

Detection of virulence genes of diarrheagenic *Escherichia coli* strains from drinking water in Khartoum State

Ehssan H. Moglad, Omima Abdl El Jalil Adam, Maram M. Alnosh and Hisham N. Altayb

ABSTRACT

This study aimed to determine the prevalence of virulence genes in all the diarrheagenic *Escherichia coli* DEC strains (EAEC, EHEC, EIEC, EPEC, and ETEC) isolated from drinking water from Khartoum State, Sudan. A total of 46 drinking water samples obtained from different water sources were analyzed for the presence of *E. coli* as fecal contamination indicator and the antimicrobial-resistant pattern of isolated *E. coli* DEC strain was investigated. The bacterial genomic DNA was used as a template for multiplex polymerase chain reaction (MPCR) for the detection of the EHEC (*stx* gene), EIEC (*ipaH* gene), EPEC (*eae* gene), and EAEC (*aggR* gene) as virulence and biomarker genes. Our results showed that *ipaH* gene was found in 41.3% (19/46) of isolates, and *aggR* gene detected in 30.4% (14/46) of isolates. Both *aggR* and *ipaH* were found positive in 9 (19.5%) isolates and as well the combination of *aggR* and *stx* genes were detected in 2 (4.3%) isolates. In conclusion, this report confirmed the presence of DEC strains in drinking water from different resources and locations. Such findings require separate future clinical research studies to examine waterborne pathogens that exist in this state's water and find a management solution to stop or avoid potential outbreaks.

Key words | diarrheagenic, fecal coliform, Khartoum State, multiplex PCR, waterborne pathogen, water-related diseases

HIGHLIGHTS

- This study reported the presence of DEC strains in drinking water from different resources and locations from Khartoum State, Sudan.
- Such findings require separate future clinical research studies to examine waterborne pathogens that exist in this state's water.
- Find a management solution to stop or avoid potential outbreaks.
- Also, this study reported a high percentage of drug resistance between isolated *E. coli*.
- Water can also be considered as a source of transmission of drug-resistant bacteria.

INTRODUCTION

Diarrheal illnesses are a severe public health problem and a major cause of morbidity and mortality in infants and young

children, especially in developing countries (Jafari *et al.* 2012; Liu *et al.* 2012; Fakhr *et al.* 2016). To ensure good health, the drinking water supply must be clean and free of harmful turbidity, dissolved toxins, and waterborne pathogens such as *Giardia*, *Cryptosporidium*, *Campylobacter*, *Salmonella*, *Shigella*, *Vibrio*, *Hepatitis A*, and Norwalk viruses (Okeke

This is an Open Access article distributed under the terms of the Creative Commons Attribution Licence (CC BY 4.0), which permits copying, adaptation and redistribution, provided the original work is properly cited (<http://creativecommons.org/licenses/by/4.0/>).

doi: 10.2166/wh.2020.097

Ehssan H. Moglad (corresponding author)
Department of Pharmaceutics, College of Pharmacy,
Prince Sattam bin Abdulaziz University,
P.O. Box 173, Alkharj 11942,
Saudi Arabia
and
Department of Microbiology, Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), P.O. Box 2404, National Center for Research, Khartoum, Sudan
E-mail: ehssanhassn@gmail.com

Omima Abdl El Jalil Adam
Maram M. Alnosh
Department of Microbiology, College of Medical Laboratory Sciences,
Sudan University for Science and Technology, Khartoum, Sudan

Hisham N. Altayb
Biochemistry Department, Faculty of Sciences, King Abdulaziz University,
Jeddah, 21452,
Saudi Arabia

2009; Cowan & Herzog 2014). Water sources can be investigated for detection of fecal contamination; high fecal levels can mean that water contains pathogens by testing for the presence of *Escherichia coli* (Cowan & Herzog 2014).

