Molecular detection of beta-lactamase *bla*$_{CTX-M}$ group 1 in *Escherichia coli* isolated from drinking water in Khartoum State

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ABSTRACT

This study aimed to detect the *bla*$_{CTX-M}$ group 1 in *Escherichia coli* (*E. coli*) isolated from drinking water in Khartoum State. Two hundred and eighty water samples were collected randomly from different areas, places, and sources from the state and examined for the presence of *E. coli* as a fecal contamination indicator. Isolation and identification of *E. coli* were performed using culture characteristics on different culture media and biochemical reactions. An antimicrobial sensitivity test was performed for all isolated *E. coli* using agar disk diffusion method. DNA was extracted by boiling method, and bacterial genomic DNA used as a template to detect *bla*$_{CTX-M}$ group 1 by PCR. Results showed 86 (30.7%) *E. coli* were isolated out of 280 water samples. Antimicrobial susceptibility testing revealed the highest resistant percentage was 59% for tetracycline, followed by 35% for gentamycin, while for chloramphenicol and cefotaxime was 22 and 20%, respectively. *bla*$_{CTX-M}$ group 1 was detected in about 40% of all isolates. This study concludes that drinking water in Khartoum State may be contaminated with feces and might be a possible source for transferring resistant bacteria. Thus, it may be one of the critical causes of increasing reports of antimicrobial resistance in Khartoum State.

Key words | beta-lactamases, *bla*$_{CTX-M-1}$ genes, drinking water, *Escherichia coli*, Khartoum

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HIGHLIGHTS

- This study reported a high prevalence of *E. coli* in drinking water from Khartoum State.
- CTX-M-1 genes group detected in a considerable number of isolated *E. coli*.
- Fecal organisms may pollute the drinking water of Khartoum State, which might cause outbreaks in the state.
- Drinking water could be a source for the transmission of drug-resistant *E. coli*.

INTRODUCTION

There is global concern about antibiotic resistance phenomena with serious significance on the treatment of infections. The increase in this problem results from widespread uses or misuses of antibiotics in human medicine as well as veterinary and agriculture spheres (WHO 2014). As a result, there is rapid growth in antibiotic resistance in bacteria that cause community infections and hospital-acquired infections (Peters *et al.* 2019). Previously, infection by *Escherichia coli* (*E. coli*) had an effective antibiotic therapy; there were no signs of *E. coli* on morbidity, mortality, and health

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care, but this condition has been altered due to accumulation of the occurrence of antimicrobial resistance by *E. coli* (Njage & Buys 2015). Although beta-lactamase has been commonly used in the treatment of *E. coli* infection in both human and animal medicine, extended-spectrum beta-lactamase (ESBLs) producing *E. coli* are increasing rapidly (Ali *et al.* 2018). ESBLs belong to class A beta-lactamases, and their genes include *TEM, SHV, CTX-M* (Hall & Barlow 2005). *TEM* and *SHV* genes were the most predominant type of ESBLs in 1990. Conversely, *CTX-M* increased recently and became the most dominant ESBL worldwide (Iovleva & Bonomo 2017). CTX-M-type has been increasingly documented in Gram-negative bacteria, predominantly in *E. coli* (Guiral *et al.* 2018). ESBL enzymes are categorized into five major classes according to the similarities between their protein sequences as follows: the CTX-M-1 cluster (CTX-M-1, -3, -10, -11, -12, -15, -28, and FEC-1), CTX-M-2 cluster (CTX-M-2, -4, -5, -6, -7, -20, and TOHO-1), CTX-M-8 cluster (CTX-M-8), CTX-M-25 cluster (CTXM-25 and -26), and CTX-M-9 cluster (CTX-M-9, -13, -14, -16, -17, -19, -21, -24, -27, and TOHO-2) (Peerayeh *et al.* 2013). The CTX-M-1 group is the most prevalent CTX-M type worldwide (Pavez *et al.* 2019). In Sudan recently, there have been increasing reports of CTX-M-15, in both hospital- and community-acquired infections (Altayb *et al.* 2018; Osman 2018; Malik & Elhag 2019).

