World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(Research Article)

Check for updates

Molecular detection of diarrheagenic *Escherichia coli* pathotypes isolated from drinking water from rural communities in Wamba, Nasarawa State, Nigeria

BILKIS MUNIR NAGOGO * and SILAS D GYAR

Department of Microbiology, Faculty of Natural and Applied Science, Nasarawa state university Keffi, Nasarawa state, Nigeria.

World Journal of Biology Pharmacy and Health Sciences, 2024, 20(02), 001–006

Publication history: Received on 19 September 2024; revised on 28 October 2024; accepted on 31 October 2024

Article DOI: https://doi.org/10.30574/wjbphs.2024.20.2.0848

Abstract

Contamination of drinking water with pathogenic *Escherichia coli* (*E.coli*) poses significant health risks, particularly in rural communities. This study was carried out to detect diarrheagenic *E.coli* pathotypes isolated from drinking water in three selected rural communities in Wamba, Nasarawa state, Nigeria. Fifteen drinking water samples comprising of stream (5), well (5) and borehole (5) were collected. *E.coli* was isolated by streaking on Eosin Methylene Blue agar. Typical green colonies with metallic sheen were Gram stained and tested biochemically. The isolates were analyzed molecularly using multiplex Polymerase chain reaction (PCR) technique with specific primers and were found to be carrying Vt2, eaeA and estA genes associated with EHEC, EPEC and ETEC pathotypes respectively. The agarose gel electrophoretic assay was used for the detection of amplified genes for different diarrheagenic *E.coli* pathotypes. The isolation of this organism possessing virulence genes from drinking water is of public health significance and therefore more attention needs to be paid to the drinking water of Wamba, Nasarawa state.

Keywords: *Escherichia coli*; Virulence genes; Drinking water; Polymerase chain reaction; Pathotypes.

1. Introduction

In Nigeria, as with other developing countries, there is relative scarcity of potable water that can be used for drinking and other domestic purposes (WHO and UNICEF, 2010). Most bacterial pathogens get into drinking water through feacal contamination either through sewage or other sources (Alalade *et al*., 2018). *E.coli* is one of the most important and widely studied etiologic agents of diarrhea worldwide and it is a normal flora of the gastrointestinal tract of animals and humans (Kotloff *et al*., 2013). Human Diarrheagenic *E.coli* (DEC) with specific combinations of virulence traits are grouped into pathotypes which includes: Enterotoxigenic *E.coli* (ETEC). Enteropathogenic *E.coli* (EPEC), Enteroinvasive *E.coli* (EIEC), Enteroaggregative *E.coli* (EAEC), Diffusely adherent *E.coli* (DAEC) and Shiga toxin producing *E.coli* (STEC). Each pathotype of DEC has a distinct set of virulence factors encoded in the plasmids or chromosome (Odetoyin *et al*., 2022).

2. Material and methods

2.1. Study area

Wamba Local Government Area is one of the 13 local Government Areas in Nasarawa state. It Coordinates 8°56’0”N 8°36’0”E. It has an estimated land area of 1,156 Km^2. Wamba is renowned for the spectacular and beautiful Farin Ruwa Falls which is one of the longest cascades in Africa.

* Corresponding author: BILKIS MUNIR NAGOGO

Copyright © 2024 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution Liscense 4.0.

2.2. Sample collection

A total number of 15 water samples were collected using 1 litre sterile bottles. 5 well water samples from Bambu community, 5 stream water samples from Kurize community and 5 borehole water samples from Gbude community. The samples were all transported to the laboratory of the Department of Microbiology, Nasarawa State University, Keffi in ice boxes for analysis.

2.3. Isolation of *E.coli*

The water samples were serially diluted and 10^6 dilution factor was streaked on Eosin Methylene Blue agar (EMB) and incubated at 44˚C for 24 hours. Colonies that were observed to be shiny green with dark centres were transferred to nutrient agar slants and then Gram stained.

2.4. Biochemical characterization of the isolates

The presumptive *E.coli* isolates were subjected to a number of conventional biochemical tests which includes Indole, citrate, methyl red, Voges-Proskauer, motility, triple sugar ion, nitrate, oxidase and urease tests.

