JIVS Journal of Istanbul Veterinary Sciences

Characterization of virulent *Escherichia coli* in healthy pet dog feces: Implications for public health

Olutayo Israel Falodun[1]*, Akeem Ganiyu Rabiu[2], Abidemi Joseph Marcus[1], Mobolaji Christiana Afolabi[1], Rotimi Ayodeji Dada[3]

1. Department of Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria. **2.** Department of Microbiology, Federal University of Health Sciences, Ila-Orangun, Osun State, Nigeria. **3.** Medical Laboratory Science Programme, College of Health Sciences, Bowen University, Iwo, Osun State, Nigeria

Falodun, O. I. ORCID: 0000-0002-6268-2532; Rabiu, A. G. ORCID: 0000-0001-6232-7680; Marcus, A. J. ORCID: 0000-0002-1239-6730; Afolabi, M. C. ORCID: 0000-0002-5677-748X; Dada, R. A. ORCID: 0000-0002-9040-3174

Research Article
Volume: 8, Issue: 1
April 2024
Pages: 5-12

ABSTRACT

The characterization of *Escherichia coli* that colonizes pets is necessary to maintain animal health and to reduce the chance of transmission to owners. In this study, we investigated the incidence of potentially virulent *E. coli* inhabiting healthy pet dogs as a risk of infection to pet owners. Antibiotic-resistant *E. coli* isolated from freshly passed dog feces were whole-genome sequenced using Illumina chemistry and classified into pathogenic lineages using pathogen-specific markers. The antimicrobial resistance genes (ARGs), virulence-associated genes (VAGs), and plasmids were respectively predicted using the ResFinder, VirulenceFinder, and PlasmidFinder. Of the 32 isolates, 13 carried resistance genes such that four, six, and 11 contained β-lactam (*bla*TEM), aminoglycoside [*aac-6*(*Ib7*)/*ant-3*(*Iia*)/*aph-3*(*Ib*)/*aph-6*(*Id*)] and tetracycline (*tet*) resistance genes, respectively. The IncF plasmids were most prevalent (n=12, 38.71%) but the highly self-conjugative IncN plasmids occurred simultaneously with the plasmid-borne [quinolones (*QnrS1*/*QnrB7*) and sulfonamide (*sul3*)] ARGs in ≥ 2 *E. coli*. One *E. coli* each was classified as avian pathogenic *E. coli*, atypical enteropathogenic *E. coli*, enterotoxigenic *E. coli*, Shiga toxin-producing enteroaggregative *E. coli*, and enteroaggregative *E. coli*. Pet feces should be carefully handled because they contain virulent and drug-resistant *E. coli*.

Keywords: *Escherichia coli*; antimicrobial resistance; virulence genes, plasmids, diarrhea

Article History
Received: 21.12.2023
Accepted: 23.01.2024
Available online: 20.04.2024

DOI: https://doi.org/10.30704/http-www-jivs-net. 1407165
To cite this article: Falodun, O. I., Rabiu, A., G., Marcus,A. J., Afolabi, M. C., Dada, R. A. (2024). Characterization of virulent *Escherichia coli* in healthy pet dog feces: Implications for public health. ***Journal of Istanbul Veterinary Sciences, 8*(1), 5-12.**
Abbreviated Title*:* J. İstanbul vet. sci.

Introduction

Escherichia coli resident in the gastrointestinal tract of humans and animals are mostly commensal (Majowicz et al., 2014) except for certain lineages that cause diarrhea (Puño-Sarmiento et al., 2013) and extra-intestinal infections (Manges et al., 2019). These pathogenic strains cause different intestinal and extra-intestinal diseases by using their virulence genes which affect several cellular processes. *Escherichia coli* infections can be challenging when pathogenic strains acquire antibiotic-resistant characteristics since this may herald a scenario where pan-resistant pathogenic lineages could emerge in the shared environment between humans, animals, and plants (Ikhimiukor et al., 2022), and this can increase the chance of human acquisition of infections that could be untreatable.

Keeping pet animals such as dogs is common in several households worldwide. The cross-infection of pet owners and diarrheic dogs by *E. coli* in households lacking appropriate animal-waste management systems (Penakalapati et al., 2017) has suggested the need to identify the lineages of the bacterium that are responsible for human infection (Robins-Browne et al.,

*Corresponding Author: Olutayo Israel Falodun
E-mail: falod2013@gmail.com

This work is licensed under the **Creative Commons Attribution 4.0 International License**.

2016; Ashbolt et al., 2018; LeCuyer et al., 2018). Little is known of the *E. coli* lineages that are responsible for shared infections between humans and dogs, besides extraintestinal *E. coli* and uropathogenic *E. coli* (LeCuyer et al., 2018; Manges et al., 2019).

