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Genetic characterization and antimicrobial susceptibility of *Escherichia coli* isolated from household water sources in northern Ghana

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ABSTRACT

The microbial quality of household water is an important issue in developing countries, especially in Ghana, where many people still depend on unimproved sources of water. The present study investigated the prevalence, genetic characteristics, and antimicrobial resistance profile of *Escherichia coli* from surface water sources. Fifty-two water samples were analyzed by using a spread plate, a biochemical test, and multiplex polymerase chain reactions. *E. coli* was isolated from each of the 52 water samples. Of these isolates, 75% were noted to possess virulence genes. Approximately 54% of the isolates were characterized as follows: enterotoxigenic *E. coli* (ETEC, 10.26%), enteropathogenic *E. coli* (EPEC, 17.95%), verotoxigenic *E. coli* (VTEC, 23.07%), and enteroinvasive *E. coli* (EIEC, 2.57%). Eighteen of the fifty-two isolates could not be characterized due to heterogeneity in banding. The disc diffusion method was used to test for antimicrobial susceptibility. The isolates were most resistant to ceftazidime, augmentin, and cefuroxime. Multidrug resistance was recorded in 48.1% of the isolates. In contrast, the isolates were most susceptible to ciprofloxacin (86.5%), nitrofurantoin (84.6%), and ofloxacin (75%). These results revealed a high diversity and widespread of *E. coli* in northern Ghana. The study provides important data for public health nationwide surveillance of *E. coli* in surface water across the country.

Key words: diarrhoea, *Escherichia coli*, multidrug resistance, surface water, virulence genes

HIGHLIGHTS

- This study explains the high occurrence of pathogenic *E. coli* in drinking water sources.
- There is a high occurrence of uncharacterized *E. coli* isolates with ambiguous banding patterns.
- A high record of multidrug resistance *E. coli* is explained.
- A reduction in the efficacy of important antimicrobials is found.

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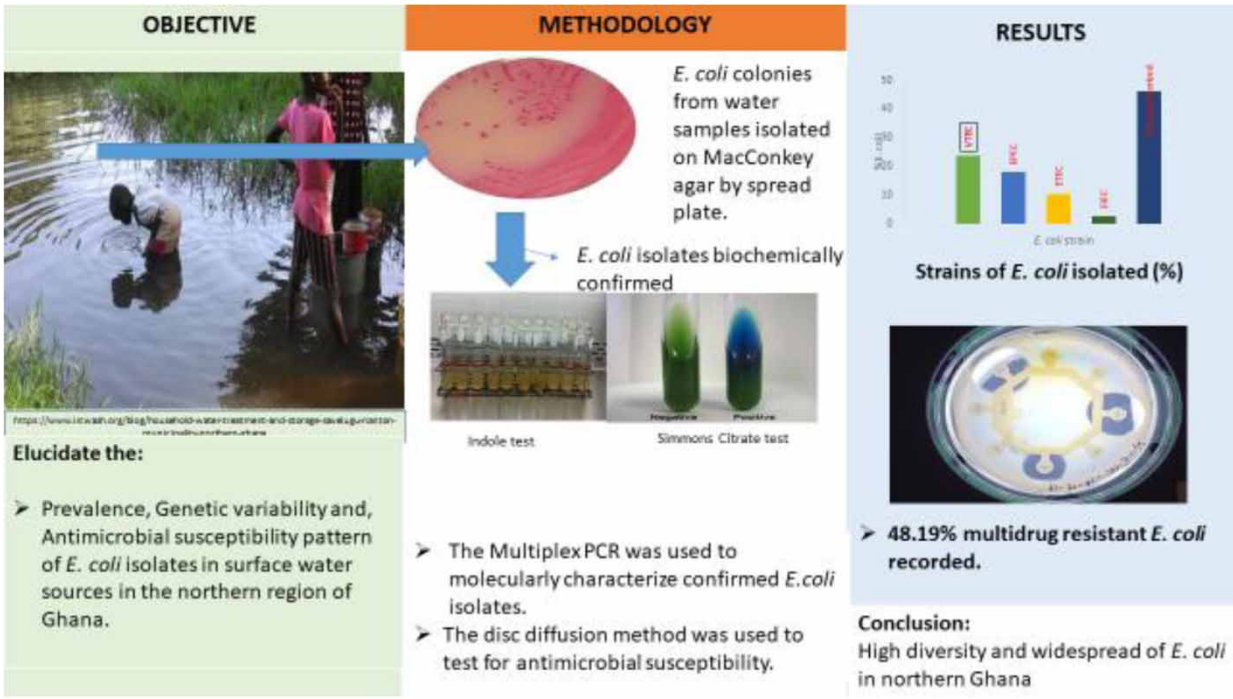
GRAPHICAL ABSTRACT



