

Research Paper

Prevalence of multidrug-resistant *Escherichia coli* in household drinking water in rural Ghana

Elvis Kichana ^{a,*}, Maame Serwa Opare-Boafoa ^b and Emmanuel Martin Obeng Bekoe ^c

^a Regional Water Quality Laboratory, World Vision International, Tamale, Ghana

^b Ghana School of Public Health, University of Ghana, Accra, Ghana

^c Water Research Institute – Council for Scientific and Industrial Research, Tamale, Ghana

*Corresponding author. E-mail: ekichana@gmail.com

 EK, 0000-0002-1816-0120; MSO, 0000-0002-4579-5332; EMOB, 0000-0001-5113-4269

ABSTRACT

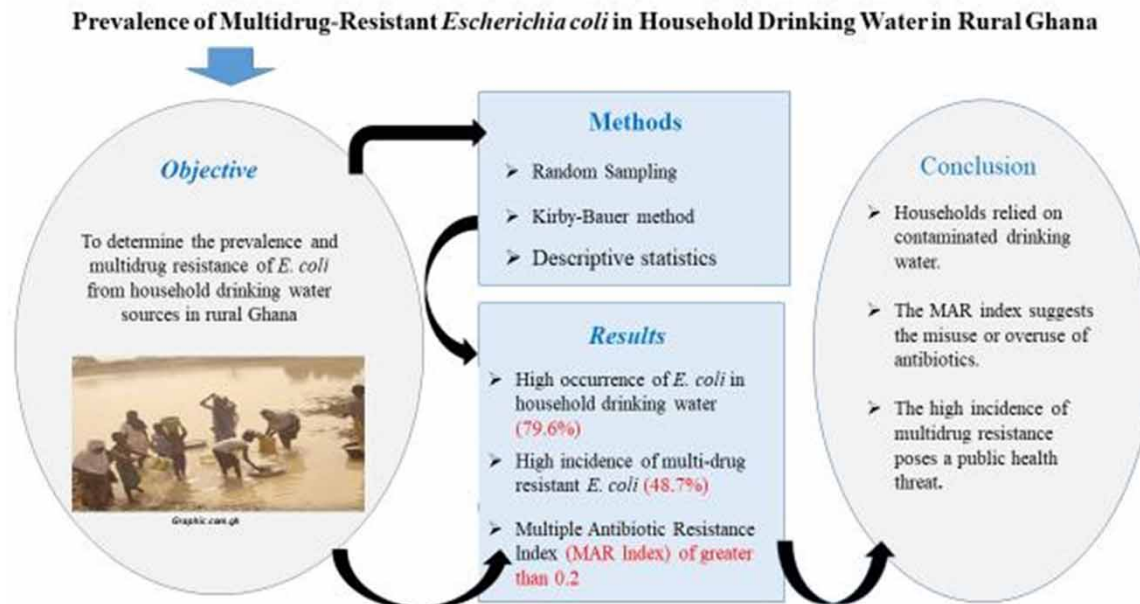
Even in the 21st century, households in rural Ghana still rely on drinking water sources that are contaminated with pathogenic *Escherichia coli*, the consumption of which results in morbidity and mortality of children and adults. The present study sought to determine the prevalence and antimicrobial susceptibility profile of *E. coli* isolated from household drinking water. A total of 49 water samples were analyzed. *E. coli* was isolated and confirmed from the water samples using the spread plate and biochemical tests, respectively. The Kirby–Bauer method was used for antimicrobial susceptibility testing. *E. coli* was isolated from 79.6% of the water samples with a mean colony growth of 15 cfu/100 µl. The isolates were absolutely resistant to ceftazidime, cefixime, augmentin, and cefuroxime. In contrast, the isolates were most susceptible to ciprofloxacin, ofloxacin, gentamicin, and nitrofurantoin. Multidrug resistance was registered in 48.7% of the isolates. *E. coli* isolates from each water sample had a Multiple Antibiotic Resistance (MAR) index greater than 0.2, indicating increased use or misuse of antibiotics in the study area. This study revealed a high occurrence of multidrug-resistant *E. coli* and a record-high reduction in the efficacy of important antimicrobials.

