Original Research Article Epidemiology and Public Health

Pathotype and virulence of *Escherichia coli* from adult human diarrheal feces and water sources in Lagos, Nigeria

Nwamaka Herrienta Igbokwe[1], Abel Olusola Idowu[1], Chijioke Emmanuel Ezeobiora[1], Bukola Aminat Oseni[2]

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria,
[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria.

*Corresponding author:
Nwamaka Herrienta Igbokwe, PhD
Department of Pharmaceutical
Microbiology and
Biotechnology, Faculty of
Pharmacy, University of Lagos,
Lagos, Nigeria.

nigbokwe@unilag.edu.ng

ABSTRACT

Objectives: The prevalence of *Escherichia coli* strains from fecal diarrheal specimens and water samples underscores the imperative of exploring the symbiotic interplay between microbial ecologies in elucidating the pathophysiology of diarrheal infections among the adult populace. This study determined the different pathotypes of *E. coli* strains in water and stool samples from a Nigerian state, Lagos, and the different virulence factors they exhibit.

Materials and Methods: *E. coli* strains were isolated and characterized by presumptive coliform tests from stool samples of patients with diarrheal and from different water sources in Lagos. Multiplex conventional and real-time polymerase chain reaction (PCR) studies that utilized a comprehensive set of 22 primers that enabled the selective amplification of 11 virulence genes, namely: *stx1, stx2, eae, bfp, lt, st11, virF, ipaH, aafII, daaE,* and *uidA* were used to detect different categories of diarrheagenic *E. coli* pathotypes.

Results: The real-time and conventional PCR analysis of the 204 *E. coli* strain from the diarrheal stool and water samples detected the six diarrheagenic *E. coli* pathotypes (enterohemorrhagic, enteropathogenic, enterotoxigenic, enteroinvasive, enteroaggregative, and diffuse adherent) and their virulence toxins; *stx2, stx1, eae, bfp, st11, lt, virF, aafII, daaE,* and *uidA* with an exception of *ipaH* toxin, the gene for enteroinvasive *E. coli,* which was not detected. Enterohemorrhagic/enteropathogenic *E. coli* toxin *eae,* 18 (32.29%), was the most detected toxin next to *uidA* which was isolated from all the samples of *E. coli* strains from Lagos.

Conclusion: The expression of these virulence genes shows that these organisms exhibit a high degree of pathogenicity, thereby presenting a substantial danger to public health.

Keywords: *Escherichia coli*, Pathotype, Virulence, Enteropathogenic, Diarrheal

INTRODUCTION

The prevalence of diarrheal illness poses a substantial public health concern.[1-3] especially in developing countries[4-6] where the disease burden is more on children[7-9] and holds precedence as the leading cause of mortality[9-11] with an attributed toll of approximately 2 million morbidities each year worldwide.[12-14] There is a compelling link between diarrheal diseases and water contamination on a global scale.[15,16] The etiologic factors associated with this disease include microbial agents which are usually transmitted through food and water contaminated with human feces.[15,17] Diarrheal disease alone causes 2.2 million of the 3.4 million water-related deaths per

year.[18] The features of acute diarrhea vary from place to place depending on geography and socioeconomic variables.[18,19] The major causes of this illness include poor quality of water, poor hand and food hygiene, and poor sanitation[18,19] In developed countries, the mortality rates have declined considerably in recent times due to improvements in general health hygiene and advances in health care. The morbidity persists in so many other countries where outbreaks of diarrheal diseases continue to affect millions of infants, young children, the immune-compromised, and adults.[2] Foreigners visiting tropical developing countries frequently experience traveler's diarrhea, caused by agents that are endemic in those countries because visitors have not had the opportunity to develop protective immunity against it.[20,21]

Most *Escherichia coli* strains are commensals,[22] however, some strains have developed the ability to cause diseases of the gastrointestinal tract, urinary tract, or central nervous systems even in the most robust human hosts. Strains that cause enteric infections are designated diarrheagenic *E. coli*, a group that includes emergent pathogens with public health relevance worldwide. Diarrheagenic *E. coli* strains can be divided into six main categories based on their distinct molecular, clinical, and pathological features.[23,24] The strains include emergent pathotypes that differ in their virulence factors (toxins). They are enterohemorrhagic *E. coli* (EHEC), a Shiga toxin-producing *E. coli* that is characterized by the production of two potent Shiga-like cytotoxins 1 and 2 (Stx1 and Stx2).[25] Infection with EHEC is a leading cause of bloody diarrhea and hemorrhagic colitis, occasionally resulting in life-threatening systemic complications including hemolytic uremic syndrome. Enteropathogenic *E. coli* (EPEC) harbors a pathogenicity island that encodes a series of proteins involved in the adhering to and expunging alterations of the intestinal microvilli of the host cell.[26] Attachment to the intestinal microvilli is mediated by an outer membrane protein called intimin, encoded by *eae*, and another adherence gene factor encoded as bundle-forming pili, *bfp*.[23] Enterotoxigenic *E. coli* produces heat-labile (LT) or heat-stable (ST) enterotoxins which are generally represented as *lt and st11* genes. Enteroinvasive *E. coli* (EIEC) has biochemical, physiological, and genetic properties similar to those of Shigella. It causes an epithelial invasion of the large bowel leading to inflammation and ulceration of the mucosa.[27] The genes (*ipa*H, *ipa*BCD, *ial*, *vir*F, *vir*B, *sig*A, *set*1A, *sep*A, *sat*, *pic*, *set*1B, and *sen*) related to invasion are located in a virulence plasmid.[28] Enteroaggregative *E. coli* (EAggEC) are a heterogeneous group and have been traditionally defined by their characteristic "stacked brick"-like aggregative adherence (AA) to HEp-2 cells.[29-31] This *E. coli* strain adheres to the intestinal epithelial cells causing biofilm formation, mucus secretion, mucosal inflammation, and cytotoxic damage.[32] The virulence factors involved include the AA fimbriae (AAF) which is coded as *aafII* gene.

