Prevalence of Diarrhoeagenic *Escherichia Coli* and Associated Risk Factors in Dug Wells in Ile-Ife, Southwestern Nigeria

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Research article

**Keywords:** Diarrhoeagenic Escherichia coli, Drinking water, Risk factors, Diversity, Contamination

**Posted Date:** August 17th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-255440/v1

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Abstract

Background

Diarrhoeagenic Escherichia coli (DEC) strains are common bacterial causes of morbidity and mortality in young children. Waterborne DEC could pose a potential health risk to humans through domestic use of contaminated water. However, epidemiological studies on DEC strains in drinking water are scarce in Nigeria. This study determined the prevalence, diversity and factors associated with the presence of DEC in dug wells in Ile-Ife, southwestern Nigeria.

Methods

We assessed 143 wells for safety by coliform count using the multiple tube technique. A standardized questionnaire was used to obtain relevant information about the wells and their owners. Contaminating isolates were identified as E. coli by amplifying their 16S rRNA gene. Five DEC pathotypes comprising enterotoxigenic E. coli (ETEC), enteroaggregative E. coli (EAEC) enteroinvasive E. coli (EIEC), enteropathogenic E. coli (EPEC) and Shiga-toxin producing E. coli (STEC) were detected using two sets of multiplex PCR assays. Isolates diversity was determined by (GTG)5 Repetitive element palindromic PCR and Shannon diversity index. Multivariate logistic regression analysis was used to identify associated risk factors.

Results

Fifty-eight (40.6%) wells were contaminated by diarrhoeagenic E. coli. Wells with dirty platforms, damaged by erosion and sited near septic tanks significantly harboured DEC (p<0.05). There was a preponderance of STEC among the isolates with nine isolates carrying multiple diarrhoeagenic genes and 10 (17.2%) wells contaminated by multiple DEC strains. The (GTG)5-PCR fingerprinting assigned all DEC strains into six clades, with an overall Shannon diversity index of 18.87. A diverse profile was obtained among and between the isolates recovered from different sources.

Conclusions

The presence of DEC strains in drinking water highlights the risk to human health associated with the use of untreated water. There was a high degree of genetic diversity among the isolates implying multiple sources of contamination. There is a need for periodic sanitation and inspection of wells for cracks to prevent seepages and possible outbreaks of waterborne diseases.

Introduction

Diarrhoeal diseases are significant public health problems in developing countries.[1] Each year, they account for 3.6% of the total global burden of diseases and 1.5 million deaths. About 88% of this burden has been ascribed to inadequate hygiene, sanitation and a lack of potable water mostly in developing countries.[1, 2] Escherichia coli, a member of faecal coliforms has a significant place in water microbiology as an indicator of faecal pollution and a pathogen in drinking water. As a pathogen, it causes a variety of diseases ranging from urinary tract infections, sepsis, meningitis and bacteraemia to diarrhoea.[3]

Diarrhoeagenic E. coli (DEC) account for about 40% of episodes of acute diarrhoea in children in developing countries. They also play a significant causative role in diarrhoea in Nigeria, in both adults and children. Currently, there are eight pathotypes of DEC strains: enterotoxigenic, enterohaemorrhagic, enteroinvasive, enteropathogenic, enteroaggregative, diffusely adherent, cytotoxic distending toxin-producing and cell detaching E. coli. Each pathotype of DEC has a distinct set of virulence factors encoded in the plasmids or chromosome. The genes that encode these factors are conserved among strains that are isolated from diverse sources in different parts of the world.[4]

DEC strains are generally spread by a faecal-oral route which includes contaminated sources of water or food and may be implicated in outbreaks of waterborne diarrhoea. Escherichia coli can enter drinking water through inadequate or failing septic or sewage systems, runoff from land treated with animal wastes or used for animal feeding activities and wildlife. Identification of the source of pollution is critical for protecting source water quality and assessing the public health risk associated with contamination from a specific host source. Consequently, much progress has been made over the years to
develop many phenotypic and genotypic microbial source tracking (MST) methods which are recommended components of faecal pollution reduction strategies.\[5, 6\]

Nigeria is one of the countries in the world where about 90 million people do not have access to potable water and 130,000 children under the age of five die each year from preventable waterborne diseases as a result of uncoordinated efforts of multiple government agencies. The larger part of the population, particularly those in the rural and suburban communities resort to water from wells and streams for domestic purposes.\[2, 7\] Those wells which are hand dug are usually around two inches in diameter and about 25 feet deep. In Ile-Ife, most of the wells are shallow because of the high water table. Such wells are more prone to contamination by runoffs from nearby farmlands and seepages from domestic sanitary sewage because of their shallow depth. Well water is an untreated source for drinking and might harbour waterborne borne diseases. Consequently, the use of these sources of water is a health risk for this population.\[7, 8\]

Despite the risk posed by exposure to \textit{E. coli} contaminated water, very little data is available on this in Ile-Ife, and the pathogenic potential, diversity of implicated isolates and factors associated with their presence in drinking water remain unknown. Therefore this study determined the prevalence, diversity and factors associated with the presence of DEC in dug wells in Ile-Ife, Southwest Nigeria.

\section*{Methods}

\subsection*{Study location and design}

The study was done in Ife East Local Government Area, Ile-Ife, Osun State, Nigeria. Ife East Local Government Area is divided into six wards which are: Moore ward, Ilode ward 1, Ilode ward 2, Okerewe ward 1, Okerewe ward 2 and Okerewe ward 3. The sampling locations are shown on the map in Fig 1. Twenty-five samples were obtained from Moore ward, 18 samples from Ilode ward 1, 49 samples from Ilode ward 2, 31 samples from Okerewe ward 1, 9 samples from Okerewe ward 2 and 11 samples from Okerewe ward 3. Ile-Ife is an ancient city in South-western Nigeria with a population of 509, 035.\[9\] The city lies on Latitudes 7°28′N and 7°45′N and longitudes 4°30′E and 4°34′E. Ile-Ife is in the tropical wet and dry climate of West Africa with an average rainfall of 1,000 to 1,250 mm between March and October and average relative humidity of 75% to 100%. The residents use dug wells for their domestic and drinking purposes.

\subsection*{Study approval and Sample Collection}

This study was approved by the Health Research Ethics Committee (HREC), Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Nigeria (HREC No: IPHOAU/12/863). Random sampling technique was used to include wells in this study. One hundred and forty-three water samples were collected from dug wells that were distributed across the wards between March and December 2019 based on the formula of Sullivan and Soe \[10\]. The wells were used by the residents for domestic purposes. Wells that have not been disinfected for two months were included in the study, while those that belonged to owners that did not give their consent, and wells that were recently disinfected were excluded. Before sample collection, informed written consent was used to obtain permission from well owners. All eligible consenting well owners were interviewed using a pretested structured questionnaire in order to obtain information on residence type, age and depth of wells, proximity of wells to septic tanks, house location, presence of septic tanks and proximity of livestock to wells. Additional demographic information including age, sex, occupation, level of education was also obtained. Two hundred ml of water were obtained by lowering a sterile bottle into each well with the aid of a rope tied around its neck. All samples were properly labelled, placed in an iced-packed box and transported to the laboratory for processing within 2hrs.