INTRODUCTION

Escherichia coli (*E. coli*) are bacteria that are commonly found in the lower gastrointestinal tract of humans and warm-blooded animals (Kittana *et al.* 2018). Most strains of *E. coli* are non-pathogenic, but some strains have acquired virulence factors and are capable of causing disease (Ramírez *et al.* 2013). *E. coli* can contaminate drinking water sources when indiscriminately excreted by humans and warm-blooded animals (Cabral 2010). Practices such as open defecation, the use of manure in agriculture, and inadequate drainage systems can result in the contamination of water sources with microbes such as *E. coli* after runoffs (Boelee *et al.* 2019).

The occurrence of *E. coli* in water is widely used as a bacteriological indicator of water quality and faecal pollution (Robert & Dirk 2017). The presence of pathogenic *E. coli* in water used for drinking, irrigation, and recreational purposes poses a potential health risk to humans and animals (Ramírez *et al.* 2013). Also, *E. coli* isolated from environmental waters can be multidrug resistant and of public health importance (Ramírez *et al.* 2013). Multidrug resistance here is defined as the acquisition of non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories (Magiorakos *et al.* 2012).

In the northern region of Ghana, of the 1.9 million inhabitants, about 50% of the people do not have access to improved drinking water sources and use unimproved water, i.e., surface water, as drinking water sources (United Nations (UN) 2020). This makes people vulnerable to waterborne diseases. Contaminated water and food have been attributed as the causes of diarrhoea (World Health Organization (WHO) 2017). Diarrhoea has been reported to account for the mortality of 525,000 infants annually (WHO 2017).

There are several etiologies responsible for infectious gastroenteritis causing acute diarrhoea (Shrivastava *et al.* 2017). However, diarrhoea outbreak among infants has largely been associated with pathogenic *E. coli* (Ochoa & Contreras 2011). Pathogenic *E. coli* capable of causing diarrhoea has been differentiated into enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAggEC), verotoxigenic *E. coli* (VTEC), diffusely adherent *E. coli* (DAEC), and enteroinvasive *E. coli* (EIEC) (Hien *et al.* 2007).

In Ghana, about half of all in-patients are exposed to one or more antibacterial therapy (Appiah-Korang *et al.* 2021), and about 90% of animal farmers purchase and administer antibiotics without prescription (Phares *et al.* 2020). Two separate multicentre point prevalence surveys involving four countries, including Ghana, and seven satellite hospitals in Ghana,

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respectively, revealed a high antibiotic use of 55 and 54.9% (Appiah-Korang *et al.* 2021; D'Arcy *et al.* 2021). In another survey, over 30% (125/400) had not received doctor's prescription during their last illness (Jimah *et al.* 2020). Antibiotics widely used in Ghana, in descending order of use, include metronidazole, amoxicillin/Clavulanic acid, ceftriaxone, cefuroxime, and ciprofloxacin (Appiah-Korang *et al.* 2021). About half of the antibiotics are mostly prescribed for the management of community-acquired infections, one-third for prophylaxis, and a tenth for no specifically documented indications (Appiah-Korang *et al.* 2021).

The inadequate availability of scientific data regarding the genetic characterization of *E. coli* from drinking water sources, the prevalence of diseases, infections and subsequent deaths caused by pathogenic *E. coli* (Ameer *et al.* 2021), and the rise in reported cases of antibiotic-resistance and pathogenic *E. coli* in Ghana is of great concern (García-Vello *et al.* 2020). It is, therefore, necessary to elucidate the prevalence, genetic variability, and antibiotic susceptibility pattern of *E. coli* isolates in surface water sources in the northern region of Ghana. This will provide important data for public health surveillance on the spread of pathogenic *E. coli*.

MATERIALS AND METHODS

Water sampling

Fifty-two water samples, upstream and downstream, from 26 sources (Figure 1), from five districts of the northern region of Ghana [Gushiegu ($n = 12$), Karaga ($n = 8$), Saboba ($n = 12$), Savelugu ($n = 8$), and Zabzugu ($n = 12$)] were aseptically collected using sterile 100 mL thio-bags (Thermo Scientific, UK). All water samples were transported in an ice chest containing ice packs to maintain the cold chain and analyzed within 24 h.

Culturing and isolation of *E. coli*

Culturing of *E. coli* was carried out as previously described by APHA (2012) and Lupindu (2017); briefly, 100 µl (0.1 ml) of each water sample was pipetted onto MacConkey agar (HiMedia, India). The water was then uniformly spread on the agar using a glass spreader and incubated at 37 °C for 24 h. Colony-forming units for each water sample ranged between 30 and 300 after incubation.