Diffuse adherent *E. coli* (DAEC) are characterized by the presence of Afa/Dr. adhesion, *daaE* genes.[33] The different *E. coli* pathotypes exhibit different virulence factors that enhance the disease burden. This study aimed at using the multiplex conventional and real-time polymerase chain reaction (PCR) to determine the virulence toxins and pathotypes of *E. coli* strains from Lagos, Nigeria.

MATERIALS AND METHODS

Ethical statement

The protocol for the study was reviewed and approved by the Ethics and Human Subjects Committee of the College of Medicine, University of Lagos, Nigeria, with Protocol number: CM/COM/08/VOL.XXV. Study participants and (or) their guardians provided written informed and/or assent and the research was conducted in compliance with the Helsinki Declaration on the conduct of human health research.

Collection of diarrheal stool samples

Adult diarrheal stool samples were collected from seven densely populated localities (Alimosho, Shomolu, Oshodi, Surulere, Lagos Mainland, Ikeja, and Mushin) in Lagos state as indicated in Figure 1. Stool samples were collected from patients suffering from Diarrhea (age range, 18–65 years) who reported at the outpatient units of general hospitals in each of the sampled localities in Lagos and some medical laboratories in the localities between April 2011 and March 2012. Fresh fecal samples were immediately placed in a specialized transport medium (Stuart's transport medium, Oxoid®, Basingstoke, United Kingdom) to maintain the freshness and the viability of microorganisms during transit. The samples were kept on ice while being transported to the laboratory for subsequent same-day analysis.

Control stool specimens were obtained from apparently healthy adults while informed consent of the patients used in this study was obtained. None of the patients reported receiving antibiotic therapy <3 weeks before sampling. Presumptive coliform tests for isolation and characterization of *E. coli* strains from diarrheal stool samples and water samples were carried out in the Department of Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Lagos and the Department of Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria. The protocol involved the isolation of one *E. coli* strain per sample.

Adult diarrheal stool samples and control (from volunteered healthy individuals), water samples, and the isolated *E. coli*

Figure 1: Map of Lagos state indicating the localities (circled in red) from where stool and water samples were collected.

strains from Lagos were transported on ice in March 2012 to the city of Milwaukee Health Department, United States of America for further investigations.

Collection of water samples

Sachet "pure water" collection

Commercial sachets "pure water produced in the locality under study were purchased from the open markets in each of the stated localities in Lagos."

Tap water collection

The tap faucets were flamed, turned on, and allowed to flow for 5 min. 100 mL of water was collected in a sterile bottle container; the bottleneck was flamed, covered, and sent to the laboratory for analysis.

Well water

The sterile bottle was dipped into the well, stirred, and pulled out carefully, avoiding the well edges when filled with water.

Canal water collection

The samples collected from open drains and canals must be representative of the areas being sampled. This was therefore achieved by collecting, pooling together, and stirring water samples from various locations and depths in the canal. The sterile screwed sample bottles were dipped into various locations of the canals, swirled, and filled with water for analysis.

Isolation of *E. coli* from diarrheal fecal samples and aqueous samples

The combined methods of the conventional Clinical and Laboratory Standards Institute and Peter *et al.*[34,35] were used in this investigation which involved culturing and sub-culturing into various diagnostic media for enteric bacterial pathogens for each of the samples (diarrheal stool and water). The samples were introduced into peptone water (Oxoid®) and then placed in an incubator set at 37°C for 24 h. Swabs of stool samples were swirled in 5 mL MacConkey broth containing Durham tubes and incubated for 24 h at 37°C. From each of the broths showing acid and gas, subcultures were made into clearly labeled MacConkey agar, blood agar, and eosin methylene blue agar (EMBA) (Oxoid, Basingstoke, England) and placed in an incubator for 24 h at 37°C. One colony from either of the plates with distinct *E. coli* morphologies was subcultured in freshly prepared EMBA and incubated for 24 h at 37°C. For the water samples, 1 mL of 1:10^6 dilution of the canal water, 1 mL of well, dechlorinated tap, and sachet water each was subcultured in 5 mL MacConkey broth and incubated for 24 h. After 24 h of incubation, swabs of each sample were streaked on MacConkey agar and incubated for 24 h. A colony from each of the samples showing clear morphology of *E. coli* was streaked on separate clearly labeled EMBA plates and incubated for 24 h. From EMBA plates, a colony of each strain was transferred into blood agar and incubated for 18 h. The isolated *E. coli* strains were typed using the analytical profile index (API)[36] which involved the use of 20 E strips (Bio Merieux®, Marcy-I'Etiole, France). The characterized *E. coli* strains were stored in duplicate motility agar and 20%v/v glycerol and trypticase soy broth (TSB).

Characterization and typing of *E. coli* strains using the API

API 20 E V 4.0 test kit (Biomerieux, USA) for Enterobacteriaceae was used for the analysis. A total of 2–5 colonies of 18-h blood agar culture of each suspected *E. coli* strain were suspended in 5 mL of normal saline. Incubation boxes (tray and lid) were prepared by distributing 5 mL of distilled water into the honeycombed wells of the tray to create a humid atmosphere and strips were placed in each of them. The identification of each isolate was recorded on the elongated flap of the designated tray. The bacterial suspension of each strain was carefully distributed into the tubes of their corresponding strips according to the manufacturer's instructions. The closed incubation boxes were incubated for 18 h at 37°C. After incubation, specific reagents were added to some of the tubes and color reactions were observed as positive or negative after 10 min. Identification was obtained using the API identification software and also referring to the manual table. The confirmed *E. coli* strains were stored in duplicates in (i) motility agar and (ii) 20%v/v glycerol + TSB.