Studying the incidence of pathogenic *E. coli* strains in healthy pet dogs is crucial (Manges et al., 2019) to enhance our understanding of the lineages responsible for shared infections between pet owners and their dogs. Previous studies that performed whole genome sequencing (WGS) of dog-derived *E. coli* either prioritized extended spectrum β-lactamases (ESBLs) investigations (Tudu et al., 2022) or weighed in on the possible role of animal feeds in the spread of resistance by pet animals (Mounsey et al., 2022). We hypothesized that certain lineages of *E. coli*, in this context, healthy pet dog-derived *E. coli* that can resist antibiotics may have evolved genetic traits to persist sufficiently to initiate infections in their host. Since carriage of these genetic traits, otherwise called virulence genes, is the basis upon which the lineages are classified, the current investigation aimed to determine the likely incidence of known *E. coli* pathotypes in freshly passed fecal samples of healthy dogs to deduce possible infection occurrence, and to know whether these pathotypes are reservoirs of mobile resistance traits.

Materials and methods

Selection of drug-resistant *Escherichia coli* for whole genome sequencing: Previously, we described drug-resistant *E. coli* isolated from fecal samples of apparently healthy dogs in Ibadan, Nigeria (Falodun et al., 2022), adhering to established guidelines (Sherwin et al., 2003) and the National Health Research Ethics Committee of Nigeria (NHREC, 2007). Briefly, fresh fecal samples (n=60) collected from dogs into sterile sample bottles were processed to isolate *E. coli* on Eosine Methylene Blue and MacConkey agars. *Escherichia coli* were identified with *uidA* primer through a polymerase chain reaction while confirmed isolates were tested against 10 antibiotics using the Kirby-Bauer disc diffusion method. Selected antibiotics are among the most commonly administered agents in veterinary practices in Nigeria (Olowe et al., 2015, Ogbolu et al., 2020). The ESBL *E. coli* phenotypic detection was performed using the double disc synergy test following CLSI (2021) while isolates showing ESBL phenotype were genotyped for ESBL *blaSHV*, *blaTEM*, and *blaCTX-M* using multiplex PCR. In this study, we retrieved 31 drug-resistant *E. coli* isolates from our previous study (Falodun et al., 2022) and included one additional *E. coli* isolate (INOF032) from the same study making the number of organisms examined equal to 32. All the isolates were subjected to whole genome sequencing using Illumina technology. The sequences generated were inspected for specific diarrheagenic virulence markers. These markers were used to classify the organisms into specific pathogenic lineages.

Extraction of DNA, preparation of library, and whole genome sequencing: The isolates' total genomic DNA was extracted using a Wizard DNA extraction kit (Promega). The extracted genomic DNA was quantified using a dsDNA Broad Range fluorometric assay (Invitrogen). The preparation of DNA libraries was done using the NEBNext Ultra II FS DNA library kit for Illumina (New England Biolabs). Libraries were sequenced at Wellcome Sanger Institute, Cambridge, United Kingdom, on the Illumina HiSeq 2500 instrument (Illumina, San Diego, CA, USA) using 2 × 250 bp paired-end reads.

Bioinformatic analyses of the sequences: Raw reads were trimmed using TrimmomaticPE, and assessed for quality using FastQC, while individual FastQC reports were aggregated into a single report file using MultiQC (Ewels et al., 2016). To further assess the quality of the sequences, reads were assigned taxonomic markers using Kraken (Wood et al., 2015) and these markers were quantified using Bracken (Lu et al., 2017). Based on the quality of the sequence, reads were assembled into contigs using Spades v3.9.0 (Bankevich et al. (2012). The genomes were annotated using Prokka v1.12 (Seemann, 2014) and reads were mapped to a reference genome (*E. coli*_042) to generate a multi-fasta alignment file. The IQTree's Model Selection option was used to determine a best-fit model (GTR+F+ASC+R4) to produce the phylogenetic tree by the IQ-tree (Trifinopoulos et al., 2016), and the tree was visualized using the Microreact tool (Berghain et al., 2018).

Assessment of whole genome sequences for resistance, virulence, and plasmid markers: The sequences were assessed for ARGs, virulence genes, and plasmid replicons using the Centre for Genomic Epidemiology Resfinder (Clausen et al., 2018), VirulenceFinder (Malberg et al., 2020), and PlasmidFinder (Carattoli and Hasman, 2020). The sequence types (STs) were determined with ARIBA using Achtman's multi-locus sequence typing scheme (Clausen et al., 2018). The phylogenetic grouping of the genomes was done by the Ezclermont in silico Clermont Phylotyper (Clermont et al., 2013).

Assignment of sequence number to novel STs: Strains with novel STs have their raw sequences uploaded to the University of Warwick Enterobase platform (https://enterobase.warwick.ac.uk/species/ecoli/upload_reads), curated at https://enterobase.warwick.ac.uk/species/ecoli/my_strains

Table 1. Criteria used for classification of the Escherichia coli strains into pathotypes