E. coli is a Gram-negative rod, facultative anaerobe, belongs to the family Enterobacteriaceae, motile by peritrichous flagella, lactose fermenter, oxidase negative, catalase-positive and widely distributed in nature (Ryan *et al.* 2010). It is found as normal flora in the gastrointestinal tract of humans and animals. A high number of *E. coli* can be shed into the environment via the feces, but most strains are harmless. However, some *E. coli* strains can cause disease, therefore, if it is detected in water, immediate action should be taken to eradicate this contamination, because waterborne pathogens are presented into drinking water supplies via contamination with human or animal feces (WHO 2012). According to their virulence properties, symptoms of the disease that they cause, species and age group where they are found, *E. coli* is classified into: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC). The last has been linked to diarrheal disease in various parts of Africa (Okeke 2009; Croxen *et al.* 2013; Adugna *et al.* 2015). The most common etiologic agent of diarrhea from water is diarrheagenic *E. coli* (DEC) (Todar 2008; Fakhr *et al.* 2016), which can be transmitted by fecal-oral contamination (Gomes *et al.* 2016). DEC strains are differentiated using DNA probes and polymerase chain reactions (PCR) (Brooks *et al.* 2010; Baylis *et al.* 2011; Hegde *et al.* 2012).

Diarrheagenic *E. coli* is defined as some pathogenic *E. coli* strains that can cause diarrhea or extraintestinal diseases both in healthy and immunocompromised individuals (Gomes *et al.* 2016). Each strain possesses characteristic virulence factors, for instance, EPEC strain usually performs localized adherence to epithelial host cells through using *eaf* gene/protein. In most cases this is associated with attaching and effacing lesion formation on enterocytes which are linked to *eae* as virulence genes (Ogata *et al.* 2002). The O157: H7 serotypes are the important EHEC strains, and *stx* is the essential virulence factor for EHEC, which is also recognized as verocytotoxin (VT) and Shiga-toxin (*stx*) (Baylis *et al.* 2011). EIEC mainly causes colon infection and has outstanding ability to invade and damage the colonic tissue through the IPaA to

IPaH plasmid invasion antigens (Hill 2013). EAEC generates aggregate adherence mediated by either aggregate adherence fimbriae (AAF/I) or (AAF/II) encoded by *aggR* genes (O'Sullivan *et al.* 2006). Fecal contamination of drinking water is a major health problem that is responsible for many cases of diarrhea, especially in infants and people from foreign countries (Shanks *et al.* 2007; Fakhr *et al.* 2016). Moreover, the DEC infection is becoming more complicated because of increasing antibiotic resistance phenomena among bacterial isolates (Ozgunus *et al.* 2007).

While many Sudanese states and cities are experiencing much higher development rates and considerably steeper population growth, Khartoum is witnessing a very different form of urbanization. The state of Khartoum is a patchwork of wealth and poverty, demographic density and widespread and robust and atrophied infrastructure (Abdellah *et al.* 2014). This results in a large community characterized by crowding, poor sanitation, and inadequate water supply. Most of the drinking water comes from rivers after treatment by chlorination method, and from wells without treatment directly to the houses through the pipeline and is consumed in its natural form. In 2015, diarrheal and gastroenteritis were the fifth leading cause of hospital admission in Khartoum State (SASRs 2015). Other studies that investigated the viral and bacterial etiology and related clinical and epidemiological factors in children with acute diarrhea in Khartoum State reported that enteroinvasive *E. coli* (EIEC) was the predominantly found bacterial pathogen, followed by enterohemorrhagic *E. coli* (EHEC), and enteropathogenic *E. coli* (EPEC) (Adam *et al.* 2018). Therefore, the main aim of this study was to detect the presence and measure the prevalence of the virulence factors, *stx* (EHEC), *eae* (EPEC), *ipaH* (EIEC), and *aggR* (EAEC) of DEC strains in drinking water in Khartoum State. Also, the antimicrobial-resistant profile for these isolated DEC strains was evaluated.

METHODOLOGY

Description of study area and collection of water samples

This study, a descriptive cross-sectional study, was conducted to determine the presence and prevalence of the

virulence factors of DEC strains in drinking water in Khartoum State (Sudan) during the period from April to May 2016.

A total of 46 samples (small sample size due to financial constraints) were collected randomly from different provinces in the state (Bahri, Khartoum, and Omdurman). Samples were collected from different places in provinces (houses, dormitory, company, pharmacy, and cafeteria) and different types of drinking water source (tap water, cooler, and tanks) because these are the sources people regularly drink directly from. Fifty mL of water was collected in sterile screw cap bottles containing about 50 mL of lauryl tryptose broth from places suspected of having fecal contamination in different parts of Khartoum State. All collected water samples in this study come from river water after treatment with chlorine.