\subsection*{Determination of well water quality}
The quality of the water samples was determined by coliform count using the multiple tube fermentation technique as described by Cheesbrough.[11] A three-tube most probable number (MPN) method was used to determine faecal contamination of well water using MacConkey broth (Oxoid Ltd., Basingstoke Hampshire, England) as the culture medium. Samples of 50ml, 10ml and 1ml of water were inoculated into corresponding dilution tubes with inverted Durham's tubes and incubated at 37°C for 24 hours. The tubes were observed for growth and gas production, and the MPN of coliforms in 100ml of water was determined by referring to McCrady's table and interpreted as “Excellent”, “Acceptable”, “Unacceptable” and “Grossly polluted”.

Detection of *Escherichia coli* in water samples

The Eijkman method was used to detect the presence of *E. coli* in the samples.[12] All positive bottles from the previous test were subcultured into fresh double strength and single strength MacConkey broth (Oxoid Ltd., Basingstoke Hampshire, England) and peptone water (Oxoid Ltd., Basingstoke Hampshire, England) and incubated at 37°C for 24 hours. The MacConkey bottles were checked after incubation for lactose fermentation (yellow colouration) and gas production (presence of a bubble in the Durham tubes). All positive MacConkey bottles were noted and three drops of Kovac's reagent were added to their corresponding peptone water bottles to detect indole (indicated by a red coloured ring). All positive samples were cultured on Eosin Methylene Blue Agar (Oxoid Ltd., Basingstoke Hampshire, England) plates and incubated aerobically at 37°C for 24 hours. Up to three distinct colonies showing green metallic sheen were aseptically picked and streaked onto Nutrient agar (NA) (Oxoid Ltd., Basingstoke Hampshire, England) plates which were, in turn, incubated aerobically at 37°C for 24 hours.[13] All suspected *E. coli* isolates were stored at -20°C in 20% glycerol broths for further examination.

Isolate resuscitation and DNA extraction

All isolates were subcultured from 20% glycerol broths on nutrient agar plates and incubated at 37°C for 24 hours. Three colonies were picked from each culture with the aid of a sterile inoculating loop and suspended in 50ul of sterile distilled water in an Eppendorf tube (Eppendorf AG, Hamburg, Germany) to extract the DNA of the isolates. The suspension was boiled for 10 minutes, kept on ice for 10 minutes, and centrifuged in a microcentrifuge (Haraues Sepatech GmBH, Germany) at 10,000 rpm for 10 minutes. [14] The supematant was transferred to a new Eppendorf tube after centrifugation and was used as a DNA template in polymerase chain reaction (PCR).

Molecular Identification of isolates by amplifying their 16SrRNA gene

All organisms suspected to be *E. coli* by their phenotypic characteristics were confirmed as *E. coli* by amplifying their 16S rRNA gene as described by Hassan *et al.* (Table1).[15] *E. coli* strain ATCC 25922 was used as the positive control while sterile distilled water was used as the negative control. A 25μl reaction mixture contained 12.5μL of One *Taq* Quick-Load 2XMaster mix with Standard Buffer (Bio Labs, New England), 10 pmol each of the primers (Inqaba, Biotec, South Africa), 2.4 μl of the DNA template and made up with Nuclease Free Water (BioConcept, Switzerland). Amplification conditions were as follows: Initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 45 s, annealing at 45°C for 45s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 5 min. Each amplicon (10μL) was electrophoresed on a 1.5% agarose gel (Cleaver Scientific, United kingdom) pre stained with 0.5μg/mL Ethidium bromide in 1X Tris-Acetate-EDTA (TAE) buffer and viewed with a UVitec transilluminator (Avebury, Cambridge UK).

Detection of Diarrhoeagenic genes in the isolates

All isolates were screened for virulence genes characteristic of five pathotypes of diarrhoeagenic *E. coli* comprising enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC)
and shiga toxin producing *E. coli* (STEC) as described by Aranda et al. [16] with modifications (Table 1). PCR was performed with a 20 µl reaction mixture containing 12.5µl One Taq Quick-Load 2X Master mix with Standard Buffer (Bio Labs, New England), 10 pmol each of PCR primers (Inqaba, Biotec, South Africa), 2.4 µl of the DNA template and made up with Nuclease Free Water (BioConcept, Switzerland).

Two PCR reaction assays were used to amplify the eaeA (intimin of EPEC), *bfpA* (bundle-forming pilus of EPEC), *stx1* and/or *stx2* (shiga toxins 1 and 2 of STEC), *eltB* and/or *estA* (enterotoxins LT and ST of ETEC), *ipaH* (invasion plasmid found in EIEC and *Shigella*) and pCVD (pCVD432 of EAEC). *E. coli* strains E2348/69, O42, H10407, EDL 933 and E137 served as positive controls for EPEC, EAEC, ETEC, STEC and EIEC respectively while sterile water was used as a negative control. All EPEC isolates that harbour *bfp* gene is classified as Typical EPEC (tEPEC). For PCR 1 (eae, CVD432, stx1, ipaH, ST): Amplification conditions were as follows: Initial denaturation at 95°C for 3 mins; 37 cycles of denaturation at 94°C for 30 s, annealing at 45°C for 30 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 7 min. For PCR 2 (stx2, bfp, LT): Amplification conditions were as follows: Initial denaturation at 94°C for 3 mins; 37 cycles of denaturation at 94°C for 45s, annealing at 39°C for 30 s, and extension at 72°C for 54 min; followed by a final extension at 72°C for 7 min. Each PCR product (10 µl) was electrophoresed on a 1.5% (w/v) agarose gel (Cleaver Scientific, United kingdom) in 1X Tris-Acetate-EDTA (TAE). Each amplicon (10µL) was electrophoresed on a 1.5% agarose gel (Cleaver Scientific, United kingdom) pre stained with 0.5µg/mL Ethidium bromide in 1X Tris-Acetate-EDTA (TAE) buffer and viewed with a UVitec transilluminator (Avebury, Cambridge UK).

