Antibiotic resistance of E. coli isolates from different water sources in Mbarara, Uganda

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Antibiotic resistance of *E. coli* isolates from different water sources in Mbarara, Uganda

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ABSTRACT

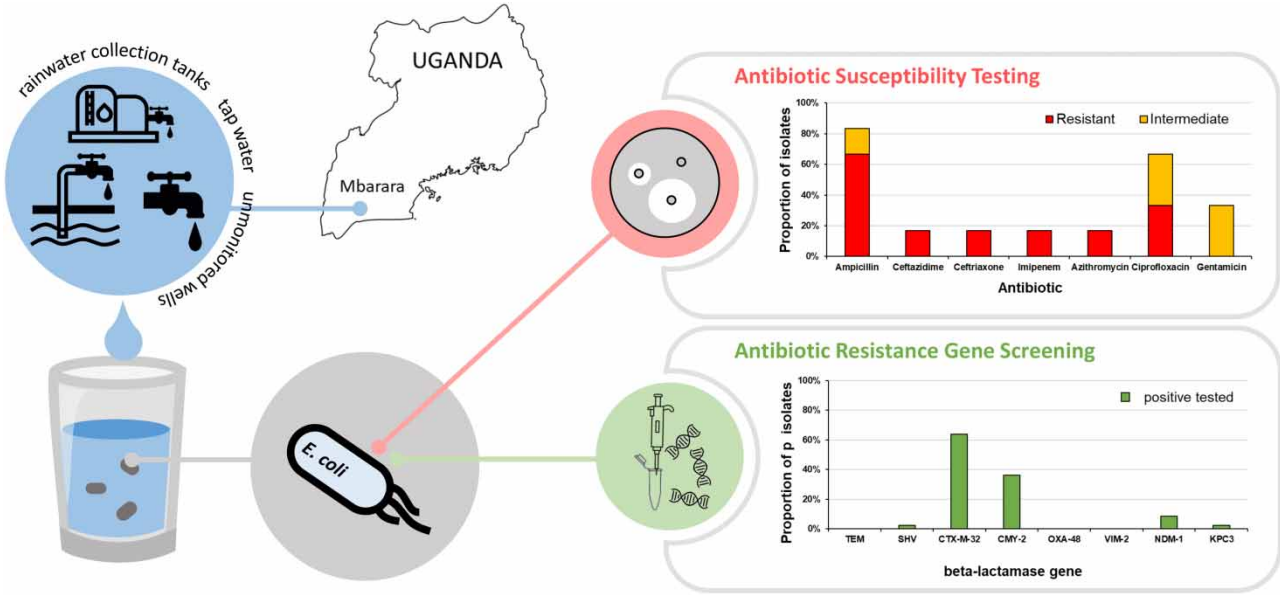
Escherichia coli is widely used as an indicator of recent faecal pollution of water. Most *E. coli* strains are commensals; however, isolates in water samples have been shown to carry antibiotic resistance determinants. In total, 47 *E. coli* were isolated from selected drinking water sources in Mbarara, Uganda. The isolates were examined for their susceptibility to seven antibiotics and the presence of nine antibiotic-resistance genes (mostly β-lactamase genes) and class 1 integrons. Isolates showed a high resistance to ampicillin of 55.5% and a high sensitivity to azithromycin and gentamicin at 98 and 96%, respectively. PCR analysis showed the presence of extended-spectrum β-lactamase genes $bla_{\text{CTX-M-32}}$ and $bla_{\text{CMY-2}}$ in 64 and 36% of the isolates. The carbapenemase genes $bla_{\text{OXA-48}}$, $bla_{\text{VIM-2}}$, $bla_{\text{NDM-1}}$, and $bla_{\text{KPC-3}}$ were either not detected or only in a very small number of the isolates, whereas class 1 integrons were present in 68% of the isolates. This study proves that antimicrobial resistance exists in *E. coli* in water used for drinking purposes in Mbarara city. There is a need for public health actors to improve the surveillance of microbiological quality of drinking water to minimize health risks.

Key words: antibiotic resistance, antibiotic resistance genes, *E. coli*, extended β-lactamase genes, multi-drug resistance, water

HIGHLIGHTS

- High rate of ampicillin resistance in *E. coli* isolated from different drinking water sources.
- 75% of isolates carried extended-spectrum β-lactamase genes.
- Isolates showed no resistance against last-resort antibiotic imipenem.
- Carbapenemase genes were detected rarely.

