Antibiotic Resistance Profile and Clonality of *E. coli* Isolated from Water and Paediatric Stool Samples in the North-West, Province South Africa

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**Abstract**

This study investigated the antibacterial resistance profiles of *E. coli* pathotypes isolated from children under five years and drinking water samples collected from the North West Province of South Africa and ascertained the clonality of the isolates. Two hundred and forty-one *E. coli* isolates were recovered from stool samples of diarrhoeic and non-diarrhoeic children under five years old, and drinking water, using the Colilert-18® Quanti-tray/2000 and Eosin methylene blue agar. The presence of enteropathogenic (*eaeA*), enterohaemorrhagic (*eaeA,stx1, stx2 and flicH7*), enteroaggregative (*eagg*), enteroinvasive (*ippH*) and enterotoxigenic (*ST and LT*) *E. coli* pathotypes were also investigated using PCR. Antibiotic susceptibility was carried out through the disk diffusion method. The presence of *blaCTX-M, blaSHV, blaCMY* and *blaDHA* genes that code for β-lactamases was investigated using real-time PCR. Similarities between human and water isolates were tested using ERIC-PCR. Overall, EHEC (35.8%), EPEC/EHEC (22%), ETEC (21.6%) and EIEC (20.2%) were detected. The highest antibiotic resistance was detected to Clarithromycin (100%) and Erythromycin (100%) while the lowest resistance was against Gentamicin (0.4%). Also, 100% sensitivity was recorded to imipenem and meropenem. Multi-antibiotic resistance was observed in all the pathotypes, and the ESBL genes were detected in 71.6% of the pathotypes. ERIC-PCR indicated 100% similarities in some water and human samples. Pathogenic *E. coli* is amongst the diarrhoea-causing agents in the North West Province with EHEC being the most identified pathotype. The clonal relatedness of the human and water isolates suggests that domestic water might be a route of transmission.

**Keywords:** *E. coli*, Stool samples, Antibiotic resistance, children.

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INTRODUCTION

Escherichia coli is a normal flora of warm-blooded animals, including humans. However, some strains have acquired pathogenic potentials allowing them to cause diarrhea (intestinal pathotypes) and other systemic illnesses involving the urinary tract, brain and blood (extra intestinal pathotypes). Intestinal or diarrhoeagenic E. coli (DEC) strains constitute the primary diarrhoea aetiologic agents in children younger than five years old and in most cases, they are associated with growth defects. About 760,000 children lose their lives every year to diarrhoeal diseases worldwide. Diarrhoeagenic E. coli pathotypes are ubiquitous in the environment, especially in aquatic environments and are most often responsible for waterborne diarrhoeal disease outbreaks both in developed and developing countries. For example, they were responsible for the second largest waterborne diarrhoea outbreak in the world reported in 2011 in Germany, which recorded over 3,000 hospitalisations and 36 fatalities. DEC strains are separated into different pathotypes based on specific virulence factors, their invasive capability and the clinical symptoms produced. These pathotypes include the EPEC (Enteropathogenic E. coli), STEC (Shiga toxin-producing E. coli or EHEC (Enterohaemorrhagic E. coli)), ETEC (Enterotoxigenic E. coli), EIEC (Enteroinvasive E. coli), EAEC (Enteroaggregative E. coli) and recently, DAEC (Diffusely-adherent E. coli). Each pathotype has its unique virulence-associated gene(s) (VGs) contributing to its disease-causing potentials.

EPEC members are a significant cause of infantile diarrhea and are frequently associated with public places like schools and hospitals. Members of this pathotype are responsible for approximately 5-10% of pediatric diarrhoea episodes in developing countries. An infectious dose of 10⁵ CFU can trigger infection even in a healthy adult. EPEC strains contain a cluster of VGs on a the Locus Enterocyte Effacement (LEE) which is a pathogenicity island involved in severe diarrhoea. EPEC members could be classified into atypical EPEC ((aEPEC; lacking the adherence effector plasmid (EAF) but containing the LEE) or typical EPEC (tEPEC; possessing the EAF plasmid). Characteristic EPEC pathotypes produce peculiar histopathological lesions on intestinal epithelial cell surfaces. The mechanism of infection in EPEC involves numerous virulence factors, including intimin, bundle-forming pilus (BFP), paa and Long polar fimbriae (LPF).