**Table 1**

| PCR primers for diarrhoeagenic *Escherichia coli* and 16srRNA gene |  |  |
Determination of isolates relatedness and diversity

(GTG)5-PCR fingerprinting was performed as described by Khare et al.[17] The 15-mer primer (5’-GTGGTGGTGGTGGTGTGG-3’) was used to amplify the repetitive sequences in chromosomal DNA of *E. coli*. PCR was performed with a 25 µl reaction mixture containing 12.5uL One Taq Quick-Load 2X Master mix with Standard Buffer (Bio Labs, New England), 10 pmol each of the 15-mer primer (Inqaba Biotec, South Africa), 2.4 µl of the DNA template and made up with Nuclease Free Water (BioConcept, Switzerland). Amplification conditions were as follows: Initial denaturation at 95°C for 5 mins; 35 cycles of denaturation at 95°C for 60 s, annealing at 40°C for 60 s, and extension at 68°C for 8 min; followed by a final extension at 68°C for 8 min. Each PCR product (10 µl) was electrophoresed on a 1.5% (w/v) agarose gel (Cleaver Scientific, United kingdom) in 1X Tris Acetate-EDTA buffer (TAE).[18] Gels containing 5ul of 10ug/ml of ethidium bromide were visualized under ultraviolet (UV) light using a UVitec transilluminator (Avebury, Cambridge UK). Genetic relationships among *Escherichia coli* isolates were analysed using GelJ (Version 1.0).[19] The dendrogram was drawn with Paleontological Statistics (PAST) (Version 4.0) software using neighbour-joining clustering method.[20]

The genetic diversity of DEC isolates was calculated using the Shannon diversity index \(H\) formula.[21]
\[ H' = - \sum_{i=1}^{s} p_i \ln p_i \]

\( i \) is the total number of isolates, \( s \) is the number of unique genotypes and \( p_i \) is the number of isolates sharing the same genotype.

**Data Analysis**

Data analysis was done with R statistical software (Version 4.0.3). Cross tables were produced with the Grammar of Tables in R package. Pearson chi-square and binomial logistic regression models were used to test for association of variables with the presence of DEC in water.[22] The P-value for a significant association was set at \( \leq 0.05 \).

**Results**

**Baseline Characteristics of the wells and their owners**

Most of the wells were covered (\( n = 108; 75.5\% \)), some were partially covered (\( n = 20; 13.99\% \)), and a few were not covered (\( n = 15; 10.5\% \)). The majority of well owners were Christians (111, 78.7\%), artisans (100, 69.9\%), with secondary education (63, 50\%) and lived in tenement (81, 56.6\%). The mean age of the wells was 21 years and the average depth was 29.3 feet (Table 2).
Table 2
Baseline characteristics of wells and Owners

| CHARACTERISTICS                        | OVERALL (N = 143) |
|----------------------------------------|-------------------|
| WARDS                                  |                   |
| ILODE 1                                | 18 (12.6%)        |
| ILODE 2                                | 49 (34.3%)        |
| MOORE                                  | 25 (17.5%)        |
| OKEREWE 1                              | 31 (21.7%)        |
| OKEREWE 2                              | 9 (6.3%)          |
| OKEREWE 3                              | 11 (7.7%)         |
| AGE OF WELLS (mean ± SD) (Months)      | 20.6 ± 21.7       |
| DEPTH OF WELLS (mean ± SD) (Feet)      | 29.3 ± 22.1       |
| AGE OF OWNERS (mean ± SD) (Years)      | 45.8 ± 17         |
| NUMBER OF YEARS IN RESIDENCE (mean ± SD) (Years) | 14.2 ± 16.4       |
| RELIGION                               |                   |
| CHRISTIANITY                           | 111 (78.7%)       |
| ISLAM                                  | 26 (18.4%)        |
| TRADITIONALIST                         | 4 (2.8%)          |
| OCCUPATION OF OWNERS                   |                   |
| ARTISAN                                | 100 (69.9%)       |
| CIVIL SERVANT                          | 28 (19.6%)        |
| RELIGIOUS LEADER                       | 5 (3.5%)          |
| STUDENT                                | 6 (4.2%)          |
| UNEMPLOYED                             | 4 (2.8%)          |
| LEVEL OF EDUCATION                     |                   |
| PRIMARY                                | 24 (19.0%)        |
| SECONDARY                              | 63 (50.0%)        |
| TERTIARY                               | 39 (31.0%)        |
| RESIDENCE TYPE                         |                   |
| FLAT                                   | 62 (43.4%)        |
| TENEMENT                               | 81 (56.6%)        |
| COVERED                                |                   |
| COVERED                                | 108 (75.5%)       |
| OPEN                                   | 15 (10.5%)        |
| PARTIALLY COVERED                      | 20 (14.0%)        |
| PRESENCE OF SEPTIC TANK                |                   |
| CHARACTERISTICS                              | OVERALL (N = 143)     |
|---------------------------------------------|-----------------------|
| NO                                          | 94/140 (67.1%)        |
| YES                                         | 46/140 (32.9%)        |
| KEEPING OF PETS                              |                       |
| NO                                          | 96/138 (69.6%)        |
| YES                                         | 42/138 (30.4%)        |
| DIRTY PLATFORM                               |                       |
| NO                                          | 104 (72.7%)           |
| YES                                         | 39 (27.3%)            |
| PROXIMITY OF LIVESTOCK TO WELL               |                       |
| NO                                          | 102 (71.3%)           |
| YES                                         | 41 (28.7%)            |
| PROXIMITY OF WASTE DUMP SITE TO WELL         |                       |
| NO                                          | 137 (95.8%)           |
| YES                                         | 6 (4.2%)              |
| PROXIMITY OF WELL TO FARM                   |                       |
| NO                                          | 133 (93.0%)           |
| YES                                         | 10 (7.0%)             |
| WELL DAMAGED BY EROSION                      |                       |
| NO                                          | 116 (81.1%)           |
| YES                                         | 27 (18.9%)            |

Flat: is a self-contained housing unit; Tenement: a type of building shared by multiple dwellings; Traditionalist: someone who believes in and follows tradition; Dirty platform: a dirty concrete slab that covers the well

Contaminated wells and Isolated *Escherichia coli* strains

One hundred and ten (110, 76.9%) wells were contaminated with coliforms bacteria. Ilode ward 2 (36; 32.7%) had the highest number of contaminated wells while Okerewe ward 3 (6; 5.5%) had the fewest (Table 3).

