte, Cuba. NGEK21-05-2023 and NGEK22A-05-2023 were of the exact ancestry origin and were closely associated with *E. coli* GCA_048222295.1 and GCA_020883315.1 from human samples in the United Kingdom and the Netherlands, respectively. However, they are distantly associated with GCA_024224055.1 from a chicken sample from Switzerland and GCA_019584715.1 from wastewater in Ibadan, Nigeria.

E. coli GCA_024225755.1, GCA_019357435.1, and GCA_022695865.1 from chicken in Switzerland, in Jiaxing, Zhejiang, China, and Athens, USA, were distantly associated with NGEK7-04-2023 and NGEK13-04-2023 obtained from the environment (water) and animal (chicken), respectively, which were of the exact ancestry origin. Lastly, *E. coli* NGEK1-04-2023 and NGEK2-04-2023, which were from the same branch, were closely associated with *E. coli* GCA_041877505.1 and GCA_002416915.1 from human samples in Accra, Ghana, and KwaZulu-Natal, South Africa, respectively.

Possible Transmission Dynamic Model for 8 *E. coli* isolates from WGS Data.

Figure 5 shows the constructed transmission model for *E. coli* based on WGS data. The result shows six possible models. Model 1: A human-to-environment-transmission route (NGEK1-Humans to NGEK2-Environment), Model 2: An environment-to-human transmission route (NGEK2-Environment to NGEK1-Humans), Model 3: An environment-to-animals transmission route (NGEK13-Environment to NGEK17-Animals), Model 4: An animals-to-environment transmission route (NGEK17-Animals to NGEK13-Environment), Model 5: An animals-to-human transmission route (NGEK22A-Animals to NGEK21-Humans), and Model 6: A human-to-animal transmission route (NGEK21-Humans to NGEK22A-Animals).

Discussion

The transmission of *E. coli* is dynamic, including moving from humans to animals, a process referred to as reverse zoonosis or anthroponosis and also the established nature of *E. coli* transmission through zoonotic, i.e, from animals to humans through food contamination, domestication or compassionate use [40, 41]. Environmental contamination with pathogenic *E. coli* occurs when animals and humans improperly shed waste into the environment. The environment thereafter became the reservoir for these pathogenic *E.coli* isolates [42, 43]. This study integrates multilayered genomic data, including resistome and virulome profiling and phylogenetic analysis.

In this study, we focused on unravelling the whole genetic and genetic relatedness of the 8 *E. coli* isolates that were obtained from matched samples obtained from three different collection cohort sites (cohort 1:hospitalised patients and their animals and environment, cohort 2:animal farms, animal handlers, their animals and the environment, including their water sources and cohort 3: slaughterhouses: Buchters, slaughtered animals and their environment including the water sources used in the slaughterhouses).

In our study, we identified a large pool of antibiotic resistance genes that could confer distinct phenotypic resistance in the 8 *E. coli* isolates. Our study identified diverse resistance genes that confer multiple antibiotic resistances, including carbapenem resistance. There were 32 antibiotic resistance genes (*bla*TEM, *bla*CTX-M-15, *qnr*S1, *aph*(3'')-Ib, *aph*(6)-Id, *sul*2, ARGs *aac*(3)-IId, *mph*(A), *qep*A4, *tet*(A), *aad*A2, *aac*(6')-Ib-cr, *bla*OXA-1, *cat*B3, *dfr*A17 and *qnr*B19, *aad*A5, *sul*1, *dfr*A17, *aph*(3')-IId, *aad*A10, *bla*CMY-2, *bla*OXA-181, *bla*OXA-1, *fos*A, *cat*B7, *flo*R, *cat*B3, *crp*P, *qnr*VC1, *tet*(B) and *dfr*B5).The predominant resistance genes found in the study were *bla*TEM, *bla*CTX-M-15, and *qnr*S1. All matched samples (NGEK1 vs NGK2, NGK13 vs NGK17, NGK21 vs NGK22A) have the same resistomes and phenotypic characteristics, indicating that each *E. coli* isolate in a pair is likely of the same origin. Genomic evidence from the 6 *E. coli* isolates obtained from the three cohort sites provided a compelling snapshot of the transmission dynamics of MDR and virulent *E. coli* across human, animal, and environmental interfaces in Nigeria. The expectation is that the *E. coli* isolates in the matched pair (NGEK7 and NGK16), based on their similar phenotypic susceptibility patterns, would have the same resistomes; however, the WGS result showed that they were not of the same origin as the phenotypic result, and the closeness of the sample collection point suggested. This shows the superiority of the WGS method over the phenotypic method for epidemiological and surveillance purposes.

