

Research Application Summary

**Genetic variability and antimicrobial susceptibility of *Escherichia coli* isolates from household water sources in Northern Ghana**

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**Abstract**

In Ghana, especially the Northern Region, a significant proportion of the population rely on surface and other unimproved water sources. Consumption of water from such sources leads to the contraction of infectious diseases of public health importance, which accounts for high morbidity and mortality among children and adults. Previous works conducted in Ghana focused on isolation of *Escherichia coli* from surface waters. However, the genetic variability and antimicrobial resistance profile of *Escherichia coli* isolates in Ghana remains unknown. The present study demonstrated genetic variability and antimicrobial profiles of *E. coli* isolated from 26 water sources from five districts in the northern Ghana region. Biochemical test was employed to confirm *E. coli* isolates, multiplex PCR was used to characterize the isolates, and disk diffusion methods was used to test for antimicrobial susceptibility. The study recorded 75% of the isolates as pathogenic and were categorized as ETEC, EPEC, VTEC, and EIEC. However, few of the pathogenic isolates remain uncharacterized. Testing of the susceptibility of the pathogenic isolates against WHO's list of essential medicines revealed multiple drug resistance with some isolates being resistant to seven out of the eight tested antibiotics. This is alarming and raises serious public health concern. Sequencing the isolates would provide a useful information on the uncharacterized isolates and mechanism for the multidrug resistance.

Keywords: Antimicrobial agents, diarrhea, *Escherichia coli*, genetic variability, Ghana, surface water.

**Résumé**

Au Ghana, en particulier dans la région du Nord, une proportion importante de la population dépend de l'eau de surface et d'autres sources non traitées. La consommation d'eau provenant de ces sources entraîne la contraction de maladies infectieuses d'importance pour la santé publique, ce qui explique la morbidité et la mortalité élevées chez les enfants et les adultes. Les travaux précédents menés au Ghana se sont concentrés sur l'isolement d'*Escherichia coli* dans les eaux de surface. Cependant, la variabilité génétique et le profil de résistance aux antimicrobiens des solutés d'*E. coli* au Ghana restent inconnus. La présente étude a concerné la variabilité génétique et les profils antimicrobiens des *E. coli* isolés de 26 sources d'eau provenant de cinq districts de la région nord du Ghana. Un test biochimique a été utilisé pour confirmer les isolats d'*E. coli*, la PCR multiplex a été utilisée pour caractériser les isolats, et des méthodes de diffusion sur disque ont été utilisées pour tester la sensibilité aux antimicrobiens. L'étude a enregistré 75% des isolats comme pathogènes qui ont été classés comme ETEC, EPEC, VTEC, et EIEC. Cependant, quelques isolats pathogènes ne sont pas encore caractérisés. Les tests de sensibilité des isolats pathogènes à la liste des médicaments essentiels

de l'OMS ont révélé une résistance multiple aux médicaments, certains isolats étant résistants à sept des huit antibiotiques testés. Cette situation est alarmante et soulève de sérieuses inquiétudes en matière de santé publique. Le séquençage des isolats fournirait des informations utiles sur les isolats non caractérisés et le mécanisme de la résistance multiple.

Mots clés : Agents antimicrobiens, diarrhée, *Escherichia coli*, variabilité génétique, Ghana, eau de surface.

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## Introduction

Globally, there are nearly 1.7 billion cases of childhood diarrheal diseases every year especially in children under five (5) years old (WHO, 2017). A report by WHO (2017) revealed that most of the diarrhea disease cases observed were due to contaminated food and water bodies which claims the lives of 525,000 children annually (WHO, 2017). Despite global efforts to provide safe and clean drinking water, individuals still depend on surface water. In Ghana, approximately six million people (nearly 22 percent) rely on surface water to meet their daily water needs, leaving them vulnerable to water-related illnesses and diseases.

Further, about 67 percent of Ghanaians lack access to improved sanitation or are entirely without toilet facilities (WHO, 2000). The unimproved nature of these water sources coupled with improper sanitation and poor hygienic practices have made water sources unwholesome as a result of microbial contamination, particularly by *Escherichia coli*. Indeed, *E. coli* contamination of drinking water sources is a major challenge especially in rural communities (Samantha, 2009). Although a lot of research has been carried out in most developed and some developing countries, little is known on the presence, distribution and antibiotics susceptibility of pathogenic *E. coli* in Ghana (Opintan *et al.*, 2010; Tagoe *et al.*, 2011). As such, the aim of this study was to investigate the presence of pathogenic *E. coli* in drinking water sources especially to determine the presence of *E. coli* in unimproved water sources, undertake genetic differentiation of *E. coli* isolates, and investigate antimicrobial susceptibility isolated strains.

