Occurrence and antibiotic resistance profile of shiga toxin-producing Escherichia coli (STEC) and enteropathogenic Escherichia coli (EPEC) from sources of water in Mubi Region, Adamawa State, Nigeria

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ABSTRACT

INTRODUCTION The pathogenic *Escherichia coli* bacterium that cause diseases within the intestine is called diarrheagenic *E. coli* (DEC) and is responsible for *E. coli* gastroenteritis. The present study aimed to identify the occurrence and antibiotic resistance profile of STEC/EHEC and EPEC from water sources in the Adamawa-north Senatorial zone, Nigeria.

METHODS Water samples from hand-dug wells and rivers/streams from the study area were screened for the presence of *E. coli*, and diarrheagenic *E. coli* (DEC) by polymerase chain reactions. The antimicrobial susceptibility pattern of the DEC pathotypes was determined by Kirby-Bauer method.

RESULTS Of the 256 water samples analyzed, 63 (24.6%) *E. coli* were isolated. Of these, 44 (69.8%) and 19 (30.2%) were isolated from river and well water sources, respectively. Of the 63 *E. coli* isolates recovered from the water sources, 27 (42.9%) were non-sorbitol fermenting *E. coli*. Of these, 24 (88.9%) isolates were of STEC/EHEC pathotypes, 2 (7.4%) were of EPEC pathotype, while shiga toxins and intimin genes were not amplified in 1 (3.7%) isolate. All the STEC and EPEC exhibited complete resistance (100%) to cotrimoxazole, ampicillin, nalidixic acid, augmentin, and ceftriaxone. However, while 13 (54.2%) out of the 24 STEC isolates demonstrated resistance to imipenem, all the EPEC isolates remained susceptible to imipenem. The resistant profile showed that the DEC pathotypes were resistant to 7–12 antibiotics with 8 (30.8%) of the 26 DEC resistant to 12 antibiotics.

CONCLUSIONS The findings of this study contribute to a better knowledge of the occurrence of STEC/EHEC and EPEC in the major water sources of the study area and may serve as reference point data for future use and epidemiological surveillance.

INTRODUCTION

The *Escherichia coli* bacterium is typically Gram-negative, characterized by its rod-shaped morphology, motility, lack of spore formation, absence of oxidase activity, production of indole, and inability to produce urease. It is capable of utilizing lactose and, as a result of glucose fermentation, generates both acid and gas. Additionally, it can thrive in environments with or without oxygen, particularly at a temperature of 37°C[1,2].

The species *E. coli* is broadly dispersed and constitutes the major commensal of the human intestine including other warm-blooded animals and is used as a reference bacterium in many laboratory investigations[1]. *E. coli* serotypes can be isolated from various samples aside fecal material of warm-blooded animals. As such different serotypes of this organism are particularly introduced into the environment

where they contaminate different foods and water sources without significant harmful consequences on human health[3]. However, the organism becomes opportunistic when it enters into some sensitive parts of the human body (such as urinary tract, blood, meninges, among others), especially in immunocompromised individuals or after involvement in surgery where it multiplies extensively and causes numerous illnesses[3,4]. Although most *E. coli* strains may be normal flora of the gut, other strains nonetheless may be major pathogens with an improved tendency to cause diseases. This may be as a result of the acquisition of virulent determinants. *E. coli* bacteria that are pathogenic can be grouped according to variable criteria which include virulence factors, pathogenicity mechanisms, clinical signs, and serotype[5]. The virulence factors that enhanced the pathogenicity of *E. coli* consist of toxins, invasins, adhesins, capsular and effacement factors[6,7]. Disease-causing strains of *E. coli* can be grouped into those that cause diseases within and outside the intestine[8,9].

The pathogenic *E. coli* that causes disease within the intestine is called diarrheagenic *E. coli* (DEC) or intestinal pathogenic *E. coli* (IPEC)[4] and is responsible for gastroenteritis[8]. The DEC pathotypes are categorized based on their virulence factors and phenotypic traits, and each pathotype has unique host preferences, prevalence, route of transmission, as well as disease burden[4]. As such, DEC is categorized as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC)[7]. The most considerable and significant virulent determinants for the detection of STEC/EHEC are the intimin protein (*eaeA*), and shiga toxins (*stx1* and *stx2*), while for EPEC are intimin protein (*eaeA*) and bundle-forming pilus (*bfp*), among others[2].