Furthermore, the presence of *E. coli* in drinking water or the environment is an indicator for fecal contamination (WGO 2012). Antimicrobial-resistant bacteria can contaminate natural and drinking water sources by some human activities such as generating wastewater settlements, farms, and hospitals. Also, increased risk of groundwater contamination, as well as natural activities such as storm water runoff, may be washed into rivers or groundwater since the soil contains bacteria as a result of using cow dung extensively as fertilizer (Rashid *et al.* 2015). In addition, wastewater usage plant sites offer a promising situation for the multiplication of resistant bacteria (Rizzo *et al.* 2013). Thus, water does not only serve as a reservoir for the distribution of antibiotic-resistant microorganisms among human and animal populations but also is used as a system by which the resistance genes are presented into the natural bacteria ecosystem, spreading resistant genes to non-resistant bacteria (Rizzo *et al.* 2013).

Consequently, this will lead to a wide epidemic and endemic spread of multidrug-resistant bacteria (Coleman *et al.* 2012, 2013). Antimicrobial-resistant *E. coli* has been reported in several areas, including drinking water. Therefore, the ingestion of this polluted water can lead to the spread of resistant strains in humans (Coleman *et al.* 2012). In Sudan, attention has not been given to the potential role of drinking water in the dissemination of antibiotic-resistant bacteria in the population, although evidence shows that it may be an essential pathway for gene transfer to human pathogenic and commensal strains (Sanganyado & Gwenzi 2019). Moreover, drinking water in Khartoum State comes from groundwater that might be polluted from septic tanks and pit latrines, and surface water comes from municipal water systems which distribute water through old pipelines after being treated by chlorination (Moglad *et al.* 2020). This study highlights the possible role of drinking water in the transmission of antimicrobial-resistant bacteria to humans. Subsequently, this study aimed to detect the $bla_{CTX\text{-}M\text{-}1}$ group in *E. coli* bacteria isolated from drinking water in Khartoum State and determine their antimicrobial susceptibility pattern.

MATERIAL AND METHODS

Study design and sample collection

In this cross-sectional study, 280 water samples were collected randomly from three localities in Khartoum State (Khartoum 145, Khartoum North 70, and Omdurman 65), during the period September to November 2017.

Water samples were collected under aseptic conditions from various water sources (160 tap water and 120 house tanks). The tap spout was sterilized first by burning around the pipe using a cotton swab moistened with alcohol to avoid contamination from the environment. Then, the water was allowed to run down for 2 to 3 minutes, after which about 50 mL of water was collected into sterile screw cap bottles containing about 50 mL of lauryl tryptose broth media and an inverted Durham tube. Collected samples were transported immediately in plastic boxes with icepacks (Cheesbrough 2005).

Bacteriological analysis

The bottles containing water samples and lauryl tryptose broth were incubated at 37 °C for 48 hours aerobically. After incubation the bottle with gas production was subcultured into two bottles of Brilliant Green Bile Broth (BGBB) media (Himedia, India) with Durham tube; one bottle was incubated for 24 hours at 37 °C to detect the coliform and the other bottle was incubated at 44 °C to detect the presence of heat-tolerant coliform.

The presence of turbidity and gas in the Durham tubes was considered a positive test for coliforms. For isolation of coliforms, 10 μ from positive tubes was further cultured on Eosin Methylene Blue (EMB) agar (Himedia, India) and incubated at 37 °C for 24 hours aerobically. The appearance of green metallic sheen, Gram stain, and biochemical tests was used for confirmation of *E. coli* (Bartram & Ballance 1996; Bumadian *et al.* 2013).

Antimicrobial susceptibility profile

Antimicrobial sensitivity testing was conducted for all isolates from overnight culture, according to disk diffusion method of EUCAST (EUCAST 2015) using Mueller–Hinton (MH) agar and the following sets of antibiotic disks: cefotaxime (30 μg), gentamycin (10 μg), tetracycline (25 μg), and chloramphenicol (30 μg) (Himedia, India).