Pink-red colony growths with bile precipitate on MacConkey agar were morphologically and presumptively identified as *E. coli*, and subsequently, the presumptive *E. coli* isolates were biochemically confirmed using indole and citrate tests. A red-pink colony with bile precipitate on MacConkey agar, which was indole-positive and citrate-negative, was identified

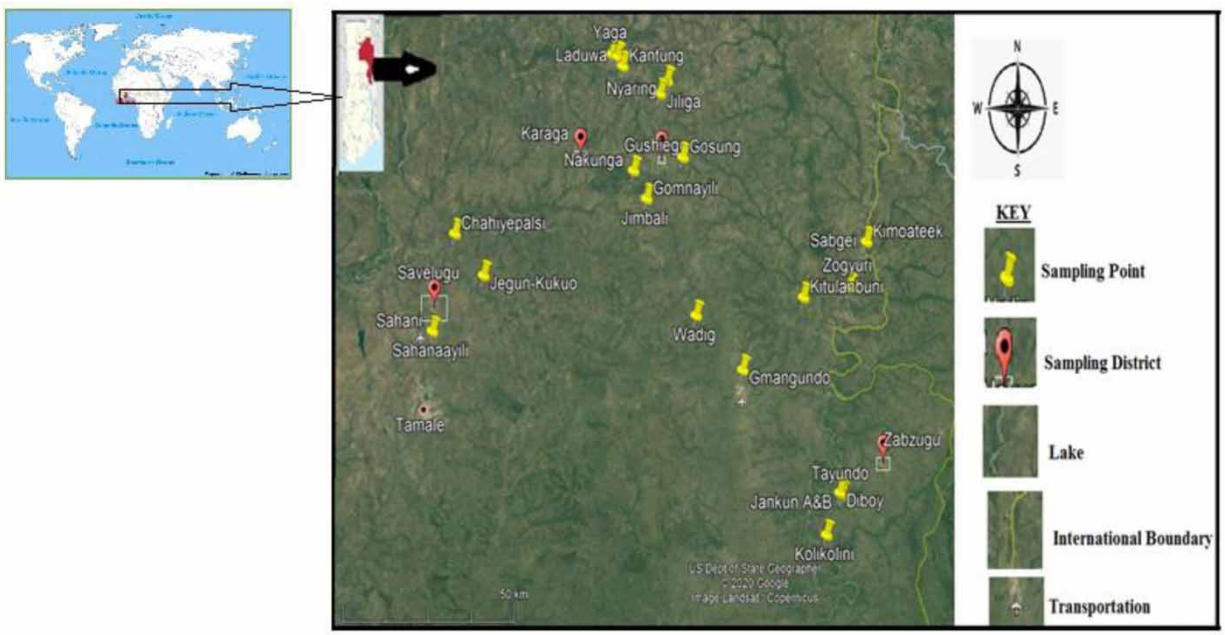

Figure 1 | Geographical positions of water sources in the districts of the northern region of Ghana from which water samples were taken.

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as *E. coli* (Lupindu 2017). The confirmed colonies were then aseptically picked and streaked on nutrient agar and incubated at 37 °C for 24 h to obtain pure culture isolates. The pure isolates were then used for the genetic characterization and antimicrobial susceptibility test.

Molecular characterization of *E. coli* isolates

Multiplex polymerase chain reactions (PCRs) were carried out with eight primer pairs as previously employed by Hien *et al.* (2007) (see Table 1 for primer sequences and genes for strain determination).

Each pure *E. coli* isolate harvested from nutrient agar plates was suspended in 30 µl Tris buffer (pH 7.9) to 1 McFarland standard (3×10^8 bacteria suspension/ml) and boiled for 30 min at 99 °C to obtain genomic DNA for PCR amplification. Two microliters of lysate from each isolate was used as a DNA template source in the PCR amplification. The PCR was carried out in a final reaction volume of 25 µl containing 2 µl DNA template, 12.5 µl Taq polymerase (OneTaq 2 × master mix buffer) (0.2 mM dNTPs, 1.8 mM $MgCl_2$, 20 mM Tris–HCI (pH 8.9), 22 mM NH_4Cl, 22 mM KCl, 0.05% Tween 20, and 0.06% IGEPAL CA-630), and 3.0 µM of forward and reverse mix (New England Biolabs). The PCRs were carried out using the peqSTAR 96 Universal Gradient thermal cycler (VWR, USA). The PCR cycling conditions were set at 5 min for initial denaturation at 95 °C, 45 cycles of 94 °C for 40 s (denaturation), 53 °C for 60 s (annealing), and 72 °C for 60 s (extension). The amplified PCR products were then analyzed on 2.0% (w/v) agarose gel and visualized under a UV transilluminator (Thermo Fisher Scientific, UK) after ethidium bromide staining.