The purpose of the present study was to identify the occurrence and antibiotic resistance profile of diarrheagenic *E. coli* from sources of water in the Adamawa North senatorial zone, Nigeria. This is particularly important because the study area lacks potable pipe-borne water supply, and the populace relies solely on alternative water sources with questionable microbiological quality[10]. Of utmost concern is the fact that data on the occurrence of DEC pathotypes in these water sources are lacking.

METHODS

Study area

The study area was Adamawa North senatorial zone commonly known as the Mubi region (Figure 1). Mubi region comprises five Local Government Areas (LGAs): Madagali, Michika, Mubi South, Mubi North, and Maiha with a land area of 4494 km^2 and a population of 682026 (NPC, 2010). The area has a tropical wet and dry climate. Dry season lasts for a minimum of six months (November–March), while the wet season spans between May and October. The mean annual rainfall ranges 700–1050 mm[11].

Sampling plan

From each Local Government Area, 2 wards were chosen for hand-dug well (HDW) water sample collection. From each ward, water from four HDWs was chosen at random and sampled in duplicate for the period of sampling. A river/stream was also selected from each Local Government Area for sampling. For each river/stream, two samples were collected at random (upstream and downstream) in quadruples for the period of sampling.

Period of sampling

Water samples were collected aseptically from upstream and downstream of 4 rivers, and 32 hand-dug wells (HDWs) between June 2019 and April 2020.

Water sampling

A total of 256 water samples (comprising 128 each from HDW and river water sources) were taken from 4 local government areas of Adamawa North senatorial zone. Hand-dug well water samples were taken from 8 locations, 2 from each LGA as follows: Lokuwa and Kolere (Mubi North), Wuro-patuji and Nassarawo (Mubi South), Michika and Bazza (Michika LGA), and Maiha and Pakka (Maiha LGA). River water samples were taken from 4 rivers, one from each LGA as follows: river Yadzaram (Mubi North and South), river Dilchim (Michika LGA) and river Mayonguli (Maiha LGA).

Isolation of bacteria

Bacteria were isolated by membrane filtration technique using, a sterile 47 mm, 0.45 µm mixed cellulose ester (MCE) membrane filter (Merck, Bangalore). At the end of the filtration, sterile forceps were used to pick the filter onto the surface of MacConkey agar (MCA) and replicated on Eosin methylene blue (EMB) agar. Recovered *E. coli* isolates were further streaked on the surface of sorbitol MacConkey agar for the presumptive detection of some strains of pathogenic *E. coli* that can ferment sorbitol. The plates were incubated at 35–37°C for 18–24 h. Discrete bacterial colonies were re-cultured and stored in nutrient agar slant for identification and further use.

Identification of isolates

After the Gram stain, each discrete bacterial colony was subjected to other biochemical tests such as Simmon's citrate test, reaction on triple sugar iron (TSI) agar, and oxidase test before they were identified with Microgen GN A kit and 16SrRNA.

Identification of bacterial isolates using Microgen Gram negative-A (GN-A) ID kits

After Gram-staining, each bacterial isolate was identified on the Microgen A kit (Gold Standard Diagnostic, Hungary)[11]. Each of these test kits is a plastic strip containing 12 microwells with dehydrated constituents that could identify 12 biochemical characteristics, namely lysine,

ornithine, hydrogen sulfide production, glucose, mannitol, xylose, indole, urease production, Voges Proskauer, citrate utilization, tryptophan deaminase (TDA), and ortho-nitrophenol-β-galactoside (ONPG). The outcomes of the 12 (GN-A kit) microwell test strips after the addition of test isolate and 24-h incubation were converted to a 4-digit octal code that was used to validate the identity of the tested bacterial isolate using Microgen ID computer software version 2.0.8.33.

Molecular identification of bacterial isolates

After genomic DNA extraction, the 16S rRNA gene was amplified and purified. The amplified fragments were cleaned using ethanol, and their reliability was proven on 1% agarose gel. The sequencing of the amplified fragments was achieved on a Genetic Analyzer 3130 × l sequencer (Applied Biosystems) at Inqaba Diagnostic, South Africa. The isolates' identity was confirmed by subjecting the sequences to analysis with the Basic Local Alignment Search Tool (BLAST)[11] (available at https://www.ncbi.nlm.nih.gov/blast).