A total of 169 *E. coli* strains were isolated from 98 wells of 110 contaminated wells. As shown in Table 3, 30 strains were isolated from the wells in Moore ward, 19 strains from Ilode ward 1, 56 strains from Ilode ward 2, 37 strains from Okerewe ward 1, 12 strains from Okerewe ward 2 and 15 strains from Okerewe ward 3.
| Wards | Locations | Number of wells | Number of contaminated wells | E. coli Isolated | No of wells with E. coli |
|-------|-----------|----------------|-------------------------------|-----------------|------------------------|
| Moore | Moore     | 6              | 4                            | 7               | 4                      |
|       | Opa       | 5              | 4                            | 3               | 2                      |
|       | Iloromu   | 1              | 0                            | 0               | 0                      |
|       | Mokuro    | 12             | 11                           | 17              | 8                      |
|       | Olopo     | 1              | 1                            | 3               | 1                      |
| Subtotal |           | 5              | 25                           | 20              | 30                     | 15                  |
| Ilode 1 | Oke atan  | 7              | 7                            | 12              | 5                      |
|         | Lokore    | 10             | 9                            | 6               | 4                      |
|         | Ayelabowo | 1              | 1                            | 1               | 1                      |
| Subtotal |           | 3              | 18                           | 17              | 19                     | 10                  |
| Ilode 2 | Oke ogbo  | 31             | 22                           | 27              | 17                     |
|         | Omitoto   | 7              | 5                            | 10              | 5                      |
|         | ogooluwatan | 10              | 9                            | 19              | 8                      |
| Subtotal |           | 3              | 49                           | 36              | 56                     | 30                  |
| Okerewe 1 | Iloro     | 3              | 0                            | 0               | 0                      |
|         | okesoda   | 5              | 4                            | 5               | 4                      |
|         | ayetoro   | 16             | 13                           | 21              | 12                     |
|         | Oke ayetoro | 3                | 3                            | 3               | 2                      |
|         | gbodo     | 4              | 4                            | 8               | 3                      |
| Subtotal |           | 5              | 31                           | 24              | 37                     | 21                  |
| Okerewe 2 | Ita agbon | 1              | 1                            | 1               | 1                      |
|         | Otutu     | 2              | 2                            | 4               | 2                      |
|         | Ajamopo   | 2              | 2                            | 2               | 1                      |
|         | Iakanye   | 2              | 2                            | 5               | 2                      |
|         | Itakogun  | 1              | 0                            | 0               | 0                      |
| Subtotal |           | 5              | 9                            | 7               | 12                     | 6                   |
| Okerewe 3 | ogbonya   | 11             | 6                            | 15              | 8                      |
| Subtotal |           | 1              | 11                           | 6               | 15                     | 8                   |
| Total   |           | 22             | 143                          | 110             | 169                    | 98                  |

Prevalence of Diarrhoeagenic Escherichia coli
The diversity and prevalence of diarrhoeagenic *E. coli* are illustrated Fig. 2, Tables 4 and 5.

Fifty-eight (40.6%) out of the 143 wells sampled for diarrhoeagenic *E. coli* were positive, yielding a total of 69 diarrhoeagenic *Escherichia coli* strains. Okerewe 1(14) had the highest number of wells that were contaminated with DEC, while Okerewe 3(5) had the least number. All detected EPEC isolates that harboured *bfp* gene was classified as Typical EPEC (tEPEC). There was a preponderance of STEC (n = 38; 55.1%) among the strains, followed by ETEC (n = 10; 14.5%). Five and two strains were both STEC/tEPEC and ETEC/STEC respectively. Multiple pathotypes of DEC were recovered from 10 (17.2%) wells in the sampled locations.

### Table 4

| Locations | Total sampled wells | No of wells with DEC Isolates | DEC EAEC | ETEC | EIEC | STEC | EPEC | STEC AND tEPEC | ETEC AND STEC | tEPEC, ETEC AND STEC | EAEC, tEPEC |
|-----------|---------------------|--------------------------------|---------|------|------|------|------|----------------|----------------|----------------------|-----------|
| Moore     | 25                  | 13                             | 15      | 0    | 1    | 0    | 12   | 2              | 0              | 0                    | 0         |
| Ilode 1   | 18                  | 7                              | 7       | 0    | 0    | 0    | 5    | 0              | 1              | 1                    | 0         |
| Ilode 2   | 49                  | 13                             | 14      | 0    | 0    | 1    | 9    | 2              | 1              | 1                    | 0         |
| Okerewe 1 | 31                  | 14                             | 16      | 0    | 6    | 2    | 3    | 2              | 0              | 3                    | 0         |
| Okerewe 2 | 9                   | 6                              | 9       | 1    | 2    | 0    | 5    | 0              | 0              | 0                    | 1         |
| Okerewe 3 | 11                  | 5                              | 8       | 0    | 1    | 1    | 4    | 1              | 0              | 0                    | 0         |
| Total     | 143                 | 58                             | 69      | 1    | 10   | 4    | 38   | 7              | 2              | 5                    | 1         |

STEC- Shiga toxin producing *E. coli*, ETEC- Enterotoxigenic *E. coli*, EAEC-Enteroaggregative *E. coli*, EIEC – Enteroinvasive *E.coli*, EPEC- Enteropathogenic *E. coli* and Shiga-toxin producing *Escherichia coli*, tEPEC- typical EPEC ( Isolates with only *bfp*)
| S/N | Strain number | Pathotype | Genes | Locations | Wards |
|-----|---------------|-----------|-------|-----------|-------|
| 1.  | 111a          | STEC      | Stx2 + Eae | Ayelabola | Ilode 1 |
| 2.  | 92w           | STEC AND tEPEC | Stx2 + Bfp | Lokore | Ilode 1 |
| 3.  | Ds85cii       | ETEC AND STEC | ST + Stx2 | Lokore | Ilode 1 |
| 4.  | Ds94dii       | STEC      | Stx2    | Okeatan   | Ilode 1 |
| 5.  | Ds96cii       | STEC      | Stx2    | Oke Atan  | Ilode 1 |
| 6.  | Ds97dii       | STEC      | Stx2    | Oke Atan  | Ilode 1 |
| 7.  | Ds99eii       | STEC      | Stx2    | Oke Atan  | Ilode 1 |
| 8.  | 13bw          | STEC      | Stx1    | Omitoto   | Ilode 2 |
| 9.  | 18aw          | STEC      | Stx2    | Oke Ogbo  | Ilode 2 |
| 10. | 37wi          | STEC AND tEPEC | Stx2 + Bfp | Ogooluwatan | Ilode 2 |
| 11. | 64ssbi        | STEC      | Stx2    | Oke Ogbo  | Ilode 2 |
| 12. | 6ew           | STEC      | Stx2    | Ogooluwatan | Ilode 2 |
| 13. | 7350ml        | STEC      | Stx2    | Omitoto   | Ilode 2 |
| 14. | 7b            | STEC      | Stx2    | Ogooluwatan | Ilode 2 |
| 15. | Ds50c         | STEC      | Stx2    | Oke Ogbo  | Ilode 2 |
| 16. | Ds65aii       | ETEC AND STEC | ST + Stx2 | Oke Ogbo | Ilode 2 |
| 17. | Ds73e         | tEPEC     | Bfp     | Omitoto   | Ilode 2 |
| 18. | Ds76aiii      | STEC      | Stx2    | Ogooluwatan | Ilode 2 |
| 19. | Ds79ci        | EIEC      | Ipah    | Ogooluwatan | Ilode 2 |
| 20. | Ds80a         | tEPEC     | Bfp     | Ogooluwatan | Ilode 2 |
| 21. | Ds80aiii      | STEC      | Stx1    | Ogooluwatan | Ilode 2 |
| 22. | 115           | tEPEC     | Bfp     | Mokuro    | Moore |
| 23. | 117           | STEC      | Stx2    | Mokuro    | Moore |
| 24. | 126           | STEC      | Stx1    | Olopo     | Moore |
| 25. | 108a          | STEC      | Stx2    | Mokuro    | Moore |
| 26. | 109a          | ETEC      | ST      | Moore     | Moore |
| 27. | 109b          | STEC      | Stx2    | Mokuro    | Moore |
| 28. | 114c          | STEC      | Stx1    | Mokuro    | Moore |
| 29. | 116a          | STEC      | Stx2    | Mokuro    | Moore |
| 30. | 119b          | STEC      | Stx2    | Opa       | Moore |
| 31. | 123a          | STEC      | Stx1    | Mokuro    | Moore |
| 32. | 123b          | STEC      | Stx2    | Mokuro    | Moore |