2.5. DNA Extraction

Isolation of DNA was performed using the Rebecca *et al*. (2011) method. The isolates were cultured and incubated at 37°C for 48 hours. From the culture plate, the cells was transferred into Eppendorf tubes containing 0.2 ml of sterile distilled water and mixed. Then, the samples were frozen at −80°C for about 20 min; after incubation it was transferred into boiling water for about 10 min. Then, the cell lysates were centrifuged at 10,000 rpm for 10s and the obtained supernatant (15µL) was used as a DNA template in PCR analysis.

2.6. PCR Procedure

Master Mix (12.5µl) was added to each of the Samples extracted using DNase/RNase free tubes. 1µl each of the forward and reverse primers was added to the mixture and 3.5µl of Nuclease free water was also added to the solution using micropipette. About 18µl of the extracted DNA sample was distributed to each of the PCR tubes and 7µl of the DNA mixture was then added to the solution and span down at 5000rpm for 30 seconds to bring out any hanging fluid by the side or cover of the PCR tubes. Finally, the preparation was laid over the reaction mixture. The reaction was performed in a DNA thermal Cycler for 40 cycles. Each amplification cycle consisted of denaturation for 30seconds at 96˚C, annealing for 30 cycles at 55˚C and extension of the annealed Oligonucleotide primers were allowed for 1 minute at 74˚C Rebecca *et al*. (2011).

2.7. Primer design

The oligonucleotide primer sequence used in this study was as used by Trabulsi *et al*., (2002) as show in table 1.

Table 1 Primers and amplicon size of *Escherichia coli* pathotypes

Primer	Target gene	Primer sequence	Amplicon size (bp)	Reference
LT	eltB	5-TCTCTATGTGCATACGGAGC-3 5-CCATACTGATTGCCGCAAT-3	322	Trabulsi et al., 2002
ST	estA	5-GCTAAACCAGTAGAGGTCTTCAAAA-3 5-CCCGGTACAGAGCAGGATTACAACA-3	147	Trabulsi et al., 2002
VT1	vt1	5-GAAGAGTCCGTGGGATTACG-3 5-AGCGATGCAGCTATTAATAA-3	130	Trabulsi et al., 2002
VT2	vt2	5-ACCGTTTTTCAGATTTTGACACATA-3 5-TACACAGGAGCAGTTTCAGACAGT-3	298	Trabulsi et al., 2002
Eae	eaeA	5-CACACGAATAAACTGACTAAAATG-3 5-AAAAACGCTGACCCGCACCTAAAT-3	372	Trabulsi et al., 2002
SHIG	Ial	5-CTGGTAGGTATGGTGAGG-3 5-CCAGGCCAACAATTATTTCC-3	320	Trabulsi et al., 2002

AA	pCVD 432	5'-CTGGCGAAAGACTGTATCAT-3' 5'-CAATGTATAGAAATCCGCTGTT-3'	630	Trabulsi et al., 2002

LT= Enterotoxigenic *E. coli* (ETEC), ST=Enterotoxigenic *E. coli* (ETEC); VT=Enterohemorrhagic *E. coli* (EHEC), EAE =Enteropathogenic *E. coli* (EPEC); SHIG=Enteroinvasive *E. coli* (EIEC), AA= Enteroaggregative *E. coli* (EAEC).

2.8. Amplification of *Escherichia coli* Genes

The amplification of DEC genes was done by PCR assay of the DNA extracted from *E. coli* isolates as described by Nguyen *et al.* (2005). The DNA templates were subjected to multiplex PCR with specific primers for the detection of the following virulence markers: *eaeA* (structural gene for intimin of EHEC and EPEC), *vt1* and/or *vt2* (Shiga toxins 1 and 2 of EHEC), *eltB* and/or *estA* (enterotoxins of ETEC), *ial* (invasion-associated locus of the invasion plasmid found in EIEC and *Shigella*) and *pCVD* (the nucleotide sequence of the EcoRI-PstI DNA fragment of *pCVD432* of EAEC).

The PCRs was performed with a 25µl reaction mixture containing 5 µl of template DNA, 0.2µl of 18x PCR buffer II, 1.6µl of a 1.25 mM mixture of deoxynucleoside triphosphates, 1.6µl of 25 mM $MgCl_2$, 0.1µl of 5 U of AmpliTaq Gold DNA polymerase per µl and a 0.2 µM concentration of each primer except primer VT1, which was used at a concentration of 0.4µM. The thermocycling conditions used are as follows: 95°C for 5 min (Initial denaturation), 94°C for 20 sec. (denaturation) 55°C for 30 sec. (Annealing) and 72°C for 30 sec. (initial extension) for 30 cycles, with a final 7 min extension at 72°C (Nguyen *et al.,* 2005).