Each resistance gene in each *E. coli* isolate could be responsible for the observed antibiotic resistance patterns. For example, *bla*TEM, *bla*CTX-M-15, *bla*CMY-2, *bla*OXA-181, and *bla*OXA-1 were found to be associated with resistance to beta-lactam antibiotics, although their resistance spectra differ. The *bla*TEM gene is associated with resistance to antibiotics like ampicillin and amoxicillin, which are the first-line antibiotics commonly used in clinical settings in Nigeria and other settings [44–46], while the *bla*OXA-181 gene is associated with carbapenem resistance.

One of the significant findings of our study was the genomic agreement observed among *E. coli* isolates from matched clinical, household, animal, handler, and shared environment samples. For example, the identical Sequence Types (STs), core genome MLST (cgMLSTs), serotypes, and phylogroups between human-animal (NGEK21/NGEK22A), human-environment (NGEK1/NGEK2), and animal-environment (NGEK13/NGEK17) pairs provided potential evidence for the zoonotic or anthroponotic and environmental transmission of pathogenic *E. coli* isolates. It is important to note that the near-identical core genomes identified in our study could serve as genomic fingerprints for surveillance. This assumption of genomic fingerprinting provides strong evidence for a specific route of transmission, which is vital for tracing the origin of an infection. It can be inferred from the available evidence in our study that zoonotic or anthroponotic transmission may be occurring, given the present tangible, genetically verified events at the study locations, as evidenced by the WGS data.

The findings of our study on the possible transmission dynamics align with evidence in the literature, for example, a study by Mitra *et al.* [15] on MDR *E.coli* isolates from humans(animal handlers), animals, and the environment, which was also WGS-based, found a similar relationship in some of the sample cohorts in their study. In Nigeria, a study conducted by

Aworh et al. (2021) in the FCT found relatedness among three sample pairs: poultry, their handlers, and the environments. However, further analysis found that they were not clonal. The SNP phylogenetic and core genome analyses of the six *E. coli* strains discussed in this study revealed clonality, making this one of the first reports of such findings in the study settings. This piece of evidence suggests the possible route of transmission and the movement of pathogenic *E. coli* isolates across the different settings and further support the need for enforcement of biosecurity guideline in farms where animals are rare and also the need to keep a clean environment as this suggests that the environment could be a significant vehicle that transmit pathogenic, virulent and multi-drug resistance *E. coli* isolates and also serves as reservoir for them.

The results of our study also reveal that the *E. coli* isolates obtained from the environment, water samples, and poultry (chicken) were not merely commensal, as they harboured antimicrobial resistance genes and virulence factors. For example, the presence of clinically relevant antimicrobial resistance genes (ARGs), such as *bla*CTX-M-15, associated with extended-spectrum beta-lactamases (ESBLs), *bla*OXA-181, associated with carbapenemases, and *qnr*S1, associated with quinolone resistance, is a public health concern. Also, the co-occurrence of all those ARGs with virulence factors related to extraintestinal infection, such as *hlyE* and *iss*, which were particularly found in ExPEC-associated phylogroups B2 and D, raises a concern about strains that are both capable of causing severe infection and difficult to treat. This implies that those who get infected with such *E. coli* pathogens experience extended hospitalisation and an increased burden on the healthcare system [47]. Equally, this can lead to low productivity as a result of loss of work time due to the time spent managing those types of infection [48, 49]. Also, since the health insurance system in Nigeria is in a primitive stage, where most individuals who work in the animal sector do not have coverage, they will have to pay for their treatment out of pocket [50]. This will deplete their finances, which could have been channelled into other areas of their lives. Also, shedding of these pathogenic *E. coli* isolates into the environment could contaminate water bodies, as open defecation remains widespread in Nigeria, although it has been outlawed in several states; implementation remains a significant concern.