Results of susceptibility testing were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2016). *E. coli* ATCC 25922 was used as quality control strain.

DNA extraction

DNA was extracted by the boiling method (Al-Gallas *et al.* 2002). A pure colony of *E. coli* was cultured on nutrient agar and incubated overnight at 37 °C. Several colonies were picked up and suspended in 200 μL of sterile deionized water in a 1.5 mL Eppendorf tube. A bacterial suspension was made by using a vortex and boiled at 95 °C for 15 min. After centrifugation at 10,000 g for 5 min, the supernatant was transferred into a new tube for subsequent PCR analysis. The purity and integrity of extracted DNA were checked by 2% agarose gel electrophoresis.

Polymerase chain reaction (PCR)

Conventional PCR was performed for the detection of $bla_{\text{CTX-M-1}}$ group. Amplification was carried out in a thermocycler (Techne/TC-312, UK). PCR was carried out using group-specific primer; CTX-MA-1 5′-SCSATGTGCAGYACCAGTAA-3′ and CTX-MA-2 5′-CCGCRATATGRTTGGTGGTG-3′ (Saladin *et al.* 2002) (where S is G or C, Y is C or T, and R is A or G) (Macrogen, Korea). The following reaction mixtures were used in a volume of 25 μL; 5 μl Master mix (iNtRON Biotechnology, Seongnam, Korea), 0.6 μL of forward primer, 0.6 μL of reverse primer (10 pmol/μL), 2 μL DNA and 16.8 μL deionized sterile water. This mixture then underwent initial denaturation at 94 °C for 5 min, then proceeded to 30 cycles of denaturation at 94 °C for 45 seconds, primer annealed at 57 °C for 45 seconds, and elongation at 72 °C for 60 seconds. A final elongation step was accomplished at 72 °C for 5 minutes. Five microliters of the products were examined on a 2.0% agarose gel in TBE 1X containing 2.5 μL of (20 mg/mL) ethidium bromide, using the electrophoresis apparatus at 100 V for 40 min. Band size was visualized under UV transilluminator (UVitec, UK) and compared to 100 base pairs (bp) standard DNA ladder (Figure 1).

Statistics analysis

Data were analyzed by using the statistical package for the Social Sciences (SPSS) version 20. Chi-squared tests were performed to find the probable relationship between

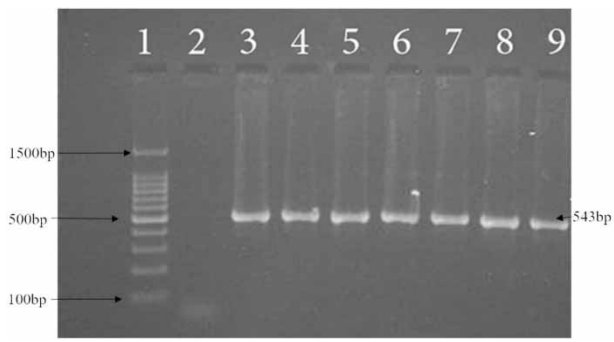

Figure 1 | PCR amplification of $bla_{\text{CTX-M-1}}$ group on 2% agarose gel electrophoresis. Lane 1, DNA ladder: MW 100–1,500 bp fragments. Lane 2, negative control (DW). Lanes 3 to 9 are positive (543 bp) samples.

variables and p-value <0.05 was considered statistically significant.

RESULTS

E. coli isolation

In the present study, a total of 280 water samples were collected from Khartoum State (145 from Khartoum, 70 from Khartoum North, and 65 from Omdurman).

From 280 water samples, 86 (30.7%) *E. coli* were isolated and are distributed as follows: Khartoum locality 29% (42/145), Khartoum North locality 29% (20/70), and Omdurman locality 37% (24/65).