2.9. Agarose Gel Electrophoresis

The agarose gel electrophoretic assay for detection of amplified genes for different DEC pathotypes was carried out as described by Nguyen *et al.* (2005). Briefly, 8µl of PCR products stained with ethidium bromide was loaded into 1.0% (wt/vol) agarose gel wells with a molecular marker run concurrently at 120 V for 30 min. The DNA bands were visualized and photographed under UV light595nm.

3. Results

Table 2 Cultural, Morphological and Biochemical Characteristics of *E.coli* isolated from different Water Sources

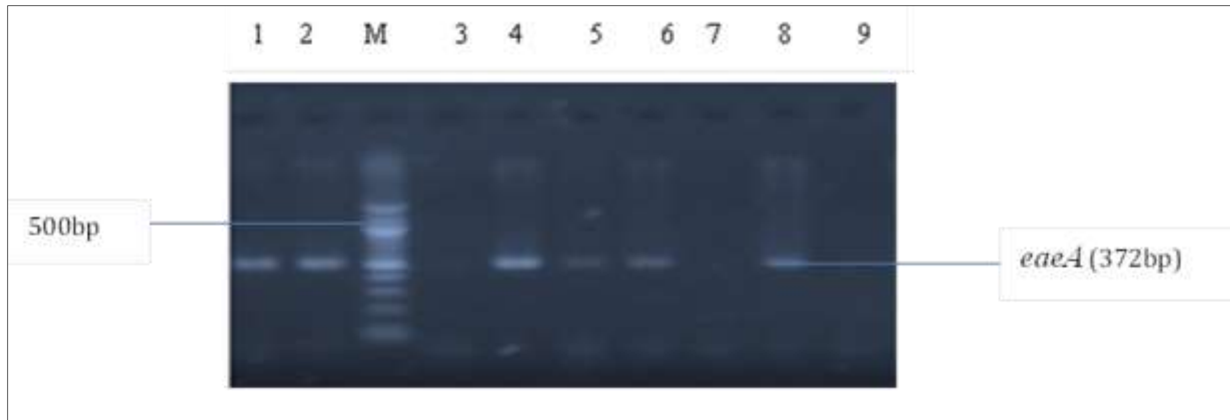
Cultural morphology	MP	GS	Biochemical Characteristics											Inference
			CAT	COA	IN	VP	MR	OX	CIT	MO	UR	TSI	NIT	
Greenish on EMB	Rod	-	+	-	+	-	+	-	-	+	-	+	+	*Escherichia coli*

KEY: MP=Morphology; GS=Gram Staining; CAT=Catalase, COA=Coagulase; IN=Indole; VP=Voges Proskauer; MR= Methyl Red; OX=Oxidase; CT= Citrate, MO= Motility; UR= Urease; TSI= Triple sugar ion; NIT= Nitrate; + = Positive; - = Negative, EMB= Eosin Methylene Blue

Table 3 Occurrence of *E.coli* from the three Different Water Sources

Bacteria	No. Isolated (%)					
	No. Sample	Well Water	No. Sample	Stream Water	No. Sample	Borehole water
E. coli	5	3 (60)	5	4 (80)	5	2 (40)

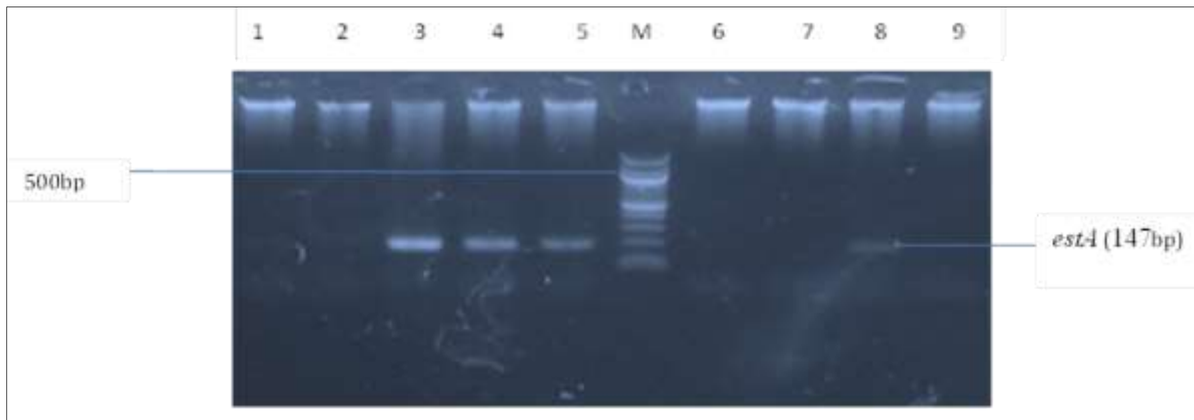
From table 3 above, stream water had the highest percentage occurrence of 80% *E.coli* followed by well water with 60% occurrence and borehole water with 40%