The analysis of mobile genetic elements (plasmid- and non-plasmid-mediated) examines the presence of extrachromosomal elements that can rapidly disseminate to other bacteria, even when those bacteria may not initially have that capacity. Particularly of interest were the presence of *bla*TEM on a plasmid (*IncFII*) and *qnr*S1 and *bla*OXA-181 on an insertion sequence (*ISKpn19*). These findings provide a possible explanation for the movement of the resistance factor across species or bacteria. It also suggests that the plasmid and other MGEs act as vehicles for distributing resistance and virulence factor traits among different bacterial hosts [51]. The isolate NGEK16 from the environment harboured the highest number of MGEs. This highlights how a single environmental strain can serve as a potent reservoir for these MGEs, further fuelling the AMR crisis. Such an *E. coli* isolate can easily transfer its resistance genes via its MGEs to other bacteria in the environment. This observation aligns with the findings of Bethke *et al.* [52], Tokuda and Shintani [53] and recently Ku *et al.* [54], who have also observed that *E. coli* isolates from the environment have several MGEs that could serve as vehicles transmitting resistance and virulence factors to non-resistant, virulent, and pathogenic bacteria, and then make them resistant, virulent, and pathogenic.

In the global context, the phylogenetic analysis reveals that the isolates in our study did not cluster only with isolates from Nigeria; they also cluster with isolates from Africa, Europe, Asia, and the Americas. This finding underscores the connection between local transmission cycles in the settings where the samples were obtained and the global epidemiology of AMR *E. coli* isolates. For example, the relatedness of NGEK21/NGEK22A to human isolates from the United Kingdom and the Netherlands could reflect the global spread of successful high-risk clones. As exemplified in very highly dispersed clones like the ST 131, which has been found in epidemiological studies in clinical and non-clinical settings globally [55–58], although ST131 was not found in this study.

Strengths and Limitations of this study

The strength of this study is based on the sampling techniques, which were matched samples, which is essential in investigating transmission dynamics and also the focus on unravelling the whole genetic features and genetic relatedness, which includes the resistome, virulome, the MGEs, and extensive phylogenomic analysis in this study, which provide great insight into the creatures of the 8 *E.coli* isolates investigated. However, the small sample size means that, while the evidence in this study strongly supports the transmission event, it represents only a snapshot rather than comprehensive epidemiological evidence. Therefore, the conclusions are robust for these specific isolates but cannot be generalised to the entire region or setting without a larger-scale surveillance. Also, while the genomic data strongly suggest a transmission link, it cannot definitively determine the direction of transfer. The six proposed models (human-to-environment vs. environment-to-human, human-to-animal vs. animal-to-human, and animal-to-environment vs. environment-to-animal) are equally plausible based on the genetic data alone; therefore, resolving these limitations would require longitudinal sampling and detailed epidemiological data.

Despite these limitations, our findings provide a valuable, data-driven foundation. It highlights specific, genetically linked instances of cross-contamination between humans, poultry, water sources, and their immediate environment. The evidence strongly suggests the need for a multifaceted intervention that addresses antibiotic stewardship in both human and veterinary medicine, improves community sanitation, ensures biosecurity in animal farming, and implements other strategies to break this transmission cycle.

Conclusion

Our study used a genome-wide analysis to investigate the resistome, virulome, MGEs, and phylogenomic relatedness, providing insight into zoonotic, arthropod, and environmental transmission of pathogenic and virulent *E. coli* isolates from matched samples using the One Health approach in Ekiti and Ondo states, Nigeria. We observed that the *E. coli* isolates have diverse ARGs, virulence factors, *STs*, and MGEs. However, samples obtained from the same cohort showed relatedness and clonality, as indicated by genomic data. The presence of antibiotic resistance, virulence factors, and MGEs in isolates from poultry (chicken), water samples, and the environment demonstrates their capacity to cause severe infection in humans. Phylogenomic analysis revealed a close snapshot of the local isolates' relationship to *E. coli* isolates from Africa, Asia, Europe, and the Americas, collected from humans, poultry, water, and the environment. The presence of such potential strain in hosts other than humans is a key factor in the spread of pathogenic *E. coli* isolates. Our study emphasises the need to enforce antibiotic stewardship and biosecurity in animal husbandry and poultry farming, as well as environmental sanitation and continuous surveillance of water sources used in homes and farms.

Declarations

DATA AVAILABILITY AND WGS DATA

The WGS data generated in this study have been deposited at NCBI under the project accession PRJNA1295602, and all other associated data are available in the supplementary file.

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Author Contributions

ULI-Conceptualisation, investigation, software, formal analysis, write first draft, OBS-Supervision, data validation, Editing, Writing. OTM-Supervision, data validation, Editing, Writing, OOE-Supervision, data validation, Editing, Writing, ATA-

investigation, formal analysis, writing, project administration, AFA-investigation, formal analysis, writing, project administration, OB-investigation, formal analysis, writing, project administration, AEC-Supervision, data validation, Editing, Writing and OSK-Supervision, data validation, Editing, Writing

Funding Declaration

There was no external funding associated with this study.