Total and thermotolerant coliforms

The cultures were used for the detection of coliform bacteria in water using the presence-absence coliform test (Clark 1980). The bottle content (50 mL) of sterile selective culture broth containing lactose and an indicator Laury tryptose (lactose) broth was added to an equal volume (50 mL) of the water sample with a Durham tube. After incubation (48 hours at 37 °C), the positive culture (lactose fermentation with acid and gas production) was cultured in two bottles of Brilliant Green Bile Broth (BGBB) media with a Durham tube. One bottle was incubated for 48 hours at 37 °C to detect the coliform and the other bottle at 44 °C for the detection of heat-tolerant coliforms (faecal coliforms) (Alraheem 2000). Then, the bacteria were purified and identified by Gram stain, culture on EMB media, and biochemical tests (Kligler's Iron Agar (KIA), indole, urease, and citrate utilization test).

Antimicrobial susceptibility test

Antibiotic susceptibility test was done for all isolated *E. coli* by Kirby-Bauer disk diffusion technique (Biemer 1973) for chloramphenicol 30 µg, ceftriaxone 30 µg, ciprofloxacin 5 µg, gentamicin 10 µg, and tetracycline 30 µg, as per the recommendation of Clinical and Laboratory Standards Institute (CLSI). Briefly, bacterial suspension was prepared

by using a sterile loop, four to five colonies of similar appearance were taken and suspended in 2 mL of sterile saline, and the saline tube was vortexed to create a smooth suspension. The suspension was compared with the turbidity standard of McFarland 0.5 to adjust the density of the test suspension. A sterile cotton swab was immersed into the inoculum tubes, and the swab was rotated against the side of the tubes using firm pressure to remove excess fluid. The swab was streaked all over the surface dried Mueller–Hinton plate three times. Finally, the swab was passed around the edge of the agar surface. Inoculums were left to dry for a few minutes at room temperature with the lid closed. Sterile forceps were used to place the anti-microbial disk uniformly and slightly pressured. The plates were then incubated at 35 °C overnight. After overnight inoculation, the zone size of inhibition was measured and recorded in mm by using a ruler. Zone size was interpreted according to CLSI guidelines (CLSI 2017). The results of the susceptibility test were reported as susceptible, intermediate, and resistant.

DNA extraction

The DNA was extracted from all isolated *E. coli* by boiling centrifugation method, as described by Al-Gallas *et al.* (2002). Briefly, several colonies of the isolated organism (*E. coli*) were subcultured in nutrient agar media, and after overnight incubation at 37 °C, one to three colonies were washed with 1 mL sterile normal saline (NS) in a sterile 1.5 mL Eppendorf tube. The tube was centrifuged at 10,000 r/min for 1–2 min. The supernatant was discarded. The pellet was re-suspended in 200 µL of distilled water. Then the tube was boiled at 100 °C for 15 min, followed by centrifugation of the lysate at 13,000 r/min for 3 min. A 150 µL sample of the supernatant was stored at –20 °C (in aliquot) as a template DNA stock and used as the target for PCR assays.

Measurement of DNA concentration

The concentration of extracted DNA was read using gel electrophoresis to show the presence and quality of DNA in the sample when compared with a DNA marker of known concentration.

Multiplex polymerase chain reaction (MPCR)

Multiplex PCR (Techne Tc.312 Thermal cycle, UK) was used for the detection of target genes, *ipaH* for EIEC, *aggR* for EAEC, *stx* for EHEC, and *eae* for EPEC, by using specific primer pairs as shown in Supplementary material, Table S1 (Ogata *et al.* 2002; Phantouamath *et al.* 2003; Saad *et al.* 2011).

Preparation of reaction mixture

The following reagents were used for each reaction in volumes of 25 μ L in 0.2 mL Eppendorf tubes as follows: 17 μ L distilled water, 5 μ L master mix (iNtRoN Biotechnology, Korea), 0.5 μ L forward primer (Macrogen Company, Korea), 0.5 μ L reverse primer (Macrogen Company, Korea), and 2.0 μ L DNA (Template DNA). The PCR mixture was subjected to an initial denaturation step at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 30 s, primer annealing at 55 °C for 30 s, extension at 72 °C for 30 s and the final extension at 72 °C for 5 min.