Note: lane 1, 2, 3 is *E. coli* from well water 4,5,6,7 from streams 8 and 9 from borehole.

Figure 1 Agarose Gel Electrophoresis of the amplified eaeA gene

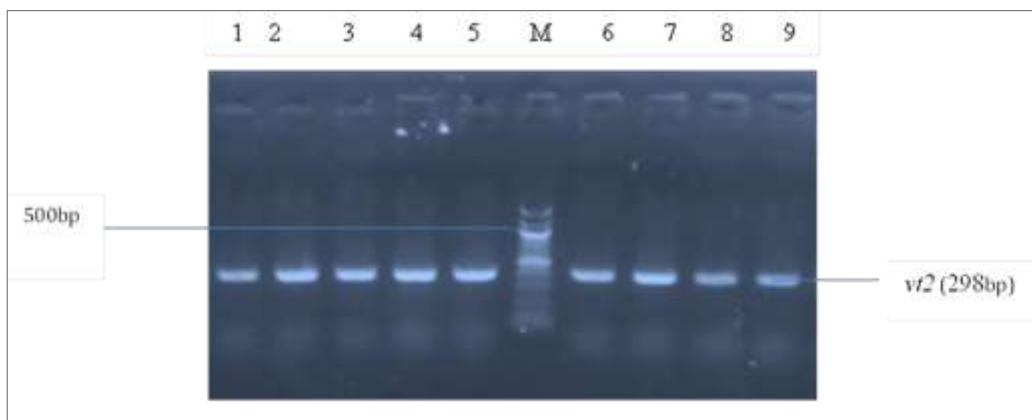
Figure 1: Agarose gel electrophoresis of the amplified *eaeA* gene. Lanes 1, 2, 4, 5, 6 and 8 represent the *eaeA* bands at 372bp while Lane M represents the 500bp molecular ladder.



Note: lane 1, 2, 3 is E. coli from well water 4,5,6,7 from streams 8 and 9 from borehole.

Figure 2 Agarose Gel Electrophoresis of the amplified estA gene

Figure 2: Agarose gel electrophoresis of the amplified *estA* gene. Lanes 3, 4, 5 and 8 represent the *estA* bands at 147bp while Lane M represents the 500bp molecular ladder.



Note: lane 1, 2, 3 is *E. coli* from well water 4,5,6,7 from streams 8 and 9 from borehole

Figure 3 Agarose Gel Electrophoresis of the amplified vt2 gene

Figure 3: Agarose gel electrophoresis of the amplified *vt2* gene. Lanes 1-9 represent the *vt2* bands at 298bp while Lane M represents the 500bp molecular ladder.

4. Discussion

In this study, there was 80% occurrence of *E.coli* from stream water, 60% from well water and 40% from borehole water which is a high and significant finding because of the pathogenicity of the organism. WHO states that *E.coli* should not be present in drinking water. This study concerned 3 pathotypes of *E.coli* (EPEC, EHEC and ETEC) responsible for diarrhea in children and adults in most developing countries.

7 virulence genes were examined by PCR and 3 were present; vt2, eaeA and estA genes associated with EHEC, EPEC and ETEC pathotypes respectively. The vt2 gene of EHEC was the most occurring gene as it was found to be present in all the *E.coli* isolates from the three different water sources this is similar with a studies in Nigeria by Iwalokun et al. (2017). The eaeA gene associated with EPEC was found in 2 *E.coli* isolates from well water sample, 4 *E.coli* isolates from stream water and 1 *E.coli* isolate from borehole water, a previous study in Ethiopia by Beyene et al. (2019) found EPEC in drinking water samples. The presence of estA gene associated with ETEC is alarming and it was detected in 1 *E.coli* isolates from well water, 2 *E.coli* isolates from stream water and 1 *E.coli* isolate from borehole water, this finding is similar to a study by Rodriguez et al. (2020) and another study in South Africa by Moyo et al (2019) that recorded presence of ETEC in drinking water samples. In this study EHEC pathotype is the most prevalent among the detected pathotypes and this finding is similar to a research by Silva et al. (2018) which also reported a higher prevalence of EHEC. The presence of DEC in drinking water sources highlights the risk to human health associated with the use of untreated water; there is need for sanitation and inspection of water sources to prevent possible outbreaks of waterborne diseases.