Nucleic acid extraction from *E. coli* strains

DNA from *E. coli* was extracted from whole organisms by boiling using the previously described method[23] with slight modification. *E. coli* strains were sub-cultured onto blood agar and incubated for 18 h. One colony of each strain was suspended in 500 µL of PCR-grade water in a capped tube and boiled at 100°C for 10 min. The tubes were removed from boiling and placed directly on ice for 2 min. The lysate was centrifuged at 4°C, 14000 rpm for 10 min. 400 µL of the supernatant was stored at −80°C for further investigations.

The methodology outlined by Vidal *et al.*[23] was utilized for the amplification and identification of virulence toxin genes through conventional multiplex real-time PCR.

Single multiplex PCR detection of *E. coli* virulence toxins

The elaboration and detection of the genes encoding toxins and factors contributing to pathogenicity were done by real-time PCR using a conventional multiplexing technique previously described.[23] Diarrheagenic *E. coli* target toxins include enterohemorrhagic, Shiga toxin-producing (stx_1, stx_2) genes, enteropathogenic (*eae, bfp*) genes, enterotoxigenic (*stII* and *lt*) genes, enteroinvasive (*virF* and *ipaH*) genes, enteroaggregative (*aafII*) gene, and diffuse adherent (*daaE*) gene. This technique utilized a comprehensive set of 22 primers that enabled the selective amplification of 11 virulence genes as shown in Table 1. Diarrheagenic *E. coli* reference strains, 011 (stx_1 and stx_2), 2348/69 (bfp and *eae*), H10407 (*st and lt*), EI-34 Nal-R (*ipaH and virF*), EI 34

Table 1: Primers used in the multiplex PCR for amplification of diarrheagenic *E. coli* Genes, their sizes, and references.

Strain	Toxin	Primers	Amplicon size (bp)	Reference
	*UidA*F	5'd CAGTCTGGATCGCGAAAACTG 3'	180	[37]
	*UidA*R	5'd ACCAGACGTTGCCCACATAATT 3'		
EHEC	*St×1*F	5'd CAGTTAATGTGGTGGCGAAGG 3'	348	[9]
	*St×1*R	5'd CACCAGACCATGTAACCGCTG 3'		
EHEC	*St×2*F	5'd ATCCTATTCCCGGGAGTTTACG 3'	582	[9]
	*St×2*R	5'd GCGTCATCGTATACACAGGAGC 3'		
EPEC	*Eae*F	5'd TCAATGCAGTTCCGTTATCAGTT 3'	482	[38]
	*Eae*R	5'd GTAAAGTCCGTTACCCCAACCTG 3'		
EPEC	*Bfp*F	5'd GGAAGTCAAATTCATGGGGGTAT 3'	300	[9]
	*Bfp*R	5'd GGAATCAGACGCAGACTGGTAGT 3'		
ETEC	*LT*F	5'd GCACACGGAGCTCCTCAGTC 3'	218	[39]
	*LT*R	5'd TCCTTCATCCTTTCAATGGCTTT 3'		
ETEC	*STII*F	5'd AAAGGAGAGCTTCGTCACATTTT 3'	129	[9]
	*STII*R	5'd AATGTCCGTCTTGCGTTAGGAC 3'		
EIEC	*Vir*F	5'd AGCTCAGGCAATGAAACTTTGAC 3'	618	[23]
	*Vir*R	5'd TGGGCTTGATATTCCGATAAGTC 3'		
EIEC	*IpaH*F	5'd CTCGGCACGTTTTAATAGTCTGG 3'	933	[23]
	*IpaH*R	5'd GTGGAGAGCTGAAGTTTCTCTGC 3'		
DAEC	*DaaE*F	5'd GAACGTTGGTTAATGTGGGGTAA 3'	542	[23]
	*DaaE*R	5'd TATTCACCGGTCGGTTATCAGT 3'		
EAggEC	*AafII*F	5'd CACAGGCAACTGAAATAAGTCTGG 3'	378	[23]
	*AafII*R	5'd ATTCCCATGATGTCAAGCACTTC 3'		

F: Forward, R: Reverse, Bp: Base pairs, PCR: Polymerase chain reaction, *E. coli*: *Escherichia coli*, EHEC: Enterohemorrhagic *Escherichia coli*, EPEC: Enteropathogenic *Escherichia coli*, ETEC: Enterotoxigenic *Escherichia coli*, EIEC: Enteroinvasive *Escherichia coli*, EAggEC: Enteroaggregative *Escherichia coli*, DAEC: Diffuse adherent *Escherichia coli*

Strep-R (*ipaH and virF*), O42 (*aafII*), 933J (*stx1, stx11, eae*), and F-1845 (*daaE*), were used as positive controls while *Shigella flexneri* and PCR grade water were used as negative controls [Figure 1].

The reaction mixture (PCR master mix), 25 μL, was prepared with OmniMix HS (contains 3U TaKaRa hot start Taq polymerase, 200 μM dNTP, 4 mM MgCl₂, 25 mM HEPES buffer pH 8.0 ± 0.1), 2 pmol of each primer, and 5 μL of template DNA. The PCR process involved 35 cycles, where each cycle comprised 1.5 min at 94°C for denaturation, followed by 1.5 min at 60°C for primer annealing, and finally, 1.5 min at 72°C for strand elongation.