Target marker(s)	Other diagnostic target(s)	Predicted Pathotypes	References
eilA/stx1B	*ehxA*, *stx1B*, *eilA*	Shiga toxin-Enteroaggregative *E. coli*	(Robins-Browne et al., 2016)
STA	*tibC*, *east-1*, *F17a*, *F17d*	Enterotoxigenic *E. coli*	(Robins-Browne et al., 2016)
eilA	*fyuA*, *irp2*, *chuA*, *traT*, *ompT*	Enteroaggregative *E. coli*	(Sheikh et al., 2006)
eae	*nleA-2*, *nleA-8*, *nleC-3*, *nleB-14*, *lifA*, *astA*	Atypical Enteropathogenic *E. coli*	(Mercado et al., 2016)
hlyF	*iss*, *iroN*, *ompT*, *cvaC*, *hlyF*, *papC*	Avian Pathogenic *E. coli*	(Ovi et al., 2023)

eilA, *Salmonella enterica hilA*-like gene; *fyuA*, yersiniabactin receptor; *chuA*, haem receptor; *ompT*, outer membrane protein; *ehxA*, enterohemolysin; *stx1B*, Shiga toxin 1B; *F17a, F17d, F17* fimbriae; *iss*, increased serum survival; *cvaC*, colicin V production; *hlyF*, hemolysin F; *eae*, intimin gene; *papC*, P fimbrial gene; nle genes, pro-inflammatory inhibitory proteins; *iroN*, enterobactin siderophore receptor protein; *astA/STA/east-1*, heat-stable enterotoxin; *tibC*, tib adhesin/invasion; *lifA*, lymphocyte inhibitory factor.

and STs were assigned based on the Achtman 7 gene multilocus sequence typing (MLST) scheme. Classification of strains into pathotypes: Genome sequences were inspected for the carriage of specific markers, and the pathotypes were determined using previously recommended protocols outlined in Table 1 (Sheikh et al., 2006; Mercado et al., 2016; Robins-Browne et al., 2016; Ovi et al., 2023).

Availability of whole genome sequence data: Raw sequence datasets generated during this study were deposited at the European Nucleotide Archive with bioproject number PRJEB8667. Accessions are available at the https://www.ebi.ac.uk/ena/browser/view/PRJEB8667.

Results

Detection of antibiotic resistance genes: Thirteen of the 32 *E. coli* isolates bore ARGs and the most prevalent was tetracycline (*tet*) found in 11 (34.36%) isolates followed by sulphonamide (*sul*) and aminoglycoside [*aac-6*(*Ib7*)/*ant-3(Iia)*/*aph-3(Ib)*/*aph-6* (*Id*)] resistance genes carried each by 6 (18.75%) isolates. Plasmid-mediated quinolone resistance (PMQR) genes *qnrs1* (n=3) and *qnrb7* (n=1) were borne by the isolates without co-occurrence. Simultaneous reading of the resistance determinants showed that the isolates carrying qnrs1 harboured *aph-6-ld, aac-6* (*Ib7*), and *blaTEM* while those carrying *aph-6-ld*, *aac-6* (*Ib7*), and *blaTEM-95* also possessed *dfrA14, mef-B, sul3*, and *tetA* genes. Other *ARGs – aadA5, dfrA17*, and *sul2* were found in one and two isolates accordingly (Table 2 and https://microreact.org/project/iEWL5GYVXBcvuje3kAyjkJ-escherichia-coli-antimicrobial-resistance-genes).

Carriage of virulence-associated genes by the isolates: The mean value of VAGs was nine and the range was from five to 28. The glutamate decarboxylase gad- and tellurite resistance terC genes were found in all the isolates while 30 (93.75%) isolates carried the long polar fimbriae *lpfA*. At least a gene responsible for bacterial iron metabolism (iron acquisition, yersiniabactin biosynthesis, and aerobactin) *-iroN, irp2, fyuA, chuA, iutA, iucC- and traT* - were detected in the strains, but *traT*- and *iroN* (n=7) occurred the most. The microcin (*mchC-, mchB-, mcmA-, mchF-, mchF-*) and colicin genes (*cba, cvaC, cma, cvaC*) were correspondingly detected in four and two isolates. A strain carried *iha*, an O157:H7 EHEC nonhemagglutinin, and *efa1* (*lifA*), a lymphocyte inhibitory factor: the toxin gene *astA* and *sitA* were accordingly found in nine and 17 isolates. Immunoglobulin repeat protein (*air*) and *eilA*, a *Salmonella enterica HilA-l*ike regulator seen in EAEC were present in two isolates. However, nine and 11 isolates carried outer membrane protein (*OmpT-*) and increased serum survival (*iss*) genes. Shiga-toxin-related gene (*stx1B*) was found in one *E. coli* containing extra 12 VAGs (Table 2 and https://microreact.org/project/mwV3AQPd59fJrXk8EetChZ-escherichia-coli-virulence-associated-genes).

Detection of plasmid replicons in *E. coli*: Seventeen strains (53.13%) carried various plasmid types while the rest of the isolates did not carry any plasmid replicons. The most occurring plasmid type was IncF found in 12 (37.5%) isolates. The IncF replicons were IncFI- (n=18), IncFII- (n=7), IncX (n=7), IncI-1-α (n=5), IncN (n=3), IncY (n=3), and Col-MG82 (n=1) (Table 2 and https://microreact.org/project/kgLorSjVnomL1Y5baXn3ga-escherichia-coli-plasmids).