Antimicrobial susceptibility testing of *E. coli* isolates

The disc diffusion method, also called the Kirby–Bauer method as recommended by CLSI (2015), was employed for the antimicrobial susceptibility test. The following antimicrobial agents were used to test for antimicrobial susceptibility, such as ceftazidime, cefuroxime, gentamicin, cefixime, ofloxacin, augmentin, nitrofurantoin, and ciprofloxacin, based on the European Committee on Antimicrobial Susceptibility Testing breakpoints (EUCAST 2018). The diameter of the zone of inhibition of each antibiotic was measured from the back of the plate against a dark background using a ruler, graduated in millimeters.

RESULTS

Distribution and prevalence of *E. coli* in surface water

All 52 water samples were found positive for *E. coli*.

Table 1 | Primer pairs used in the PCR identification of *E. coli* strains (Echeverria *et al.* 1993; Svenungsson *et al.* 2000; Hien *et al.* 2007)

E. coli strain	Target gene	Primer	Primer sequence (5′–3′)	Amplicon size (bp)
ETEC	*eltB*	LT1	TCTCTATGTGCATACGGAGC	
		LTr	CCATACTGATTGCCGCAAT	322
		STI2 l	GCTAAACCAGTARGGTCTTCAAAA	
	estB	STI2 r	CCCGGTACARGCAGGATTACAACA	147
VTEC	*vtx* 1	VT1 l	GAAGAGTCCGTGGGATTACG	
		VT1 R	AGCGATGCAGCTATTAATAA	130
	vtx 2	VT2 l	ACCGTTTTTCAGATTTTRCACATA	
		VT2r	TACACAGGAGCAGTTTCAGACAGT	298
EPEC	*Eae*	eae u	CACACGAATAAACTGACTAAAATG	
		eae l	AAAAACGCTGACCCGCACCTAAAT	376
	bfp A	bfp A2 u	TTCTTGGTGCTTGCGTGTCTTTT	
		bfp A2 l	TTTTGTTTGTTGTATCTTTGTAA	367
EIEC	*ipaH*	IpaH III	GTTCCTTGACCGCCTTTCCGATACCGTC	
		IpaH IV	GCCGGTCAGCCACCCTCTGAGAGTAC	620
EAggEC	*aatA*	EA 1	CTGGCGAAAGACTGTATCAT	
		EA 2	CAATGTATAGAAATCCGCTGTT	630

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Molecular variability of *E. coli* isolates

Thirty-nine out of the fifty-two confirmed *E. coli* isolates from the water samples were found to carry virulence genes in different proportions (Figure 2).

Based on the pattern of detected virulence genes (Figure 2), *E. coli* isolates were characterized into four apparent strains (Figure 3). However, 46.15% of *E. coli* isolates that carried virulence genes were not characterized due to unusual banding patterns (Figure 3).

Antimicrobial susceptibility pattern of *E. coli* isolates

All the 52 *E. coli* isolates from each of the water samples were resistant to at least two of the essential antimicrobials used. However, multidrug resistance was recorded in 25/52 (48.1%) of the isolates. The pattern of susceptibility is shown in Table 2), and the proportion of multidrug resistance of *E. coli* to categories and individual antimicrobials are shown in Table 3 and Figure 4), respectively.

DISCUSSION

Access to safe drinking water is a human right (UN 2010). Drinking water is required to be free from *E. coli* (WHO 2017). In this study, however, a wide-spread distribution of *E. coli* was observed in drinking water sources. The widespread *E. coli* distribution in the water was attributed to runoff from environments with poor sanitation practices such as open defecation