PCR products were observed through the utilization of 1.5% agarose gel electrophoresis combined with ethidium bromide staining for visualization, and the gel pictures taken. A 100 base pair (bp) size ladder (1500–100 bp) was used to identify PCR amplicons. The positive controls used were 011: 011 (*stx₁ and stx₂*), 2348/69 (bfp and *eae*), H10407 (*st and lt*), EI-34 Nal-R (*ipaH and virF*), EI 34 Strep-R (*ipaH and virF*), O42 (*aafII*), and F-1845 (*daaE*) while *S. flexineri* and PCR grade water were used as negative controls.

Statistical analysis

Data were analyzed using the Z-test involving the use of the unweighted-pair group method. The statistical significance threshold was established at $P \leq 0.05$ also in accessing the manifestation of toxin genes by the various strains of *E. coli*.
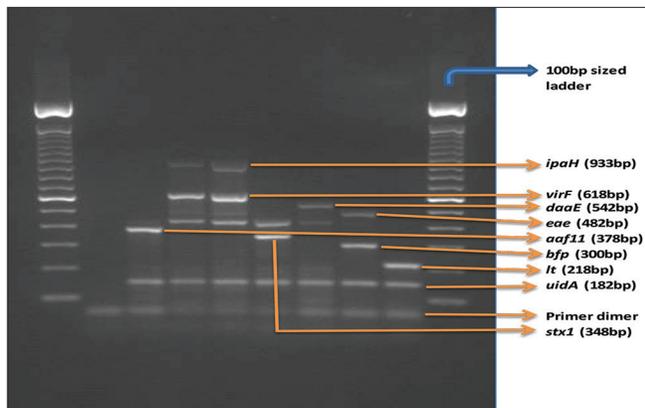

Figure 2: Multiplex PCR gel analysis of the control [positive (reference strains) and negative strains]. The positive strains: 011 (*stx₁* and *stx₂*), 2348/69 (bfp and *eae*), H10407 (*st* and *lt*), EI-34 Nal-R (*ipaH* and *virF*), EI 34 Strep-R (*ipaH* and *virF*); O42 (*aafII*) and F-1845 (*daaE*). Lanes 1 and 10, 100bp sized ladder; lane 2, negative control (PCR grade water); lane 3, 042; lane 4, EI-34 Nal-R; lane 5, EI 34 Strep-R; lane 6, 933J; lane 7, F-1845; lane 8, 2348/69 and lane 9, H10407.

Table 2: Percentage number of toxins expressed by the *E. coli* strains.

E. coli pathotype	Toxins expressed	% Number of each toxin expressed+uidA
EHEC	st×1	7 (4.90)
	st×2	11 (7.69)
EPEC	Eae	85 (59.44)
	Bfp	10 (6.99)
ETEC	Lt	20 (13.99)
	st11	4 (2.79)
EIEC	virF	2 (1.39)
	ipaH	0
EAggEC	aaf11	2 (1.39)
DAEC	daaE	2 (1.39)
Total=6	10+uidA	143+uidA

E. coli: *Escherichia coli*, EHEC: Enterohemorrhagic *Escherichia coli*, EPEC: Enteropathogenic *Escherichia coli*, ETEC: Enterotoxigenic *Escherichia coli*, EIEC: Enteroinvasive *Escherichia coli*, EAggEC: Enteroaggregative *Escherichia coli*, DAEC: Diffuse adherent *Escherichia coli*
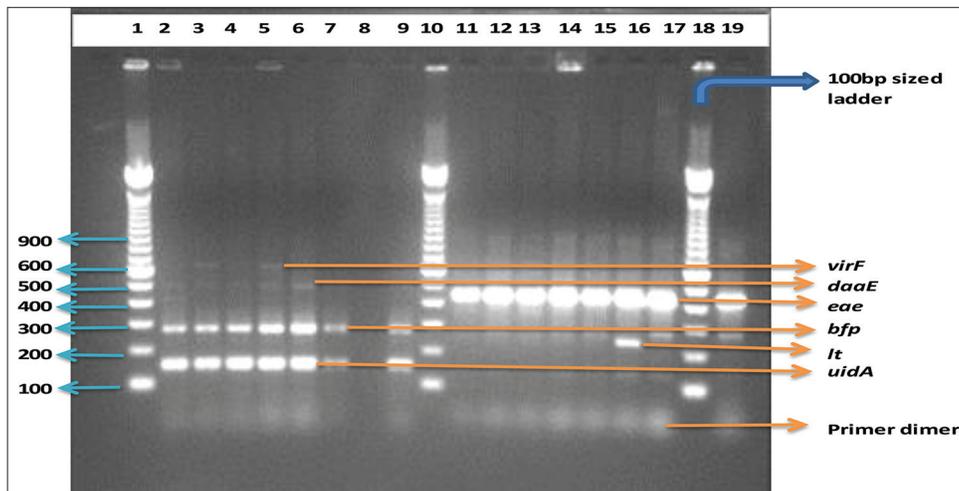

Figure 3: Multiplex PCR gel analysis of diarrhoeagenic *E. coli* strains from Lagos and some selected reference strains. Lanes 1, 10 and 18 are 100-bp sized ladder; lane 19, *bfp* and *eae* positive control while lane 8 is the negative control (PCR grade water)

RESULTS

Detection of *E. coli* pathotypes and their virulence toxins using real-time and conventional PCR