GRAPHICAL ABSTRACT



1. INTRODUCTION

In Uganda, 57% of the urban population has access to safe drinking water, according to the Ministry of Water and Environment of the Republic of Uganda (Ministry of Water and Environment (MWE) Uganda 2020). However, in addition to safely managed water services, other sources of water are used for consumption. Human and animal faeces are a major source of water pollution, especially in low-income countries. Drinking this contaminated water increases the risk of exposure to bacterial, viral, and protozoal pathogens that can cause serious human illness (World Health Organization 2014b).

In particular, the emergence and spread of antimicrobial-resistant (AMR) bacteria is considered a major threat to human health (World Health Organization 2014a). AMR reduces the effectiveness of antibiotic treatment, leading to increased morbidity, mortality, and associated healthcare costs (World Health Organization 2014a). Antibiotic-resistant bacteria (ARB) in contaminated water sources are thought to contribute to the increased prevalence of these bacteria in low-income countries (Okeke *et al.* 1999; Roberts *et al.* 2009; Dekker *et al.* 2015). Therefore, the occurrence of ARB and antibiotic-resistant genes (ARGs) in the aquatic environment is of increasing concern (Diwan *et al.* 2010; Stange *et al.* 2016; Stange *et al.* 2019). In particular, the ARBs that are ranked as priority 1: critical on the World Health Organization (WHO) list of ARB must be taken into account. These include carbapenemase-producing *Acinetobacter baumannii* and *Pseudomonas aeruginosa,* as well as carbapenemase and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae (Tacconelli *et al.* 2018). ESBL confers resistance to all penicillins and 1st to 4th generation cephalosporins. The carbapenemases are β-lactamases that can also hydrolyze carbapenems, a group of last-resort antibiotics used to treat severe, multidrug-resistant infections.

Escherichia coli is a bacterial species of the family Enterobacteriaceae and is commonly found in the intestines of humans and warm-blooded animals (Tenaillon *et al.* 2010). Most strains of *E. coli* are harmless, but some strains have acquired pathogenic potentials that allow them to cause diarrhoea and other systemic diseases that affect the urinary tract, brain, and blood (Youmans *et al.* 2015). In addition, *E. coli* has a high capacity to accumulate and transmit resistance genes via horizontal gene transfer (Maal-Bared *et al.* 2013; Middleton & Salierno 2013). It is used as a common indicator of faecal contamination of water (Edberg *et al.* 2000), and its isolation from a water source indicates that the quality and safety of that water source are compromised and therefore unsafe for human consumption (Khan & Gupta 2020). However, the use of this indicator has been questioned due to the potential for *E. coli* to become naturalized in the environment (Walk *et al.* 2007; Ishii & Sadowsky 2008; Jang *et al.* 2017). Nevertheless, *E. coli* is still considered to be the best faecal indicator bacterium (Edberg *et al.* 2000) and is still recommended by the WHO to assess water contamination and the associated risk of diarrheal diseases (WHO 2017).

Data on the incidence of AMR in Africa are scarce compared to Europe, America, and China. For Uganda, there are some studies on AMR infectious agents in clinical settings in urban areas (Okoche *et al.* 2015; Najjuka *et al.* 2020; Wekesa *et al.* 2020; Johnson *et al.* 2021), but the occurrence of resistance in rural areas and especially in the aquatic environment is poorly studied. In this study, we investigated the prevalence of antibiotic-resistant *E. coli* from different water sources in Mbarara, Uganda. We screened the *E. coli* isolates for the presence of selected ARGs. In the long term, knowledge of water contamination levels will help to develop measures to reduce the risk of waterborne disease transmission and the spread of AMR.