Ethics and consent to participate

Ethical Clearance from the National Health Research Ethics Committee of Nigeria (NHREC) (NHREC/01/01/2007-21/03/2023), the Federal Teaching Hospital, Ido-Ekiti (ERC/2023/09/20/1034B) and the Ondo State Ministry of Agriculture (MNR/V.384/64) were obtained for this study. Consent was gotten from the participants to participate in the study.

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

Clinical Trial Number

Not applicable

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Tables

Tables are available in the Supplementary Files section.

Figures

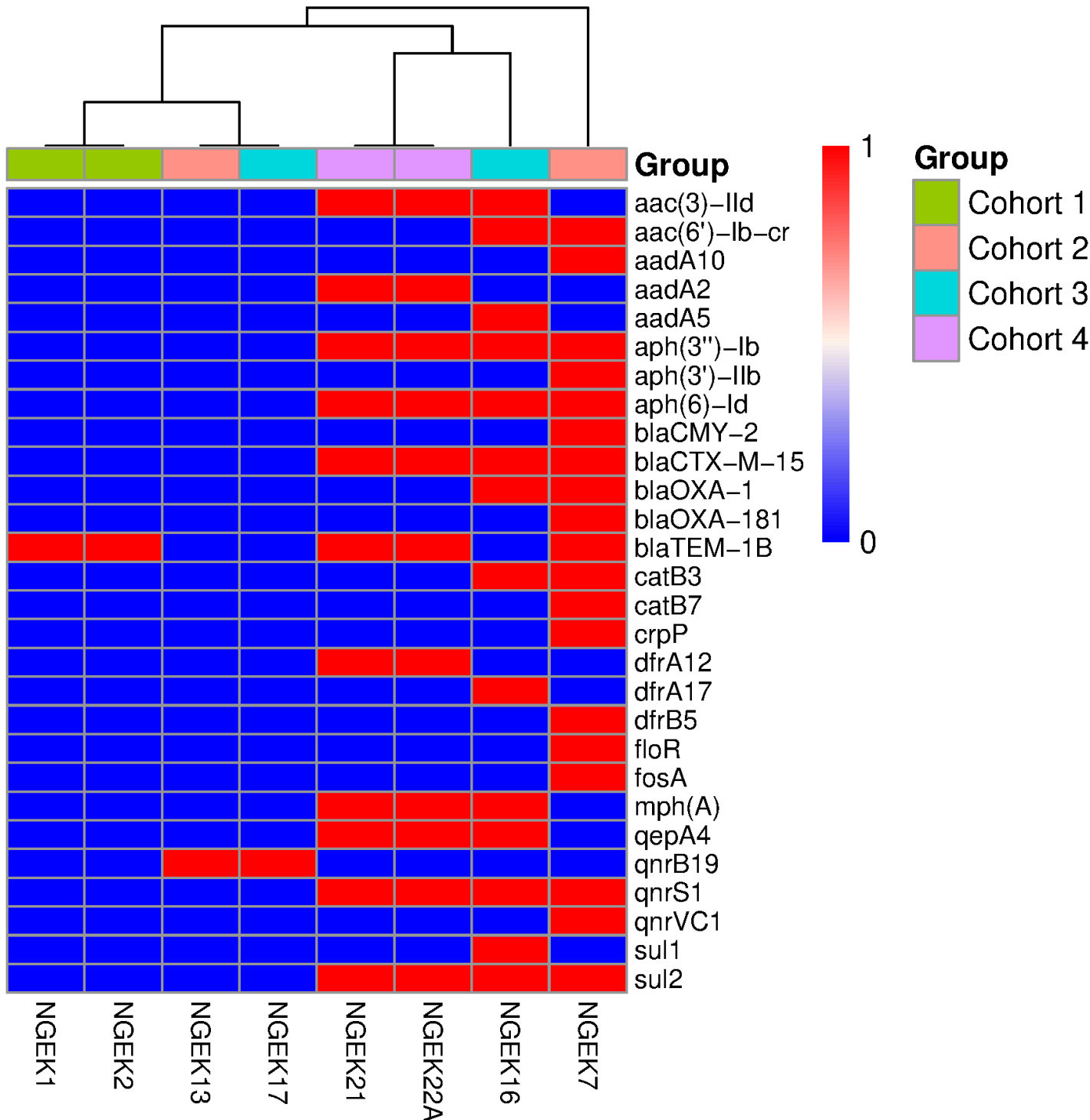


Figure 1

Antimicrobial resistance gene (ARG) profiles of eight matched *Escherichia coli* isolates. The heatmap displays the presence (red) and absence (blue) of ARGs across isolates recovered from clinical, household, animal, handler, and environmental sources in Ekiti and Ondo states, Nigeria. Each column represents an isolate, while each row corresponds to a specific ARG detected using whole-genome sequencing. This distribution illustrates both shared and unique resistome