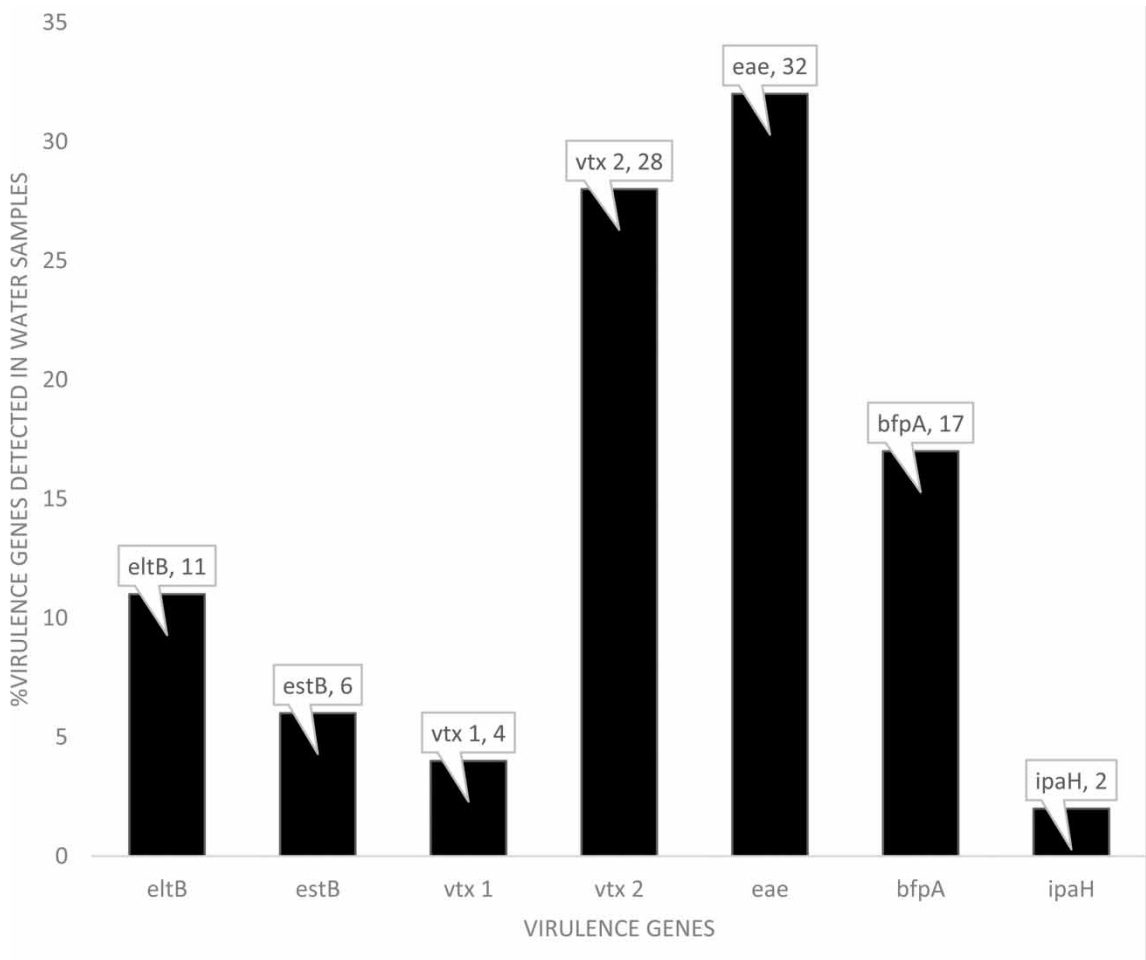

Figure 2 | Percentage distribution of *E. coli* virulence genes (*elt* B, *est* B, *vtx* 1, *vtx* 2, *eae*, *bfp*A, and *ipa*H) detected in surface water sources in the northern region of Ghana.