2. MATERIALS AND METHODS

2.1. Study site

This study was conducted in Mbarara city. Mbarara city is the commercial and administrative capital of Mbarara district in southwestern Uganda. It is located 270 km southwest of the capital city, Kampala. Mbarara city covers an area of 1,778.4 km^2. It has a population of 91,867 (Uganda Bureau of Statistics 2022) and is divided into six divisions. The city receives an average annual rainfall of 1,200 mm with two rainy seasons during the months of September–December and February–May. Temperature ranges between 17 and 30 °C, humidity of 80–90%. The topography is a mixture of fairly rolling and sharp hills and mountains, shallow valleys and flat land. Mbarara city is provided, operated and maintained with safe water supply technologies and sanitation facilities for all communities of the district. Mbarara district recorded an increase in access to safe and clean water from 45% in 2000 to about 63% in the villages and 65% for the municipality in 2007. The safe water coverage is 65.9% in rural areas and 95.7% in urban, while accessibility to safe water lies between 29 and 95% (Mbarara District Local Government 2023).

2.2. Study design and sample collection

This was a quantitative cross-sectional study on selected drinking water sources in Mbarara city. Mbarara city has a total of 23 wards spread across six divisions and constituencies (Figure 1). Judgmental sampling was employed (Sarker & Al-Muaalemi - 2022). Administrative clearance was obtained from the district, city, parish, National Water and Sewerage Cooperation, and the Ministry of Water, Lands, and Environment authorities. The protocol was reviewed and approved by the Mbarara University of Science and Technology Institutional Review Committee (MUST-2021-39) and the National Council of Science and Technology (HS1469ES). Permission was obtained from the district, local council leaders, and household heads, especially for water harvest tanks, before the commencement of data collection. Three divisions of Kakoba, Kakiika, and Nyakayojo were randomly selected. A ward was randomly selected from each of the three selected divisions. From each of the selected parishes (Nyarubanga, Rubiri, Lugazi, Kaburangiire, Katebe, and Katukuru), a village was selected. In total, six villages were selected and surveyed to identify the water sources. The selected communities were mapped, and all the drinking water sources used by them were listed. From each of the listed water sources, approximately 50% were sampled in selected wards and divisions between May and June 2022. However, all the wells, boreholes, and rainwater tanks in each selected village were sampled since there were very few. Samples were collected aseptically from the selected water sources into sterilized 250-mL glass bottles. Tap nozzles were flamed, the water was allowed to run for approximately 2 min, and then samples were collected. Boreholes were pumped for up to 15 min to purge the aquifers and minimize contamination prior to sample collection. 1% sodium thiosulfate was used to neutralize any chlorine in chlorinated water samples. The standard operating procedures were diligently adhered to during the study.

2.3. Culture methods

Water samples were delivered to the Microbiology Laboratory of the Department of Microbiology of Mbarara University of Science and Technology (MUST) in an ice-cooled box. Microbiological quality testing was carried out in accordance with standard microbiological procedures following standard operating procedures (Cheesbrough 2005). Using aseptic procedures, water samples were diluted tenfold down to 10^4 in sterile phosphate buffer. A volume of 1 mL of the dilutions was inoculated into a petri dish and 18 mL of molten plating medium Eosin Methylene Blue Agar (Levine) was added. Eosin methylene blue is a media that selectively grows Gram-negative organisms, while at the same time inhibiting Gram-positive organisms and some yeast (Patras *et al.* 2020). This agar is used in water quality testing to differentiate between coliforms and faecal coliforms (Whitlock *et al.* 2002; Abu-Sini *et al.* 2023). The sugars present (lactose and sucrose, but not glucose) promote the growth of Gram-negative bacteria, especially coliforms, while the methylene blue dye inhibits the growth of Gram-positive bacteria. In addition, the growth of lactose-fermenting Gram-negative bacteria leads to acidification of the medium, resulting