patterns, highlighting potential transmission events and reservoirs of antimicrobial resistance within and between human, animal, and environmental compartments. The Venn diagram was created from <http://www.bioinformatics.com.cn/>

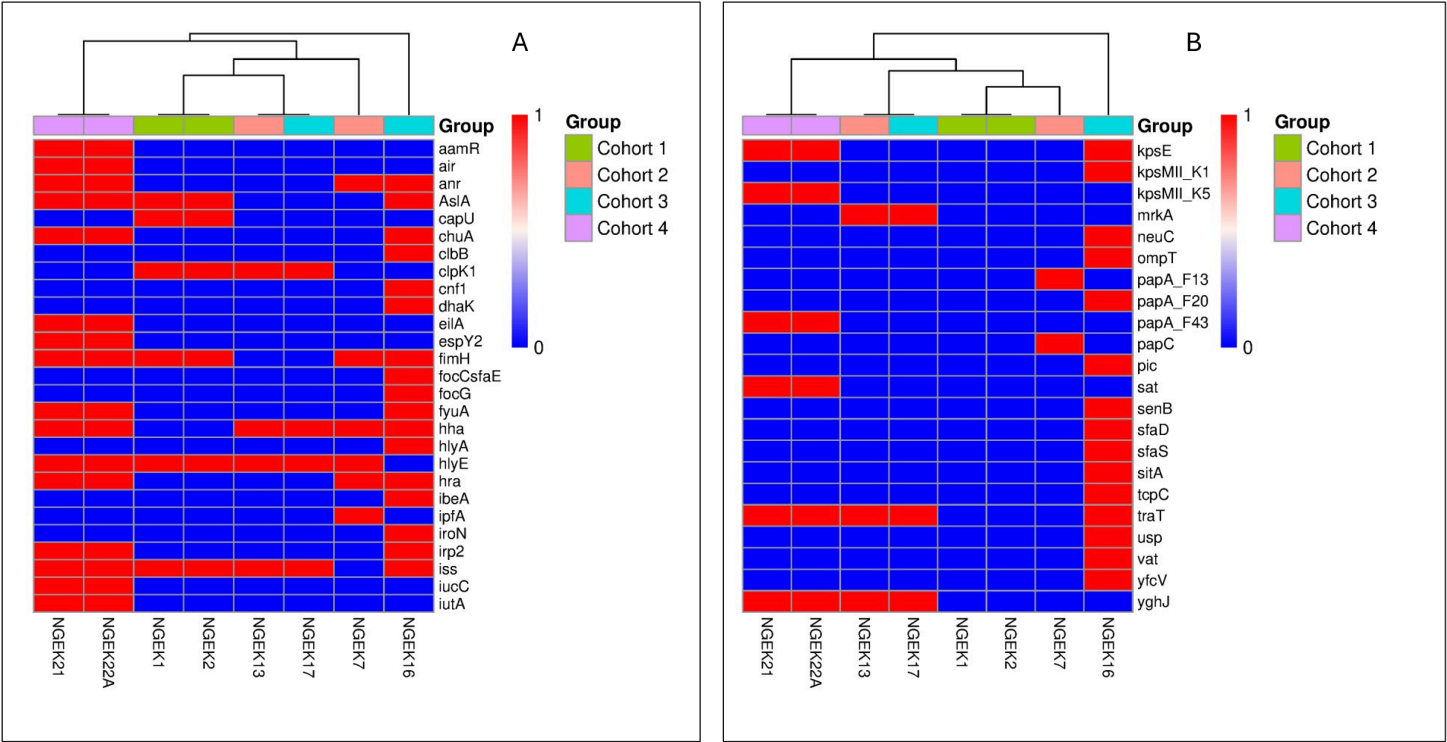


Figure 2

A and B. Virulence and Antimicrobial Resistance Gene Profiles of Eight Matched *Escherichia coli* Isolates. These figures illustrate the distribution of virulence factors and antimicrobial resistance genes (ARGs) among eight *E. coli* isolates, each obtained from matched human, animal, and environmental samples in Ekiti and Ondo states, Nigeria. The heatmaps display the presence (red) and absence (blue) of specific genes across the isolates, enabling direct comparison of both shared and unique genetic features. This visualisation highlights the complexity of the resistome and virulome within and between different compartments, underscoring the potential for cross-transmission and the importance of integrated surveillance in addressing antimicrobial resistance risks. The Venn diagram was created from <http://www.bioinformatics.com.cn/>

