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

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**ABSTRACT**

This study aimed to detect the $bla_{\text{CTX-M}}$ 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. Antimicrobial sensitivity testing 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 $bla_{\text{CTX-M}}$ 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. $bla_{\text{CTX-M}}$ 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, $bla_{\text{CTX-M-1}}$ genes, drinking water, *Escherichia coli*, Khartoum

**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<sub>CTX-M-1</sub> 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 μL 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. 2015).

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 blaCTX-M group. Amplification was carried out in a thermocycler (Techne/TC-312, UK). PCR was carried out using group-specific primer; CTX-MA-1 5’-SCSAGTGCAYACCAG-TAA-3’ and CTX-MA-2 5’-CCGRATATGRTTGTTGGTG-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
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<sub>CTX-M-1</sub> group by PCR

Out of 86 E. coli isolates subjected to PCR for the detection of bla<sub>CTX-M-1</sub> 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<sub>CTX-M-1</sub> 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<sub>CTX-M-1</sub> 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 water.

Table 1 | Association of bla<sub>CTX-M-1</sub> group and antimicrobial susceptibility testing

| CTX-M-1 gene   | Cefotaxime | Gentamicin | Tetracycline | Chloramphenicol |
|----------------|------------|------------|--------------|-----------------|
|                | 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.

Table 2 | Distribution of bla<sub>CTX-M-1</sub> group according to the area of water samples

| Provinces | Water source |
|-----------|--------------|
|           | Tap water | Tanks |
| Khartoum | 13 | 18 |
| Omdurman | 10 | 16 |
| Khartoum North | 11 | 23 |

p-value 0.188 0.485
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 \( \text{bla}_{\text{CTX-M-1}} \) 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 \( \beta \)-lactamase-encoding gene was \( \text{bla}_{\text{CTX-M}} \) group 1 (75%) followed by \( \text{bla}_{\text{CTX-M}} \) 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 \( \text{CTX-M} \) 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 \( \text{CTX-M} \). The high prevalence of \( \text{bla}_{\text{CTX-M-1}} \) carrier \( E. coli \) in Khartoum State drinking water could be one of the main reasons for the dissemination of \( \text{CTX-M} \) positive clinical isolates of \( E. coli \) in Khartoum State (Altaby et al. 2018; Osman 2018; Malik & Elhag 2019).

Although cefotaxime is one of the cephalosporins which can hydrolyze by \( \text{CTX-M} \) gene, this study showed that 77% (26/34) of cefotaxime sensitive isolates were positive for \( \text{CTX-M} \) gene. This finding may be due to a lack of or a low level of expression of \( \text{CTX-M} \) 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 \( \text{CTX-M} \), 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 \( \text{bla}_{\text{CTX-M-1}} \). 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.

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