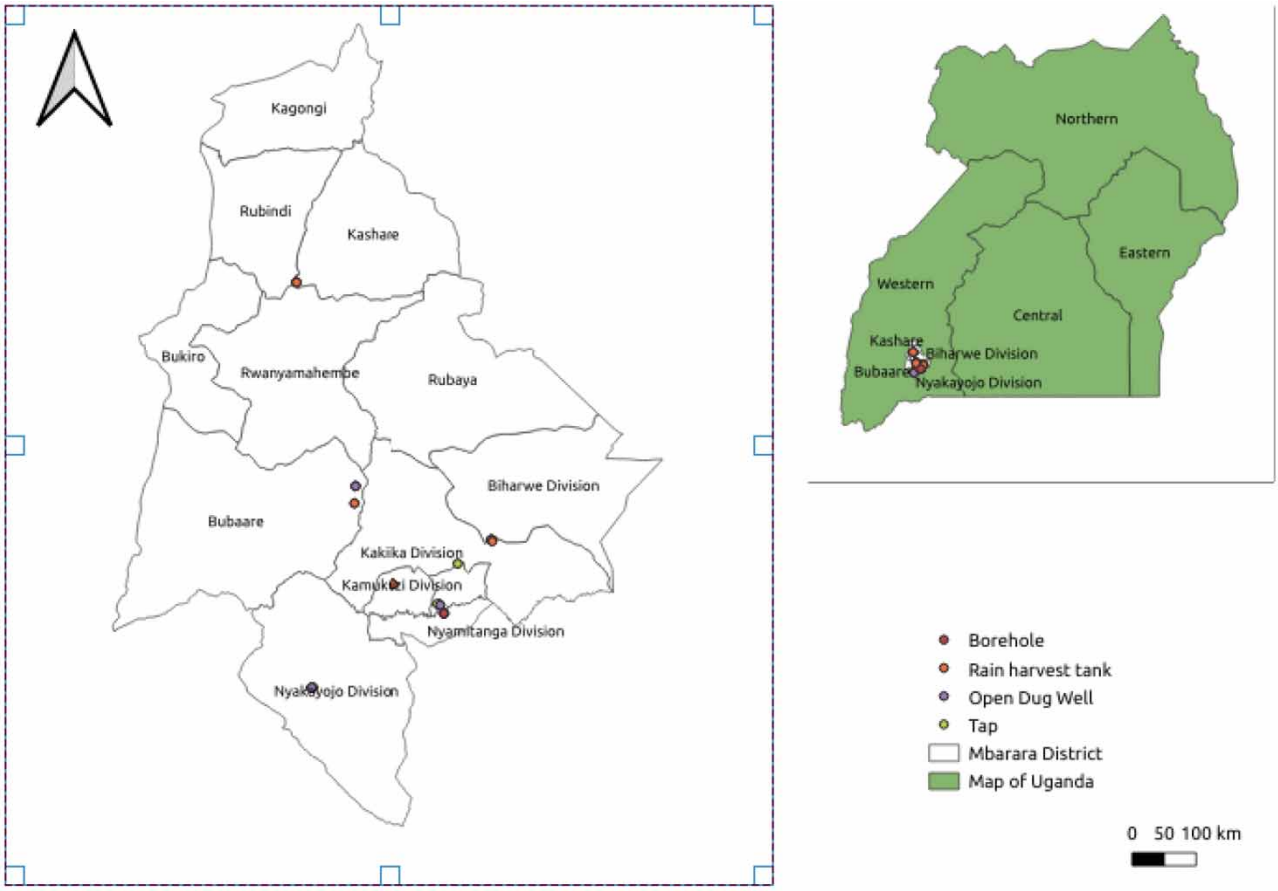


Figure 1 | Map of study area in Mbarara city showing the location of the selected drinking water sources ('figure is similar but not identical to the original image and is therefore for illustrative purposes only').

in the formation of a dark purple complex associated with a green metallic sheen due to the pH indicator dyes eosin Y and methylene blue. The colonies could be differentiated by the degree of colour change combined with colony morphology. *E. coli* colonies grow with a metallic sheen with a dark centre. However, additional biochemical tests are required after isolation for confirmation of *E. coli*. Media was mixed, left to solidify and incubated at 37 °C for up to 48 h. The grown colonies were counted depending on their colour. The suspected *E. coli* were sub-cultured on MacConkey agar plates and biochemical tests (indole, citrate, motility, methyl red and Voges–Proskaeur) were performed to identify *E. coli*. *E. coli* has the ability to produce indole from the metabolism of tryptophane, ferments glucose to high acid as detected by the methyl red pH indicator, produce neutral products 2,3-butanediol and/or acetoin from glucose metabolism and utilize citrate as a sole carbon source. Confirmed *E. coli* isolates were suspended in tryptic soy broth with 15% glycerol and frozen at −80 °C.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility of *E. coli* isolates was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar according to the local clinical context and a standard method recommended by the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute 2020; Humphries *et al.* 2021). After overnight incubation, cultures of *E. coli* isolates were placed into 5 mL of sterile normal saline and adjusted to be uniform with a 0.5 MacFarland standard. Bacterial suspensions were used to homogenously cover the surfaces of Mueller–Hinton agar (Biolab) using disposable sterile swabs. The plates were allowed to dry for 3–5 min: then discs (Oxoid LTD) were evenly distributed on the inoculated plate using a disc dispenser, ensuring appropriate spacing between discs and incubated at 37 °C for 18–24 h. The diameter of the zone of inhibition around the disc was measured using a ruler. Results were interpreted as Sensitive, Intermediate, or Resistant. The following antibiotic discs of the routinely used antibiotics were used: ampicillin, ceftazidime,