Specific primers for detection of diarrheagenic *E. coli* pathotype

Specific primer sets were used to detect *stx1*, *stx2,* and *eaeA* coding genes on the presumptive pathogenic *E. coli* isolates in separate PCR reactions as shown in Table 1. The PCR profile settings were: preliminary denaturation for 5 min at 94°C, which was attended with 30 rounds of denaturation for 30 s at 94°C, hardening for 60 s at 50°C and lengthening for 30 s at 72°C with a concluding termination for 10 min at 72°C.

A volume of 5 µL of each PCR product was electrophoresed in 2% agarose gel containing 5 µL of 10 mg/mL ethidium bromide at 100V for 45 min. The molecular marker used was A 1 kb plus DNA marker. DNA amplifications were examined under an ultraviolet (UV) transilluminator and results were documented[12,13].

Antimicrobial susceptibility testing of DEC isolates

The disc diffusion method, also known as the Kirby-Bauer method was employed for the antimicrobial susceptibility test as recommended by CLSI[14]. Each DEC isolate was used to test for its susceptibility to the following antimicrobial agents; pefloxacin (10 µg), gentamicin (10 µg), ofloxacin (10 µg), imipenem (10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), streptomycin (30 µg), ciprofloxacin (10 µg), amoxillin-clavulanic acid (30 µg), ampicillin (30 µg), nalidixic acid (30 µg), and cotrimoxazole (30 µg). The inhibition zone diameter was measured in millimeters and was interpreted based on the diameter of interpretative standard breakpoints[14]. MDR phenotype was determined when an isolate was resistant to at least one antibiotic in three classes of antibiotics[11].

Statistical analysis

All the data were analyzed by descriptive statistics using simple percentages. All data were analyzed using the Statistical Package for Social Sciences (SPSS) statistics for windows (Chicago) version 17.

RESULTS

The results in Table 2 show the total number of *E. coli* isolated from the water sources. Of the 256 water samples analyzed, 63 (24.6%) *E. coli* were isolated. Of these, 44 (69.8%) and 19 (30.2%) were isolated from river and well water sources, respectively. The sequences of some of the *E. coli* isolates documented in this study have been assigned accession numbers (Table 3) and have equally been added in the NCBI GenBank.

Of the 63 *E. coli* isolates recovered from the water sources, 27 (42.9%) were non-sorbitol fermenting *E. coli*. Of these, shiga toxins (*stx1* and *stx2*) genes (typical characteristics of STEC/EHEC) were detected in 24 (88.9%) isolates, with *stx1* and *stx2* genes detected in 13 (48.2%) and 22 (81.5%) *E. coli* isolates, respectively. The *eaeA* gene along with other virulent genes was documented in 21 (77.8%) *E. coli* isolates. However, *eaeA* gene alone (a typical characteristic of an EPEC) was detected only in two *E. coli* isolates. Whereas 11 (40.7%) of the STEC/EHEC harbored a combination of *stx1* and *stx2* genes, the combination of *stx1*, *stx2,* and *eaeA* genes was documented in 10 (37.0%) STEC/EHEC isolates (Table 4).

The resistant pattern of the DEC pathotypes (STEC and EPEC) is shown in Table 5. Resistance to cotrimoxazole, ampicillin, nalidixic acid, amoxicillin-clavulanic acid, and ceftriaxone was 100% for both STEC and EPEC. However, 95.5%, 87.5%, and 83.3%, of STEC were resistant to ceftazidime, ciprofloxacin, and pefloxacin, respectively. Whereas 13 (54.2%) of STEC were resistant to imipenem, all the EPEC isolates were susceptible to imipenem.

The resistant profile showed that the DEC pathotypes

Table 1. Primers used for the detection of STEC/EHEC and EPEC

Targeted gene	Primer sequence	Amplicon size (bp)	Reference
stx1	F: (ACACTGGATGATCTCAGTGG) R: (CTGAATCCCCCTCCATTATG)	614	(Tahamta and Namavari[12], 2014)
stx2	F: (GGCACTGTCTGAAACTGCTCC) R: (TCGCCAGTTATCTGACATTCTG)	255	Paton and Paton[12]
eaeA	F: (GACCCGGCACAAGCATAAGC) R: (CCACCTGCAGCAACAAGAGG)	384	Paton and Paton[12]