Visualization of PCR products

Agarose (2%) was used to make an agarose casting tray flooded by 10X TBE buffer near the gel cover surface, then 5 μ L of amplified PCR products of each sample was put into each well. To the first well of the casting tray 3 μ L of DNA ladder (100 bp) was injected for each run. The gel electrophoresis apparatus was connected to the power supply (primer, 125 V, 500 MA, UK). The electrophoresis was done at 100 V/cm for 30 min, after that, the gel was removed from the gel holder and visualized by a UV transilluminator (Uvite-UK), and the gel results were photographed using Polaroid film.

Statistical analysis

Statistical analysis for the prevalence of *E. coli* virulent genes in different water samples was performed using a Microsoft Excel spreadsheet and presented as a percentage or graph.

RESULTS

Distribution of isolated organisms

All water samples ($n = 46$) collected from Khartoum State were positive for the presence of coliform bacteria as well as fecal coliforms, thus, 46 *E. coli* bacteria were isolated from drinking water. The distribution of water samples is as follows: 44% (20/46) were from Khartoum province, 35% (16/46) from Omdurman, and 21% (10/46) from Bahri province. Most of the isolates were from tap water 40/46 (87%), 11% (5/46) from water coolers, and 2% (1/46) from tanks, and of these 78% (36/46) were from houses and the rest from markets 22% (10/46).

Antimicrobial susceptibility testing

From 46 isolated *E. coli*, 17% were resistant to chloramphenicol, 9% to ceftriaxone, 24% to ciprofloxacin, and 24% to gentamicin with a high resistance rate to tetracycline 98% (Table 1).

Genotyping of DEC virulent genes

Out of 46 isolates, 27 (58.6%) were positive for one or two of *ipaH*, *stx*, and *aggR* genes, while the *eae* gene was negative for all isolates. We found 19 (41.4%) isolates negative for all DEC virulent genes used in this study. *ipaH* gene was detected in 10 (12.7%), *aggR* gene detected in 3 (6.5%), and *stx* gene detected in 3 (6.5%). Both *aggR* and *stx* co-existed in 9 (19.5%) and both *aggR* and *ipaH* co-existed in 2 (4.3%) (see Supplementary material, Table S2).

Table 1 | The antimicrobial susceptibility test of isolated *E. coli*

Antibiotics	Sensitive number (%)	Resistance number (%)	Intermediate number (%)
Chloramphenicol	31 (68%)	8 (17%)	7 (15%)
Ceftriaxone	29 (63%)	4 (9%)	13 (28%)
Ciprofloxacin	24 (52%)	11 (24%)	11 (24%)
Gentamicin	19 (41%)	11 (24%)	16 (35%)
Tetracycline	1 (2%)	45 (98%)	0 (0.0%)

Prevalence of diarrheagenic *E. coli* virulence genes according to the source of water and the place can be seen in Figures 1 and 2.

DISCUSSION

In the present study, we found a high prevalence of EIEC (*ipaH* gene) in the drinking water of Khartoum State, mostly from tap water (32.6%) in houses (30.4%); however, this finding is in disagreement with a study in Japan that reported EPEC was the most common strain in tap water (Yatsuyanagi *et al.* 2003). Also, another study reported that the most common strain was EAEC in the tank water (Dobrowsky *et al.* 2014). This finding may indicate that the tap water was provided with inefficient chlorination. According to WHO guidelines, *E. coli* must not be

detectable in any 100 mL of drinking water sample (WHO 2011). A commonly used risk classification is based on the number of indicator organisms in a 100-mL sample, which includes: <1, 'very low risk'; 1–10, 'low risk'; 10–100, 'medium risk'; >100, 'high risk' or 'very high risk' (Bain *et al.* 2014). Also, this study found that the prevalence of DEC is higher in Khartoum province (43.5%) and lower in Bahri province (21.7%). This result may be due to the facts that Khartoum providence is bigger than Bahri province and consists of many wards, and Khartoum has an old sanitation system with multiple broken pipelines. There was no previous study from Sudan to compare the results with, so this is the first study in the country to report this result.