From 280 water samples, 160 were from tap water and 120 were from tank water; 29% (47/160) and 33% (39/120) of tap and tank water were positive for *E. coli*, respectively. Out of 86 isolates, 55% (47/86) was from tap water and 45% (39/86) from water tanks. The distribution according to the area was as follows: 49% (42/86) were from Khartoum, 28% (24/86) were from Omdurman, and 23% (20/86) were from Khartoum North.

Antimicrobial susceptibility

All *E. coli* isolates (86) were subjected to antimicrobial susceptibility testing by the disk diffusion technique. The results showed that 20% (17/86) of all isolates were resistant to cefotaxime, 35% (30/86) were resistant to gentamicin, 59% (51/86) were resistant to tetracycline, and 22% (19/86) were resistant to chloramphenicol (Table 1).

Detection of the $bla_{\text{CTX-M-1}}$ group by PCR

Out of 86 *E. coli* isolates subjected to PCR for the detection of $bla_{\text{CTX-M-1}}$ group, 40% (34/86) were positive. Among them, 31% (13/42) were from Khartoum, 55% (11/20) were from Khartoum North, and 42% (10/24) were from Omdurman. Eighteen were from tap water and 16 were from tanks. From 34 $bla_{\text{CTX-M-1}}$ positive isolates, 38% (18/47) were from tap water and 41% (16/39) were from tank water (Table 2).

There was no statistically significant association between the presence of $bla_{\text{CTX-M-1}}$ group and different variables (Khartoum State provinces, sources of water samples, and antimicrobials susceptibility) (p-value >0.05).

DISCUSSION

According to the recommendations by the World Health Organization (WHO 2012), the presence of *E. coli* in drinking water was used as an indicator for testing water quality and fecal contamination. In this study, 30.7% (86/280) isolates of *E. coli* were detected in Khartoum State drinking

Table 2 | Distribution of $bla_{\text{CTX-M-1}}$ group according to the area of water samples

	Provinces			Water source	
$bla_{\text{CTX-M-1}}$ group	Khartoum	Omdurman	Khartoum North	Tap water	Tanks
Positive ($n=34$)	13	10	11	18	16
Negative ($n=52$)	29	14	9	29	23
Total ($n=86$)	42	24	20	47	39
p-value	0.188			0.485	

Table 1 | Association of $bla_{\text{CTX-M-1}}$ group and antimicrobial susceptibility testing

	Cefotaxime		Gentamicin		Tetracycline		Chloramphenicol	
CTX-M-1 gene	S	R	S	R	S	R	S	R
Positive ($n=34$)	26	8	20	14	12	22	28	6
Negative ($n=52$)	43	9	36	16	23	29	39	13
Total ($n=86$)	69	17	56	30	35	51	67	19
p-value	0.3		0.08		0.4		0.4	

S = sensitive, R = resistant.

water; this might be due to the fecal contamination of the state's drinking water (Alraheem 2000), and the absence or inefficiency of the pre-distribution of water treatment (Moglad *et al.* 2020).

In the present study, we detected a high prevalence of $bla_{\text{CTX-M-1}}$ group in *E. coli* isolated from drinking water (40%). This result is less than an earlier study which isolated *E. coli* from water environments in northern Thailand, which stated that the most common extended-spectrum β-lactamase-encoding gene was $bla_{CTX\text{-}M}$ group 1 (75%) followed by $bla_{CTX\text{-}M}$ group 9 (13.2%) (Assawatheptawee *et al.* 2017). Furthermore, this finding is more than a previous report in northern Tanzania; there it was found that the *CTX-M* gene was present in 17.7% of *E. coli* isolated from drinking water (Lyimo *et al.* 2016). Furthermore, in China, a study conducted by Gao *et al.* (2014) reported 14.8% of ESBL-producing *E. coli* from downstream water. In South Africa, Muringani *et al.* (2016) found that 28% (18/65) of water samples were positive for *CTX-M*. The high prevalence of $bla_{\text{CTX-M-1}}$ carrier *E. coli* in Khartoum State drinking water could be one of the main reasons for the dissemination of *CTX-M* positive clinical isolates of *E. coli* in Khartoum State (Altayb *et al.* 2018; Osman 2018; Malik & Elhag 2019).