Table 2. Total *Escherichia coli* isolated from the water sources

Water source	Number of samples collected	Number of E. coli isolates
Well	128 (50.0)	19 (30.2)
River	128 (50.0)	44 (69.8)
Total	256 (100)	63 (24.6)

Table 3. Identification of the selected sequenced *Escherichia coli* using BLAST

Sample ID	Scientific name	Maximum score	Total score	Query cover %	E-value	Percentage identity %	Accession number
97	*E. coli*	1880	1880	100	0	100	MZ437057
107	*E. coli*	1868	1305	100	0	98.95	MZ437064
109	*E. coli*	1868	1305	100	0	98.95	MZ437065
131	*E. coli*	1880	1880	100	0	100	MZ437078
132	*E. coli*	1880	1880	100	0	100	MZ437079

Table 4. Prevalence of DEC virulence genes

Pathotype	Virulence markers				
	stx1	stx2	eaeA	stx1+2	stx+eaeA
STEC	13	22	19	11	10
EPEC	0	0	2	NA	NA
Total	13	22	21	11	10

NA: not applicable.

Table 5. Antimicrobial susceptibility pattern of DEC pathotype

Antibiotics	STEC (N=24)		EPEC (N=2)	
	Susceptible n (%)	Resistant n (%)	Susceptible n (%)	Resistant n (%)
Cotrimoxazole	0	24 (100)	0	2 (100)
Streptomycin	5 (20.8)	19 (79.2)	0	2 (100)
Ampicillin	0	24 (100)	0	2 (100)
Pefloxacin	4 (16.7)	20 (83.3)	1 (50.0)	1 (50.0)
Gentamicin	5 (20.8)	19 (79.2)	0	2 (100)
Ofloxacin	9 (37.5)	15 (62.5)	2 (100)	0
Nalidixic acid	0	24 (100)	0	2 (100)
Augmentin	0	24 (100)	0	2 (100)
Ciprofloxacin	3 (12.5)	21 (87.5)	2 (100)	0
Ceftriaxone	0	24 (100)	0	2 (100)
Ceftazidime	1 (4.2)	23 (95.8)	0	2 (100)
Imipenem	11 (45.8)	13 (54.2)	2 (100)	0

Table 6. Antibiotic resistance profile of DEC

SN	Number of antimicrobials	Resistance profile	Numbers observed	MDR status
1	12	sxt,s,pn,cpx,aug,cn,pef,na,ofx,cro,caz,ipm	8	MDR
2	11	sxt,s,pn,cpx,amc,cn,pef,na,ofx,cro,caz	2	MDR
3	11	sxt,s,pn,pef,cn,na,aug,cpx,cro,caz,ipm	3	MDR
4	11	sxt,s,pn,pef,ofx,na,aug,cpx,cro,caz,ipm	1	MDR
5	10	sxt,s,pn,aug,cn,pef,na,cro,caz,ipm	1	MDR
6	10	sxt,pn,cpx,aug,cn,pef,na,ofx,cro,caz	2	MDR
7	9	sxt,s,pn,cpx,aug,cn,na,cro,caz	3	MDR
8	9	sxt,s,pef,ofx,na,aug,cpx,cro,caz	1	MDR
9*	9	sxt,s,pn,pef,cn,na,aug,cro,caz	1	MDR
10	9	sxt,pn,pef,cn,ofx,na,aug,cro,caz	1	MDR
11*	9	sxt,s,pn,cn,ofx,na,aug,cro,caz	1	MDR
12	7	sxt,pn,cpx,aug,na,cro,caz	1	MDR
13	7	sxt,pn,cpx,aug,na,cpx,cro	1	MDR

*EPEC isolates. sxt: cotrimoxazole. s: streptomycin. pn: ampicillin. pef: pefloxacin. cn: gentamicin. ofx: ofloxacin. na: nalidixic acid. aug: augmentin. cpx: ciprofloxacin. cro: ceftriaxone. caz: ceftazidime. ipm: imipenem. MDR: multidrug resistance.

were resistant to from 7 to 12 antibiotics with 8 (30.8%) of the 26 DEC resistant to 12 antibiotics (Table 6).

DISCUSSION

The availability of potable drinking water is paramount to life forms, especially humans. That is why water meant for domestic purposes is expected to be devoid of microbial agents like *E. coli* which serves as an indicator for the presence of potential pathogens[15]. The results of this study, on the other hand, have recorded the existence of *E. coli* in water sources meant for domestic purposes. The observed presence and spread of *E. coli* in these water sources can be linked to a variety of factors including, but not restricted, to poor sanitation practices in the vicinity of these water sources[16].