The eae-encoded intimin protein is an essential EPEC virulence gene which allows for attaching and effacing (A/E) lesions on the host’s intestinal epithelial cell surface. Other virulence factors found in tEPEC are the bfpA and perABC genes which encode the bundle-forming pili used for localised attachment to epithelial cells. STEC, also referred to as EHEC, consists of pathotypes that can cause hemorrhagic colitis (HC) and most severe cases, infection may lead to hemolytic uremic syndrome (HUS). The most notorious serotype in this group is the O157:H7 serotype, often implicated in most foodborne diseases outbreaks globally. The pathogenesis of STEC infections involves two Shiga-like toxins (stx1 and stx2) and other VGs that aid in attachment like the intimin (eae) gene. STEC/EHEC members are the primary cause of foodborne illnesses and can initiate foodborne diarrhoea even at minimal infective doses.

ETEC pathotypes are the cause of childhood diarrhoea in many developing countries. Also, a few foodborne outbreaks due to ETEC have been recorded in some developed countries. ETEC pathotypes induce infection by attaching to the intestinal cell surface, and secreting either the heat stable (ST) or labile (LT) enterotoxin (or both) which are plasmid encoded. The ST toxin is a 2kDa single peptide toxin; its mode of action is the activation of guanylyl cyclase C to increase the cyclic guanosine monophosphate (cGMP) level. An increase in cGMP leads to a surge in bicarbonate and chloride ion secretion which inhibits the intake of sodium and chloride ions resulting in loose diarrhoea. LT, on the other hand, is an 86kDa protein. It deregulates the host’s adenylate cyclase, thus, enhancing attachment to intestinal epithelial cells which results in diarrhoea.

EIEC pathotypes, like Shigella, cause of watery diarrhoea or bacillary dysentery in severe cases. The process of infection depends on some essential virulence chromosomal-encoded and plasmid-encoded determinants, including the ipaH gene. They are highly invasive pathogens capable of attaching to intestinal epithelial cells and replicating to the large intestine.
pathotypes are the most commonly encountered aetiologies of mild and persistent diarrhoea\(^3\). They are frequently isolated from both diarrhoeic and non-diarrhoeic individuals, especially in low-resource settings\(^1\).

EAEC members have been reported to cause inflammation of the intestines in children and impairing their growth and negatively impacting their development even without diarrhoea\(^4\). EAEC virulence genes are heterogeneous. The pathogenesis and genes encoding virulence factors in these strains are not uniform but show a considerable degree of variability among strains\(^4,14\). Nonetheless, the plasmid-borne aggR gene has been characterised and reported as the most common gene associated with EAEC pathotypes, and it regulates the virulence genes encoded in the chromosomes, plasmid and the type VI secretion system\(^4\).

Most diarrhoea cases due to DEC are self-limiting. Oral rehydration and electrolyte replacement have been advised and reported to be effective in some paediatric cases\(^30\). Antibiotics such as fluoroquinolones, azithromycin and rifampicin have been used in severe diarrhoea cases and have reportedly reduced the duration of diarrhoea symptoms\(^31\). However, because of the excessive use of numerous antibiotics against these pathogens, resistant strains have emerged\(^21,31\). There is a global increase in antibiotic-resistance among pathogenic \(E. coli\) strains against commonly prescribed antibiotics, even to newly discovered and more potent antibiotics\(^2,31\). Multi-drug resistant strains are another public health hazard associated with these DEC pathotypes. It has been reported that \(E. coli\) infections caused by multi-drug resistance strains are problematic, as they are usually severe and take a longer time to clear especially in infections in children\(^12,32,33\).

Phenotypic multi-drug resistance of some organisms including pathogenic \(E. coli\) emerged because of a metallo-\(β\)-lactamase gene possessed by these strains. This gene plays an active role in hydrolysing all \(β\)-lactam antibiotics except aztreonam\(^34\) and it is said to confer resistance to other antibiotics in \(E. coli\)\(^11,21\). In pathogenic \(E. coli\), the most essential resistance mechanism against \(β\)-lactams involves plasmid-encoded extended-spectrum \(β\)-lactamases (ESBLs)\(^13,33\). ESBL-producing \(E. coli\) hydrolyses penicillin, extended spectrum Cephalosporins and the monobactams\(^19,21\) but cannot efficiently degrade Cephamycin, Carbapenems and \(β\)-lactamase inhibitors\(^19,36\). However, resistant strains have emerged\(^11\). There are different groups of ESBLs including CTX-M, CMY, SHV and DHA\(^37–39\). The CTX-M are reported to be a product of plasmid transfer from pre-existing chromosomal ESBL genes\(^38\). They are the most frequently reported group in most parts of the world\(^21,40\).