Although cefotaxime is one of the cephalosporins which can hydrolyze by *CTX-M* gene, this study showed that 77% (26/34) of cefotaxime sensitive isolates were positive for *CTX-M* gene. This finding may be due to a lack of or a low level of expression of *CTX-M* gene (Cantón *et al.* 2012; Kjeldsen *et al.* 2015). This finding agrees with Muringani *et al.* (2016), who found that from 65 water samples, 18 were positive for *CTX-M*, and all samples were sensitive to cefotaxime.

Moreover, the present study showed that there was a high resistance rate (59%) to tetracycline compared with the other antimicrobials used in this study. This result entirely disagrees with Adesoji & Ogunjobi (2016), who reported that the resistance to tetracycline was 29% of Gram-negative bacteria isolated from drinking water in Nigeria, and other studies reported lower resistant rates of tetracycline for *E. coli* isolated from surface waters. For instance, Nontongana *et al.* (2014) reported 13% of tetracycline resistance for *E. coli* recovered from the Kat River in South Africa. Rashid *et al.* (2015) found that all of the *E. coli* isolates from water were resistant to at least one or more classes of antimicrobials, and the most common resistant phenotypes were to ampicillin and tetracycline. This difference in resistance may be due to differences in the study area and sample size.

There are some recommendations drawn from this study's findings. (1) Appropriate measures are urgently needed to be enforced in order to decrease the burden of antibiotic resistance in the environment, such as judicious use of antibiotics in human and veterinary medicine as well as in agriculture. (2) Improvement of water status is of primary concern, such as more stringent chlorine disinfection needs to be taken into consideration to prevent resistant bacteria from being released into the aquatic environment. (3) Following the spread of antibiotic-resistant bacteria in human drinking water, such as taps and well water, policymakers should develop management strategies for the prevention of such cases.

The limited class of antimicrobial agents, as well as the limited number of resistant genes used in this study, were considered as a limitation of this study.

CONCLUSIONS

This is a first study to report the presence of the antimicrobial resistance gene in *E. coli* bacteria isolated from drinking water in Khartoum State. *E. coli* was present in 30.7% (86/280) of Khartoum State water samples, and 40% (34/86) of these isolates were carriers of $bla_{\text{CTX-M-1}}$. Health workers need to be aware of the spread of antibiotic-resistant bacteria through drinking water. They should improve the process of water filtration. Furthermore, drinking water in Khartoum State may be contaminated with feces and might be a source of transmission of antimicrobial-resistant genes.

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DATA AVAILABILITY STATEMENT

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

REFERENCES

Adesoji, A. T. & Ogunjobi, A. A. 2016 Detection of extended spectrum beta-lactamases resistance genes among bacteria isolated from selected drinking water distribution channels in southwestern Nigeria. *BioMed Research International* **2016**, 7149295.

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, T., Ali, I., Khan, N. A., Han, B. & Gao, J. 2018 The growing genetic and functional diversity of extended spectrum beta-lactamases. *BioMed Research International* **2018**, 1–14.

Alraheem, M. A. A. A. 2000 Pollution in the water supply wells of Khartoum, Sudan. *Bulletin of Engineering Geology and the Environment* **58**, 257–264.

Altayb, H. N., Sissig, M. A., El Amin, N. M., Maowia, A. I. H. & Mukhtar, M. 2018 Molecular characterization of CTX-M ESBLs among pathogenic Enterobacteriaceae isolated from different regions in Sudan. *Global Advanced Research Journal of Microbiology (GARJM)* **7**, 040–047.

Assawatheptawee, K., Tansawai, N., Kiddee, A., Thongngen, P., Punyadi, P., Romgaew, T., Kongthai, P., Sumpradit, T. & Niumsup, P. R. 2017 Occurrence of extended-spectrum and AmpC-type β-lactamase genes in *Escherichia coli* isolated from water environments in northern Thailand. *Microbes and Environments* **32**, 293–296.