The detection of 27 (42.9%) *E. coli* bacteria that were sorbitol-non-fermenting suggests that they were pathogenic. The frequency, however, was lower than 75%, 68.0%, and 67.5% of pathogenic *E. coli* documented in water sources from Ghana[15], Côte d'Ivoire[17], and South Africa[18], respectively.

In causing diseases, strains of *E. coli* that are pathogenic usually employ a series of multifaceted machinery embracing a number of virulence determinants which eventually leads to the destruction of the target host cells. As such, the expression of one or more virulent determinants in appropriate combinations determines the pathogenic capability of a particular *E. coli* isolate[19].

The pathogenic *E. coli* bacteria encountered in this study belong to two diarrheagenic pathotypes; shiga toxin-producing *E. coli* (STEC) also known as enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC). While the STEC/EHEC constitute the majority of the DEC pathotype, EPEC constitutes only 7.4% of the DEC pathotypes encountered in this study.

The STEC/EHEC cause several types of disease indications in humans, which include mild diarrhea to severe disease forms like hemolytic uremic syndrome (HUS), and hemorrhagic colitis (HC) mediated primarily by shiga toxins (*stx1* and *stx2* genes) and intimin (*eaeA* gene). Though each gene of the shiga toxins (*stx1* or *stx2*) possesses the ability to cause acute diarrhea as earlier reported[7], the detection of both *stx1* and *stx2* genes in some strains of STEC/EHEC isolates, as shown in this study, is of grave consequence. This is because *E. coli* carrying a mixture of shiga toxin genes was reported to cause more complicated diarrhea in humans[7,12]. Also, the high prevalence of the *stx2* genotype in STEC/EHEC strains of this study is of public health significance. This is because *stx2* is reported to cause more severe clinical outcomes than *stx1*[20]. More so, studies have shown that EHEC that causes HUS expresses *stx2* in more cases than the *stx1* genotype[21,22].

More so, in this study, the combination of *stx2* and *eaeA* genes was more pronounced than *stx1* and *eaeA* genes among the STEC/EHEC strains. This observation is in contrast to a similar study in Iran[2] which reported the preponderance of *stx1* and *eaeA* over *stx2* and *eaeA* genes. Studies have shown that STEC/EHEC strains with the *eaeA* gene are more virulent when compared with *eaeA*-negative STEC/EHEC strains[2]. The intimin gene was reported to be accountable for the in-depth adhesion of the STEC/EHEC and EPEC pathotypes to

the epithelial cells of the intestinal mucosa; this subsequently gives rise to attaching and effacing lesion at the point of attachment[19,23].

The two (7.4%) *E. coli* isolates that lacked other virulent genes, which are typical of the STEC/EHEC pathotype but harbored only the *eaeA* gene, are known as EPEC. This observation was similar to a previous study in South-Western Nigeria where 4.0% of *E. coli* isolates from river sources were reported to harbor only *eae*A[7]. The detection of EPEC strain with only the *eaeA* gene is of public health concern. This is because the EPEC pathotype that carries only the *eaeA* gene was reported mainly to cause an outbreak of gastroenteritis globally[18,24]. In general, EPEC is reported to cause infantile diarrhea more often in underdeveloped countries like Nigeria[25].

Quite similar to the findings of this study, the detection of DEC pathotype isolates with a characteristic that is typical of STEC/EHEC and EPEC was previously reported in Nigeria[7,23] and beyond[26,27]. The detection of these DEC pathotypes in surface and groundwater sources meant for domestic purposes constitutes a risk of outbreaks with possibly grave consequences if left unchecked.

In this study, a high level of DEC resistant to ampicillin (100%), cotrimoxazole (100%), nalidixic acid (100%), augmentin (100%), and ceftriaxone (100%) was documented. High resistance of DEC to ampicillin and cotrimoxazole in this study was consistent with the reports of various studies in South Africa[8], Peru[28], and Iran[29].

The high rate of DEC resistant to cotrimoxazole and ampicillin in this study could be because these antimicrobials are the most frequently used antibiotics for therapy against diarrheoa[30,31]. The common use of these classes of antimicrobials could be because they are relatively inexpensive, have ease of accessibility, and initially are very effective with a broad spectrum of activity against a wide range of infections, especially against diarrhoea[32].