Drinking poorly treated or untreated polluted water or consuming raw or partially-cooked meat and vegetables have been implicated in the transmission of pathogenic \(E. coli\) strains to humans\(^41,42\). Molecular techniques like the enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) have been used to detect similarity between Gram-negative bacteria by comparing their genetic fingerprints\(^40,43\). This genotyping approach ensures strain-specific fingerprinting which allows for the evaluation of the genetic diversity in pathogenic \(E. coli\) and other organisms\(^44,45\). The outstanding reproducibility and discriminatory power of this technique in detecting similarity between strains\(^45,46\) warranted its application in the current study to evaluate the genetic relatedness of \(E. coli\) isolates from human stool and water samples. Given that \(E. coli\) has not been identified as a contributing aetiology of diarrhoea in the North West Province of South Africa, especially among the paediatric group, the current study, therefore, sought to investigate if there was any clonal relationship between water and clinical \(E. coli\) isolates within the community. These results would identify the possible source of the \(E. coli\), thus stimulating the need for control measure to be implemented to safeguard the health of children within the Province.

MATERIALS AND METHODS

Sample collection

Three hundred and fifty (350) stool samples were collected from diarrhoeic and 155 samples from non-diarrhoeal cases in children under five years attending the Brits District Hospital, Oukasie Clinic, Lethabeleng and Bopang Clinics. Also, 88 water samples, including eight directly from a municipal tap and 80 from water storage containers, were collected from different homes around the sampled clinics. Of the stored
water samples, 38 were fetched from the municipal tap while 42 were from a well/underground water or rainwater harvested from rooftops. Also, four samples were collected from the Crocodile River. Sampling was done between September 2016 and December 2017 and transported to the Council for Scientific and Industrial Research (CSIR), for processing. Approval to conduct the study was obtained from the College of Agriculture and Environmental Sciences (CAES) UNISA (2016/CAES/033) and North West Department of Health.

Written informed consent was obtained from the participants’ parents/guardians. Details of the participants’ age, gender, and clinical signs were recorded.

**Isolation of E. coli from water and stool samples**

Isolation of *E. coli* from water and stool samples was carried out using the Colilert-18®/Quanti-tray/2000 (IDEXX Laboratories, Inc., Johannesburg, South Africa) following the manufacturer’s instructions. Briefly, the Colilert-18° reagent was added to 100 mL of a water sample, mixed properly, sealed in a Quanti-Tray 2000 and incubated for 18 – 24 h. For stool samples, 2g of stool sample was thoroughly mixed with 100 ml of distilled water in a sterile vessel. The supernatant was then extracted and analysed as the water sample. After incubation at 37°C, presumptive *E. coli* isolates were harvested from fluorescent Quanti-trays wells and streaked onto EMB agar to obtained pure colonies as previously described.

Purified colonies were stored at -80°C in a 50% glycerol for further analysis.

**Confirmation of pathogenic potentials of *E. coli* isolated strains**

**DNA extraction and detection of virulence genes (VGs)**

Two hundred and forty-one randomly selected *E. coli* isolates (136 human and 105 isolates) were inoculated onto nutrient agar and incubated for 24 h at 37°C. DNA was extracted from selected colonies after 24 h, by the heat lysis method as previously described.

Extracted DNA templates were first examined for the presence of the malate dehydrogenase (*mdh*) gene to confirm the isolates as being *E. coli*. Isolates harbouring the *mdh gene* were further separated into five DEC pathotypes, EPEC, EAEC, EIEC, ETEC, EHEC targeting specific VGs through multiplex and singleplex PCR assays and using primers and PCR-cycling conditions described by Abia et al. Multiple PCR Group 1 contained the *stx1* and *flich7* genes, Group 2 contained *eaeA, ipaH,* and *eagg* genes and Group 3 contained the *stx2* gene.

*ST* and *LT* genes were detected through Singleplex PCR. The *ST* gene was amplified using primer sequence Forward: TTTCCCTCTTTTGCAGTCAA and reverse: GCAGGATTACAACACAATTCCACGCA with the temperature conditions used for the detection of *mdh* gene. The PCR assay for *LT* gene was performed using a 218 bp primer (Forward: GCACACGGAGCTCTCAGGTC) and (Reverse: TCTTCTACCTTTCAATGCGTTT) with an initial activation at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 1 min and an extension at 72°C for 1 min. A melt curve was prepared as described for the other VGs using the Rotor-Gene™ real-time analysis software, version 6.1 (build 93) (Corbett Life Science (Pty) Ltd., Sydney, Australia). All reactions were performed in a total volume of 20µl made up of 10µl of 2x SensiFAST™ high-resolution melt (HRM) mix (SF) (Bioline GmbH, Germany), 0.5µl of primer (forward and reverse; final concentration 0.5µM each), 5µl of nuclease-free water (NF H2O) and 4µl DNA template. All assays included a positive control consisting of DNA from a reference strain obtained from the Microbiology Laboratory of the NRE, CSIR and previously characterised by Abia et al. (2016). A reaction mixture void of template DNA was also added in each PCR assay as a No Template Control.