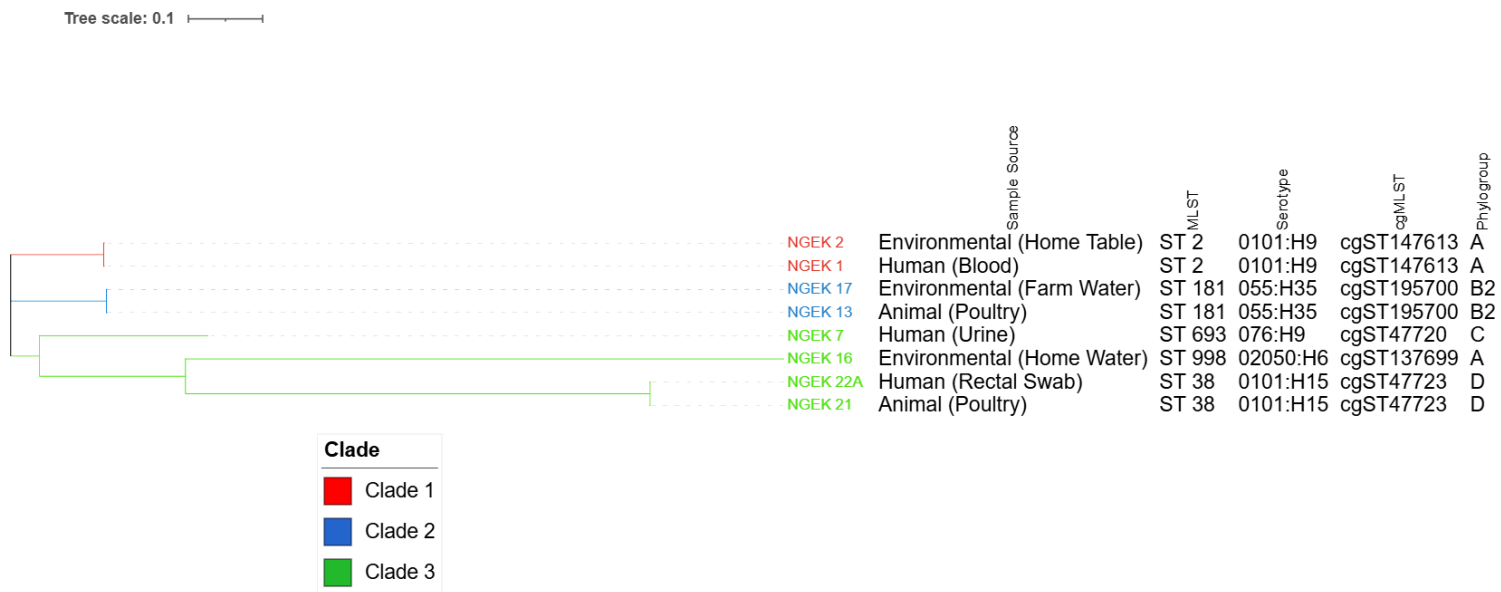


Figure 3

SNP Phylogenetic Tree for the Eight Recovered *E.coli* Isolates from Ekiti and Ondo State. The figure showed three clades (1, 2, and 3). NGEK1 is an *E. coli* isolate from human blood, and they were found to be in the sample clade with similarity in their MLST, serotypes, cgMLST, and also to belong to the same phylogroup. *E.coli* NGEK2 from the home table of Isolates NGEK1. *E.coli* isolate NGEK 17 was obtained from farm water with a similar genetic makeup to *E. coli* isolate *E.coli* NGEK12 from an animal (poultry). The same pattern was found in the *E. coli* isolate (NGEK22A) from a rectal swab from an animal handler (human) and an animal (poultry) (NGEK21). However, there was a difference in the genetic makeup of the *E. coli* isolate (NGEK 7) from the human urine sample and the *E.coli* isolate (NGEK16) gotten from the patient's environment (home water). The colour code showed isolates in the same clade.