ceftriaxone, imipenem, azithromycin, ciprofloxacin, and gentamicin (Pulingam *et al.* 2002). The concentration of antibiotic used in this study and the diameter of inhibition zones surrounding the antibiotic discs were interpreted as outlined in Table 1. For antimicrobial susceptibility, *E. coli* ATCC 25922, which is sensitive to all antibiotics tested, was used as a control strain.

2.5. Quality control and quality assurance

All glassware was thoroughly washed and rinsed with deionized water and dried in an oven. Media and all the reagents were purchased from authorized companies in Uganda and Germany and they were within their shelf life. Media were sterilized at 15 lbs for 15 min. Standard operating procedures were prepared by the principal investigator according to manufacturer's instructions and were adhered to throughout the sample collection, transportation and processing. Research assistants were trained on standard operating procedures before the commencement of the study. Quality control checks were conducted as stipulated in the standard operating protocol for every procedure.

2.6. DNA extraction

E. coli isolates from the cryostocks were recultured on nutrient agar plates. The cultured bacteria were washed with molecular biology-grade water. The cell suspensions were centrifuged at 4,500 rpm for 5 min at 4 °C, and the pellets obtained were used for DNA extraction using boiling lysis according to Moore *et al.* (2004), but without bead-beating. In brief, the bacterial pellets were resuspended in 40 µL of molecular biology-grade water, subjected to boiling in a water bath at 100 °C for 10 min and then cooled on ice. The mixture was centrifuged at 15,000 × *g* for 10 min and stored at −20 °C in the microbiology laboratory of MUST until transport to the water microbiology department of the Technologiezentrum Wasser.

2.7. PCR analysis

E. coli isolates were confirmed by PCR amplification of the *yccT* gene using primers published by Clifford *et al.* (2012). Subsequently, the isolates were screened for the presence of class 1 integrons and nine ARGs. The focus was on *β*-lactamase genes (bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}32}$, $bla_{CMY\text{-}2}$ $bla_{OXA\text{-}48}$, $bla_{VIM\text{-}2}$, $bla_{NDM\text{-}1}$, and $bla_{KPC\text{-}3}$), including ESBLs and carbapenemases, as *β*-lactam antibiotics are the most widely used antibiotics worldwide. In addition, carbapenemase and ESBL-producing Gram-negative bacteria are an emerging and growing global health problem (Tacconelli *et al.* 2018). An attempt has been made to include representatives of most *β*-lactamase types. The polymyxin resistance gene *mcr-1* was considered as a further resistance gene mediating resistance to an antibiotic of last resort. The screening was performed using PCR assays already established within other studies. Therefore, previously published primer sets were used (see Table 2).

All endpoint PCR assays were performed using a thermocycler (Biometra) and MyTaq Red Mix (Biocat). Final reaction volume of 25 µL that consisted of 12.5 µL of 2x MyTaq Red Mix, 1.25 µl of 10 µM forward and reverse primer (ThermoScientific), 2 µL of template DNA and 8 µL of nuclease-free water. Positive controls and no-template controls were included in each run.

Cycling conditions were as follows: 1 min 95 °C (initial phase for enzyme activation), 35 cycles of 15 s at 95 °C (denaturation), 20 s at a primer-specific annealing temperature (T_A), and a fragment length-dependent elongation time (t_E) at 72 °C,

Table 1 | List of antibiotics and diameter of zone inhibition standard

			Zone of inhibition (mm)		
Antibiotic	**Antibiotic class**	**Concentration**	**Sensitive**	**Intermediate**	**Resistant**
Ampicillin	Penicillin	10 µg	≥17	14–16	≤13
Ceftazidime	Third-generation cephalosporin	30 µg	≥21	18–20	≤17
Ceftriaxone	Third-generation cephalosporin	30 µg	≥23	20–22	≤19
Imipenem	Carbapenem	10 µg	≥23	20–22	≤19
Azithromycin	Macrolide	30 µg	≥13	None	≤12
Ciprofloxacin	Fluoroquinolone	5 µg	≥26	22–25	≤21
Gentamicin	Aminoglycoside	30 µg	≥15	13–14	≤12