**Determination of the antibiotic-susceptibility of *E. coli***

Antibiotic sensitivity testing was performed using the disk diffusion method following the CLSI guidelines. Twenty antibiotics, selected based on their common use in treating *Enterobacteriaceae* infections, were screened in the current study (Table 1). *E. coli* ATCC 25922 and ATCC 35218 were used as positive and negative controls respectively. Breakpoints recommended by the CLSI were used.

**Determining the Minimum Inhibitory Concentration (MIC) of the Antibiotics**

MICs were assessed following the CLSI recommendation and results were interpreted as per the guideline described by the CLSI for *Enterobacteriaceae*.
Detection of antibiotic resistance genes

The presence of the genes conferring resistance to the β-lactamase antibiotics: \textit{blaCTX-M}, \textit{blaSHV}, \textit{blaCMY}, and \textit{blaDHA} was investigated using primer sequences detailed in Table 2.

Amplifications of the \textit{blaSHV}, \textit{blaCMY}, and \textit{blaDHA} genes were carried out in 15µL reaction volumes containing 10µL of SF, 0.5µL of each primer sets (Forward and reverse) final concentration, 0.5µM of each primer), 3µL of template DNA and 1µL of NF H2O, using the same PCR cycling conditions as for the \textit{mdh} gene. The \textit{blaCTX-M} gene was amplified in a 15µL reaction volume and optimized cycling conditions consisting of an initial denaturation at 99°C for 50 s, followed by denaturation at 98°C for 10 s, annealing at 58.5°C for 30 s, extension at 72°C for 15 s and a final extension at 72°C for 1min. A melt curve was prepared as described for the other genes using the Rotor-Gene™ real-time analysis software, version 6.1 (build 93) (Corbett Life Science (Pty) Ltd., Sydney, Australia).

**Detection of Clonality using ERIC-PCR**

Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) was performed using primers ERIC1 (52-ATGTAAG CTCTGGGGGATTCAC-32) and ERIC2 (52-AAGTAAAGT GACTGGG GTAGCG-32)\textsuperscript{50}. DNA was extracted using the GeneJET Genomic DNA purification kit (ThermoFisher Scientific) according to the manufacturer’s protocol. PCR was carried out on the T100 Thermal Cycler (Bio-Rad, USA) in an overall volume of 25µL made up of 12.5µL of DreamTaq Green PCR Master mix (2X) (ThermoFisher Scientific), 0.1µL of 100µM primers ERIC 1 and ERIC 2, 9.3µL of NF H2O and 3µL of

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**Table 1. Antibiotics used for \textit{E. coli} susceptibility profile**

| Class of antibiotic | Antibiotics             | Antibiotics code | Concentration (µg) |
|---------------------|-------------------------|------------------|--------------------|
| Macrolides          | Clarithromycin          | CLR              | 15                 |
|                     | Erythromycin            | ERY              | 15                 |
| Carbapenem          | Meropenem               | MEM              | 10                 |
|                     | Imipenem                | IMI              | 10                 |
| β-lactam/β-lactamase inhibitor combination | Amoxicillin/clavulanic acid | AMC | 30 |
| Penicillin          | Ampicillin              | AMP              | 10                 |
| Folate pathway inhibitors | Cotrimoxazole | SXT              | 25                 |
| Sulfonamides        | Trimethoprim            | TMP              | 5                  |
| Nitrofurans         | Nitrofurantoin          | NIT              | 300                |
| Fluoroquinolones    | Ciprofloxacin           | CIP              | 5                  |
|                     | Norfloxacin             | NOR              | 10                 |
| Aminoglycosides     | Amikacin                | AMK              | 30                 |
|                     | Gentamicin              | GEN              | 10                 |
|                     | Streptomycin            | STR              | 10                 |
| Polypeptides        | Colistin sulfate        | CST              | 25                 |
| Tetracycline        | Tetracycline            | TET              | 25                 |
|                     | Tigecycline             | TGC              | 15                 |
| Cephalosporine      | Cephalolin              | CFZ              | 30                 |
|                     | Cefuroxime              | CXM              | 30                 |
|                     | Cephalothin             | CPD              | 10                 |