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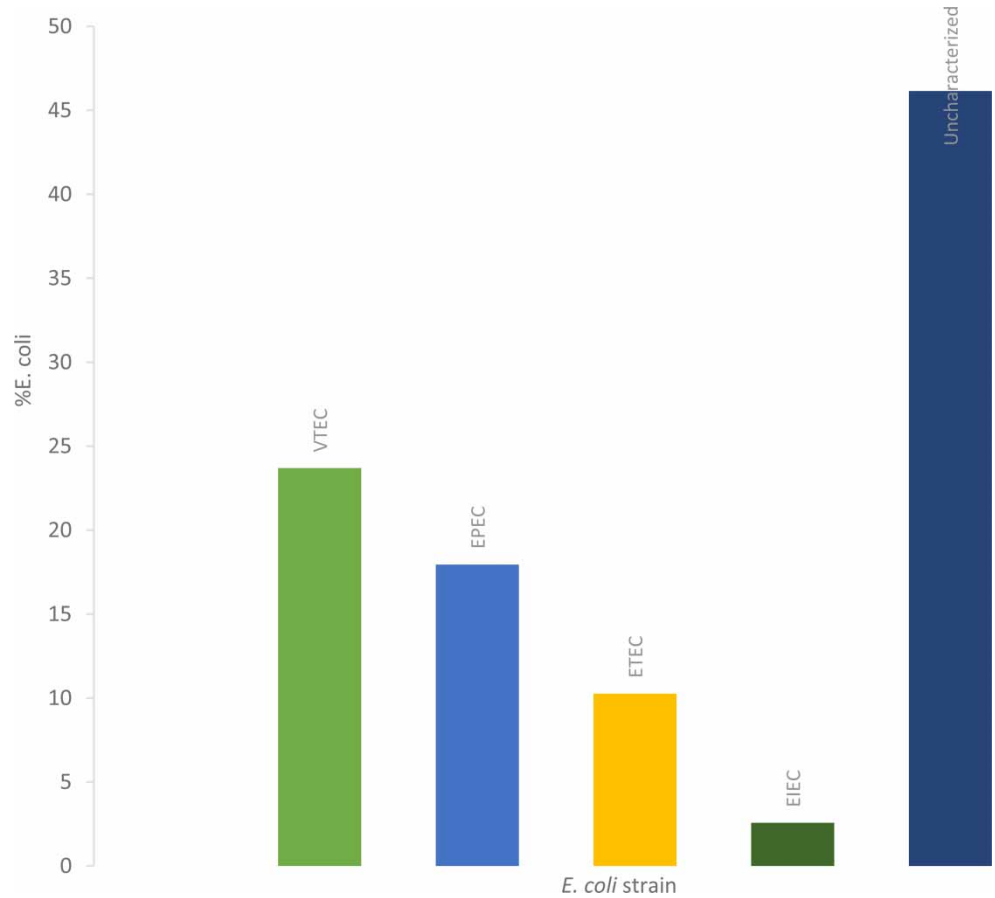

Figure 3 | Proportion of strains of *E. coli* isolated from surface water sources in the northern region of Ghana.

Table 2 | Pattern of susceptibility of *E. coli* isolates to the antibiotics used based on EUCAST (2018)

	ZDM breakpoints (mm)		Susceptibility		
Antibiotics	**S≥**	**R<**	**Susceptible (S) (%)**	**Resistance (R) (%)**	**ATU (%)**
Ceftazidime (30 μg)	22	19	0	100	–
Cefuroxime (30 μg)	19	19	3.8	96.2	–
Gentamicin (10 μg)	17	14	73	27	–
Cefixime (5 μg)	17	17	55.8	44.2	–
Ofloxacin (5 μg)	24	22	75	25	–
Augmentin (30 μg)	16	16	0	100	–
Nitrofurantoin (300 μg)	11	11	84.6	15.4	–
Ciprofloxacin (5 μg)	25	22	86.5	5.8	7.7

ZDM, zone diameter breakpoint; ATU, area of uncertainty; mm, millimeters.

(Osumanu *et al.* 2019). The consumption of water contaminated with *E. coli* leads to the outbreak of waterborne diseases and infections, which subsequently results in morbidity and mortality in children and adults (Ameyaw *et al.* 2017).

The occurrence of pathogenic *E. coli* (75%) as identified in this study was higher than what was formerly identified in some African countries such as in Côte d'Ivoire (68%) (Kambire *et al.* 2017) and South Africa (67.5%) (Obi *et al.* 2004). The

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Table 3 | Resistance of *E. coli* isolates to antimicrobial categories

***E. coli* isolates from each water sample/52 resistant to antimicrobial categories**	**No. of antimicrobial category resistance**	**Percentage *E. coli* resistance (no. of *E. coli* isolates/total no. of samples × 100)**
27	2	51.9
16	3	30.8
6	4	11.5
3	5	5.8