Phylogroup and multilocus sequence type of *E. coli*: The phylogroup B1 (n= 22, 68.75%) was predominant as against phylogroup A (n=8, 25%) while one isolate belonged to phylogroup E and the cryptic clade. The most frequently occurring STs were ST2541 and ST7483 (n=5), and ST295 (n=3). The detected novel STs were ST13336 (n=2) and ST13327 (n=1). ST/phylogroup showed that 21 isolates belonged to group B1 while ST2541, ST206, and ST13336 belonged to phylogroup A. A novel ST (ST13327) belonged to group E (Table 2).

Table 2. WGS analysis of *E. coli* showing antimicrobial resistance, virulence, plasmid types, phylogroups, and sequence types

ST/ Phylogroup	Virulence-associated genes	Plasmids	AMR genes	Classifications
ST224/B1	*terC-5, terC-23, lpfA, gad-, hra-11, hra-6, F17a(6), F17a(7), mchC-4, afaA-2, mchB-2,* mcmA-3, afaC-7, mchF-8, mchF-2, f17G-5, hra-2, afaB-6		nil	ETEC
ST13336/E	*terC-5, terC-23, gad-, air-4, iss-3, air-3, chuA-50, eilA-3, air-2, ompT-8, ompT-, sitA, traT-*	IncFIC-FII, IncFIB	nil	EAEC
ST665/B1	*terC-5, terC-23, lpfA, gad-, efa1(7), esp(1), espA(11), tir(30), eae (5), eae(44), eae(7), eae-42, astA-8, iutA-20, tccP-15, iha-14, gad-, nleA-8, nleA-2, nleC-3, nleB-14, espB-7, espF-3, iucC-1, ompT-, traT*	nil	nil	aEPEC
ST7428/B1	*terC-5, terC-23, lpfA, gad-, ompT-8, fyuA-78, irp2, traT, iss-9*	IncFIA-HI1, IncFII-pCoo, IncFIB-pB171	nil	Commensal
ST13336/A	*terC-5, terC-23, lpfA, gad-, iss-4, cba-5, cvaC-10, papC-34, papC-29, iroN-6, mchF-11, ompT-240, cma-12, cvaC-6, hlyF-15, ompT-, sitA, traT-*	IncFII, IncFIB	nil	APEC
ST2467/ cryptic	*terC-5, terC-23, lpfA, gad-, air-4, ehxA(1), sta1-2, astA-4, air-3, stx1B-14, eilA-3, yfcV-42, sitA*	IncFII-pSE11, IncFIB	nil	ST-EAEC
ST2541/A	*terC-5, terC-23, lpfA, gad-, astA-4, sitA*	IncI1-I-α		Commensal
ST2541/A	*terC-5, terC-23, lpfA, gad-, sitA, tsh-3*	Col-MG828, IncFIC-FII, IncI1-I-α		Commensal
ST224/B1	*terC-5, terC-23, lpfA, gad-, hra-11, hra-6, F17a(7), mchC-4, afaA-2, mchB-2,mcmA-3,* afaC-7, mchF-8, f17G-5, hra-2, afaB-6			Commensal
ST155/B1	*terC-5, terC-23, lpfA, gad-, sitA*			Commensal
ST295/B1	*terC-5, terC-23, lpfA, gad- sitA*	IncN, IncX1.1, IncX1.2, IncY	*aac-6(Ib7), ant-3 (Iia), aph-6(Id), qnrS1, bla$_{TEM-95}$, dfrA14, mef-B, sul3, tetA*	Commensal
ST295/B1	*terC-5, terC-23, lpfA, gad-, sitA*	IncN, IncX1.1, IncX1.2, IncY	*aac-6(Ib7), aph-6 (Id), qnrS1, bla$_{TEM-84}$, dfrA14, mef-B, sul3, tetA*	Commensal
ST164/B1	*terC-5, terC-23, lpfA, gad-, hra-11, hra-6, iss-8, ompT-8, ompT-, sitA, traT-*	*IncFIC-FII, IncFIA, IncFIB*		Commensal

ST-EAEC, Shiga toxin/Enteroaggregative *E. coli*; ETEC, Enterotoxigenic *E. coli*; EAEC, Enteroaggregative *E. coli*; APEC, Avian Pathogenic *E. coli*; aEPEC, atypical Enteropathogenic *E. coli*

Classification of the Escherichia coli strains into pathotypes: One *E. coli* isolate each was grouped into five pathotypes, viz. highly contagious APEC, ETEC, EAEC, ST-EAEC, and aEPEC. The isolates possessed an average of nine VAGs, even though most of them (n=27/32) were unclassifiable into specific pathotypes based on our stringent criteria and were therefore regarded as commensals.

Continuous reading of plasmids, antimicrobial resistance, and virulence genes: The investigation of the co-occurrence of ARGs, VAGs, and plasmids using a Venn diagram revealed that none of the isolates carried only ARGs or plasmids (Fig. 1). However, 10 *E. coli* isolates contained VAGs only without harbouring ARGs and plasmids, while separate strains (n=10) carried both VAGs and plasmids. Five *E. coli* isolates carried both ARGs and VAGs as against seven isolates that carried ARGs, VAGs, and plasmid replicons altogether (Figure. 1).