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**Table 2. Gene-specific primers for ESBL detection**

| Primer | Sequence (5'- 3') | Ref. |
|--------|-------------------|------|
| BlaDHA | F: AACTTTTACAGGTGCTCGGT |
|        | R: CCCTACCTTACTTGCTTTC | 37   |
| CMY-2  | F: GATTCTTTGACTCTTCA |
|        | R: TAAAACCGGTTCCTCCAGATAGC | 38   |
| SHV    | F: TTAACCTCCGTGATAGCC |
|        | R: GATTTTGCTGATTCGCCCC | 39   |
| CTX-M  | F: GGTTAAAAATCATCGGCTC |
|        | R: TTGGTGACGATTTTACCGC | 38   |
template DNA. PCR conditions were optimized to an initial denaturation at 94°C for 3 min, followed by 30 denaturation at 94°C (30 s), annealing at 50°C (1 min), extension at 65°C (8 min) and a final extension at 65°C (16 min). The PCR amplicons were separated through electro-phoresis on a 1.0% (w/v) agarose, after staining in 0.1 mg/ml ethidium bromide for 20 min. The gels were visualised and the images captured using a Gel Doc™ XR+ System (Bio-Rad, South Africa).

The freely available DNA fingerprint gel images analysis tool, GelJ Version 2.0 (https:// sourceforge.net/projects/gelj/) was used to analyse the gel images following a previously described protocol. The normalisation of the DNA fragments was performed using a Quick Load 1 kb DNA molecular weight marker (Inqaba Biotec, South Africa) and the Jacqaud and Unweighted Pair Group Method with Arithmetic mean (UPGMA) cluster analysis was used to produce a dendrogram.

Data analysis
Results were analysed using the statistical package for social sciences (SPSS) (IBM SPSS Statistics Version 20) for windows. The Spearman’s correlation was used to assess the relationship between the diarrhoea status and the various DEC pathotypes. All relationships were considered significant at $p \leq 0.05$.

RESULTS
Identification of *E. coli* pathotypes using the virulence genes

One hundred and thirty-six (136) *E. coli* isolates were obtained from the stool, of which 125 (91.9%) isolates were positive for at least one of the targeted VGs. The most detected gene was the *eaeA* gene of EPEC/EHEC (33/125; 26.4%) while the least detected gene was the *LT* (7/125; 5.6%) gene. None of the human isolates was positive for the *stx2* (EHEC) and *eagg* (EAEC) (Table 3).

For the water samples, 105 isolates were obtained, and 87 of these (82.8%) expressed at least one of the VGs tested. Like the human samples, the *eaeA* gene was the most detected of all the VGs tested (Table 3). No virulence gene was found in water samples collected directly from the municipal tap.

There were differences in the detection of the various DEC pathotypes among the children showing different clinical symptoms (Fig. 1). Antibiotic susceptibility profiles of the human and water *E. coli* isolates

Two hundred and forty-one (241) *E. coli* isolates comprising all those screened for VGs, were further assessed for their susceptibility to 20 antibiotics. The highest recorded resistance was against clarithromycin and erythromycin (100%) and total susceptibility (100%) was recorded against meropenem and imipenem (Table 4). Human isolates showed an overall higher resistance compared to the water isolates. Also, a low percentage of isolates showed resistance to Gentamicin (0.4%) and Norfloxacin (2.4%). The antibiotic resistance rate in individual pathotypes is shown in Table 5. From the human samples, resistance to Amoxicillin/clavulanic acid was recorded only in the EHEC and ETEC pathotypes, while, all the water isolates were resistant to Amoxicillin/clavulanic acid. Also, only the EIEC pathotype from the water was resistant to Norfloxacin.

| Virulence genes (Pathotypes) | Human samples | Water samples | Total |
|-----------------------------|--------------|---------------|-------|
| *eaeA* (EPEC/EHEC)          | 33 (26.4%)   | 14 (16%)      | 47 (22.2%) |
| *eagg* (EAEC)               | 0            | 0             | 0     |
| *ipaH* (EIEC)               | 25 (20%)     | 18 (20.6%)    | 43 (20.3%) |
| *ST* (ETEC)                 | 20 (16%)     | 9 (10.3%)     | 29 (13.6%) |
| *LT* (ETEC)                 | 7 (5.6%)     | 10 (11.4%)    | 17 (8%) |
| *stx1* (EHEC)               | 16 (12.8%)   | 14 (16%)      | 30 (14.2%) |
| *stx2* (EHEC)               | 0            | 2 (2.3%)      | 2 (2.3%) |
| *flicH* (EHEC)              | 24 (19.2%)   | 20 (22.95%)   | 44 (20.7%) |
| Total                       | 125 (91.9%)  | 87 (82.8%)    | 212 (87.9%) |
Determination of Multiple-Antibiotic Resistance (MAR)

Multi-resistance in this study was defined as the resistance of *E. coli* to three or more antibiotics. MAR was observed in 87.5% and 80.8% of isolates from human and water samples respectively. Twenty-nine (21.3%) isolates from human samples demonstrated simultaneous resistance to four antibiotics while 23.2% from water samples were resistant to five antibiotics concurrently. No isolate was resistant to more than 11 antibiotics (Fig. 2).