The real-time and conventional PCR analysis of the 204 *E. coli* strains from the diarrheal stool and water expressed different compositions of toxins: *Stx2, stx1, eae, bfp, st11, lt, virF, aafII, daaE,* and *uidA* with an exception of *ipaH* toxin, as also observed from the positive standard [Figure 2], the gene for EIEC, which was not detected [Figure 3]. A total of 143 toxins (101 from fecal and 42 from water samples) and *uidA* were detected from the screened *E. coli* strains. The six categories of diarrheagenic *E. coli* with their corresponding toxins (enterohemorrhagic (*stx$_1$, stx$_2$*), enteropathogenic (*eae, bfp*), enterotoxigenic (*stII* and *lt*), enteroinvasive (*virF* and), enteroaggregative (*aafII*), and diffuse adherent including the less common DAEC with its corresponding toxin (*daaE*)) were identified [Table 2]. EAggEC toxin, *aafII* 2/143 (1.39%), EIEC
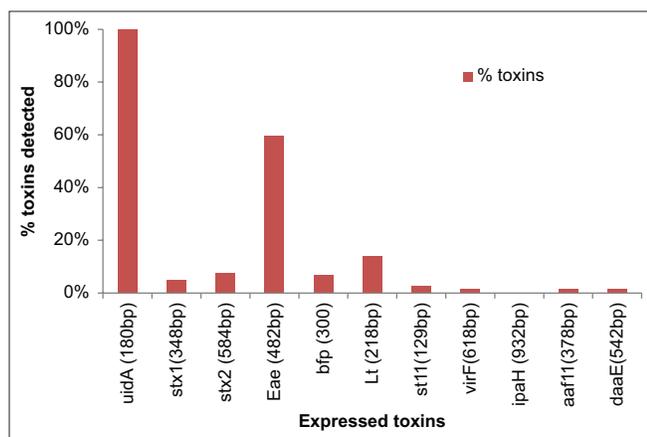
toxin *virF* 2/143 (1.39%), and DAEC toxin *daaE* 2/143 (1.39%) were the least toxins detected as shown in Table 2. *AafII* 2/143 (1.39%) *and virF* 2/143 (1.39%) toxins were detected only from water isolates. *UidA* toxins were detected in all (stool and water) isolates, [Figure 1] followed by EPEC toxin, 85/143 (59.44%) of which 68 (33.33%) were from stool strains while 17 (8.33%) were from water strains [Figure 3]. While *bfp* was found only in stool *E. coli* strains, *eae, lt,* and *st11* were found more in stool strains. More of the Shiga toxins (*stx2*) were isolated from the water strains 9/143 (21.42%) than the stool strains 2/143 (1.98%). Out of the 143 (4.90%) detected toxins, 7 were *Stx1* of which 2/143 (1.98%) were detected in stool strains while 5 (11.90%) were detected in water strains. The percentage of virulence toxins detected from all the samples (stool and water) are in the following decreasing order: *UidA* 204/204 (100%) > *eae* 85/143 (59.44%) > *lt* 20/143 (13.99%) > *stx2* 11/143 (7.69%) > *bfp* 10/143 (4.49%%) > *stx1* 7/143 (4.90%) > *st11* 4/143 (2.79%) > *virf* 2/143 (1.39%) and *aaf11* 2/143 (1.39%), and *daaE* 2/143 (1.39%) as shown in Figure 4. All the toxins under investigation except *ipaH* were detected from water strains while *virF, aaf11,* and *ipaH* were not detected from the stool strains [Table 2]. The toxins detected from fecal samples are in the decreasing order of *uidA* 204/204 (100%) > *eae* 68/101 (67.33%) > *lt* 16/101 (15.84%) > *bfp* 9/101 (8.91%) > *st11* 3/101 (2.97%) > *stx2* 2/101 (1.98%) and *stx1* 2/101 (1.98%) > *daaE* 1/101 (0.99%) while the toxins detected from water samples are in the decreasing order of *uidA* (100%) > *eae* 17/42 (40.48%) > *stx2* 9/42 (21.42%) > *stx1* 5/42 (11.9%) > *lt* 4/42 (9.52%) > *VirF* 2/42 (4.76%) and *aaF11* 2/42 (4.76%), *bfp* 1/42 (2.38%), *st11* 1/42 (2.38%), *daaE* 1/42 (2.38%) [Figure 5]. The virulence toxins were detected most from enteropathogenic strains 95/143 (66.43%), followed by enterotoxigenic strains 24/143 (16.78%) and enterohemorrhagic strains 18/143 (12.59%) as evident in Table 2. The EIEC strain with *virF*, 2/143 (1.39%); EAggEC strains with *aaf11*, 2/143 (1.39%); and DAEC strains with *daaE*, 2/143 (1.39%) had the same percentage degree of occurrence [Figure 4].

Figure 4: Percentage Toxins detected from *Escherichia coli* isolated from all (Stool and water) samples.
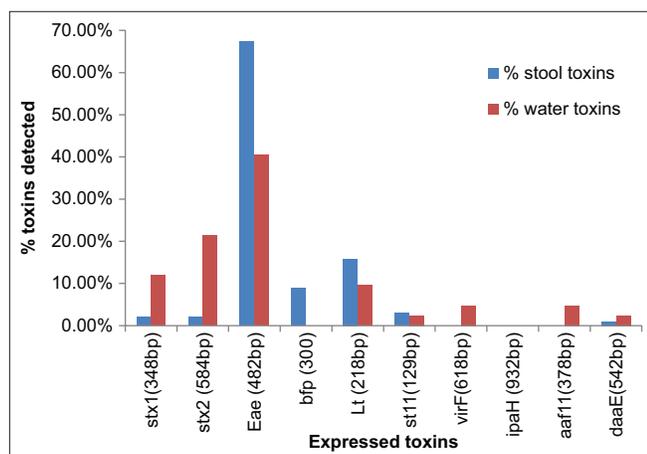

Figure 5: Percentage Toxins detected from *Escherichia coli* isolated from water and diarrhoeal stool. *uidA* was 100% detected in both stool and water strains.