Table 2 | Target genes, primer sequences, and amplicon sizes for the investigated genes

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Amplicon length in bp	T_A in °C	t_E in s	Reference
intl1	GCCTTGATGTTACCCGAGAG	GATCGGTCGAATGCGTGT	196	60	20	Barraud *et al.* (2010)
bla_{TEM}	TTCCTGTTTTTGCTCACCCAG	CTCAAGGATCTTACCGCTGTTG	112	60	20	Bibbal *et al.* (2007)
bla_{SHV}	TCGCCTGTGTATTATCTCCC	CGCAGATAAATCACCACAATG	822	55	35	Maynard *et al.* (2004)
$bla_{CTX\text{-}M\text{-}32}$	CGTCACGCTGTTGTTAGGAA	CGCTCATCAGCACGATAAAG	155	60	20	Stalder *et al.* (2014)
$bla_{CMY\text{-}2}$	CGTTAATCGCACCATCACC	CGTCTTACTAACCGATCCTAGC	172	60	20	Kurpiel & Hanson (2011)
$bla_{OXA\text{-}48}$	TGTTTTTGGTGGCATCGAT	GTAAMRATGCTTGGTTCGC	177	55	20	Monteiro *et al.* (2012)
$bla_{VIM\text{-}2}$	GTTTGGTCGCATATCGCAAC	AATGCGCAGCACCAG GATAG	382	60	20	Li *et al.* (2015)
$bla_{NDM\text{-}1}$	ATTAGCCGCTGCATTGAT	CATGTCGAGATAGGAAGTG	154	60	20	Naas *et al.* (2011)
$bla_{KPC\text{-}3}$	CAGCTCATTCAAGGGCTTTC	GGCGGCGTTATCACTGTATT	196	59	20	Szczepanowski *et al.* (2009)
mcr-1	GGGCCTGCGTATTTTAAGCG	CATAGGCATTGCTGTGCGTC	183	60	20	Hembach *et al.* (2017)

followed by melting curve analysis. The T_A and t_E used are listed for each gene in Table 2. The amplicons were analysed by gel electrophoresis using the QIAxcel® Advanced system (Qiagen).

3. RESULTS AND DISCUSSION

3.1. Enumeration of *E. coli* and coliform bacteria in different water sources

In this study, the majority of the isolated organisms were *Citrobacter divergenes* (62.2%) in the wet season while in the dry season, it was majorly *Enterobacter cloacae* (61.8%). A higher percentage of *E. coli* (35.14%) and *Klebsiella* species (8.11%) was isolated in the wet season as compared to 26.5 and 2.9%, respectively, during the dry season. The average concentration of coliform bacteria in the wells was 5.3 ($\pm$2.5), in the tanks 5.0 ($\pm$3.7) and in the tap water samples 5.0 ($\pm$2.2) log/100 mL. There was no significant difference in the distribution of mean log coliform/100 mL across the sampled water sources and during the dry and wet seasons ($p \leq 0.05$). *E. coli* was not present in all water samples. However, in total six isolates were obtained from the two tank water samples (two isolates) and six tap water samples (four isolates), while in total 35 *E. coli* strains were isolated from the 29 well samples during the dry and wet seasons.

In temperate regions such as Europe and North America, *E. coli* is a widely accepted indicator of faecal contamination. In recent years this indicator has been called into question for tropical countries (Walk *et al.* 2007; Ishii & Sadowsky 2008; Jang *et al.* 2017). However, for the climatic conditions prevailing in Uganda, especially the comparatively low water temperatures compared to other tropical countries, *E. coli* has been confirmed as the most appropriate parameter for the detection of faecal pollution. The detected *E. coli* are of faecal origin. *E. coli* bacteria were isolated from drinking water from sources in Mbarara town. Most *E. coli* were isolated from wells. Contamination of the wells could be due to the low water table execrated by the presence of pit latrines within the vicinity and lack of fence, allowing easy accessibility to animals (Wamyil *et al.* 2023). These findings indicate that the water from these sites is not directly suitable for human consumption and poses a threat to human health. This has also been shown in other studies (Lukubye & Andama 2017).