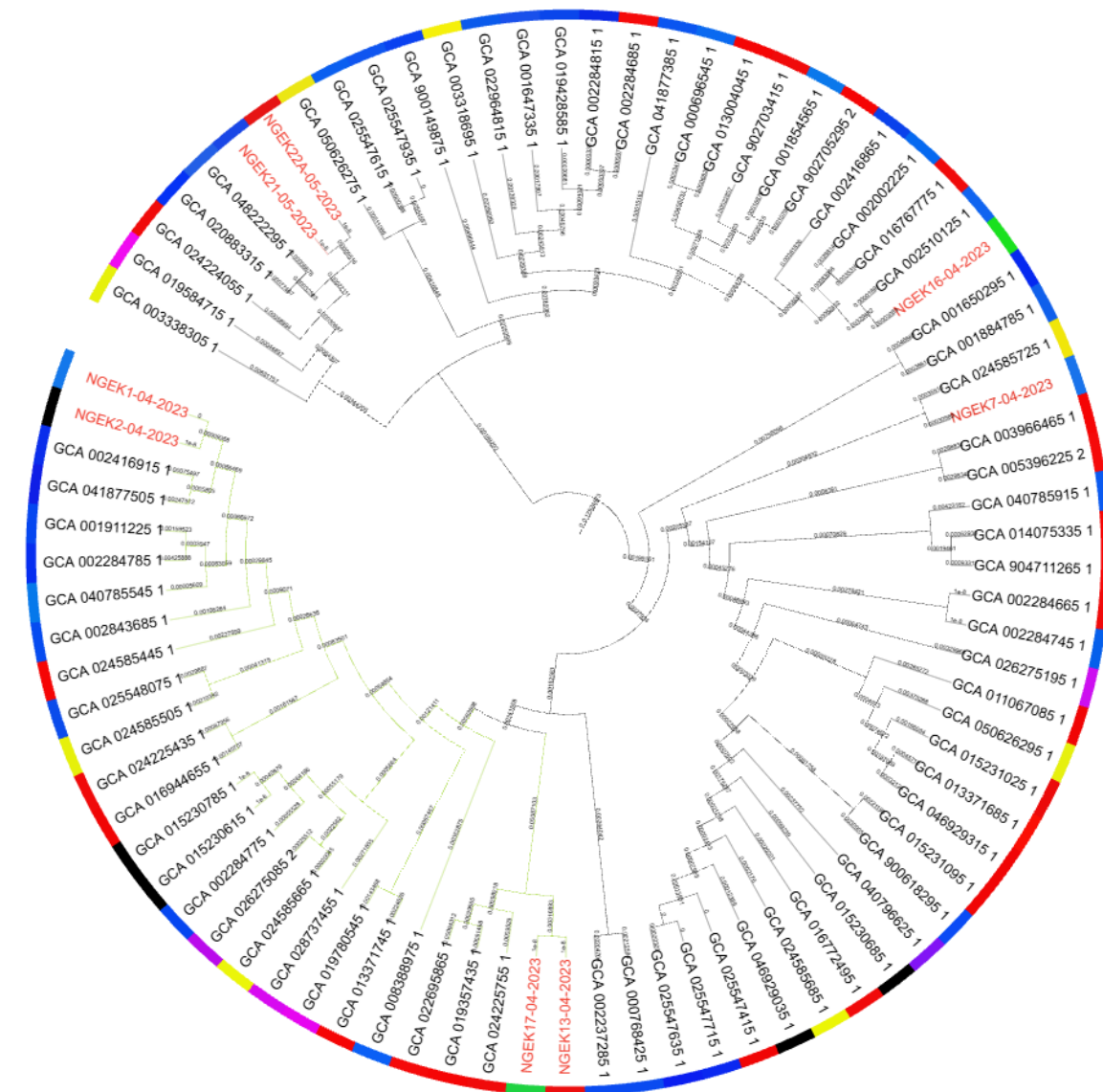


Figure 4

Comparative phylogenomic tree of the 8 *E. coli* isolates (local) and 78 *E. coli* isolates (global) obtained from different sample types across Africa, Asia, Europe, and the Americas. The colour code showed the sample types from which the isolates were obtained. (The list of the isolates and their corresponding accession number is in the supplementary file A).