Prevalence of ESBLs encoding genes

Identified *E. coli* pathotypes were tested for the presence of ESBLs encoding genes. Out of the 212 tested, ESBLs was detected in 152 (71.6%). The genes were detected more in water samples (85%) compare to human samples (62.4%). The *blaDHA* gene was the most detected gene in both human and water samples. ESBL genes were expressed more in EHEC pathotypes compared to other pathotypes (Table 6). Different antibiotic resistance phenotypes were associated with different ESBLs encoding genes. The most frequent resistance pattern observed was to Clarithromycin-Erythromycin-Cephalothin-Ampicillin-Cefuroxime-Cephazolin-Trimethoprim-Colistin.

Determination of the clonality (ERIC-PCR analysis) of *E. coli* pathotypes

The dendrogram revealed that all the clusters contained mixed isolates from water and human samples, except for cluster V which was made up of water samples alone and cluster IX which contained only stool samples (Fig. 3). There was a 100% similarity between some water samples and human samples (S40, S39, W8, and S34). There was also a 100% similarity between water samples of different clusters (W15, W14,
In all the clusters (apart from the clusters with 100% similarities), the similarity patterns between some of the water and human samples were ≤ 80%.

**DISCUSSION**

**Identification and distribution of DEC pathotypes**

Diarrhoeagenic *E. coli* pathotypes are among the most significant aetiologic agents of diarrhoea worldwide, particularly in children from developing countries. In this study, the frequency, virulence markers and antibiotic resistance patterns of DEC from diarrhoeic and non-diarrhoeic paediatric stools and water samples were evaluated. The frequency of DEC was high in paediatric specimens compared to the water sample. These findings could be because *E. coli* is a normal inhabitant of the human intestinal tract and the participants included diarrhoeic children. A high frequency of DEC in water samples could have indicated pollution. Several studies have reported a high prevalence of DEC in different aquatic bodies in South Africa. Detection of DEC in water, particularly domestic waters, should be regarded

**Table 4. Number of *E. coli* isolates resistant to selected antibiotics**

| Antibiotics | Human (%) | Water (%) | Total (%) |
|-------------|-----------|-----------|-----------|
| CLR         | 136 (100%)| 105 (100%)| 241 (100%)|
| ERY         | 136 (100%)| 105 (100%)| 241 (100%)|
| MEM         | 0         | 0         | 0         |
| IMI         | 0         | 0         | 0         |
| AMC         | 13 (9.5%) | 31 (29%) | 34 (18.2%)|
| CPD         | 70 (51.4%)| 81 (77%)  | 151 (62.5%)|
| AMP         | 119 (87.5%)| 105 (100%)| 224 (92.9%)|
| SXT         | 36 (26.5%)| 32 (30.4%)| 68 (28.2%) |
| TMP         | 35 (25.7%)| 44 (41.9%)| 79 (32.7%) |
| NIT         | 10 (7.3%) | 3 (2.8%)  | 13 (5.3%)  |
| CIP         | 6 (4.4%)  | 6 (5.7%)  | 12 (4.9%)  |
| NOR         | 4 (2.9%)  | 2 (1.9%)  | 6 (2.4%)   |
| AMK         | 14 (10.3%)| 8 (7.6%)  | 22 (9%)    |
| GEN         | 1 (0.7%)  | 0         | 1 (0.4%)  |
| STR         | 31 (22.7%)| 44 (41.9%)| 75 (31%)  |
| CST         | 30 (22%)  | 40 (38%)  | 70 (29%)  |
| STR         | 10 (7.3%) | 13 (12.3%)| 23 (9.5%) |
| TGC         | 6 (4.4%)  | 12 (11.4%)| 18 (7.4%) |
| CFZ         | 28 (20.6%)| 35 (33.3%)| 43 (17.8%)|
| CXM         | 33 (24.2%)| 38 (36%)  | 71 (29.4%)|