The MDR phenotype exhibited by DEC in this study was similar to those of previous studies in Southwestern Nigeria[7], and South Africa[31,33]. Resistance to 7–12 antibiotics by DEC in this study implies excessive, indiscriminate, and inappropriate use of these antimicrobials in the study area. It could also be due to the acquisition of resistance genes through horizontal gene transfer[31,34]. So, the use of surface or groundwater sources for domestic activities and/or irrigation may further increase the chances for the spread of MDR DEC in the study area, and in economy-restrained countries[29].

In this study also, resistance to imipenem was relatively lower than to other antimicrobials. This corroborates studies that documented low resistance to carbapenem antibiotics by DEC isolates in Nigeria[35,36] and in Asian countries[37]. The high susceptibility of the DEC isolates to imipenem in this study might be due to the no or low prescription and usage of the antimicrobial, especially in treating diarrhea-related illness. The results obtained from this study may be valuable in building strategies that will reduce the risk associated with the spread of DEC isolates to the public through water sources.

Limitations

The limitation of this study was its inability to differentiate the two EPEC isolates into typical and atypical EPEC due to other requirements which were not captured in the course of this study.

Also, the inability to screen for other virulent genes characteristics of both EHEC and EPEC was another limitation.

CONCLUSIONS

The occurrence of STEC/EHEC in water sources of the study area is remarkable, and it highlights the fact that these sources may serve as significant avenues through which microbial agents of diarrhea are disseminated. Because there was a paucity of data (or there were no data) that reported the presence of STEC/EHEC and EPEC from water sources in the study area, the findings of this study contribute to a better knowledge of the occurrence of pathogenic *E. coli* in the major water sources of the study area and may serve as reference point data for future use and epidemiological surveillance. Also, the documented and significant high MDR phenotypes of the DEC isolates in this study call for concern, and it underscores the necessity for better and robust practical measures to be put in place that will help in curbing the menace of antimicrobial resistance.

REFERENCES

1. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. Recent advances in understanding enteric pathogenic Escherichia coli. Clin Microbiol Rev. 2013;26(4):822-880. doi:10.1128/CMR.00022-13
2. Shojaei M. Virulence Factors of Shiga-Toxigenic Escherichia coli in drinking water of Shahrekord, Iran. Electronic Journal of Biology 2017; 13(1): 18-21
3. Kuhnert P, Boerlin P, Frey J. Target genes for virulence assessment of Escherichia coli isolates from water, food and the environment. FEMS Microbiol Rev. 2000;24(1):107-117. doi:10.1111/j.1574-6976.2000.tb00535.x
4. Gomes TA, Elias WP, Scaletsky IC, et al. Diarrheagenic Escherichia coli. Braz J Microbiol. 2016;47 Suppl 1(Suppl 1):3-30. doi:10.1016/j.bjm.2016.10.015
5. Lim JY, Yoon J, Hovde CJ. A brief overview of Escherichia coli O157:H7 and its plasmid O157. J Microbiol Biotechnol. 2010;20(1):5-14.
6. Galane PM, Le Roux M. Molecular epidemiology of Escherichia coli isolated from young South African children with diarrhoeal diseases. J Health Popul Nutr. 2001;19(1):31-38
7. Titilawo Y, Obi L, Okoh A. Occurrence of virulence gene signatures associated with diarrhoeagenic and non-diarrhoeagenic pathovars of Escherichia coli isolates

from some selected rivers in South-Western Nigeria. BMC Microbiol. 2015;15:204. doi:10.1186/s12866-015-0540-3