The antimicrobial susceptibility profile of *E. coli* revealed a high rate of antibiotic resistance to tetracycline (97.8%) and similar findings to this study have been reported in South Africa (Adefisoye & Okoh 2016). This resistance may be due to long-term usage of tetracycline (introduced in 1948). However, *E. coli* was highly susceptible to chloramphenicol (67.4%), ceftriaxone (63%), ciprofloxacin (52.2%), and gentamicin (41.3%). This finding was different from another study in Europe which reported that antimicrobial resistance to ciprofloxacin, gentamicin, and ceftriaxone is on the rise in European countries (Allocati *et al.* 2013). Another study reported that *E. coli* was resistant to all the antibiotics above (Adugna *et al.* 2015). In Khartoum State, some studies have investigated the sensitivity profile of *E. coli* as the causative agent for urinary tract infection, and reported that most *E. coli* were resistant to ciprofloxacin, gentamicin, tetracycline, chloramphenicol, and ceftriaxone (Hamdan *et al.* 2015; Saeed *et al.* 2017). This indicated the antibiotic resistance by *E. coli* strains that has increased in recent years could mainly be due to high consumption of antibiotics, irrational use, incomplete course of therapy, and use of treatment without prescription from physicians.

The prevalence of virulence factors (*ipaH*, *aggR*, *stx*, and *eae*) associated with EIEC, EAEC, EHEC, and EPEC, respectively, was tested using PCR. It was found that the most prevalent gene detected during this study was *ipaH* (EIEC) ($N = 10/46$) (12.7%), followed by *aggR* (EAEC) and *stx* (EHEC) ($N = 3/46$) (6.5%). This finding might explain the causative agent of acute diarrhea among children in Khartoum State, where EIEC was the predominantly

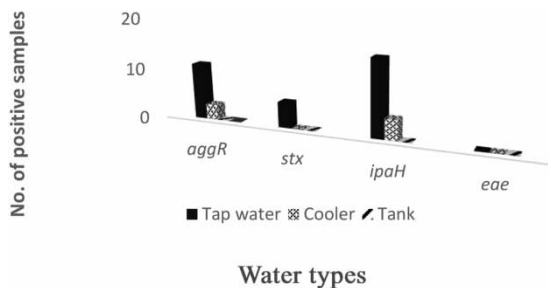


Figure 1 | The incidence of virulence genes from *E. coli* isolates according to the type of drinking water source.

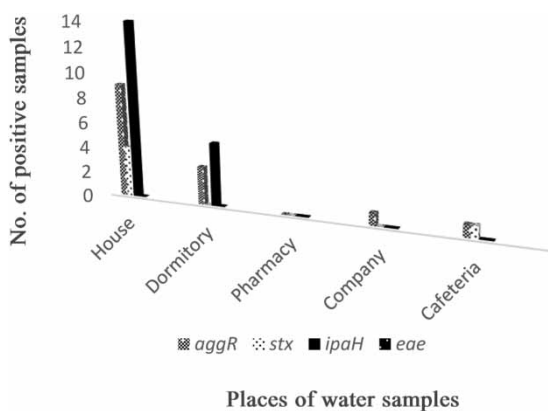


Figure 2 | The presence of virulence genes of *E. coli* isolates according to the place.

detected bacterial pathogen, followed by EHEC and EPEC (Adam *et al.* 2018). This finding is unlike a previous study which reported that the most common pathogen isolated was EPEC followed by EAEC (Canizalez-Roman *et al.* 2013), while others reported that EHEC and EPEC types were the most prevalent (Sidhu *et al.* 2013). Furthermore, Gutiérrez-Jiménez *et al.* (2014) reported that ETEC was the most common, indicating that the water purification system, hygiene practices, and source of water might differ from region to region and from country to country.

Moreover, the results showed a two-combination *E. coli* strain isolated from two samples: *aggR* (EAEC), *stx* (EHEC) ($N = 2/46$) (4.3%). This finding was entirely in agreement with a study reporting that 4% of isolates is observed to have a combination of genes from both EHEC and EAEC path types (Sidhu *et al.* 2013), and another study found one sample (0.5%) that contained *stx1* (EHEC) and *aggR* (EAEC) (Trung *et al.* 2016). This finding may reflect recent fresh fecal contamination of surface water with diarrheagenic *E. coli*.

Interestingly, this was the first study to report a combination between the strains of *E. coli* isolated from nine samples that were *aggR* (EAEC) and *ipaH* (EIEC) ($N = 9/46$) (19.5%), which was not found in any other previous studies.

The detection of the *eae* gene which encodes intimin which is essential for the intimate attachment of the EPEC to the enterocytes was not found in any drinking water sample. The EPEC pathotype can be classified into two groups, typical EPEC (tEPEC) and atypical EPEC (aEPEC). Humans are the only reservoir for tEPEC, which is transmitted by inter-human contact (Baylis *et al.* 2011). All previous studies that detected this gene were from food samples (Canizalez-Roman *et al.* 2013) and surface water (Sidhu *et al.* 2013). This negative result may be due to all the isolates were from drinking water which might not be contaminated by inter-human contact.