Key words: antibiotic stewardship, antimicrobial, drinking water, *E. coli*, multidrug resistance

HIGHLIGHTS

- Households in rural Ghana rely on water contaminated with faecal matter for their livelihoods.
- High multidrug resistant *E. coli* in household drinking water.
- Suggestive evidence of increased use or misuse of antibiotics in the study area.
- Record of reduced efficacy of important antimicrobials.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Escherichia coli (*E. coli*) is a member of the Enterobacteriaceae family that exists predominantly in the lower gastrointestinal tract of mammals as a non-pathogenic commensal (Jang *et al.* 2017). Although mostly found in humans and animals, *E. coli* also occurs in water. The presence of *E. coli* in water is due to anthropogenic activities such as indiscriminate waste disposal, open defecation, and agricultural manuring of farmlands (Channah 2014). *E. coli* isolated from water sources have been found to be pathogenic and antibiotic-resistant (Odonkor & Addo 2018). This is attributed to the dynamic ability of *E. coli* to exchange genetic-resistant genes among themselves and as a result of other factors including mutations, use, and misuse of antibiotics (Fodor *et al.* 2020). Pathogenic and antibiotic-resistant *E. coli* cause diseases such as the notable deadly hemorrhagic uremic syndrome (HUS), hemorrhagic colitis, diarrhea, and, in some cases the cause of medical treatment failures (Kuhnert *et al.* 2000).

The surge in resistance of *E. coli* to existing essential antibiotics is a global health threat (WHO 2020). Before the advent of antibiotics, infections and diseases caused by pathogenic *E. coli* and other related bacteria led to a prolonged illness, with accompanying deaths (Ventola 2015). However, the discovery of antibiotics and the therapeutic advancements made in the use of antibiotics for the treatment of infections and diseases caused by *E. coli* have drastically reduced the number of deaths resulting from bacterial infections (Ventola 2015). Unfortunately, however, over the years, bacteria, including *E. coli* have developed rapid resistance against many essential antibiotics that were used in the successful and effective treatment of bacterial infections and diseases (Puvaca 2021).

Each year, nearly 700,000 individuals worldwide pass away from illnesses that are resistant to treatment (Rosini *et al.* 2020). By 2030, drug resistance might push over 24 million people into poverty and result in around 10 million yearly fatalities in 2050 (WHO 2019). With rising antibiotic resistance, organ and tissue transplants could become all but impossible (Rosini *et al.* 2020).

The global health threat posed by antibiotic resistance (WHO 2020) calls for international, regional, and national surveillance. Unfortunately, in Ghana, and in the Talensi district, not much is known and documented about the antimicrobial resistance of *E. coli* isolated from drinking water.

The sanitation situation in the Talensi district is inadequate (Yin-Anaab 2017). Apart from a few public places such as markets, health facilities, school and government quarters, bungalows, and offices, where improved latrines are found, the district is generally lacking such facilities as toilets, slaughterhouses, urinals, sanitary equipment, and waste disposal sites (Yin-Anaab 2017). As a result, most people resort to open defecation.

The majority of the communities in the Talensi district rely on unimproved water sources like rivers, dams, ponds, and dug-outs for their livelihoods (Chegbeleh *et al.* 2020). These sources are prone to fecal contamination as a result of the inadequate sanitation situation in the district.

The number of health facilities in the district is not sufficient, and those available are poorly equipped (Yin-Anaab 2017). As a result, most people resort to using chemical shops and Traditional Healers (Yin-Anaab 2017). These are normally the first point of call for many ill people as many people self-medicate (Yin-Anaab 2017). Self-medication mostly leads to the use and misuse of antimicrobials and other medicines.

This study sought to determine the prevalence and multidrug resistance of *E. coli* from household drinking water sources in rural Ghana. The study will provide important scientific data for public health surveillance of antibiotic resistance in Ghana, and will also provide information for antibiotic stewardship programs.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted in the Talensi District, in the Upper East Region, Ghana. The district is located within the boundaries of longitudes 0°31' and 1°05' west and latitudes 10°35' and 10°60' north of the Greenwich Meridian. The district shares boundaries with Nabdam District to the north, Bolgatanga Municipal to the west, East Mamprusi Municipal to the south-east, West Mamprusi Municipal to the south-west, and Bawku West District to the east. The population of the district according to the 2021 population and housing census stands at 87,021 with 43,849 males and 43,172 females. It has a total land area of about 845.3 km² with a population density of 102.9/km². The majority of the inhabitants (about 90%) in the district are peasant farmers. Small-scale mining, artisanal stone crushing, agro-processing, charcoal burning, firewood harvesting, and irrigation farming constitute the other sources of income in the district (Yin-Anaab 2017).

2.2. Water sampling

Water samples were aseptically and randomly collected from 49 households, from four satellite communities in the Talensi district of the upper east region of Ghana (see Figure 1). The number of households selected was based on the total number of households in each community. Each community had an average of 22 households. Water samples were collected using pre-sealed 125-ml sterile sodium thiosulfate sampling bottles (Thermo Scientific, UK). All the water samples were transported in an ice chest containing ice packs and analyzed within 24 h.