8. Lamprecht C, Romanis M, Huisamen N, Carinus A, Schoeman N, Sigge GO. Escherichia coli with virulence factors and multidrug resistance in the Plankenburg River. South African Journal of Science 2014; 110 (9-10), 1–6. doi.10.1590/sajs.2014/20130347
9. Rojas-Lopez M, Monterio R, Pizza M, Desvaux M, Rosini R. Intestinal pathogenic Escherichia coli: insights for vaccine development. Front Microbiol. 2018;9:440. doi:10.3389/fmicb.2018.00440
10. Tula MY, Enabulele OI, Ophori EA, Aziegbemhin AS. A comprehensive study on the physicochemical characteristics of two water sources from the Adamawa North senatorial zone, Nigeria. Public Health and Toxicology. 2022;2(4):18. doi:10.18332/pht/158012
11. Tula MY, Enabulele OI, Ophori EA, Aziegbemhin AS, Iyoha O, Filgona J. Phenotypic and molecular detection of multidrug-resistant Enterobacteriaceae species from water sources in Adamawa-North senatorial zone, Nigeria. Dysona-Life Science, 2022;3(2):57-68. doi:10.30493/DLS.2022.351097
12. Tahamtan Y, Namavari M. Prevalence of O157:H7 and non-O157 E. coli in Iranian domestic sheep. Pak J Biol Sci. 2014;17(1):104-108. doi:10.3923/pjbs.2014.104.108
13. Paton AW, Paton JC. Detection and characterization of Shiga toxigenic Escherichia coli by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic E. coli hlyA, rfbO111, and rfbO157. J Clin Microbiol. 1998;36(2):598-602. doi:10.1128/JCM.36.2.598-602.1998
14. Bitrus Y, Duru BN, Ali UD. Public health risk of O157 and Non-O157 Shiga toxin-producing Escherichia coli strains in a semi-urban environment in Nigeria. African Journal of Microbiology Research 2011; 5(31):5636-5643. doi:10.5897/AJMR11.802
15. CLSI. Performance Standards for Antimicrobial Susceptibility Testing, 27th ed. CLSI supplement M100; USA: Wayne PA, 2017: Clinical and Laboratory Standards Institute.
16. Kichana E, Addy F, Dufailu OA. Genetic characterization and antimicrobial susceptibility of Escherichia coli isolated from household water sources in northern Ghana. J Water Health. 2022;20(5):770-780. doi:10.2166/wh.2022.197
17. Osumanu IK, Kosoe EA, Ategeeng F. Determinants of open defecation in the Wa municipality of Ghana: empirical findings highlighting socio-cultural and economic dynamics among households. Journal of Environmental and Public Health 2019, 10. doi:10.1155/2019/3075840
18. Kambire O, Adingra AA, Yao KM, Koffi-Nevry R. Prevalence of virulence genes associated with diarrheagenic pathotypes of Escherichia coli isolates from water, sediment, fish, and crab in Aby Lagoon, Côte d'Ivoire. Int J Microbiol. 2017;2017:9532170. doi:10.1155/2017/9532170
19. Obi CL, Green E, Bessong PO, De-Villiers B, Hoosen AA, Igumbor EO. Gene encoding virulence markers among Escherichia coli isolates from diarrhoeic stool samples and river sources in rural Venda communities of South Africa. Water South Africa 2004; 30(1):37–42. doi:10.4314/wsa.v30i1.5024
20. Sidhu JP, Ahmed W, Hodgers L, Toze S. Occurrence of virulence genes associated with Diarrheagenic pathotypes in Escherichia coli isolates from surface water. Appl Environ Microbiol. 2013;79(1):328-335. doi:10.1128/AEM.02888-12
21. Kargar M, Homayoon M. Prevalence of shiga toxins (stx1, stx2), eaeA and hly genes of Escherichia coli O157:H7 strains among children with acute gastroenteritis in southern of Iran. Asian Pac J Trop Med. 2015;8(1):24-28. doi:10.1016/S1995-7645(14)60182-6
22. Friedrich AW, Bielaszewska M, Zhang WL, et al. Escherichia coli harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. J Infect Dis. 2002;185(1):74-84. doi:10.1086/338115
23. Mark Taylor C. Enterohaemorrhagic Escherichia coli and Shigella dysenteriae type 1-induced haemolytic uraemic syndrome. Pediatr Nephrol. 2008;23(9):1425-1431. doi:10.1007/s00467-008-0820-3
24. Alalade OM, Ameh JB, Abdullahi IO, Whong CMZ, Alalade OO. Screening for virulence genes in Escherichia coli O157:H7 obtained from drinking water from Ikara, Kaduna State, Nigeria. Ife Journal of Science 2018; 20(1):139-144. doi:10.4314/ijs.v20i1.14
25. Begum YA, Talukder KA, Nair GB, Qadri F, Sack RB, Svennerholm AM. Enterotoxigenic Escherichia coli isolated from surface water in urban and rural areas of Bangladesh. J Clin Microbiol. 2005;43(7):3582-3583. doi:10.1128/JCM.43.7.3582-3583.2005
26. Yatsuyanagi J, Saito S, Sato H, Miyajima Y, Amano K, Enomoto K. Characterization of enteropathogenic and enteroaggregative Escherichia coli isolated from diarrheal outbreaks. J Clin Microbiol. 2002;40(1):294-297. doi:10.1128/JCM.40.1.294-297.2002
27. Lauber CL, Glatzer L and Sinsabaugh RL. Prevalence of pathogenic Escherichia coli in recreational waters. Journal of Great Lakes Research 2003; 29:301-306. doi:10.1016/S0380-1330(03)70435-3
28. Chern EC, Tsai YL, Olson BH. Occurrence of genes associated with enterotoxigenic and enterohemorrhagic Escherichia coli in agricultural waste lagoons. Appl Environ Microbiol. 2004;70(1):356-362. doi:10.1128/AEM.70.1.356-362.2004
29. Ochoa TJ, Ruiz J, Molina M, Del Valle LJ, Vargas M, Gil AI. High frequency of antimicrobial drug resistance of diarrheagenic Escherichia coli in infants in Peru. The American Journal of Tropical Medicine and Hygiene 2009; 81(2); 296–301. doi:10.4269/ajtmh.2009.81.296
30. Alikhani MY, Hashemi SH, Aslani MM, Farajnia S. Prevalence and antibiotic resistance patterns of diarrheagenic Escherichia coli isolated from adolescents and adults in Hamedan, Western Iran. Iran J Microbiol. 2013;5(1):42-47.
31. Nguyen TV, Le PV, Le CH, Weintraub A. Antibiotic resistance in diarrheagenic Escherichia coli and Shigella strains isolated from children in Hanoi, Vietnam. Antimicrob Agents Chemother. 2005;49(2):816-819. doi:10.1128/AAC.49.2.816-