The prevalence of diarrheagenic *E. coli* strains in drinking water in the general population of African countries is reported being as follows: (48%) EAEC in Libya (Ali *et al.* 2012), (35.3%) EPEC in Egypt (Hassanain *et al.* 2015), and (34.7%) EPEC in Chad (Bessimbaye *et al.* 2013). These results differ from the present study findings. This might be due to using a different method for treating drinking water

and to the crucial regional difference in the prevalence of categories of DEC.

CONCLUSIONS

There is a high prevalence of EIEC (*ipaH* gene) in drinking water in Khartoum State, and there is a high percentage of DEC in tap water, and this may indicate that the contamination of drinking water came from the source. Also, there is a high percentage of drug resistance between isolated bacteria, thus, the water can also be a source of transmission of drug-resistant bacteria. We recommend that drinking water must be treated and periodically screened for the presence of bacterial contaminants and, also, houses should use filters in tap spouts.

ACKNOWLEDGEMENTS

The authors would like to thank the staff of Central Public Health Lab, Khartoum, and in particular, Mr NorAldain Mahmoud Ali Ahmed, who helped access facilities to complete this study. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. All the authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

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

REFERENCES

- Abdellah, A., Abdel-Magid, H., El-Tingari, M. & Adam, F. 2014 Assessment of drinking water quality in Grand Khartoum City, Khartoum State, Sudan. *Journal of Atoms and Molecules* 4, 645–655.
- Adam, M. A., Wang, J., Enan, K.-A., Shen, H., Wang, H., El Hussein, A. R., Musa, A. B., Khidir, I. M. & Ma, X. 2018 Molecular survey of viral and bacterial causes of childhood diarrhea in Khartoum State, Sudan. *Frontiers in Microbiology* 9, 112.