Bartram, J. & Ballance, R. 1996 *Water Quality Monitoring: A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes*. CRC Press, Boca Raton, FL, USA.

Bumadian, M. M., Almansury, H. H., Bozakouk, I. H., Lawgali, Y. F. & Bleiblo, F. A. 2013 Detection and enumeration of coliform bacteria in drinking water at hospital of Benghazi/ Libya. *Journal of Experimental Biology* **1**, 6.

Cantón, R., Gonazález-Alba, J. M. & Galán, J. C. 2012 CTX-M enzymes: origin and diffusion. *Frontiers in Microbiology* **3**, 110.

Cheesbrough, M. 2005 *District Laboratory in Tropical Countries, Part 1*. 2nd edn. Cambridge University Press, Cambridge. http://dx.doi.org/10.1017/CBO9780511581304.

CLSI 2016 *Performance Standards for Antimicrobial Susceptibility Testing*. Clinical and Laboratory Standards Institute, Wayne.

Coleman, B. L., Salvadori, M. I., McGeer, A. J., Sibley, K. A., Neumann, N. F., Bondy, S. J., Gutmanis, I. A., McEwen, S. A., Lavoie, M., Strong, D., Johnson, I., Jamieson, F. B. & Louie, M. 2012 The role of drinking water in the transmission of antimicrobial-resistant E. coli. *Epidemiology and Infection* **140**, 633–642.

Coleman, B. L., Louie, M., Salvadori, M. I., McEwen, S. A., Neumann, N., Sibley, K., Irwin, R. J., Jamieson, F. B., Daignault, D., Majury, A., Braithwaite, S., Crago, B. & McGeer, A. J. 2013 Contamination of Canadian private drinking water sources with antimicrobial resistant Escherichia coli. *Water Research* **47**, 3026–3036.

EUCAST 2015 *EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing, Version 5.0*. European Committee on Antimicrobial Susceptibility Testing, Sweden.

Gao, L., Hu, J., Zhang, X., Ma, R., Gao, J., Li, S., Zhao, M., Miao, Z. & Chai, T. 2014 Dissemination of ESBL-producing escherichia coli of chicken origin to the nearby river water. *Journal of Molecular Microbiology and Biotechnology* **24**, 279–285.

Guiral, E., Pons, M. J., Vubil, D., Marí-Almirall, M., Sigaúque, B., Soto, S. M., Alonso, P. L., Ruiz, J., Vila, J. & Mandomando, I. 2018 Epidemiology and molecular characterization of multidrug-resistant Escherichia coli isolates harboring blaCTX-M group 1 extended-spectrum β-lactamases causing bacteremia and urinary tract infection in Manhiça, Mozambique. *Infection and Drug Resistance* **11**, 927.

Hall, B. G. & Barlow, M. 2005 Revised Ambler classification of β-lactamases. *Journal of Antimicrobial Chemotherapy* **55**, 1050–1051.

Iovleva, A. & Bonomo, R. A. 2017 The ecology of extended-spectrum β-lactamases (ESBLs) in the developed world. *Journal of Travel Medicine* **24**, S44–S51.

Kjeldsen, T. S., Overgaard, M., Nielsen, S. S., Bortolaia, V., Jelsbak, L., Sommer, M., Guardabassi, L. & Olsen, J. E. 2015 CTX-M-1 β-lactamase expression in Escherichia coli is dependent on cefotaxime concentration, growth phase and gene location. *Journal of Antimicrobial Chemotherapy* **70**, 62–70.

Lyimo, B., Buza, J., Subbiah, M., Smith, W. & Call, D. R. 2016 Comparison of antibiotic resistant Escherichia coli obtained from drinking water sources in northern Tanzania: a cross-sectional study. *BMC Microbiology* **16**, 254.

Malik, I. & Elhag, K. 2019 Characterisation of extended-spectrum β-lactamases among multidrug resistant Enterobacteriaceae from Sudan. *Journal of Pure and Applied Microbiology* **13**, 61–68.