However, *E. coli* bacteria have been detected in the tap water and tank water. The town of Mbarara is a newly established town in Uganda that is struggling with increasing population growth and urbanisation and the associated problems of encroachment on protected water catchment areas and poor waste management (Catherine *et al.* 2023). Mbarara's tap water is drawn from the Rwizi River. The water is treated by flocculation, sedimentation, and chlorination and then distributed. The chlorination ensures microbiologically safe water quality. The reason for the presence of these bacteria in tap water could be contamination in the distribution systems by leaks or physical breaches. Regarding the water quality of harvested rainwater, there are already studies showing the presence of *E. coli* bacteria in these waters in Uganda (Nalwanga *et al.* 2018).

As the drinking water samples examined showed faecal contamination, which also indicated the presence of other pathogenic micro-organisms, measures would be desirable. These include sensitising the population on the installation and use of

sanitary facilities, regular monitoring of water quality by the authorities, maintenance of the water distribution system to repair leaks, and the installation of protection zones/fences around the wells to keep animals away.

3.2. Antibiotic susceptibility of *E. coli* isolates

While there have been general studies on the occurrence of microbiological water quality indicators in such waters, there has been little research on the occurrence of antibiotic resistance in bacteria isolated from these waters. The analysis of *E. coli* isolates revealed a high resistance to ampicillin of 55.5% (see Table 3). This could be due to the fact that penicillins – and especially ampicillin – are commonly prescribed in human and veterinary medicine in Uganda (Obakiro *et al.* 2022; Mambula *et al.* 2023). In addition, β-lactams are among the largest group of antibiotics used in veterinary medicine in Uganda (Nayiga *et al.* 2020). However, other studies have reported the presence of ampicillin-resistant *E. coli* isolated from waters in countries around the world, such as South Africa (Nontongana *et al.* 2014), Tanzania (Lyimo *et al.* 2016), and Nicaragua (Amaya *et al.* 2012).

However, resistance to the third-generation cephalosporins, ceftazidime and ceftriaxone could be observed in six or 15% of the investigated isolates (see Table 3). None of the isolates was resistant to imipenem and gentamicin, and only one and two isolates was resistant to azithromycin and ciprofloxacin, respectively; 17% of the isolates were multi-drug resistant against two, three, and five antibiotics (10.6, 4.3, and 2.1%, respectively). The multi-drug resistance was against ampicillin, ceftazidime, ceftriaxone, and ciprofloxacin.

Looking at the occurrence of resistance in *E. coli* from the different water sources, ampicillin-resistant bacteria were detected in all three types of water samples (see Figure 2). Ceftazidime- and ceftriaxone-resistant *E. coli* were detected only in tap and well water, not in the tank samples. However, only a very limited number of *E. coli* isolates from tank water was tested.

This study aimed at assessing the occurrence of antibiotic resistance genes in *E. coli* isolated from drinking water sources in Mbarara city southwestern Uganda. *E. coli* is a commensal inhabitant of the gastrointestinal tract of all animal species and humans. It is genetically flexible making it possible to adapt to changing environments hence acquiring a great number of antimicrobial resistance (Baquero *et al.* 2021). *E. coli* is not only important as an indicator bacterium but also as a pathogen, as it is common in various infections, including hospital-acquired urinary tract infections. Ceftriaxone and ceftazidime are the most commonly used antibiotics to treat *E. coli* infections (Jahani *et al.* 2017). Ceftriaxone and other cephalosporins are also used extensively in Uganda. Recent studies show that prescription rates in human medicine range from 11 to 37% (Kiggundu *et al.* 2022; Obakiro *et al.* 2022; Mambula *et al.* 2023), although they are rarely used for animal treatment (Nayiga *et al.* 2020). The cephalosporin resistances are of particular medical relevance, and third-generation cephalosporin-resistant Enterobacteriaceae are on the WHO priority list of ARB as priority 1: critical (Tacconelli *et al.* 2018). The presence of third-generation cephalosporin-resistant *E. coli* has been demonstrated in isolates from humans (Oteo *et al.* 2006; Yuan *et al.* 2016), animals (Tello *et al.* 2020), food (Daz-Gavidia *et al.* 2021), and environmental sources (Daz-Gavidia *et al.* 2021) on a global level. For example, in wound swabs from 109 caesarean section surgical site infections at Mulago Hospital in Kampala (Uganda), resistance to ceftriaxone was detected in all isolated *E. coli* ($n = 11$) (Wekesa *et al.* 2020). In recent years, an alarming increase in third-generation cephalosporin-resistant *E. coli* has been observed, particularly in the human sector (Oteo *et al.* 2006; Yuan *et al.* 2016). The presence of cephalosporin resistance in the isolates from this study is particularly noteworthy, as a survey shows that the use of these antibiotics is very limited in Uganda (Nayiga *et al.* 2020).