- Adefisoye, M. A. & Okoh, A. I. 2016 Identification and antimicrobial resistance prevalence of pathogenic *Escherichia coli* strains from treated wastewater effluents in Eastern Cape, South Africa. *Microbiologyopen* **5**, 143–151.
- Adugna, A., Kibret, M., Abera, B., Nibret, E. & Adal, M. 2015 Antibioqram of *E. coli* serotypes isolated from children aged under five with acute diarrhea in Bahir Dar town. *African Health Sciences* **15**, 656–664.
- Al-Gallas, N., Ben Aissa, R. R., Attia Annabi, T., Bahri, O. & Boudabous, A. 2002 Isolation and characterization of shiga toxin-producing *Escherichia coli* from meat and dairy products. *Food Microbiology* **19**, 389–398.
- Ali, M. M., Mohamed, Z. K., Klena, J. D., Ahmed, S. F., Moussa, T. A. & Ghenghesh, K. S. 2012 Molecular characterization of diarrheagenic *Escherichia coli* from Libya. *Am. J. Trop. Med. Hyg.* **86** (5), 866–871.
- Allocati, N., Masulli, M., Alexeyev, M. F. & Di Ilio, C. 2013 *Escherichia coli* in Europe: an overview. *International Journal of Environmental Research and Public Health* **10**, 6235–6254.
- Alraheem, M. 2000 Pollution in the water supply wells of Khartoum, Sudan. *Bulletin of Engineering Geology and the Environment* **58**, 7.
- Bain, R., Cronk, R., Wright, J., Yang, H., Slaymaker, T. & Bartram, J. 2014 Fecal contamination of drinking-water in low- and middle-income countries: a systematic review and meta-analysis. *PLOS Medicine* **11**, e1001644.
- Baylis, C., Uyttendaele, M., Joosten, H. & Davies, A. 2011 *The Enterobacteriaceae and Their Significance to the Food Industry*. International Live Science Institute European, Brussels, Belgium.
- Bessimbaye, N., Tidjani, A., Moussa, A. M., Brahim, B. O., Mbanga, D., Sangare, L., Barro, N. & Traore, A. S. 2013 Gastroenteritis with *Escherichia coli* in pediatric hospital in N'Djamena-Chad. *Journal of Applied Biology & Biotechnology* **1**, 4.
- Biemer, J. J. 1973 Antimicrobial susceptibility testing by the Kirby–Bauer disc diffusion method. *Ann. Clin. Lab. Sci.* **3** (2), 135–140.
- Brooks, G. F., Carrollk, G., Butel, J. S., Morse, S. A. & Mietzner, T. A. 2010 *Jawetz, Melnick and Adelberg's Medical Microbiology*, 25th edn. McGraw Hill, New York, USA.
- Canizalez-Roman, A., Gonzalez-Nunez, E., Vidal, J. E., Flores-Villasenor, H. & Leon-Sicairos, N. 2013 Prevalence and antibiotic resistance profiles of diarrheagenic *Escherichia coli* strains isolated from food items in northwestern Mexico. *International Journal of Food Microbiology* **164**, 36–45.
- Clark, J. A. 1980 The influence of increasing numbers of nonindicator organisms upon the detection of indicator organisms by the membrane filter and presence–absence tests. *Canadian Journal of Microbiology* **26**, 827–832.
- CLSI 2017 *Preference Standard for Antimicrobial Susceptibility Testing. CLSI Supplement M100*, 27th edn. Clinical and Laboratory Standards Institute, Wayne.
- Cowan, M. K. & Herzog, J. 2014 *Microbiology: A System Approach*, 4th edn. McGraw Hill, New York, USA.
- Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M. & Finlay, B. B. 2013 Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clinical Microbiology Reviews* **26**, 822–880.
- Dobrowsky, P. H., Van Deventer, A., De Kwaadsteniet, M., Ndlovu, T., Khan, S., Cloete, T. E. & Khan, W. 2014 Prevalence of virulence genes associated with pathogenic *Escherichia coli* strains isolated from domestically harvested rainwater during low- and high-rainfall periods. *Applied and Environmental Microbiology* **80**, 1633–1638.
- Fakhr, A. E., Gohar, M. K. & Atta, A. H. 2016 Impact of some ecological factors on fecal contamination of drinking water by diarrheagenic antibiotic-resistant *Escherichia coli* in Zagazig City, Egypt. *International Journal of Microbiology* **2016**, 1–9.
- Gomes, T. A., Elias, W. P., Scaletsky, I. C., Guth, B. E., Rodrigues, J. F., Piazza, R. M., Ferreira, L. C. & Martinez, M. B. 2016 Diarrheagenic *Escherichia coli*. *Brazilian Journal of Microbiology* **47** (Suppl. 1), 3–30.
- Gutiérrez-Jiménez, J., Cassasuce, F., Cruz, L., Aquino-López, J., Hernández-Shilón, J., Schlie, M. & Vidal, J. 2014 Evaluation of a point-of use water purification system (Llaveoz) in a rural setting of Chiapas, Mexico. *Journal of Microbiology and Experimentation* **1**, 00015.
- Hamdan, H. Z., Kubbara, E., Adam, A. M., Hassan, O. S., Suliman, S. O. & Adam, I. 2015 Urinary tract infections and antimicrobial sensitivity among diabetic patients at Khartoum, Sudan. *Annals of Clinical Microbiology and Antimicrobials* **14**, 26.
- Hassanain, N. A., Shaapan, R. M., Hassanain, M. A. & Khalil, F. A. M. 2015 Significant zoonotic enteropathogens associated with childhood diarrhea in Egypt. *Middle-East Journal of Scientific Research* **23**, 6.
- Hegde, A., Ballal, M. & Shenoy, S. 2012 Detection of diarrheagenic *Escherichia coli* by multiplex PCR. *Indian Journal of Medical Microbiology* **30**, 279–284.
- Hill, S. 2013 Virulence factors in fecal *Escherichia coli* from humans and animals. Thesis. University of Guelph, Environmental Sciences, Ontario, Canada.
- Jafari, A., Aslani, M. M. & Bouzari, S. 2012 *Escherichia coli*: a brief review of diarrheagenic pathotypes and their role in diarrheal diseases in Iran. *Iranian Journal of Microbiology* **4**, 102–117.
- Liu, L., Johnson, H. L., Cousens, S., Perin, J., Scott, S., Lawn, J. E., Rudan, I., Campbell, H., Cibulskis, R., Li, M., Mathers, C. & Black, R. E. 2012 Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet* **379**, 2151–2161.
- Ogata, K., Kato, R., Ito, K. & Yamada, S. 2002 Prevalence of *Escherichia coli* possessing the *eaeA* gene of enteropathogenic *E. coli* (EPEC) or the *aggR* gene of enteroaggregative *E. coli* (EAaggEC) in traveler's diarrhea diagnosed in those returning to Tama, Tokyo from other Asian countries. *Japanese Journal of Infectious Diseases* **55**, 14–18.