## Materials and Methods

Fifty two (52) water samples were aseptically taken from 26 water sources (communities) from five districts of the Northern Region of Ghana and transported under controlled conditions to the laboratory for analysis.

**Culturing and isolation of *E. coli*.** Culturing was carried out as previously described by Athumani (2017). 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 spreader and incubated at 37 °C for 24 hours. Single colony growths characteristic of *E. coli* on MacConkey agar (HiMediaIndia) was confirmed as described by Athumani (2017), using indole and citrate tests. The positive colonies were aseptically picked and streaked on nutrient agar and incubated at 37 °C for 24 hours to obtain pure culture isolates.

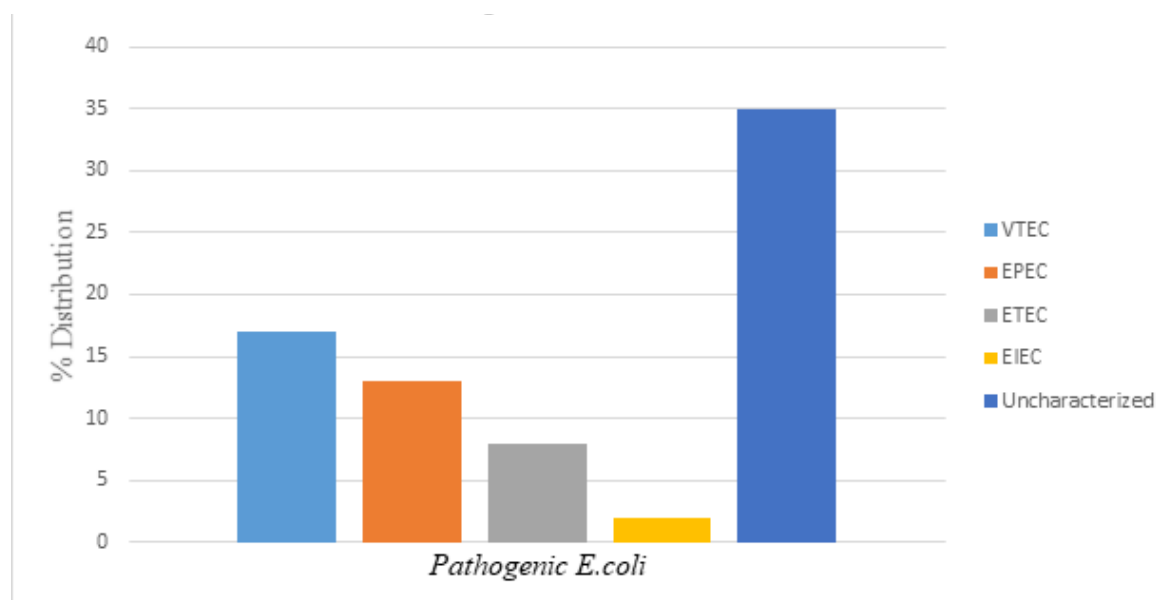
**Molecular characterization of *E. coli* isolates.** Conventional Multiplex Polymerase Chain Reaction was used for the molecular characterization of all *E. coli* isolates based on the protocol described by Hien *et al.* (2007). The following primer pairs were used to detect and differentiate the various strains of pathogenic *E. coli*: LTI and LTr, ST12 1 and ST12 r, VTI I I and VTI I r, VT2 1 and VT2 r, eae u and eae l, bfp A2 u and bfp A2 l, IPaH 111 and IPaH IV, EAI and EA2.

**Antimicrobial susceptibility testing of *E.coli* isolates.** The Disc Diffusion Method, also called Kirby-Bauer method as recommended by the Clinical and Laboratory Standards Institute (2010) was employed for antimicrobial susceptibility test. Eight (8) antimicrobial agents which are listed as part of the World Health Organization's list of essential medicines (*Ceftazime, Cefuroxime, Gentamicin, Cefixime, Ofloxacin, Augmin, Nitrofurantoin* and *Ciprofloxacin*) were used to test for antimicrobial susceptibility and resistance based on the European Committee on Antimicrobial Susceptibility Testing breakpoints, 2019.