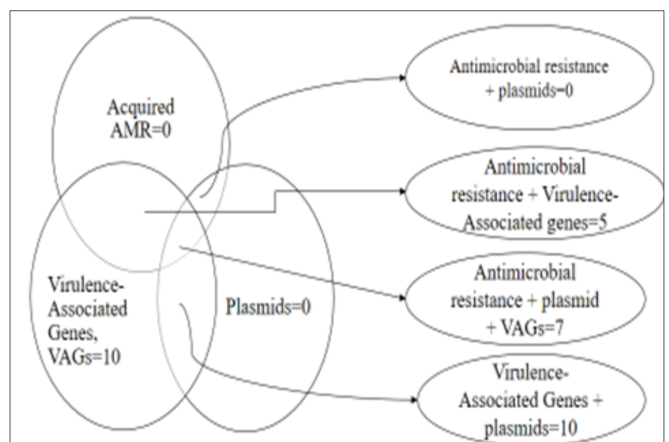

Figure 1. Representation of detected antimicrobial resistance genes (ARGs), virulence-associated genes (VAGs), and plasmids in *E. coli* (n=32) isolated from dog fecal samples. Additional information on accessions and other metadata are available in the Supplementary files.

Table 2 continuation. WGS analysis of *E. coli* showing antimicrobial resistance, virulence, plasmid types, phylogroups, and sequence types.

ST/Phylogroup	Virulence-associated genes	Plasmids	AMR genes	Classifications
ST211/B1 (n=2)	*terC-5, terC-23, lpfA, gad-, astA-4*	IncFII, IncFIB, IncI1-I-α	*aph-3(Ib), aph-6(Id), sul2, tetR, tetB*	Commensal
ST13327/A	*terC-5, terC-23, lpfA, gad-, iss-4, cba-5, cvaC-10, papC-34, iroN-6, mchF-11, ompT-240, cma-12, cvaC-6, hlyF-15, ompT -, sitA, traT-*	IncFII, IncFIB		Commensal
ST162/B1	*terC-5, terC-23, lpfA, gad-, iss-8, ompT-, sitA*			Commensal
ST297/B1	*terC-5, terC-23, lpfA, gad-, iss-8, ompT-8, sitA, traT-*	IncFIA-HI1, IncFII-pCoo, IncFIB-pB171		Commensal
ST2541/A (n=2)	*terC-5, terC-23, lpfA, gad-, hra-11, hra-6, F17a(5), f17G-6*			Commensal
ST13029/B1	*terC-5, terC-23, lpfA, gad-, astA-4, tsh-3, ompT-, sitA*	IncI1-I-α, IncX1.4	*bla*$_{TEM-84}$, *tetA*	Commensal
ST2541/A	*terC-5, terC-23, lpfA, gad-, hra-11, hra-6, F17a(5), f17G-6*			Commensal
ST295/B1	*terC-5, terC-23, lpfA, gad-, sitA*	IncN, IncX1.1, IncX1.2, IncY	*aac-6(Ib7), aph-6 (Id), qnrS1, bla*$_{TEM-95}$, *dfrA14, mef-B, sul3, tetA*	Commensal
ST2067/B1	*terC-5, terC-23, lpfA, gad-, hra-11, iss-3, ompT-147, ompT-, sitA*			Commensal
ST297/B1	*terC-5, terC-23, lpfA, gad-, iss-3, ompT-8, ompT-, sitA*	IncFIC_FII, IncFIB		Commensal
ST206/A	*terC-5, terC-23, gad-*	IncFIB-pHCM2	*aac-6(Ib7), qnrB7, aadA5, dfrA17, sul2*	Commensal
ST7483/B1(n=5)	*terC-5, terC-23, lpfA, gad-, astA-4, iroN-3*		*tetR, tetB*	Commensal

ST-EAEC, Shiga toxin/Enteroaggregative *E. coli*; ETEC, Enterotoxigenic *E. coli*; EAEC, Enteroaggregative *E. coli*; APEC, Avian Pathogenic *E. coli*; aEPEC, atypical Enteropathogenic *E. coli.*

Discussion

We recently reported multiple phenotypic antibiotic resistance for *E. coli* isolates retrieved from the fecal sample of healthy dogs in Ibadan (Falodun et al., 2022). In the current investigation, we leveraged the awareness that pets constitute channels for the spread of highly contagious diseases (Jacob et al., 2015) to sequence the antibiotic-resistant isolates and assess their genomes for resistance markers. We found *blaTEM (*n=4) as the sole ESBL gene that encoded resistance to β-lactam antimicrobials; no other ESBL genes were found in the strains. In 75% of the strains that contained *blaTEM*, there was a co-occurrence of *qnrS1, tetA, sul3, aac-6(Ib7), aph-6(Id)* genes with the highly versatile self-mobilizable IncN and host-specific IncX replicons. Our study is concordant with that of Sumrall et al. (2014) who reported that the occurrence of PMQR with IncX in *E. coli* attracted multiple and transmissible ARGs. The dosing of pets with antibiotics such as tetracycline, sulphonamide, and β-lactam antibiotics by veterinarians is a precursor for the different resistance genes found in the isolates. The presence of mobile antimicrobial resistance genes alongside self-conjugative plasmids in the isolates increases the risk of their contagion and this constitutes a major threat to public health.