Moglad, E. H., Jalil Adam, O. A. E., Alnosh, M. M. & Altayb, H. N. 2020 Detection of virulence genes of diarrheagenic Escherichia coli strains from drinking water in Khartoum State. *Journal of Water and Health*. https://doi.org/10.2166/wh.2020.097.

Muringani, B., A, T. & Nziramasanga, P. 2016 β-lactamase genes produced by E. coli isolated from water and stool samples in Mthata Region Eastern Cape Province of South Africa. *EC Microbiology* **3**, 548–552.

Njage, P. M. K. & Buys, E. M. 2015 Pathogenic and commensal Escherichia coli from irrigation water show potential in transmission of extended spectrum and AmpC β-lactamases determinants to isolates from lettuce. *Microbial Biotechnology* **8**, 462–473.

Nontongana, N., Sibanda, T., Ngwenya, E. & Okoh, A. I. 2014 Prevalence and antibiogram profiling of Escherichia coli pathotypes isolated from the Kat River and the Fort Beaufort abstraction water. *International Journal of Environmental Research and Public Health* **11**, 8213–8227.

Osman, H. S. A. 2018 *Evaluation of Multi-Drug Resistant Echerichia Coli Isolated From Patients with Urinary Tract Infection and Phylogenetic Analysis of CTX-M Gene, In Om-Algora Locality, Gezira State, Sudan (2017)*. *M.Sc dissertation*, University of Gezira, Sudan.

Pavez, M., Troncoso, C., Osses, I., Salazar, R., Illesca, V., Reydet, P., Rodríguez, C., Chahin, C., Concha, C. & Barrientos, L. 2019 High prevalence of CTX-M-1 group in ESBL-producing enterobacteriaceae infection in intensive care units in southern Chile. *The Brazilian Journal of Infectious Diseases* **23**, 102–110.

Peerayeh, S. N., Eslami, M., Memariani, M. & Siadat, S. D. 2013 High prevalence of blaCTX-M-1 group extended-spectrum β-lactamase genes in Escherichia coli isolates from Tehran. *Jundishapur Journal of Microbiology* **6** (7), e6863. doi:10.5812/jjm.6863.

Peters, L., Olson, L., Khu, D. T., Linnros, S., Le, N. K., Hanberger, H., Hoang, N. T., Tran, D. M. & Larsson, M. 2019 Multiple antibiotic resistance as a risk factor for mortality and prolonged hospital stay: a cohort study among neonatal intensive care patients with hospital-acquired infections caused by gram-negative bacteria in Vietnam. *PloS One* **14** (5), e0215666. doi:10.1371/journal.pone.0215666.

Rashid, M., Rakib, M. M. & Hasan, B. 2015 Antimicrobial-resistant and ESBL-producing Escherichia coli in different ecological niches in Bangladesh. *Infection Ecology & Epidemiology* **5**, 26712.

Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M. C., Michael, I. & Fatta-Kassinos, D. 2013 Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Science of the Total Environment* **447**, 345–360.

Saladin, M., Cao, V. T. B., Lambert, T., Donay, J.-L., Herrmann, J.-L., Ould-Hocine, Z., Verdet, C., Delisle, F., Philippon, A. & Arlet, G. 2002 Diversity of CTX-M β-lactamases and their promoter regions from Enterobacteriaceae isolated in three Parisian hospitals. *FEMS Microbiology Letters* **209**, 161–168.

Sanganyado, E. & Gwenzi, W. 2019 Antibiotic resistance in drinking water systems: occurrence, removal, and human health risks. *Science of the Total Environment* **669**, 785–797.

WGO 2012 *Acute Diarrhea in Adults and Children: A Global Perspective*. World Gastroenterology Organisation Global Guidelines, February 2012, Milwaukee, WI, USA.

WHO 2012 *Acute diarrhea in adults and children: a global perspective. World Gastroenterology Organization Global, Guidelines February 2012*. World Health Organization, Geneva, Switzerland.

WHO 2014 *Antimicrobial Resistance: Global Report on Surveillance*. World Health Organization, Geneva, Switzerland.

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