* Isolates that are highlighted are from the same well
| S/N | Strain number | Pathotype | Genes | Locations | Wards   |
|-----|---------------|-----------|-------|-----------|---------|
| 33  | 126c          | STEC      | Stx2  | Olopo     | Moore   |
| 34  | 23cwii        | STEC      | Stx2  | Opa       | Moore   |
| 35  | 4aw           | tEPEC     | Bfp   | Moore     | Moore   |
| 36  | Ds122a        | STEC      | Stx2  | Mokuro    | Moore   |
| 37  | 124c          | ETEC AND STEC | ST + Stx2 | Gbodo | Okerewe 1 |
| 38  | 125a          | STHEC     | Stx2 + Eae | Gbodo | Okerewe 1 |
| 39  | 130c          | STHEC     | Stx1 + Eae | Ayetoro | Okerewe 1 |
| 40  | 131b          | tEPEC     | Bfp   | Ayetoro   | Okerewe 1 |
| 41  | 132b          | ETEC AND STEC | ST + Stx2 | Oke Soda | Okerewe 1 |
| 42  | 138b          | tEPEC     | Bfp   | Ayetoro   | Okerewe 1 |
| 43  | 139b          | ETEC      | ST    | Ayetoro   | Okerewe 1 |
| 44  | 142a          | ETEC      | ST    | Ayetoro   | Okerewe 1 |
| 45  | 142di         | ETEC      | LT    | Ayetoro   | Okerewe 1 |
| 46  | 143c          | ETEC      | ST    | Oke Soda  | Okerewe 1 |
| 47  | 154a          | EIEC      | Ipah  | Ayetoro   | Okerewe 1 |
| 48  | 154b          | STEC      | Stx2  | Ayetoro   | Okerewe 1 |
| 49  | 69wii         | ETEC AND STEC | ST + Stx2 | Ayetoro | Okerewe 1 |
| 50  | Ss145eii      | ETEC      | ST    | Oke Ayetoro | Okerewe 1 |
| 51  | 142diii       | ETEC      | ST    | Ayetoro   | Okerewe 1 |
| 52  | Ds144cii      | EIEC      | Ipah  | Oke Ayetoro | Okerewe 1 |
| 53  | 107a          | ETEC      | ST    | Lakanye   | Okerewe 2 |
| 54  | 127a          | STEC      | Stx1  | Otutu     | Okerewe 2 |
| 55  | 127b          | STEC      | Stx2  | Otutu     | Okerewe 2 |
| 56  | 128a          | ETEC      | ST    | Otutu     | Okerewe 2 |
| 57  | 128b          | tEPEC, ETEC AND STEC | Bfp + St + Stx2 | Otutu | Okerewe 2 |
| 58  | 128c          | STEC      | Stx2  | Otutu     | Okerewe 2 |
| 59  | 148a          | STEC      | Stx2  | Ajamopo   | Okerewe 2 |
| 60  | 150b          | STEC      | Stx1  | Itakogun  | Okerewe 2 |
| 61  | Ds42c         | EAEC      | Cvd432 | Itakogun | Okerewe 2 |
| 62  | 101a          | STEC      | Stx1  | Ogbonya   | Okerewe 3 |
| 63  | 101b          | EIEC      | Ipah  | Ogbonya   | Okerewe 3 |
| 64  | 102b          | tEPEC     | Bfp   | Ogbonya   | Okerewe 3 |
| 65  | 103b          | STEC      | Stx2  | Ogbonya   | Okerewe 3 |

* Isolates that are highlighted are from the same well
Factors associated with diarrhoeagenic Escherichia coli contamination of wells

Of the wells that were contaminated by DEC, 16 (28.6%) were undercut by erosion, 26 (46.4%) were sited near septic tanks, 24(42.9%) had dirty platforms, 22 were owned by those who keep pets, 39(69.6%) were used by those in a tenement, 19(33.9%) were sited near livestock and 40(71.4%) were owned by artisans. The average age and depth of the wells were 17.5 ± 22.2 (mean ± SD; Years) and 31.5 ± 23.5 (mean ± SD; Feet) respectively (Table 6).

Univariate analysis revealed that wells that were undercut by erosion (p = 0.018), sited near septic tanks (p = 0.005), had dirty platforms (p = 0.001), owned by those who kept pets (p = 0.035), used by those in tenement (p = 0.012) significantly harboured diarrhoeagenic E. coli.

The associated factors were further subjected to multivariate analysis using the binomial logistic regression model. Wells that were undercut by erosion (OR = 2.616, CI = 1.019–6.716, p = 0.046), sited near septic tank (OR = 2.611, CI = 1.131–6.027, p = 0.025), had dirty platforms (OR = 3.125, CI = 1.232–7.924, p = 0.016) were significantly associated with the presence of DEC in wells. However, there was no significant association between wells that were owned by those who kept pets (OR = 0.884, CI = 0.335–2.329, p = 0.803) and those used in tenement (OR = 1.115, CI = 0.418–2.977, p = 0.828) and the presence of diarrhoeagenic E. coli. (Table 7)

| S/N | Strain number | Pathotype | Genes          | Locations | Wards       |
|-----|---------------|-----------|----------------|-----------|-------------|
| 66. | 105a          | ETEC      | ST             | Ogbonya   | Okerewe 3   |
| 67. | 105b          | STEC      | Stx2           | Ogbonya   | Okerewe 3   |
| 68. | 152a          | STEC      | Stx2           | Ogbonya   | Okerewe 3   |
| 69. | 152b          | EPEC, EAEC| Cvd432 + Bfp   | Ogbonya   | Okerewe 3   |