DISCUSSION

A broad spectrum of bacterial, viral, and parasitic pathogens can colonize the intestine irrespective of the presence or absence of pronounced dehydrating diarrhea.[11] The death burden associated with diarrhea can be comprehended in terms of the systematic malnutrition resulting from persistent diarrheal occurrences and chronic enteropathy induced by recurrent gastrointestinal infections. This phenomenon is conspicuous within developing nations, where the prevalence of diarrheal infections correlates with substandard sanitary infrastructure, notably reflected in the hygiene standards of ingested food and water sources, frequently compromised by diverse intestinal pathogens.[11] *UidA* was detected in all the *E. coli* strains, and its presence being an indicator

toxin further confirms the studied strains as *E. coli*.[40,41] The detection of *E. coli* which is an indicator organism and its survival in any medium suggests contamination with other dangerous organisms such as *Salmonella*, *Shigella, Vibrio* species, *Campylobacter* species, *Clostridium welchii,* and *Entamoeba histolytica* enterovirus. Detection of the six diarrheagenic *E. coli* pathotypes (enterohemorrhagic, enteropathogenic, enterotoxigenic, enteroinvasive, enteroaggregative, and diffuse adherent) and their virulence toxins (*uidA* [100%], *eae* [42%], *lt* [9.8%], *stx2* [5.4%], *bfp* [5%], *stx1* [3.4%], *st11* [2%], *Virf* [1%], *Aaf11* [1%], and *daaE* [1%]) from the stool and water strains is a clear indication that more pathogenic organisms could be common in the environment. This poses a serious threat to people living in Lagos and to public health at large. Detection of 10 toxins (*uidA* [100%], *eae* 17/117 [14.52%], *stx2* 9/117 [7.69%], *lt* 4/117 [3.42%], *stx1* 5/117 [4.27%], *VirF* 2/117 [1.71%], *aaF11* 2/117 [1.71%], *bfp* [0.85%], *st11* 1/117 [0.85%], *daaE* 1/117 [0.85%]) out of 11 targeted *E. coli* toxins from water strains against the detected eight toxins (*uidA* [100%], *eae* 68/87 [78.16%], *lt* 16/87 [18.39%], *bfp* 9/87 [10.36%], *stx2* 2/87 [2.29%], *st11* 3/87 [3.45%], *stx1* 2/87 [2.29%], and *daaE* 1/87 [1.15%]) from the stool strains also exposes the degree of contamination in the studied environment. More of the *stx2* toxins were expressed by the water strains 9/143 (6.29%) than in stool strains 2/142 (1.39%), a call for urgent attention to the need for various disinfection/inspection techniques and provision of potable water by government authorities and concerned agencies since only about 4 million of the 15 million population in Lagos have access to piped water.[42,43] It was observed that the toxins *eae*, *lt,* and *bfp* were significantly detected in stool (clinical) strains than the environmental (water) strains, indicating the strains were not just commensals but pathogenic which might have enhanced the disease burden of the patients. The prevalence of the eae toxin gene, responsible for encoding intimin, a pivotal intestinal adherence factor, exhibited extensive distribution across both clinical and environmental specimens. The identification of 8 diarrheagenic *E. coli* virulence toxin genes (*uidA, eae, lt, bfp, stx2, stx1, ST11,* and *daaE*) in the diarrheal stool samples and 10 virulence toxin genes (*uidA*, *eae*, *stx2*, *lt*, *stx1*, *VirF*, *aaF11*, *bfp*, *st11*, *daaE*) from water isolates [Figure 3] is of great interest. It is scientifically sound to postulate that in the context of potable water procurement, the application of relative filtration techniques may occur without substantial alteration to bacterial concentrations within the water source.

The city of Lagos exhibits compelling indications of the widespread and indiscriminate utilization of antibiotics.[44] which is a major factor that predisposes antibiotic resistance.[45,46] The discovery of extensive toxin gene expression by *E. coli* isolates in both environmental and fecal samples raises significant public health concerns. These findings indicate an augmented burden on patients suffering from the affliction of virulence toxin genes. There is a great necessity for understanding the prevalence and expression patterns of these genes for establishing targeted interventions to mitigate the health risks associated with these pathogens. Great significance is attached to this result because of the prominent position of diarrheagenic *E. coli* among bacterial pathogens contributing to diarrheal illness.

CONCLUSION

The conventional and real-time PCR studies of *E. coli* strains from Lagos, Nigeria, detected *uidA* as an inherent gene in all the strains of *E. coli*. Enteroinvasive (*VirF* and *ipaH*) *E. coli* pathotype is not commonly detected but *virF* was detected in environmental strain, an indication of an increased unhygienic environment. The environmental *E. coli* strains exhibited more toxins than the stool strains. Shiga toxins (*Stx1* and *Stx2*) were isolated more from the environmental samples compared to the stool samples which are indications of the accessible water samples in Lagos being a threat to human health. The most predominant pathotypes detected with their expressed toxins were EPEC and ETEC.

Ethical approval

The research/study was approved by the Institutional Review Board at the Research Grants and Experimentation Ethics Committee, number CM/COM/08/VOL.XXV, dated February 20, 2014.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

REFERENCES

1.   Bouzari S, Aslani MM, Oloomi M, *et al.* Comparison of multiplex PCR with serogrouping and PCR-RFLP of fliC gene

for the detection of enteropathogenic *Escherichia coli* (EPEC). *Braz J Infect Dis*. 2011;15:365-369. doi: 10.1016/S1413-8670(11)70206-9

2. Mokomane M, Kasvosve I, de Melo E, *et al*. The global problem of childhood diarrhoeal diseases: Emerging strategies in prevention and management. *Ther Adv Infect Dis*. 2018;5:29-43. doi: 10.1177/2049936117744429

3. Ugboko HU, Nwinyi OC, Oranusi SU, *et al*. Childhood diarrhoeal diseases in developing countries. *Heliyon*. 2020;6:e03690. doi: 10.1016/j.heliyon.2020.e03690

4. UNICEF. Water, sanitation and hygiene; 2021. Available from: https://www.unicef.org/wca/what-we-do/wash [Last accessed on 2021 Nov 23].