The predominant STs of the strains isolated from freshly passed dog faecal samples were ST2541 and ST7483. However, most of the known epidemic *E. coli* strains such as STs-224, 162, 155, 297, and 2067 occurred rarely. It is thus clear that most isolates recovered in this study did not fall into widely circulated *E. coli* lineages of ST131, and ST10 complex-related isolates in Nigeria (Aibinu et al., 2012; Ogbolu et al., 2020; Afolayan et al., 2022). It is well known that ST2541 and others (STs-224, 162, 155, 297) belong to the global lineage associated with ESBL in *E. coli* isolated from dogs in Asia, Europe, and America

(Salgado-Caxito et al., 2020), although, none of the epidemic STs detected in this study carried ESBL genes.

Nevertheless, the *E. coli* phylogroup structure A (n=8,25.0%) and B1 (n=21,67.74%) agree with the previous report of Vega-Manriquez and colleagues who reported a similar pattern of phylogroup A (n=5) and B1 (n=21) in *E. coli* strains isolated from dogs (Vega-Manriquez et al., 2020). The clustering of diarrheagenic *E. coli* in phylogroups A, B1, and E indicates diarrhea association (Clermont et al., 2011) implying that these organisms can cause in the healthy pet dogs and even infect the pet owners.

The detection of highly infectious and pathogenic strains of ETEC, EAEC, ST-EAEC, aEPEC, and APEC strains was worrying because these strains, especially aEPEC have been attributed to diarrhea in dogs (Puño-Sarmiento et al., 2013). EPEC virulence markers (typical or atypical) lead to diarrhea complications as they are differential diagnostic markers in dogs with enteric infections (Kjaergaard et al., 2016). ST-EAEC, cryptic *E. coli* that harbored *stx1B*, enterohemolysin *ehxA*, *hilA*-homologue *eilA*, and toxigenic *sta1* genes, are predictors for bloody diarrhea and hemolytic uremic syndrome in STEC infections (hua et al., 2021). Even though in Nigeria, there is a rarity of information on the incidence of STEC in dogs; PCR characterization of STEC genes retrieved from human clinical samples (Olowe et al., 2015) showed a moderate prevalence of *stx1* (13.9%), *stx2* (6.9%) and *hlyA* (2.0%). Therefore, given the opportunity of exposure, ST-EAEC acquisition by humans from dogs is possible.

The pathogenicity island, PAI O-122 (*lifA/efa-1 and nleA-E*), present in some strains showed that the strains can cause severe diarrhea in children (Mercado et al., 2016). These strains possibly progressed through enforcing maintenance in their host (Doumith et al., 2012) and then acquired virulence factors to emerge as new *E. coli* subtypes. Feng et al. (1988) already showed evidence of genetic re-arrangements of a novel assortment of *E. coli* virulence genes in EHEC. A key study limitation is that the number of *E. coli* isolates obtained and sequenced was small to generalize the evolution of novel *E. coli* strains in apparently healthy pet dogs. Additional studies are needed to examine the specific mechanism(s) that could be responsible for the evolution of new pathogenic subtypes especially in healthy pet animals.

Conclusions

The possible exposure to distinct *E. coli* pathogenic lineages in fecal materials of healthy dog by humans poses a risk of disease spread from pets to humans. The delineation of the strains into specific lineages afforded by the whole genome sequencing further revealed the co-incidence of antimicrobial resistance genes and mobile genetic elements such as plasmids. Therefore, pet owners are strongly advised to handle fecal samples from their household dogs with care to minimize the risk of the environmental spread of infections and diseases. Antimicrobial surveillance systems should be expanded to incorporate healthy pet dogs to reduce the chance of disease spread between pets and their owners.

Acknowledgments

We graciously acknowledge the technical assistance offered by Iruka N. Okeke of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Nigeria towards the sequencing of the isolates.

Conflict of Interest

The authors declared that there is no conflict of interest

References

Afolayan, A. O., Aboderin, A. O., Oaikhena, A. O., Odih, E. E., Ogunleye, V. O., Adeyemo, A. T., Adeyemo, A. T., Bejide, O. S., Underwood, A., Argimón, S., Abrudan, M., Egwuenu, A., Ihekweazu, C., Aanensen, D. M., & Okeke, I. N. (2022). An ST131 clade and a phylogroup A clade bearing an O101 -like O-antigen cluster predominate among bloodstream Escherichia coli isolates from South-West Nigeria hospitals. Microbial Genomics 8(12), mgen000863.

Aibinu, I., Odugbemi, T., Koenig, W., & Ghebremedhin, B. (2012). Sequence type ST131 and ST10 complex (ST617) is predominant among CTX-M-15-producing Escherichia coli isolates from Nigeria. Clinical Microbiology and Infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases 18(3), E49–E51

Ashbolt, N., Pruden, A., Miller, J., Riquelme, M.V., & Maile-Moskowitz, A. (2018). Antimicrobial resistance: fecal sanitation strategies for combatting a global public health threat. Rose, J.B., Jiménez-Cisneros, B., eds, Glob Water Pathogen Project.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology: A Journal of Computational Molecular Cell Biology 19(5), 455–477.