* Isolates that are highlighted are from the same well
Table 6
Univariate analysis of risk factors for contamination with DEC

| Characteristics                  | NO (N = 87) | YES (N = 56) | Total (N = 143) | p value |
|----------------------------------|-------------|--------------|-----------------|---------|
| WARDS                            |             |              |                 | 0.183¹  |
| ILODE 1                          | 11.0 (12.6%)| 7.0 (12.5%)  | 18.0 (12.6%)    |         |
| ILODE 2                          | 37.0 (42.5%)| 12.0 (21.4%) | 49.0 (34.3%)    |         |
| MOORE                            | 13.0 (14.9%)| 12.0 (21.4%) | 25.0 (17.5%)    |         |
| OKEREWE 1                        | 16.0 (18.4%)| 15.0 (26.8%) | 31.0 (21.7%)    |         |
| OKEREWE 2                        | 4.0 (4.6%)  | 5.0 (8.9%)   | 9.0 (6.3%)      |         |
| OKEREWE 3                        | 6.0 (6.9%)  | 5.0 (8.9%)   | 11.0 (7.7%)     |         |
| AGE OF WELL OWNERS (mean ± SD) (Years) | 44.3 ± 16.3 | 48.1 ± 17.9 | 45.8 ± 17      | 0.200   |
| NUMBER OF YEARS IN RESIDENCE (mean ± SD) (Years) | 16.58 ± 18.6 | 12.7 ± 14.8 | 14.2 ± 16.4     | 0.168   |
| AGE OF WELLS (mean ± SD) (Months)  | 25.4 ± 20.2 | 17.5 ± 22.2 | 20.6 ± 21.7     | 0.033   |
| DEPTH OF WELLS (mean ± SD) (Feet)   | 25.8 ± 19.5 | 31.5 ± 23.5 | 29.3 ± 22.1     | 0.128   |
| WELL DAMAGED BY EROSION          |             |              |                 | 0.018¹  |
| NO                               | 76.0 (87.4%)| 40.0 (71.4%) | 116.0 (81.1%)   |         |
| YES                              | 11.0 (12.6%)| 16.0 (28.6%) | 27.0 (18.9%)    |         |
| GENDER                           |             |              |                 | 0.053¹  |
| FEMALE                           | 70.0 (80.5%)| 37.0 (66.1%) | 107.0 (74.8%)   |         |
| MALE                             | 17.0 (19.5%)| 19.0 (33.9%) | 36.0 (25.2%)    |         |
| RELIGION                         |             |              |                 | 0.621¹  |
| CHRISTIANITY                     | 69.0 (80.2%)| 42.0 (76.4%) | 111.0 (78.7%)   |         |
| ISLAM                            | 14.0 (16.3%)| 12.0 (21.8%) | 26.0 (18.4%)    |         |
| TRADITIONALIST                   | 3.0 (3.5%)  | 1.0 (1.8%)   | 4.0 (2.8%)      |         |
| LEVEL OF EDUCATION               |             |              |                 | 0.334¹  |
| PRIMARY                          | 16.0 (20.5%)| 8.0 (16.7%)  | 24.0 (19.0%)    |         |
| SECONDARY                        | 35.0 (44.9%)| 28.0 (58.3%) | 63.0 (50.0%)    |         |
| TERTIARY                         | 27.0 (34.6%)| 12.0 (25.0%) | 39.0 (31.0%)    |         |
| COVERED                          |             |              |                 | 0.227¹  |
| COVERED                          | 70.0 (80.5%)| 38.0 (67.9%) | 108.0 (75.5%)   |         |
| OPEN                             | 7.0 (8.0%)  | 8.0 (14.3%)  | 15.0 (10.5%)    |         |
| PARTIALLY COVERED                | 10.0 (11.5%)| 10.0 (17.9%) | 20.0 (14.0%)    |         |

¹Pearson chi-square test; ²Student t test; mFlat: is a self-contained housing unit; Tenement: a type of building shared by multiple dwellings; Traditionalist: someone who believes in and follows tradition; Dirty platform: a dirty concrete slab that covers the well
| Characteristics                     | NO (N = 87) | YES (N = 56) | Total (N = 143) | p value |
|------------------------------------|------------|-------------|----------------|---------|
| PRESENCE OF SEPTIC TANK            |            |             |                | 0.005¹  |
| NO                                 | 64.0 (76.2%) | 30.0 (53.6%) | 94.0 (67.1%)   |         |
| YES                                | 20.0 (23.8%) | 26.0 (46.4%) | 46.0 (32.9%)   |         |
| KEEPING OF PETS                    |            |             |                | 0.035¹  |
| NO                                 | 64.0 (76.2%) | 32.0 (59.3%) | 96.0 (69.6%)   |         |
| YES                                | 20.0 (23.8%) | 22.0 (40.7%) | 42.0 (30.4%)   |         |
| PROXIMITY OF LIVESTOCK TO WELL     |            |             |                | 0.265¹  |
| NO                                 | 65.0 (74.7%) | 37.0 (66.1%) | 102.0 (71.3%)  |         |
| YES                                | 22.0 (25.3%) | 19.0 (33.9%) | 41.0 (28.7%)   |         |
| PROXIMITY OF WASTE DUMP SITE TO WELL |          |             |                | 0.578¹  |
| NO                                 | 84.0 (96.6%) | 53.0 (94.6%) | 137.0 (95.8%)  |         |
| YES                                | 3.0 (3.4%)  | 3.0 (5.4%)  | 6.0 (4.2%)     |         |
| PROXIMITY OF WELL TO FARM          |            |             |                | 0.198¹  |
| NO                                 | 79.0 (90.8%) | 54.0 (96.4%) | 133.0 (93.0%)  |         |
| YES                                | 8.0 (9.2%)  | 2.0 (3.6%)  | 10.0 (7.0%)    |         |
| RESIDENCE TYPE                     |            |             |                | 0.012¹  |
| FLAT                               | 45.0 (51.7%) | 17.0 (30.4%) | 62.0 (43.4%)   |         |
| TENEMENT                           | 42.0 (48.3%) | 39.0 (69.6%) | 81.0 (56.6%)   |         |
| OCCUPATION                         |            |             |                | 0.131¹  |
| ARTISAN                            | 60.0 (69.0%) | 40.0 (71.4%) | 100.0 (69.9%)  |         |
| CIVIL SERVANT                      | 18.0 (20.7%) | 10.0 (17.9%) | 28.0 (19.6%)   |         |
| RELIGIOUS LEADER                   | 2.0 (2.3%)  | 3.0 (5.4%)  | 5.0 (3.5%)     |         |
| STUDENT                            | 6.0 (6.9%)  | 0.0 (0.0%)  | 6.0 (4.2%)     |         |
| UNEMPLOYED                         | 1.0 (1.1%)  | 3.0 (5.4%)  | 4.0 (2.8%)     |         |
| DIRTY PLATFORM                      |            |             |                | <0.001¹ |
| NO                                 | 72.0 (82.8%) | 32.0 (57.1%) | 104.0 (72.7%)  |         |
| YES                                | 15.0 (17.2%) | 24.0 (42.9%) | 39.0 (27.3%)   |         |
| HOSPITALIZATION IN LAST YEAR       |            |             |                | 0.542¹  |
| NO                                 | 67.0 (83.8%) | 43.0 (79.6%) | 110.0 (82.1%)  |         |
| YES                                | 13.0 (16.2%) | 11.0 (20.4%) | 24.0 (17.9%)   |         |