5. Conclusion

This study highlights the presence DEC virulence genes in drinking water sources. The detection of vt2, eaeA and estA genes associated with EHEC, EPEC and ETEC respectively with a preponderance of EHEC. The presence of these pathogenic strains of *E.coli* in drinking water highlights the risk posed to human health associated with the use of untreated water. There is need to sensitize rural communities to inculcate the habit of boiling untreated water before use and the need to improve water treatment and sanitation measures to prevent waterborne diseases.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Alalade, O.M, Ameh, J.B, Abdullahi, I.O and Whong C.M.Z Screening for virulence genes in E. coli O157:H7 Obtained from Drinking Water life journal of science vol.20, no. 1 (2018).

[2] Beyene, G., Yismaw, G., Alemayehu, E., and Gebre-Selassie, S, (2019). Diarrheagenic Escherichia coli in drinking water from rural communities in Ethiopia. American Journal of Tropical Medicine and Hygiene, 100(4), 931-938.

[3] Iwalokun, B.A., Gbadamosi, I.T., Ogunjimi, A.A., Oguntoyinbo, F.A., & Osibogun, A. (2017). Prevalence of Diarrheagenic Escherichia coli from water sources in Nigeria. Journal of Environmental Health Science & Engineering, 15, 1-9.

[4] Kotloff, K.L., Nataro, J.P., Blackwelder, W.C., Nasrin, D., Farag, T.H., Panchalingam, S., Wu, Y., Sow, S.o., Sur, D., Breiman, R.F., et al. (2013). Burden and aetiology of diarrheal disease in infants and young children in developing countries (the Global Enteric Multicentre Study, GEMS): A prospective, case-control study. Lancet, 382(9888), 209-222.

[5] Moyo, S.J., Mashe, T., Ncube, N., & Ncube, B. (2019). Molecular Characterization of Enterotoxigenic Escherichia coli isolated from drinking water in South Africa. Journal of Water Research, 158, 33-341.

[6] Nguyen, T.V., Le Van, P., Le Huy, C., Nguyen, T.M., & Weintraub, A. (2005). Detection and Characterization of Diarrheagenic Escherichia coli from Acute Diarrhea cases in Vietnam Using PCR. Journal of Medical Microbiology, 54(11), 1031-1036.

[7] Odetoyin, B., Ogundipe, O., & Onanuga, A. (2022). Prevalence, diversity of diarrheagenic Escherichia coli and associated risk factors in well water in Ile-Ife, Southwestern Nigeria. One Health Outlook, 4(1), . doi:10.1186/s42522-021-00057-4. PMID: 35130987; PMCID: PMC8822758.

[8] Rebecca, O., Babalola, O.O., & Adesina O.M. (2011), PCR Detection of Escherichia coli O157:H7 in Cattle Feaces and Water Sources in Ogun State, Nigeria. Journal of Infection in Developing Countries, 5(10), 693-698

[9] Rodriguez, J.M., Rivas, M.V., Quiroga, M.P & Sanz, M.E. (2020). Detection of Diarrheagenic of Escherichia coli in drinking water samples from Argentina. Journal of Environmental Health, 82(6), 8-14.

[10] Silva, D.D., Ferreira, E.O., Francisco. W.M., Leal, N.C., & Teixeira, J.B. (2018). Molecular Characterization of Escherichia coli from drinking water in Brazil, Journal of Water and Health, 16(2), 257-265.

[11] Trabulsi, L.R., Keller, R., Gomes, T.A and Tardelli (2002). 10.321/eid0805. Typical and Atypical Enteropathogenic Escherichia coli. Emerging Infectious Disease 8(5): 508-513

Authors short biography

Bilkis Munir Nagogo holds a B.Sc. in Microbiology from Ahmadu Bello University and a M.Sc. in Food and Industrial Microbiology from Nasarawa State University. She serves as a scientific officer at the National Biosafety Management Agency. Her expertise encompasses Water analysis, food safety, risk assessment and risk management. With publications in reputable journals, Bilkis is dedicated to advancing public health through her work.