Beghain, J., Bridier-Nahmias, A., Le Nagard, H., Denamur, E., & Clermont, O. (2018). Clermont Typing: an easy-to-use and accurate in silico method for Escherichia genus strain phylotyping. Microbes 4:e000192.

Carattoli, A., & Hasman, H. (2020). PlasmidFinder and in silico pMLST: Identification and typing of plasmid replicons in whole-genome sequencing (wgs). Methods in Molecular Biology (Clifton, N.J.) 2075, 285–294.

Clausen, P.T.L.C., Aarestrup, F. M., & Lund, O. (2018). Rapid

and precise alignment of raw reads against redundant databases with KMA. BMC Bioinformatics 19(1), 307.

Clermont, O., Christenson, J. K., Denamur, E., & Gordon, D. M. (2013). The Clermont Escherichia coli phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environmental Microbiology Reports 5(1), 58–65.

Clermont, O., Olier, M., Hoede, C., Diancourt, L., Brisse, S.,

Clermont, O., Olier, M., Hoede, C., Diancourt, L., Brisse, S., Keroudean, M., Glodt, J., Picard, B., Oswald, E., & Denamur, E. (2011). Animal and human pathogenic Escherichia coli strains share common genetic backgrounds. Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases 11(3), 654–662.

Doumith, M., Dhanji, H., Ellington, M.J., Hawkey, P., & Woodford, N. (2012). Characterization of plasmids encoding extended-spectrum-lactamases and their addiction systems circulating among Escherichia coli clinical isolates in the UK. Journal of Antimicrobial Chemotherapy 67(4), 878-885.

Ewels, P., Måns, M., Sverker, L., & Max, K. (2016). MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 32(19), 3047-3048.

Falodun, O. I., Afolabi, M. C., & Rabiu, A. G. (2022). Detection of extended Spectrum β-lactamase (ESBL) genes in Escherichia coli isolated from fecal samples of apparently healthy dogs in Ibadan. Animal Gene 26, 2352-4065.

Feng, P., Lampel, K. A., Karch, H., & Whittam, T. S. (1998). Genotypic and phenotypic changes in the emergence of Escherichia coli O157:H7. The Journal of Infectious Diseases, 177(6), 1750–1753.

Hua, P., Badat, M., Hanssen, L. L. P., Hentges, L. D., Crump, N., Downes, D. J., Jeziorska, D. M., Oudelaar, A. M., Schwessinger, R., Taylor, S., Milne, T. A., Hughes, J. R., Higgs, D. R., & Davies, J. O. J. (2021). Defining genome architecture at base-pair resolution. Nature 595(7865), 125–129. https://doi.org/10.1038/s41586-021-03639-4

Ikhimiukor, O. O., Odih, E. E., Donado-Godoy, P., & Okeke, I. N. (2022). A bottom-up view of antimicrobial resistance transmission in developing countries. Nature Microbiology 7(6), 757–765. https://doi.org/10.1038/s41564-022-01124-w

Jacob, J., & Lorber, B. (2015). Diseases transmitted by man's best friend: The Dog. Microbiology Spectrum 3(4), 10.1128/microbiolspec.IOL5-0002-2015.

Kjaergaard, A.B., Carr, A.P., & Gaunt, M.C. (2016). Enteropathogenic Escherichia coli (EPEC) infection in association with acute gastroenteritis in 7 dogs from Saskatchewan. Canadian Veterinary Journal 57.

LeCuyer, T. E., Byrne, B. A., Daniels, J. B., Diaz-Campos, D. V., Hammac, G. K., Miller, C. B., Besser, T. E., & Davis, M. A. (2018). Population structure and antimicrobial resistance of canine uropathogenic Escherichia coli. Journal of Clinical Microbiology 56(9), e00788-18.

Lu, J., Breitwieser, F. P., Thielen, P., & Salzberg, S. L. (2017). Bracken: estimating species abundance in metagenomics data. PeerJ Computer Science 3: e104

Majowicz, S. E., Scallan, E., Jones-Bitton, A., Sargeant, J. M., Stapleton, J., Angulo, F. J., Yeung, D. H., & Kirk, M. D. (2014). Global incidence of human Shiga toxin-producing Escherichia coli infections and deaths: a systematic review and knowledge synthesis. Foodborne Pathogens and Disease 11(6), 447–455.

Malberg Tetzschner, A. M., Johnson, J. R., Johnston, B. D., Lund, O., & Scheutz, F. (2020). In silico genotyping of Escherichia coli isolates for extraintestinal virulence genes by use of whole-genome sequencing data. Journal of Clinical Microbiology 58(10), e01269-20.

Manges, A. R., Geum, H. M., Guo, A., Edens, T. J., Fibke, C. D., & Pitout, J. D. D. (2019). Global extraintestinal pathogenic Escherichia coli (ExPEC) lineages. Clinical Microbiology Reviews 32(3), e00135-18.