¹Pearson chi-square test; ²Student t test; mFlat: is a self-contained housing unit; Tenement: a type of building shared by multiple dwellings; Traditionalist: someone who believes in and follows tradition; Dirty platform: a dirty concrete slab that covers the well
**Table 7**
Multivariate Logistic regression models of DEC in the assessed wells

| Predictor                  | N (%)   | Odds ratio | Lower 95% Confidence Interval | Upper 95% Confidence Interval | P   |
|----------------------------|---------|------------|--------------------------------|-------------------------------|-----|
| Well damaged by erosion    |         |            |                                |                               |     |
| Yes                        | 16.0 (28.6%) | 2.616      | 1.019                          | 6.716                         | 0.046 |
| No                         | 11.0 (12.6%) |            |                                |                               |     |
| Presence of septic tank    |         |            |                                |                               |     |
| Yes                        | 26.0 (46.4%) | 2.611      | 1.131                          | 6.027                         | 0.025 |
| No                         | 20.0 (23.8%) |            |                                |                               |     |
| Dirty platform             |         |            |                                |                               |     |
| Yes                        | 24.0 (42.9%) | 3.125      | 1.232                          | 7.924                         | 0.016 |
| No                         | 15.0 (17.2%) |            |                                |                               |     |
| keeping of pets            |         |            |                                |                               |     |
| Yes                        | 22.0 (40.7%) | 0.884      | 0.335                          | 2.329                         | 0.803 |
| No                         | 20.0 (23.8%) |            |                                |                               |     |
| Residence type             |         |            |                                |                               |     |
| Tenement                   | 39.0 (69.6%) | 1.115      | 0.418                          | 2.977                         | 0.828 |
| Flat                       | 42.0 (48.3%) |            |                                |                               |     |

**Characteristics**

| Characteristics          | NO (N = 87) | YES (N = 56) | Total (N = 143) | p value |
|--------------------------|-------------|--------------|-----------------|---------|
| **MARITAL STATUS**       |             |              |                 |         |
| MARRIED                  | 75.0 (86.2%)| 54.0 (96.4%) | 129.0 (90.2%)   | 0.045   |
| SINGLE                   | 12.0 (13.8%)| 2.0 (3.6%)   | 14.0 (9.8%)     |         |

1Pearson chi-square test; 2Student t test; mFlat: is a self-contained housing unit; Tenement: a type of building shared by multiple dwellings; Traditionalist: someone who believes in and follows tradition; Dirty platform: a dirty concrete slab that covers the well

**Relatedness and Diversity of DEC isolates**

Repetitive PCR was used to determine the relatedness of the DEC isolates. A representative (GTG)5-PCR fingerprint picture is shown in Fig. 3. Isolates banding patterns ranged from 1 to 14 bands. Bands molecular weight varied from 100bp to 4706 bp. Forty-nine isolates were typed by (GTG)5 while certain isolates did not produce any band and appeared not typeable. The (GTG)5-PCR fingerprints dendrogram is shown in Fig. 4. All the isolates clustered together. Nevertheless, six clades of strains were observed along the axis from 0 to 45. Clade 5 had the highest number of strains (12/49; 24.5%), while clade 3 had the least number (3/49; 6.1%). Four STEC isolates (119b-Opa-Moore, 23cw-Opa-Moore, 96-Oke Atan-Iloide1 and 94-Oke Atan-Ilode1) from different locations and wards in the local government in Clade 5 are identical.
In all, the isolates were highly diverse as indicated by the Shannon diversity index \((H = 18.87)\). Isolates from Okerewe ward 1 \((H = 5.41)\) were the most diverse while those from Okerewe ward 3 were the least diverse \((H = 3.17)\). (Table 8)

**Table 8**

| Source of strains | Total no. of strain types | Shannon diversity index \((H)\) |
|-------------------|---------------------------|---------------------------------|
| Moore             | 13                        | 4.93                            |
| Ilode1            | 7                         | 4.68                            |
| Ilode 2           | 9                         | 4.93                            |
| Okerewe 1         | 12                        | 5.41                            |
| Okerewe 2         | 6                         | 4.60                            |
| Okerewe 3         | 2                         | 3.17                            |
| All               | 49                        | 18.87                           |

**Discussion**

Diarrhoeal disease is a significant cause of morbidity and mortality in children worldwide and a high percentage of bacterial gastroenteritis is caused by diarrhoeagenic *E. coli* (DEC).\([1]\) In Nigeria, epidemiological studies of DEC isolates in drinking water are scarce. To the best of our knowledge, this is the first study in Nigeria that will investigate the occurrence of DEC in well water.

In this study, 169 *E. coli* strains were isolated from 98 out of 110 wells that were contaminated by coliform bacteria. All the isolates were screened for eight different diarrhoeagenic genes possessed by five *E. coli* pathotypes. We detected DEC in 58 wells in the six wards of the local government area. Our observation aligns with the reports of previous investigators which observed that drinking water can be a reservoir of DEC in the environment.\([23, 24]\) The prevalence of DEC in our study (40.6\%) is relatively higher than that of da Silva *et al.*\([25]\) (28.1\%) and Taomaneso *et al.*\([23]\) (33.3\%), but similar to 48\% reported by Ali *et al.*\([4]\) The prevalence of DEC pathotypes appears to vary according to geographical region probably due to different prevailing risk factors. Largely, the presence of potentially pathogenic *E. coli* in drinking water highlights the potential risk for environmental transmissibility of these strains in different parts of the world.

In order to identify the risk factors associated with the presence of DEC in water in the study environment, we used binomial logistic regression models to test for association. Our analysis revealed a significant relationship between the presence of DEC and wells that were damaged by erosion, located near septic tanks or had unclean platforms. Findings from previous studies have also highlighted these factors to have a significant association with water contamination.\([26–29]\) Siting of septic tanks near wells may result in leakages or seepages of faecal material into the wells thereby contaminating groundwater. This was evident in a study conducted in the United States that examined the seasonal association of septic tank distance and well contamination and discovered a strong link between decreasing distance and increasing coliform between septic tanks and wells. Similarly, a review of pit latrines and their impacts on groundwater quality by Graham *et al.* concluded that in order to avoid groundwater contamination, latrines and water sources should be at least 50 m apart.\([30]\) Also, cracks in the wells can expose wells to polluted storm water and agricultural runoffs. Hence, the knowledge of associated risk factors can provide information that can generate ideas for workable interventions.
We found that the prevalence of DEC pathotypes varied by location, probably due to the prevailing associated factors in each location. Okerewe ward 1 had the highest number of wells that were contaminated with DEC, while Okerewe 3 had the least number. Furthermore, multiple DEC pathotypes were recovered from eight wells in the sampled locations. Previous studies in Burkina Faso [31], Bangladesh [32] and Brazil [33] have reported similar findings, implying multiple sources of contamination of the wells.