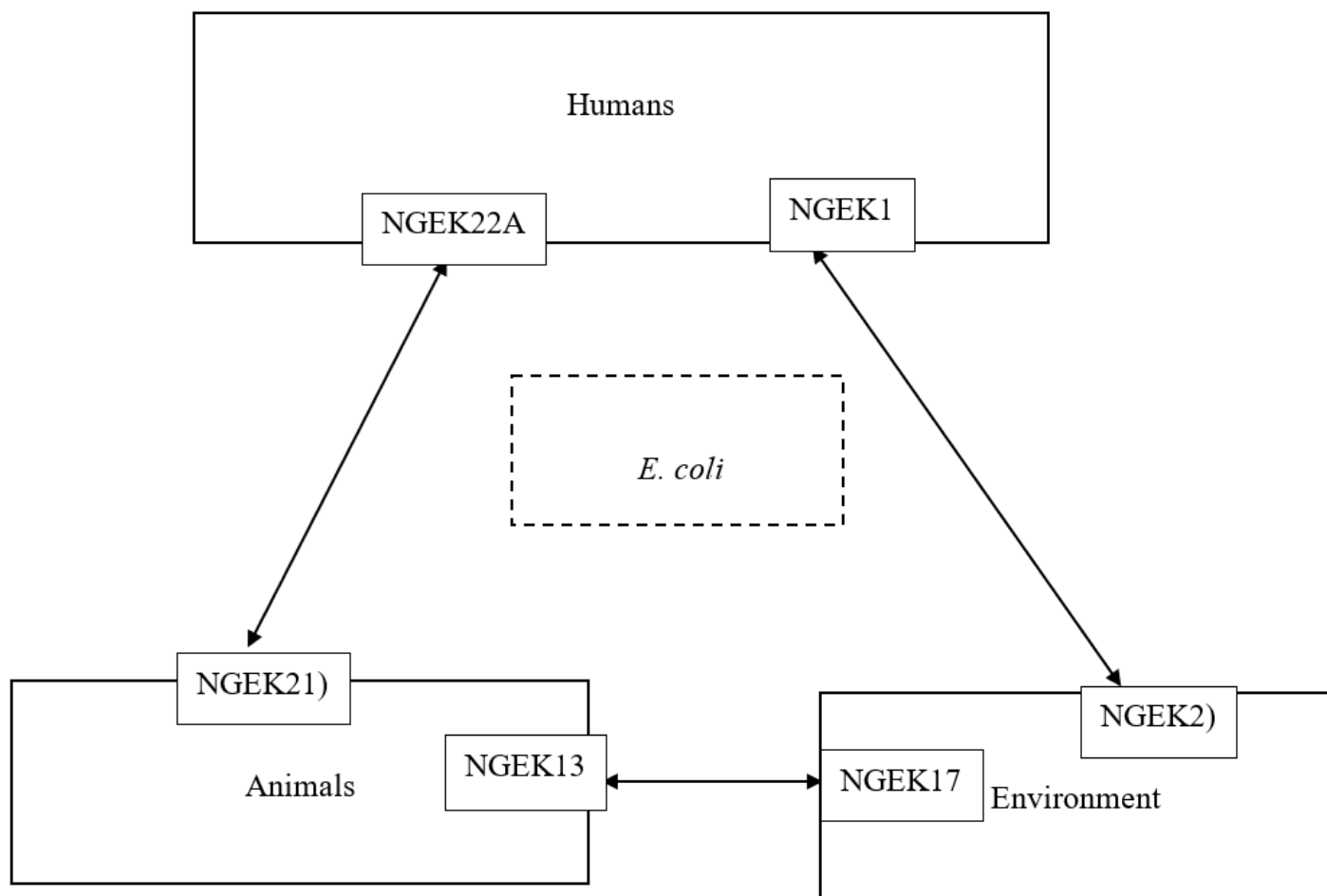


Figure 5

Transmission Dynamic Matrix Model for *E. coli* using WGS Data. The transmission dynamic matrix model illustrates the pathways and interactions involved in the dissemination of *E. coli* across ecological niches, integrating whole-genome sequencing (WGS) data to map genetic relationships and transmission events. Each cell in the matrix represents the direction and magnitude of transmission between sources, including humans, animals, and environmental reservoirs. The model incorporates key mobile genetic elements identified by WGS, such as insertion sequences and transposons, which facilitate the horizontal transfer of resistance and virulence genes. The matrix provides a visual summary of transmission dynamics, supporting the identification of critical control points and informing targeted interventions to manage antimicrobial resistance.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GenomicMetaData.csv](#)
- [Table.docx](#)