Mercado, E. H., Piscoche, C., Contreras, C., Durand, D., Riveros, M., Ruiz, J., & Ochoa, T. J. (2016). Pathogenicity Island O-122 in enteropathogenic Escherichia coli strains is associated with diarrhea severity in children from Lima Peru. International Journal of Medical Microbiology 306 (4), 231–236.

Mounsey, O., Wareham, K., Hammond, A., Findlay, J., Gould, V. C., Morley, K., Cogan, T. A., Turner, K. M. E., Avison, M. B., & Reyher, K. K., 2022, Evidence that fecal carriage of resistant Escherichia coli by 16-week-old dogs in the United Kingdom is associated with raw feeding. One Health (Amsterdam, Netherlands) 14, 100370.

National Health Research Ethics Committee of Nigeria, NHREC, 2007, Federal Ministry of Health. Available at http://www.nhrec.net (Accessed on 15 September, 2020).

Ogbolu, D. O., Piddock, L. J. V., & Webber, M. A. (2020). Opening Pandora's box: High-level resistance to antibiotics of last resort in Gram-negative bacteria from Nigeria. Journal of Global Antimicrobial Resistance, 21, 211–217.

Olowe, O. A., Adewumi, O., Odewale, G., Ojurongbe, O., & Adefioye, O. J. (2015). Phenotypic and molecular characterization of extended-spectrum beta-lactamase-producing Escherichia coli obtained from animal fecal samples in Ado Ekiti, Nigeria. Journal of Environmental and Public Health 497980.

Ovi, F., Zhang, L., Nabors, H., Jia, L., & Adhikari, P. (2023). A compilation of virulence-associated genes that are frequently reported in avian pathogenic Escherichia coli (APEC) compared to other E. coli. Journal of Applied Microbiology 134 (3), lxad014.

Penakalapati, G., Swarthout, J., Delahoy, M. J., McAliley, L., Wodnik, B., Levy, K., &

Freeman, M. C. (2017). Exposure to animal feces and human health: a systematic review and proposed research priorities. Environmental Science and Technology 51(20), 11537–11552.

Puño-Sarmiento, J., Medeiros, L., Chiconi, C., Martins, F., Pelayo, J., Rocha, S., Blanco, J., Blanco, M., Zanutto, M., Kobayashi, R., & Nakazato, G. (2013). Detection of diarrheagenic Escherichia coli strains isolated from dogs and cats in Brazil. Veterinary Microbiology 166(3-4), 676–680.

Robins-Browne, R. M., Holt, K. E., Ingle, D. J., Hocking, D. M., Yang, J., and Tauschek, M. (2016). Are Escherichia coli pathotypes still relevant in the era of whole-genome sequencing? Frontiers in Cellular and Infection Microbiology 6, 141.

Salgado-Caxito, M., Benavides, J. A., Adell, A. D., Paes, A. C., and Moreno-Switt, A. I. (2021). Global prevalence and molecular characterization of extended-spectrum β-lactamase producing-Escherichia coli in dogs and cats - A scoping review and meta-analysis. One Health (Amsterdam, Netherlands), 12, 100236.

Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. Bioinformatics (Oxford, England) 30(14), 2068–2069.

Sheikh, J., Dudley, J.E.G., Sui, B., Tamboura, B., Suleman, A., & Nataro, J.P. (2006). EilA, a HilA-like regulator in enteroaggregative Escherichia coli. Molecular Microbiology 61, 338-350

Sherwin, C. M., Christiansen, S. B., Duncan, I. J., Erhard, H. W., Lay, D. C., Mench, J. A., O'Connor, C. E., & Petherick, J. C. (2003). Guidelines for the ethical use of animals in applied ethology studies. Applied Animal Behaviour Science 81, 291–305.

Sumrall, E. T., Gallo, E. B., Aboderin, A. O., Lamikanra, A., & Okeke, I. N. (2014). Dissemination of the transmissible quinolone-resistance gene qnrS1 by IncX plasmids in Nigeria. PloS One 9(10), e110279. https://doi.org/10.1371/journal.pone.0110279

Trifinopoulos, J., Nguyen, L. T., von Haeseler, A., & Minh, B. Q. (2016). W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research, 44 (W1), W232–W235.

Tudu, R., Banerjee, J., Habib, M., Bandyopadhyay, S., Biswas, S., Kesh, S. S., Maity, A., Batabyal, S., & Polley, S. (2022). Prevalence and molecular characterization of extended-spectrum β-lactamase (ESBL) producing Escherichia coli isolated from dogs suffering from diarrhea in and around Kolkata. Iranian Journal of Veterinary Research 23(3), 237–246.

Vega-Manriquez, X. D., Ubiarco-López, A., Verdugo-Rodríguez, A., Hernández-Chiñas, U., Navarro-Ocaña, A., Ahumada-Cota, R. E., Ramírez-Badillo, D., Hernández-Díaz de León, N., & Eslava, C. A. (2020). Pet dogs potential transmitters of pathogenic Escherichia coli with resistance to antimicrobials. Archives of Microbiology 202(5), 1173–1179.

Wood, D. E., & Salzberg, S. L. (2014). Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biology 15: R46.