**Table 5. Antibiotic resistance among *E. coli* pathotypes from human samples**

| Antibiotics | Human EHEC | EPEC/EHEC* | EIEC | ETEC | Water EHEC | EPEC/EHEC* | EIEC | ETEC |
|-------------|------------|------------|------|------|------------|------------|------|------|
| CLR         | 40         | 33         | 25   | 27   | 22         | 7          | 10   | 10   |
| ERY         | 40         | 33         | 25   | 27   | 8          | 1          | 10   | 7    |
| MEM         | 0          | 0          | 0    | 0    | 12         | 8          | 9    | 10   |
| IMI         | 0          | 0          | 0    | 0    | 3          | 0          | 0    | 0    |
| AMC         | 8          | 2          | 1    | 3    | 10         | 6          | 10   | 11   |
| CPD         | 23         | 19         | 17   | 12   | 15         | 7          | 10   | 6    |
| AMP         | 38         | 32         | 25   | 26   | 36         | 14         | 18   | 19   |
| SXT         | 8          | 12         | 7    | 9    | 36         | 14         | 18   | 19   |
| TMP         | 7          | 16         | 3    | 9    | 0          | 0          | 0    | 0    |
| NIT         | 3          | 5          | 0    | 2    | 0          | 0          | 0    | 0    |
| CIP         | 4          | 4          | 2    | 0    | 13         | 8          | 1    | 3    |
| NOR         | 1          | 0          | 1    | 2    | 36         | 14         | 18   | 9    |
| AMK         | 4          | 4          | 3    | 3    | 3          | 0          | 0    | 1    |
| GEN         | 0          | 1          | 0    | 0    | 0          | 0          | 0    | 0    |
| STR         | 12         | 10         | 3    | 7    | 1          | 0          | 4    | 3    |
| CST         | 17         | 3          | 5    | 7    | 0          | 0          | 0    | 0    |
| TET         | 4          | 2          | 1    | 2    | 6          | 2          | 3    | 2    |
| TGC         | 4          | 1          | 0    | 1    | 5          | 3          | 1    | 0    |
| CFZ         | 14         | 4          | 3    | 6    | 11         | 7          | 7    | 5    |
| CXM         | 17         | 7          | 3    | 6    | 14         | 7          | 8    | 5    |

*Isolates that were only positive for the eaeA gene which is present in both EPEC and EHEC
Fig. 3. Clonal relatedness of *E. coli* pathotypes from human and water as determined by ERIC-PCR.
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as a public health concern because these waters are used for human and animal consumption and other indoor potable uses such as washing dishes and cooking. This study confirmed that DEC might have contributed to the burden of diarrhoea within the studied communities. The absence of *E. coli* in the municipal tap water samples further strengthened the findings of the study. The detection of *E. coli* in the stored water samples suggests that poor hygienic practices within the households could have led to the recontamination of the treated water from the municipal tap, as the residual chlorine concentration decreased during storage. In agreement with the present study, Chigo et al. reported a higher prevalence of DEC in human compared to water samples. In Benin, a high prevalence of DEC was equally observed in humans. Studies have confirmed the involvement of DEC in diarrhoea in paediatric groups even in the developed world. For example, DEC pathotypes were among the predominant pathogens isolated in a paediatric emergency facility in Washington. Although DEC pathotypes are a common cause of diarrhoea in children, the high prevalence reported in this study is worrisome as infections with these strains are known to present with some severe health complications such as anaemia, thrombocytopenia and renal failure.

The frequency of DEC pathotypes found in our study was similar to the reports from other developing countries. EHEC was the most detected pathotype found in both human and water samples followed by EPEC/EHEC. These pathotypes were the most detected pathotypes in children with diarrhoea in two studies performed in Iran and Spain. Usein et al. reported EHEC virulence genes in all the diarrhoea samples screened in their study. Although the detection rate in this study is slightly high compared to the 30% reported in Indian children, it should be noted that the present study characterised EHEC pathotype based on the frequency of detection of three different virulence genes. The present study correlates with other studies where the detection rate of EHEC pathotype among children under the age of 5 years was high. It also agrees with studies that used an equal number of virulence genes to characterise the EHEC pathotype. The overall prevalence of EPEC/EHEC (eaeA gene) found in our study is consistent with other studies. A higher frequency of 42.3% has been reported in India while over 50% has been reported in South African domestic water, 67% in Tennessee and 47.5% found in Iranian children.

ETEC is the important diarrhoeic agents in some African countries as reported by the Global Enteric Multicentre Study (GEMS). It has been documented as an important agent of diarrhoea in many studies. EIEC pathotype was the cause of a diarrhoea outbreak in Italy and an important diarrhoeal agent in children in Mexico, although it has also been found in stool samples of individuals without diarrhoea.