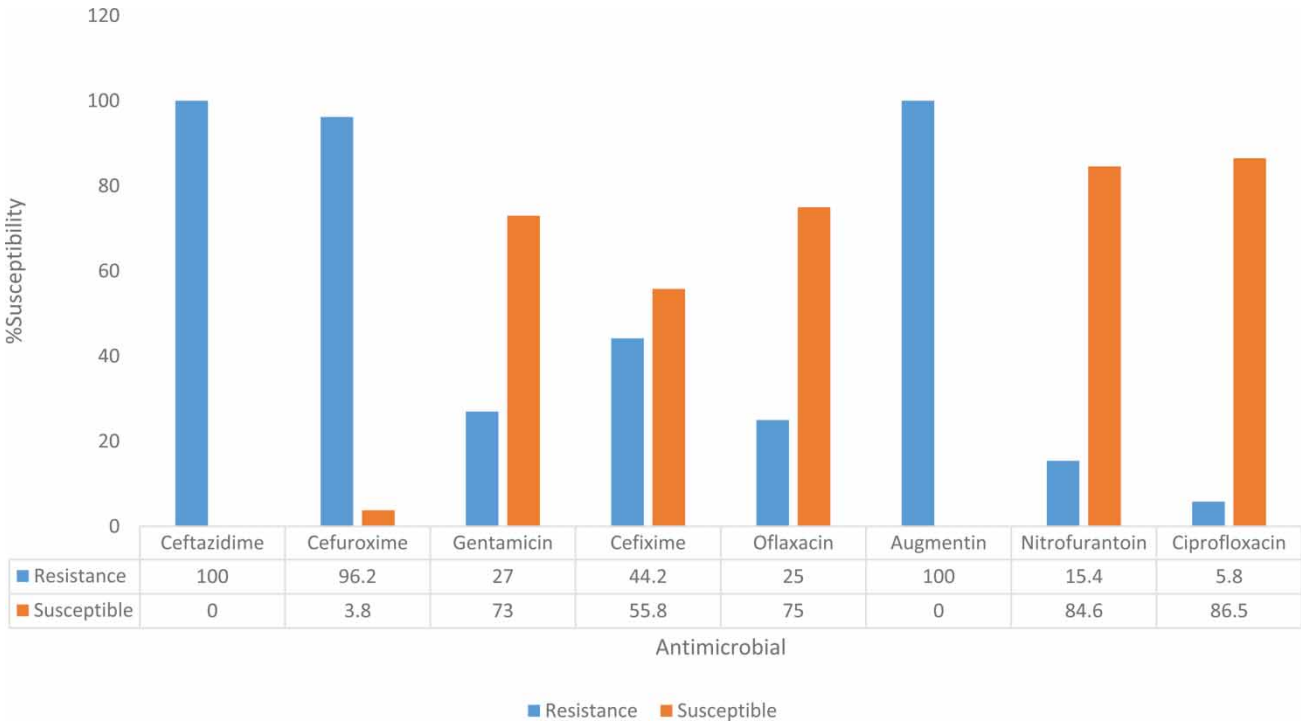

Figure 4 | Proportion of antimicrobial resistance (%) of *E. coli* isolates to individual essential antimicrobials.

occurrence of pathogenic *E. coli* in the water sources is a result of poor drainage systems and the inability of the people to access improved sanitation (WHO & UNICEF 2000).

The frequency of virulent genes in this study varied greatly than what was found in South Africa (Ndlovu *et al.* 2015) and Côte d'Ivoire (Kambire *et al.* 2017). A study conducted in Southern Africa recorded *aggR* genes as the most predominant genes (69%) in Paarl and the Berge rivers, followed by *iPaH* (31%), *stx* or *vtx* (15%), and *eae* (8%). In Côte d'Ivoire, *est* genes were higher than *elt* genes (Kambire *et al.* 2017). However, the prevalence and predominance of *elt* genes as found in this study agrees less with the previous studies conducted in South Africa and Côte d'Ivoire, but agrees more with the studies conducted in the USA and in Egypt (Shaheen *et al.* 2004; Cho *et al.* 2018).

Four strains of potential pathogenic *E. coli* were identified and characterized, namely, VTEC, EPEC, ETEC, and EIEC. According to Lupindu (2017), VTEC, ETEC, EPEC, and EIEC are part of the intestinal disease-causing *E. coli* that cause various degrees of diarrhoea in warm-blooded animals such as humans. The main potential disease-causing *E. coli* strain as identified in the study, VTEC, is known to be the cause of diseases like the most deadly hemorrhagic uremic syndrome (HUS) and hemorrhagic colitis, which are mainly observed in both the elderly and children (Kuhnert *et al.* 2000). ETEC, the second most predominant strain in this study, is the causative strain for watery diarrhoea in humans (Lupindu 2017). EPEC and EIEC, on the other hand, are also the principal causes of diarrhoea in children under 2 years (Li *et al.* 2009) and dysentery-like diarrhoea with accompanied fever, respectively (Todar 2012).

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The prevalence and predominance of potential disease-causing strains of *E. coli* as identified in this study conforms with the 2017 World Health Organization report, which opined that about 525,000 children die annually due to the consumption of contaminated food and water.

Of the 39 identified potential pathogenic *E. coli* isolates, 46.15% were not characterized into strains because of their unusual banding patterns. It remains unclear whether these are novel strains until further tests are carried out to fully elucidate the isolates.