## Results

**Pathogenic *E.coli* isolated from surface water.** Fifty two (52) water samples were analyzed for the presence of *E. coli*. All the 52 water samples recorded *E. coli* contamination. Of these, 39 of the 52 *E. coli* isolates representing 75% were pathogenic and the other 13 isolates representing 25% were non-pathogenic.

**Molecular characterization of pathogenic *E.coli*.** Out of the 75% pathogenic *E.coli* isolates identified, 17% carried virulent genes characteristic of VTEC, 13% of EPEC, 8% of ETEC, 2% of EIEC, and 35% carried uncharacterized virulent genes (Figure 1)



**Figure 1. Pathogenic distribution in surface water**

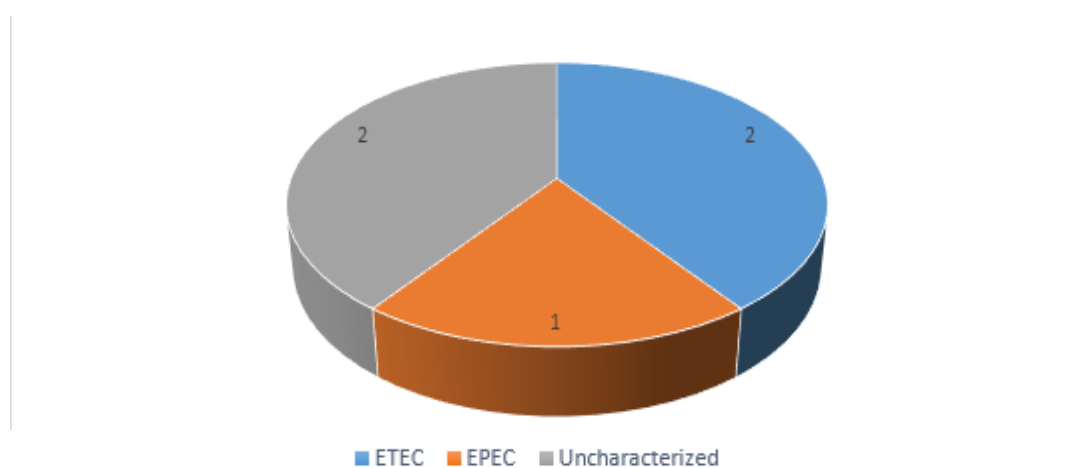
The pathogenic *E. coli* isolates were further classified as VTEC (17%), EPEC (13%), ETEC (8%), EIEC (2%), and Uncharacterized (35%).

**Multidrug-resistant *E.coli* isolates.** All the isolates were resistant to more than one antibiotics when tested against eight antibiotics listed as part of WHO (2017) list of essential medicines. The least resisted antibiotics were Ciprofloxacin and Nitrofurantoin (Table 1).

Figure 2 shows the number of *E.coli* strains resistant to 7/8 of the antibiotics used. Two ETEC, two Uncharacterized, and 1 EPEC strain (s) were resistant to 7/8 of the antimicrobials used.

**Table 1. *Escherichia coli* isolates resistant to antibiotics used**

No. of <i>E. coli</i> isolates	No. of antibiotics resisted
5	7
4	6
8	5
14	4
20	3
1	2

**Figure 2. Number of *E. coli* strains resistant to 7/8 of the antibiotics used**

### Discussion

For many years, *E. coli* was considered a commensal organism of the colon and an indicator of water contamination. This study reveals the prevalence and high genetic variability of pathogenic *E. coli* in Ghana surface waters, and its multiple antimicrobial resistance. This observation confirms the prediction by Motta *et al.* (2003) who noted global increase in prevalence of antibiotic resistance in pathogenic *E. coli* strains. The prevalence and predominance of pathogenic *E. coli* in Ghana as observed here is higher than previously reported by Obi *et al.* (2014) and Ollo *et al.* (2017). From this study, the effective antibiotics against *E. coli* infections were Ciprofloxacin and Nitrofurantoin (although some EPEC, ETEC and uncharacterized strains still indicated resistance to them). This study helps to deepen our knowledge on the genetic variability, and antimicrobial susceptibility of prevailing strains of *E. coli* in the Northern Region of Ghana.

### Conclusion and recommendation

Public health bodies and stakeholders need to take actions to reduce water source infections by *E. coli* in the Northern Region, in particular, and in Ghana as a whole.

### Acknowledgement

This paper is a contribution to the Fifteenth RUFORUM Annual General Meeting held 2-6 December 2019 in Cape Coast Ghana.

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