All the five pathotypes of DEC that we sought were identified with a preponderance of STEC. The occurrence of STEC in drinking water has been reported globally [33, 34], along with outbreaks of waterborne disease caused by this pathotype [35, 36]. STEC are a public health issue because they can cause anaemia, uraemia, and renal failure, particularly in young children. Our findings are consistent with earlier research that found STEC in drinking water. [34, 37, 38] Our prevalence is higher than that of Elmonir et al. [24] in Egypt (33.3%). In contrast, none of the E. coli isolates from water samples in France was STEC. [39] Interestingly, our previous study on the prevalence of DEC in diarrheic children in this environment revealed a predominance of STEC among the pathotypes detected. [14] Therefore, our study indicates that STEC is prevalent in this environment, and that water might serve as its reservoir.

Most of our STEC harboured stx2, which has been linked to haemorrhagic colitis and haemolytic uraemic syndrome in humans. Even though eae is a key determinant of virulence in STEC infection, most of the stx2-positive isolates lacked it, apart from three isolates that harboured eae with stx2 and stx1. In light of the reported health risk associated with STEC, the detection of eae-negative STEC strains in our study could be a public health concern, as outbreaks of bloody diarrhoea and hemolytic-uremic syndrome (HUS) caused by STEC strains lacking the eae gene have been reported, implying that Shiga toxin is the primary virulence trait responsible for HUS. [33, 35] Furthermore, the stx2 gene has been shown to be more strongly linked with severe illness in humans than the stx1 gene, indicating its relevance in human infection.

ETEC, EAEC, EPEC have been linked with waterborne outbreaks of gastroenteritis. In our study, ETEC was second to STEC in terms of prevalence. Kambire et al. [40] found that 90% of E. coli isolated from water were ETEC which differs from the prevalence of 14.5% we got in our study, but higher than Rodrigues da Silva et al. [25] that reported less than 1%. EAEC strains have been linked with outbreaks of gastroenteritis in South Korea due to consumption of contaminated groundwater. [35] In this study, EAEC was the least prevalent pathotype. Also, a study conducted in South Africa, showed that only EAEC was found of all the DEC strains. [41] The EPEC strains are of two types; atypical EPEC (aEPEC) and typical EPEC (tEPEC). Humans are the only reservoir for tEPEC, which is spread by inter-human contact. Canizalez-Roman et al. [42] and Sidhu et al. [43] detected tEPEC in food and surface water respectively. The detection of only tEPEC in our study suggests that the wells were contaminated by humans. Open wells are usually fetched with dirty drawers and many people normally stand on the wells with their shoes to draw water; these practices normally expose the wells to human contact, which will eventually lead to contamination. The detection of EPEC as the third most prevalent pathotypes in our study shows that contaminated water can be a source of infection by this pathotype in humans.

EIEC is an important E. coli pathotype that causes watery diarrhoea and dysentery similar to Shigella in terms of pathogenesis. In this study, EIEC was detected in four (5.8%) DEC isolates. Compared with our findings, higher prevalence rates of EIEC have been reported from China (9.1%) [44] and Sudan (41.3%) [45] probably due to geographical differences.

Moreover, our results showed two and three combinations of diarrhoeagenic genes of different E. coli pathotypes isolated from some water samples: STEC and tEPEC (N= 2/58) (3.4%), ETEC and STEC (N = 5/58) (8.6%), tEPEC, ETEC and STEC (1/58) (1.7%), EAEC and tEPEC (1/58) (1.7%). Remarkably, this is the first study to report these combinations in waterborne DEC isolates. Other studies reported a different combination of genes from both EAEC and EHEC. [43, 46] This finding is of a public health concern as mixed infections usually involve more dehydration compared with episodes caused by a single DEC pathotype.

In our study, DEC isolates showed a high degree of diversity using The (GTG)5 rep-PCR typing (Shannon diversity index H = 18.87). Moreover, all the DEC isolates clustered together with six clades of strains observed. The isolates recovered within same region and those from other regions showed diverse genetic profiles. This extensive diversity among the DEC strains
isolated from different sources rules out between/within location transmissibility of isolates, and may also imply multiple sources of contamination in these locations. Likewise, several independent studies have reported the existence of diverse populations of *E. coli* in several hosts and environments.[5, 47] Clade 5 had the highest number of strains (12/50; 24%), while clade 3 had the least number (3/50; 6%). Four STEC isolates from different locations and wards in the local government in Clade 5 were identical. This implies that these isolates have either been maintained or circulated within a similar source of origin.

**Conclusions And Recommendations**

This study reports a high prevalence of DEC in well water with a preponderance of STEC. The presence of these pathogenic strains of *E. coli* in drinking water highlights the risk to human health associated with the use of untreated water. There was a high degree of genetic diversity among the isolates implying multiple sources of contamination thus emphasizing the need for periodic sanitation and inspection of wells for cracks to prevent seepages, runoff and possible outbreaks of waterborne diseases. In addition, owners of dug wells should be educated about the dangers of drinking contaminated water, the importance of well sanitation and the need to repair construction faults.

**Abbreviations**

DEC: Diarrhoeagenic *Escherichia coli*

ETEC: Enterotoxigenic *E. coli*

EAEC: Enteroaggregative *E. coli*

EIEC: Enteroinvasive *E. coli*

EPEC: Enteropathogenic *E. coli*

STEC: Shiga-toxin producing *E. coli*

MPN: Most probable number

MST: Microbial source tracking

PCR: Polymerase chain reaction

**Declarations**

**Ethics approval and consent to participate**

This study approval was obtained from the Health Research Ethics Committee (HREC), Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Nigeria (HREC No: IPHOAU/12/863). There is no participation section for this study as it is not applicable.

** Consent for publication**

None. This manuscript does not contain any individual person's data.

**Availability of data and materials**

All data and materials of this study are included. If additional information is needed, please contact the author for requests.
Competing interests
The authors declare that they have no competing interests

Funding:
None

Authors’ contribution:
OB conceived the study, wrote the manuscript; FM performed the experiments, collected and analysed data, wrote the manuscript; OA analysed data, wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgment
We are grateful to the health workers of Osun state primary health centres Ile-Ife, Nigeria for their support.

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Figures

Figure 1
Figure 2

A gel picture showing diarrhoeagenic genes of isolates Lane 1: Water (Negative); Lane 2: E. coli 042 (CVD432-630bp); Lane L: 100bp ladder; Lane 3: E. coli EDL 933 (stx1-180bp, stx2-255bp); Lane 4: E. coli ; Lane 5: E. coli H10407 (LT-450bp); Lane 6: E. coli; Lane 7: E. coli E2348 (bfp-326bp); Lane 8: E. coli H10407 (ST-190bp and LT-450bp); Lane 9: E. coli; Lane 10: E. coli (LT-450bp); Lane 11: E. coli (eae-917bp); Lane 12: E. coli (stx2-255bp); Lane 13: E. coli E137 (ipaH-600bp)

Figure 3

A gel picture of GTG5 PCR fingerprints of DEC isolates
Figure 4

Neighbour-joining dendrogram clusters of (GTG)5-fingerprints of strains with their locations.