The rise in multidrug resistance of *E. coli* is a global health concern. Here, the 52 isolates from the 52 water samples were resistant to more than one of the antibiotics used. The prevalence of antibiotic resistance to more than one antibiotic as registered in this investigation was far more than the 49.48% that was earlier reported by Odonkor & Addo (2018) in southern Ghana. However, the multidrug resistance in this investigation was novel according to the recent definition of multidrug resistance (Magiorakos *et al.* 2012). Although similar to the work of Odonkor & Addo (2018) where *E. coli* isolates were most susceptible to ciprofloxacin, nitrofurantoin, and gentamicin, a decrease in the levels of susceptibility of ciprofloxacin, nitrofurantoin, and gentamicin was, however, observed in the present study. A decrease in the antimicrobial susceptibility of ciprofloxacin and gentamicin was also reported by Adzitey *et al.* (2015). The multidrug resistance revealed in this study confirmed speculation by WHO (2018) that there may be a worldwide increase in the prevalence of multidrug resistance of *E. coli* strains. It is also worrying to note that, although nitrofurantoin and ciprofloxacin were the most effective antimicrobials against the *E. coli* isolates, some of the *E. coli* isolates posed resistance to these antimicrobials.

The multidrug resistance recorded in this study could be as a result of mutations and the dynamic ability of *E. coli* to exchange genetic-resistance genes through horizontal gene transfer (Fodor *et al.* 2020). This could have also been fueled by the overuse or misuse of antimicrobials in animals and humans without expert advice (WHO 2018). An inadequate knowledge of antibiotics and the inappropriate prescription of antibiotics to patients could also be contributory factors for the surge in multidrug resistance in *E. coli* in this study (Afari-Asiedu *et al.* 2020).

The susceptibility of the *E. coli* isolates to nitrofurantoin and ciprofloxacin might be a result of the reduced prescription and usage of these antimicrobials (Appiah-Korang *et al.* 2021). However, the high antibiotic resistance recorded against ceftazidime, augmentin, and cefuroxime could be attributed to their regular prescription and usage, inexpensive nature, easy access and both use and misuse of these antibiotics by some Ghanaians.

The WHO asserted that novel antimicrobial resistance patterns are emerging and increasing all over the globe, thereby threatening the treatment of common infectious diseases, a scenario that will present an incidence/prevalence of prolonged illnesses, disability, and death (WHO 2018).

Here, our data show good sensitivity of ciprofloxacin and nitrofurantoin against the *E. coli* isolates, since most of the isolates were susceptible to these antimicrobials.

The susceptibility of the isolates to nitrofurantoin could be due to the uniqueness of the antibiotic, characterized by a hydantoin ring with a nitro-substituted furanyl side chain that is metabolized within the bacteria to produce reactive compounds that are bactericidal (Howard 2007; Sauberan & Bradley 2018). Also, the susceptibility of the isolates to ciprofloxacin could stem from the fact that ciprofloxacin is an antitoxin bacteria-killing antimicrobial of the fluoroquinolone medicine class that is able to restrain DNA replication by repressing DNA-gyrase and bacterial DNA topoisomerase (Hooper & Jacoby 2016).

CONCLUSIONS

The study revealed a 100% prevalence of *E. coli* in the surface water samples, of which 75% were classified as pathogenic. These were further characterized into four strains, namely VTEC (17%), EPEC (13%), ETEC (8%), and EIEC (2%). Notably, 18 *E. coli* harbouring virulence genes with unusual banding could not be easily characterized. A sequencing of the isolate could help in the categorization.

The study also revealed a 48.1% multidrug resistance in 25/52 of the water samples when *E. coli* isolates from the samples were tested against eight antimicrobial agents belonging to five antimicrobial categories and listed as part of the WHO's list of essential medicines. The most resisted antimicrobial agents in this study were *ceftazidime*, *augmentin*, *cefuroxime*, and *cefixime*, while the susceptible antimicrobial agents were *ciprofloxacin*, *nitrofurantoin*, *oflaxacin*, *gentamicin*, and *cefixime*.

The observed surge in multidrug resistance isolates in this study, coupled with the decrease in susceptibility against nitrofurantoin and ciprofloxacin as compared to earlier studies, is indicative of the fact that *E. coli* isolates are gradually gaining

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resistance to these all important antimicrobials. This is despite the study revealing that nitrofurantoin and ciprofloxacin were the most effective antimicrobials against the *E. coli* isolates. Further works on the antimicrobial resistance mechanism of the isolates are essential for the understanding of multidrug resistance and the management of water-related *E. coli* infections.

The current study was conducted in a resource-limited setting. As a result, only PCR, using *E. coli* virulence gene markers, was used in the genetic characterization and molecular confirmation of the *E. coli* isolates. Therefore, whole genome sequencing and/or 16S rRNA of all or some of the isolates is recommended for the confirmation of identities in future studies.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

FUNDING

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DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

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