- Okeke, I. N. 2009 Diarrheagenic *Escherichia coli* in sub-Saharan Africa: status, uncertainties and necessities. *Journal of Infection in Developing Countries* **3**, 817–842.
- O’Sullivan, J., Bolton, D. J., Duffy, G., Baylis, C., Tozzoli, R., Wasterson, Y. & Lofdahl, S. 2006 *Methods for Detection and Molecular Characterization of Pathogenic Escherichia coli*. Stockholm.
- Ozgunus, O. B., Sevim, E., Alpay Karaoğlu, Ş., Sandalli, C. & Sevim, A. 2007 Molecular characterization of antibiotic resistant *Escherichia coli* strains isolated from tap and spring waters in a coastal region in Turkey. *Journal of Microbiology (Seoul, Korea)* **45**, 379–387.
- Phantouamath, B., Sithivong, N., Insisiengmay, S., Higa, N., Toma, C., Nakasone, N. & Iwanaga, M. 2003 The incidence of *Escherichia coli* having pathogenic genes for diarrhea: a study in the People’s Democratic Republic of Lao. *Japanese Journal of Infectious Diseases* **56**, 103–106.
- Ryan, K. J., Ray, C. G. & Ahmad, W. 2010 *Sheri’s Medical Microbiology*, 5th edn. McGraw Hill, New York, USA.
- Saad, M., Edris, A., Shaltout, F. & Shimaa, N. 2011 Isolation and identification of *Salmonella* and *E. coli* isolated from cut-up meat and chicken by using PCR. *BVMJ* **22**, 152–160.
- Saeed, A., Hamid, S. A., Bayoumi, M., Shanan, S., Alouffi, S., Alharbi, S. A., Alshammari, F. D. & Abd, H. 2017 Elevated antibiotic resistance of Sudanese urinary tract infection bacteria. *EXCLI Journal* **16**, 1073–1080.
- SASRS 2015 *Sudan Annual Statistical Report*. Republic Sudan Federal Ministry of Health, Sudan. <http://www.fmoh.gov.sd/statisticalReports>
- Shanks, O. C., Domingo, J. W. S., Lu, J., Kelty, C. A. & Graham, J. E. 2007 Identification of bacterial DNA markers for the detection of human fecal pollution in water. *Applied and Environmental Microbiology* **73**, 2416–2422.
- Sidhu, J. P. S., Ahmed, W., Hodgers, L. & Toze, S. 2013 Occurrence of virulence genes associated with Diarrheagenic pathotypes in *Escherichia coli* isolates from surface water. *Applied and Environmental Microbiology* **79**, 328–335.
- Todar, K. 2008 Structure and function of bacterial cell. In: *Todar’s Online Textbook of Bacteriology*. University of Wisconsin, Madison, WI, USA.
- Trung, N. V., Nhung, H. N., Carrique-Mas, J. J., Mai, H. H., Tuyen, H. T., Campbell, J., Nhung, N. T., Van Minh, P., Wagenaar, J. A., Mai, N. T. N., Hieu, T. Q., Schultsz, C. & Hoa, N. T. 2016 Colonization of Enteroaggregative *Escherichia coli* and Shiga toxin-producing *Escherichia coli* in chickens and humans in southern Vietnam. *BMC Microbiology* **16**, 208.
- WHO 2011 *Guidelines for Drinking-Water Quality*. World Health Organization, Geneva, Switzerland.
- WHO 2012 Acute diarrhea in adults and children: a global perspective. *World Gastroenterology Organization Global, Guidelines February 2012*. World Health Organization, Geneva, Switzerland.
- Yatsuyanagi, J., Saito, S., Miyajima, Y., Amano, K.-I. & Enomoto, K. 2003 Characterization of atypical enteropathogenic *Escherichia coli* strains harboring the astA gene that were associated with a waterborne outbreak of diarrhea in Japan. *Journal of Clinical Microbiology* **41**, 2033–2039.

First received 30 April 2020; accepted in revised form 2 June 2020. Available online 24 July 2020