5. Nweze EI. Aetiology of diarrhoea and virulence properties of diarrhoeagenic *Escherichia coli* among patients and healthy subjects in southeast Nigeria. *J Health Popul Nutr*. 2010;28:245-252. doi: 10.3329/jhpn.v28i3.5551

6. Melese B, Paulos W, Astawesegn FH, *et al*. Prevalence of diarrheal diseases and associated factors among under-five children in Dale District, Sidama zone, Southern Ethiopia: a cross-sectional study. *BMC Public Health*. 2019;19:1235. doi: 10.1186/s12889-019-7579-2

7. Okeke IN, Ojo O, Lamikanra A, *et al*. Etiology of acute diarrhea in adults in Southwestern Nigeria. *J Clin Microbiol*. 2003;41:4525-4530. doi: 10.1128/JCM.41.10.4525-4530.2003

8. Amaya E, Reyes D, Vilchez S, *et al*. Antibiotic resistance patterns of intestinal Escherichia coli isolates from Nicaraguan children. *J Med Microbiol*. 2011;60:216-222. doi: 10.1099/jmm.0.020842-0

9. Amisano G, Fornasero S, Migliaretti G, *et al*. Diarrheagenic *Escherichia coli* in acute gastroenteritis in infants in North-West Italy. *New Microbiol*. 2011;34(1):45-51.

10. Sarantuya J, Nishi J, Wakimoto N, *et al*. Typical enteroaggregative *Escherichia coli* is the most prevalent pathotype among *E. coli* strains causing diarrhea in Mongolian children. *J Clin Microbiol*. 2004;42:133-139. doi: 10.1128/JCM.42.1.133-139.2004

11. Igbokwe H, Bhattacharyya S, Gradus S, *et al*. Preponderance of toxigenic *Escherichia coli* in stool pathogens correlates with toxin detection in accessible drinking-water sources. *Epidemiol Infect*. 2015;143:494-504. doi: 10.1017/S0950268814001046

12. GBD 2019 Antimicrobial Resistance Collaborators. Global mortality associated with 33 bacterial pathogens in 2019: A systematic analysis for the Global Burden of Disease Study 2019. *Lancet Lond Engl*. 2022;400:2221-2248. doi: 10.1016/S0140-6736(22)02185-7

13. Birhan TA, Bitew BD, Dagne H, *et al*. Prevalence of diarrheal disease and associated factors among under-five children in flood-prone settlements of Northwest Ethiopia: A cross-sectional community-based study. *Front Pediatr*. 2023;11:1056129. doi: 10.3389/fped.2023.1056129

14. Rahmat ZS, Zubair A, Abdi I, *et al*. The rise of diarrheal illnesses in the children of Pakistan amidst COVID-19: A narrative review. *Health Sci Rep*. 2023;6:e1043. doi: 10.1002/hsr2.1043

15. Khabo-Mmekoa CM, Genthe B, Momba MN. Enteric pathogens risk factors associated with household drinking water: A case study in Ugu District Kwa-Zulu Natal Province, South Africa. *Int J Environ Res Public Health*. 2022;19:4431.

16. Soboksa NE, Gari SR, Hailu AB, *et al*. Association between microbial water quality, sanitation and hygiene practices and childhood diarrhea in Kersa and Omo Nada districts of Jimma Zone, Ethiopia. *PLoS One*. 2020;15:e0229303. doi: 10.1371/journal.pone.0229303

17. Nantege R, Kajoba D, Ddamulira C, *et al*. Prevalence and factors associated with diarrheal diseases among children below five years in selected slum settlements in Entebbe municipality, Wakiso district, Uganda. *BMC Pediatr*. 2022;22:394. doi: 10.1186/s12887-022-03448-2

18. WHO. Diarrhoeal disease. Available from: https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease [Last accessed on 2024 Jun 05].

19. Baldi F, Bianco MA, Nardone G, *et al*. Focus on acute diarrhoeal disease. *World J Gastroenterol*. 2009;15:3341-3348. doi: 10.3748/wjg.15.3341

20. Lalani T, Maguire JD, Grant EM, *et al*. Epidemiology and self-treatment of travelers' diarrhea in a large, prospective cohort of department of defense beneficiaries. *J Travel Med*. 2015;22:152-160. doi: 10.1111/jtm.12179

21. Bodhidatta L, Anuras S, Sornsakrin S, *et al*. Epidemiology and etiology of Traveler's diarrhea in Bangkok, Thailand, a case-control study. *Trop Dis Travel Med Vaccines*. 2019;5:9. doi: 10.1186/s40794-019-0085-9

22. Ramos S, Silva V, Dapkevicius ML, *et al*. *Escherichia coli* as commensal and pathogenic bacteria among food-producing animals: Health implications of extended spectrum β-lactamase (ESBL) production. *Animals*. 2020;10:2239. doi: 10.3390/ani10122239

23. Vidal M, Kruger E, Durán C, *et al*. Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic *Escherichia coli* associated with enteric infections. *J Clin Microbiol*. 2005;43:5362-5365. doi: 10.1128/JCM.43.10.5362-5365.2005