**Table 6. Distribution of ESBL genes among the *E. coli* pathotypes**

| Source | ESBL gene | EHEC | EIEC | EHEC/EPEC | ETEC | Total |
|-------|-----------|------|------|-----------|------|-------|
| Human | CMY       | 10   | 2    | 1         | 3    | 16    |
|       | CTX-M     | 8    | 1    | 3         | 6    | 18    |
|       | DHA       | 8    | 10   | 5         | 6    | 29    |
|       | SHV       | 7    | 3    | 1         | 4    | 15    |
|       | Sub-total | 33   | 16   | 10        | 24   | 78    |
| Water | CMY       | 5    | 6    | 5         | 4    | 18    |
|       | CTX-M     | 10   | 3    | 4         | 2    | 20    |
|       | DHA       | 11   | 1    | 5         | 3    | 21    |
|       | SHV       | 3    | 3    | 0         | 9    | 15    |
|       | Sub-total | 29   | 13   | 14        | 18   | 74    |

Antibiotic resistance has been recorded in human as well as aquatic environments, and the findings of the present study correspond with the findings reported in other studies. The result of the antibiotic-resistance profile showed varying degrees of resistance in human and water samples. The results further revealed a higher resistance rate to the most frequently used antibiotics in South Africa domestic water, 67% in Tennessee and 47.5% found in Iranian children.
Africa including Clarithromycin, Erythromycin and Ampicillin. Thus, high resistance rate of DEC to these frequently used antibiotics might pose a severe threat to the public health, because the increase in resistant strains will negatively impact on the efficacy of these antibiotics which might in the long term contribute to higher morbidity and mortality. Other studies have also reported on E. coli resistance to these drugs. For example, a high resistance of DEC isolates was observed to fluoroquinolones, Ampicillin, Cotrimoxazole, and Nalidixic Acid in India. In Iran, over 50% of the paediatric patients were resistant to Ampicillin, ceftriaxone, tetracycline, Clotrimazole, and Cefixime. In the UK, E. coli isolates were resistant to Ampicillin, Cefotaxime, streptomycin, sulphonamide and Oxytetracycline and in South Africa, high resistance was observed in E. coli pathotypes isolated from harvested rainwater that was intended for domestic purposes. Antibiotic resistance is increasing worldwide as most microorganisms now exhibit resistance to a large number of antibiotics. The presence of antibiotic-resistant bacteria in water (environment and household) is a health hazard to combat because even if antibiotic consumption were reduced, the existing concentration of antibiotics and the resulting selective pressure on the bacterial communities could persist for an extended period. This study, therefore, recommends the continuous monitoring of antibiotic-resistant microorganisms in humans and water.

**Detection ESBLs gene in the study**

ESBL-positive E. coli strains are highly resistant to an array of antibiotics and infections caused by these strains are difficult to treat. In the present study, ESBL genes were found in a considerable proportion of the pathotypes screened. Isolates from water samples frequently expressed the ESBL genes (85%) compared to human samples (62.4%). The rate of expression of ESBL genes reported in clinical isolates in this study is low compared to the 85.24% reported in Egyptian children and 80% found in China. The reason might be the difference in geographical location. However, these results indicate the presence of these genes in the studied communities. The blaDHA and blaCTX-M genes were the most expressed genes, followed by blaSHV and blaCMY genes. The presence of the blaDHA gene was higher in human compared to water samples. Similar to the present study, Osinska et al. (2017) reported a high prevalence of blaCTX-M and lower prevalence of blaSHV in β-lactam resistant strains isolated from various sources. Also, 25% and 23% of blaCTX-M and blaSHV have been reported in Egyptian clinical isolates. ESBL genes are often plasmid encoded and can easily be transferred between bacteria. This possibility of transfer, therefore, highlights the need for better control mechanisms and antibiotic stewardship to prevent the spread of these resistant bacteria within communities, especially resource-poor settings like those included in the current study.

**Clonal relatedness between stool and water isolates**

ERIC-PCR technique was used to amplify the diverse regions of DNA extracted from the pathotypes identified from water and human stool samples in the present study. Studies have shown that a high degree of sequence similarities between isolates usually reflects descent from a common ancestor. The dendrogram (Fig. 3) showed 100% similarities between some isolates from water and human samples (Cluster I), and 100% similarities in some water isolates that belonged to different clusters. The overall remarkable similarities of 80-90% between the ERIC profile for the isolates strongly suggests that the domestic water played a significant role in the transmission of E. coli within the studied population. Thus, there is a need to develop strategies that will help to reduce pathogenic E. coli infections in humans, particularly from domestic waters within communities.

**CONCLUSION**

The DEC isolates screened in this study revealed the presence of E. coli pathotypes in children and household drinking water. The tested water samples could serve as a reservoir to not only DEC but also antibiotic resistant E. coli strains including MAR and ESBL-producing strains. These findings suggest that the containers used in storing the tested water samples might be reservoirs of these pathogens as water samples collected directly from the municipal tap did not harbour any pathogenic strain, and water from these storage containers should not be used without
appropriate treatment. In case of DEC infection due to the identified pathotypes within the studied communities, antibiotics such as imipenem and meropenem might be an effective treatment as DEC isolates in this study were 100% susceptible to these antibiotics. Gentamicin could also be used as a second option.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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