2.3. Culturing and isolation of *E. coli*

Isolation of *E. coli* was carried out as earlier described by Lupindu (2017), using the spread plate method; briefly, 100 µl 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. Single colony growths, characteristic of *E. coli* (pink-red colony with bile precipitate) on MacConkey agar were confirmed using indole and citrate tests (Lupindu 2017). The positive colonies were aseptically picked and streaked on nutrient agar and incubated at 37 °C for 24 h to obtain pure culture isolates. The pure 24 h old isolates were then used for an antimicrobial susceptibility test.

2.4. Antimicrobial susceptibility testing of *E. coli* isolates

The disc diffusion method also called the Kirby–Bauer method was used for the antimicrobial susceptibility test (CLSI 2015). Ceftazidime, cefuroxime, gentamicin, cefixime, ofloxacin, augmentin, nitrofurantoin, and ciprofloxacin were the antimicrobial agents used to test for the antimicrobial susceptibility of the *E. coli* isolate, 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.

2.5. Multiple antibiotic resistance index

The multiple antibiotic resistance (MAR) index refers to the number of antibiotics an isolate is resistant to (*a*), divided by the total number of antibiotics used in the study (*b*). The MAR index was calculated using the formula $MAR\ Index = a/b$.

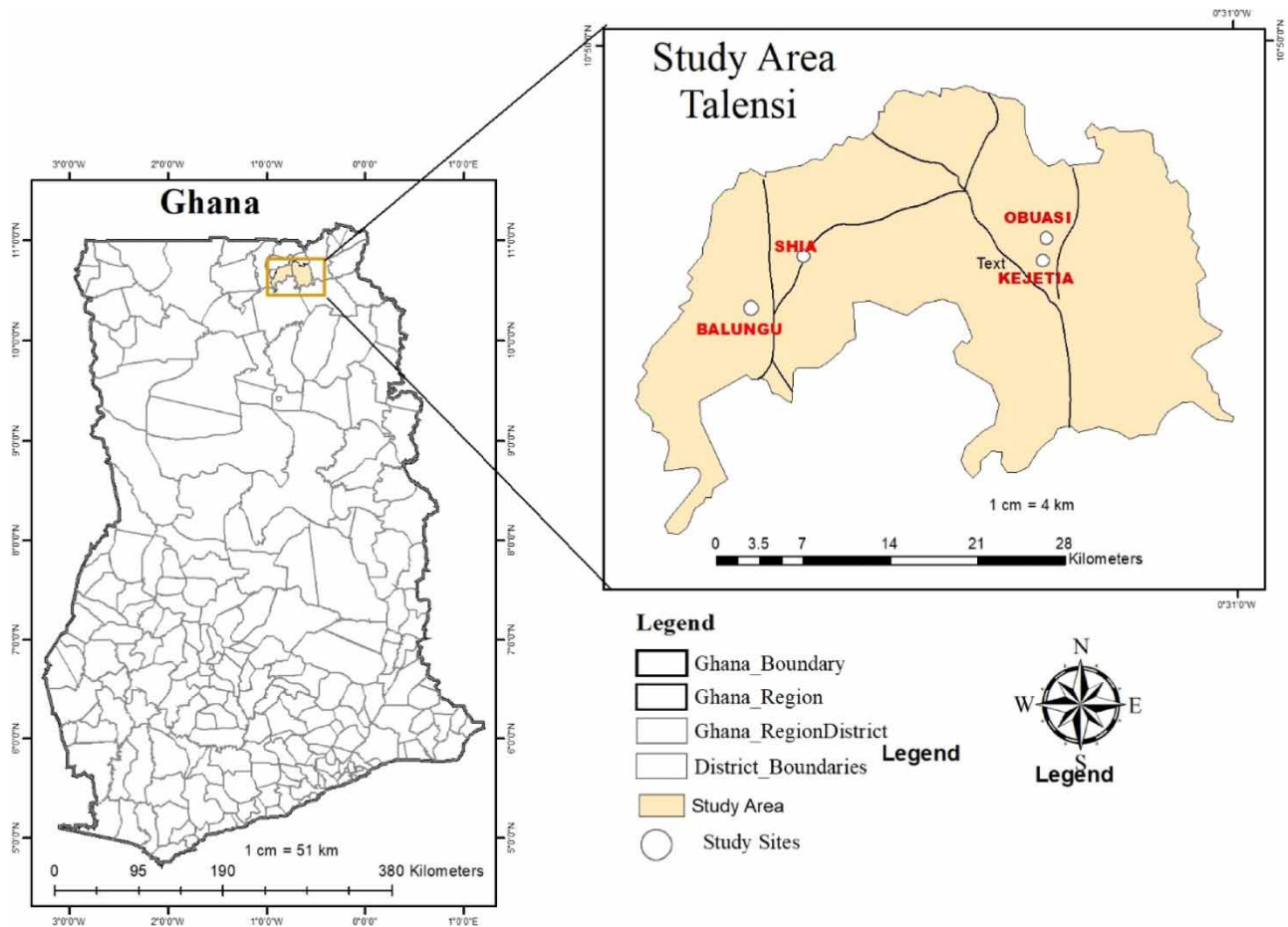


Figure 1 | Geographical positions of water sampling communities in the Talensi district of the upper east region of Ghana.