819.2005
32. Omolajaiye SA, Afolabi KO, Iweriebor BC. Pathotyping and antibiotic resistance profiling of Escherichia coli isolates from children with acute diarrhea in Amatole District Municipality of Eastern Cape, South Africa. Biomed Res Int. 2020;2020:4250165. doi:10.1155/2020/4250165
33. Natarajan M, Kumar D, Mandal J, Biswal N, Stephen S. A study of virulence and antimicrobial resistance pattern in diarrhoeagenic Escherichia coli isolated from diarrhoeal stool specimens from children and adults in a tertiary hospital, Puducherry, India. J Health Popul Nutr. 2018;37(1):17. doi:10.1186/s41043-018-0147-z
34. Msolo L, Iweriebor BC, Okoh AI. Antimicrobial resistance profiles of Diarrheagenic E. coli (DEC) and salmonella species recovered from diarrheal patients in selected rural communities of the Amathole District Municipality, Eastern Cape Province, South Africa. Infect Drug Resist. 2020;13:4615-4626. doi:10.2147/IDR.S269219
35. Karama M, Mainga AO, Cenci-Goga BT, Malahlela M, El-Ashram S, Kalake A. Molecular profiling and antimicrobial resistance of Shiga toxin-producing Escherichia coli O26, O45, O103, O121, O145 and O157 isolates from cattle on cow-calf operations in South Africa. Sci Rep. 2019;9(1):11930. doi:10.1038/s41598-019-47948-1
36. Alabi OH, Obiekezie SO, Ekeleme K. Antibiotic resistance profiling and molecular detection of Escherichia coli Pathotypes isolated from irrigated fresh vegetables. AROC in Pharmaceutical and Biotechnology. 2022;2 (1): 18-26. doi:10.53858/arocpb0201 2735
37. David EE, Yameen MA, Igwenyi IO, Okafor AC, Obeten UN, Obasi DO, Ezeilo UR, David Ch N (2020) The frequency of virulent genes and antimicrobial resistance patterns of diarrheagenic Escherichia coli isolated from stools of children presenting with diarrhea in a tertiary hospital in Abakaliki, Nigeria. Int J One Health. 2020; 6: 147-152. doi:10.14202/IJOH.2020.147-152
38. Salleh MZ, Nik Zuraina NMN, Hajissa K, Ilias MI, Deris ZZ. Prevalence of multidrug-resistant diarrheagenic Escherichia coli in Asia: a systematic review and Meta-Analysis. Antibiotics (Basel). 2022;11(10):1333. doi:10.3390/antibiotics11101333

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