24. Moyo SJ, Maselle SY, Matee MI, *et al*. Identification of diarrheagenic *Escherichia coli* isolated from infants and children in Dar es Salaam, Tanzania. *BMC Infect Dis*. 2007;7:92. doi: 10.1186/1471-2334-7-92

25. Barnett Foster D. Modulation of the enterohemorrhagic *E. coli* virulence program through the human gastrointestinal tract. *Virulence*. 2013;4:315-323. doi: 10.4161/viru.24318

26. Ochoa TJ, Contreras CA. Enteropathogenic *Escherichia coli* infection in children. *Curr Opin Infect Dis*. 2011;24:478-483. doi: 10.1097/QCO.0b013e32834a8b8b

27. Pasqua M, Michelacci V, Di Martino ML, *et al*. The intriguing evolutionary journey of enteroinvasive *E. coli* (EIEC) toward pathogenicity. *Front Microbiol*. 2017;8:2390. doi: 10.3389/fmicb.2017.02390

28. Fan W, Qian H, Shang W, *et al*. Low distribution of genes encoding virulence factors in *Shigella flexneri* serotypes 1b clinical isolates from eastern Chinese populations. *Gut Pathog*. 2017;9:76. doi: 10.1186/s13099-017-0222-9

29. Ellis SJ, Crossman LC, McGrath CJ, *et al*. Identification and characterisation of enteroaggregative *Escherichia coli* subtypes associated with human disease. *Sci Rep*. 2020;10:7475. doi: 10.1038/s41598-020-64424-3

30. Estrada-Garcia T, Navarro-Garcia F. Enteroaggregative

Escherichia coli pathotype: A genetically heterogeneous emerging foodborne enteropathogen. *FEMS Immunol Med Microbiol*. 2012;66:281-298. doi: 10.1111/j.1574-695X.2012.01008.x

31. Jenkins C, Chart H, Willshaw GA, *et al.* Genotyping of enteroaggregative *Escherichia coli* and identification of target genes for the detection of both typical and atypical strains. *Diagn Microbiol Infect Dis*. 2006;55:13-19. doi: 10.1016/j.diagmicrobio.2005.10.019

32. Okhuysen PC, Dupont HL. Enteroaggregative *Escherichia coli* (EAEC): A cause of acute and persistent diarrhea of worldwide importance. *J Infect Dis*. 2010;202:503-505. doi: 10.1086/654895

33. Mansan-Almeida R, Pereira AL, Giugliano LG. Diffusely adherent *Escherichia coli* strains isolated from children and adults constitute two different populations. *BMC Microbiol*. 2013;13:22. doi: 10.1186/1471-2180-13-22

34. CLSI. Performance standards for antimicrobial susceptibility testing. United States: CLSI; 2017.

35. Peter F, Stephen DW, Michael AG, *et al.* Enumeration of *Escherichia coli* and the Coliform Bacteria. In: FDA`s bacteriological analytical manual. Ch. 4. FDA; 2020. Available from: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria [Last accessed on 2024 Jun 12].

36. Ghita BT, Bennani L, Berrada S, *et al.* Molecular serotyping and antibiotic resistance patterns of *Escherichia coli* isolated in hospital catering service in Morocco. *Int J Microbiol*. 2020;2020:5961521. doi: 10.1155/2020/5961521

37. Fey PD, Wickert RS, Rupp ME, *et al.* Prevalence of non-O157:H7 shiga toxin-producing *Escherichia coli* in diarrheal stool samples from Nebraska. *Emerg Infect Dis*. 2000;6:530-533. doi: 10.3201/eid0605.000513

38. Nielsen EM, Andersen MT. Detection and characterization of verocytotoxin-producing *Escherichia coli* by automated 5′ nuclease PCR assay. *J Clin Microbiol*. 2003;41:2884-2893.

doi: 10.1128/JCM.41.7.2884-2893.2003

39. Mohammed MA. Molecular characterization of diarrheagenic *Escherichia coli* isolated from meat products sold at Mansoura city, Egypt. *Food Control*. 2012;25:159-164. doi: 10.1016/j.foodcont.2011.10.026

40. Martins MT, Rivera IG, Clark DL, *et al.* Distribution of uidA gene sequences in *Escherichia coli* isolates in water sources and comparison with the expression of beta-glucuronidase activity in 4-methylumbelliferyl-beta-D-glucuronide media. *Appl Environ Microbiol*. 1993;59:2271-2276. doi: 10.1128/aem.59.7.2271-2276.1993

41. Godambe LP, Bandekar J, Shashidhar R. Species specific PCR based detection of *Escherichia coli* from Indian foods. *3 Biotech*. 2017;7:130. doi: 10.1007/s13205-017-0784-8

42. Satterthwaite D. The transition to a predominantly urban world and its underpinnings. (Human settlements discussion paper series); 2007.

43. Aliyu AA, Amadu L. Urbanization, cities, and health: The challenges to Nigeria - a review. *Ann Afr Med*. 2017;16:149-158. doi: 10.4103/aam.aam_1_17

44. Chukwu EE, Oladele DA, Enwuru CA, *et al.* Antimicrobial resistance awareness and antibiotic prescribing behavior among healthcare workers in Nigeria: A national survey. *BMC Infect Dis*. 2021;21:22. doi: 10.1186/s12879-020-05689-x

45. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: A global multifaceted phenomenon. *Pathog Glob Health*. 2015;109:309-318. doi: 10.1179/2047773215Y.0000000030

46. Salam MA, Al-Amin MY, Salam MT, *et al.* Antimicrobial resistance: A growing serious threat for global public health. *Healthcare*. 2023;11:1946. doi: 10.3390/healthcare11131946