3. RESULTS

3.1. Prevalence of *E. coli* in household drinking water

E. coli was isolated from 39/49 of the water samples, representing 79.6% of the total number of samples taken, with a mean colony forming units (cfu) of 55 cfu/100 μ l of each water sample.

3.2. Antimicrobial susceptibility pattern of *E. coli* isolates

Confirmed *E. coli* isolates from each water sample were resistant to at least two antibiotics (at least 2/8 antibiotics), with a MAR index of greater than 0.2. However, multidrug resistant *E. coli* was recorded in 48.7% of the total number of water samples analyzed. The pattern of susceptibility (Table 1), and the proportion of multidrug resistance of *E. coli* to antimicrobial categories, and individual antimicrobials are shown, respectively (Table 2 and Figure 2).

4. DISCUSSION

Drinking water is required to be free from *E. coli* (WHO 2017). In this study, however, *E. coli* was found in 79.6% of household drinking water, as a possible result of poor sanitation practices (Osumanu *et al.* 2019). Consumption of water contaminated with *E. coli* results in morbidity and mortality in children and in adults (Gwimbi & George 2019). With the increased number of households drinking water contaminated with fecal matter, the incidences of morbidity and mortality could increase, most especially in children and, adults.

Resistance of *E. coli* to essential antibiotics is a global health threat. This study revealed that *E. coli* isolates were resistant to antibiotics in at least two antimicrobial classes, and had a MAR index greater than 0.2. Multidrug resistance was also

Table 1 | Pattern of susceptibility of *Escherichia coli* isolates to individual antibiotics (EUCAST 2018)

Antibiotic	ZDM breakpoints (mm)		Susceptibility		
	≥ S	< R	Susceptible (S) (%)	Resistance (R) (%)	ATU (%)
Ceftazidime (30 µg)	22	19	0	100	–
Cefuroxime (30 µg)	19	19	0	100	–
Gentamicin (10 µg)	17	14	79.5	20.5	–
Cefixime (5 µg)	17	17	0	100	–
Ofloxacin (5 µg)	24	22	87.2	12.8	–
Augmentin (30 µg)	16	16	0	100	–
Nitrofurantoin (300 µg)	11	11	74.4	25.6	–
Ciprofloxacin (5 µg)	25	22	94.9	5.1	–

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

Table 2 | Resistance of *Escherichia coli* isolates to antimicrobial categories

<i>E. coli</i> isolates from each water sample/52 resistant to antimicrobial categories	No. of antimicrobial categories resistance	Percentage <i>E. coli</i> resistance (no. of <i>E. coli</i> isolates/total No. of samples × 100)
20	2	51.3
15	3	38.5
2	4	5.1
2	5	5.1

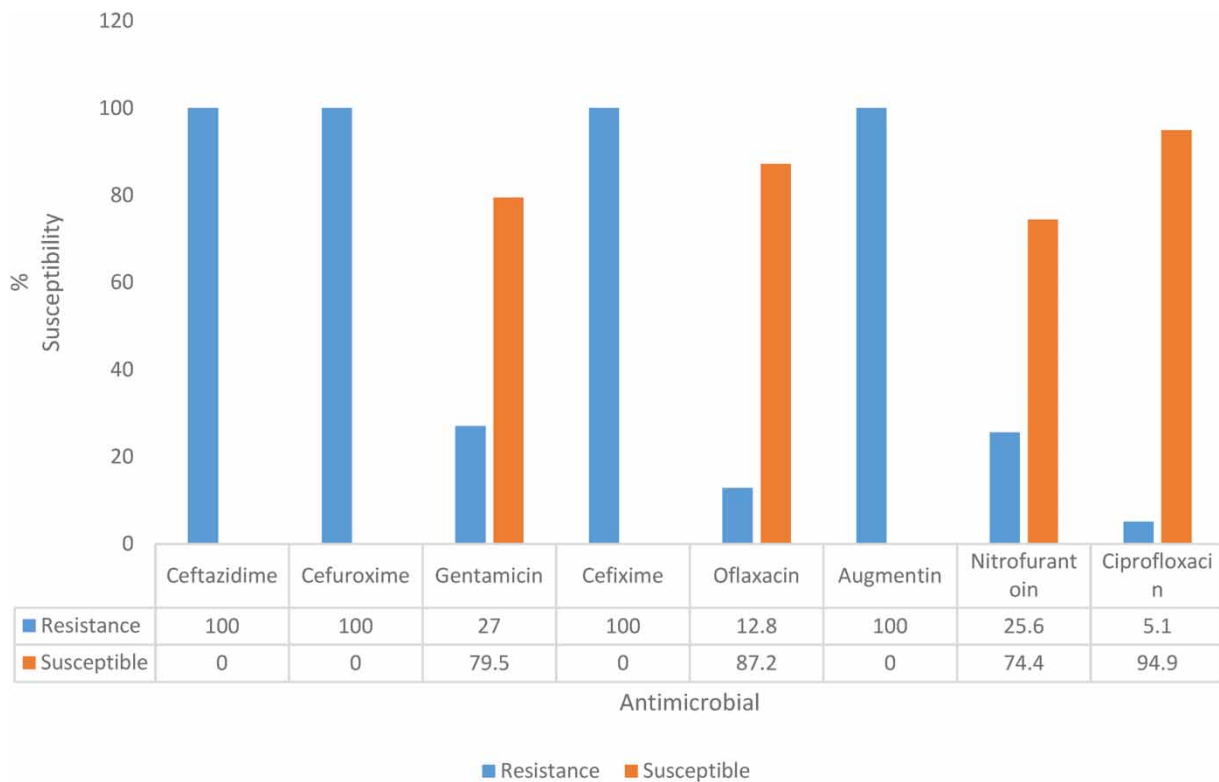


Figure 2 | Antibiotic resistance (%) of *Escherichia coli* isolates to individual essential antimicrobials.

registered in 48.7% of the isolates (Magiorakos *et al.* 2012). The MAR value of greater than 0.2, and the multidrug resistance in 48.7% of the isolates indicate an increased use or misuse of antibiotics in the Talensi district. This is evident from the assertion made by the District Assembly that many people practiced self-medication because of the inadequate availability and distribution of health facilities in the district (Yin-Anaab 2017). This also suggests a decline in the efficacy of essential antimicrobials that may lead to future medical treatment failures. The multidrug resistance revealed in this study confirmed an assertion by the World Health Organization 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 2017). The high multidrug resistance in this study could also be attributed to mutations and the dynamic ability of *E. coli* to exchange genetic-resistance genes through horizontal gene transfer (Fodor *et al.* 2020). Overuse or misuse of antimicrobials in animals and humans without expert advice (WHO 2017) and inadequate knowledge of antibiotics, coupled with the inappropriate prescription of antibiotics to patients, are also possible factors that led to the high record of multidrug-resistant *E. coli* in this study (Afari-Asiedu *et al.* 2020).

Unlike in the study conducted by (Odonkor & Addo 2018), where *E. coli* isolates were most susceptible to ciprofloxacin and nitrofurantoin, in this recent study, *E. coli* isolates were most susceptible to ciprofloxacin and ofloxacin. In this study also, the susceptibility of *E. coli* isolates to ofloxacin and gentamicin was greater than the susceptibility of the isolates to nitrofurantoin as recorded (Odonkor & Addo 2018). A similar study in the northern region of Ghana confirms the susceptibility of *E. coli* to Gentamicin and Ciprofloxacin (Kichana *et al.* 2022). This suggests that *E. coli* from different geographical areas respond differently to different essential antimicrobials.

With our findings, ciprofloxacin and ofloxacin are suggested for the management and treatment of *E. coli*-induced waterborne infections and diseases in the study area, since most of the isolates were most susceptible to these antimicrobials.

5. CONCLUSIONS

The surge in prevalence of multidrug-resistant isolates in this study is indicative that *E. coli* are gradually gaining resistance to essential antimicrobials. Although the study revealed ciprofloxacin and ofloxacin as the most effective antimicrobials against the *E. coli* isolates, the study was however limited by financial constraints to explore the resistance mechanisms of the *E. coli* isolates that resulted in the high multidrug resistance. Further work on the antimicrobial resistance mechanism of the isolates is essential for the understanding of multidrug resistance and the management of water-related *E. coli* infections in Ghana.

With this surge in antibiotic and multidrug resistance, the development of new antimicrobials is highly recommended, in order to prevent future medical treatment failures.

FUNDING

No funding was received for this work.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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