Only a few of the isolates showed phenotypic resistance to azithromycin and ciprofloxacin (2 and 4%). Azithromycin belongs to the group of macrolide antibiotics. Macrolide antibiotics are used in both human and veterinary medicine. In human medicine, the main areas of use are the treatment of respiratory tract infections (Pechère 1993), gonorrhoea (Mensforth & Ross 2019) and multi-drug-resistant Enterobacteriaceae (van der Paardt *et al.* 2015). In this area, an increase

Table 3 | Antimicrobial susceptibility testing results of the isolated *E. coli*

	Antibiotic						
	Ampicillin	**Ceftazidime**	**Ceftriaxone**	**Imipenem**	**Azithromycin**	**Ciprofloxacin**	**Gentamicin**
Sensitive	11 (23%)	40 (85%)	40 (85%)	45 (96%)	46 (98%)	34 (72%)	45 (96%)
Intermediate	10 (21%)	4 (9%)	–	2 (4%)	–	11 (23%)	2 (4%)
Resistant	26 (55%)	3 (6%)	7 (15%)	–	1 (2%)	2 (4%)	–

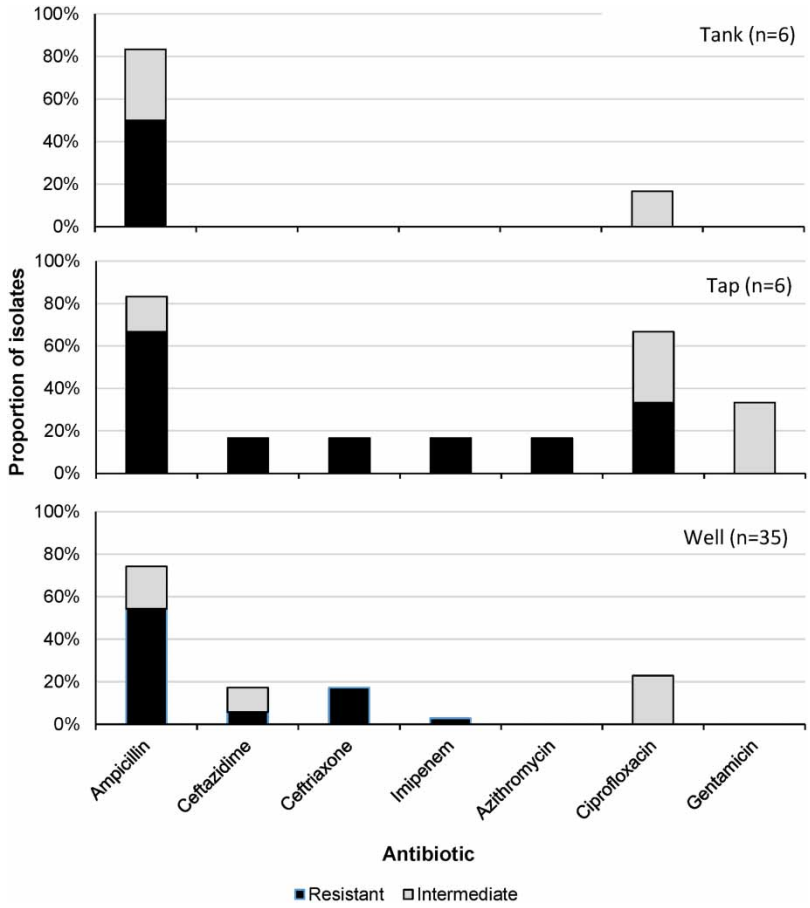


Figure 2 | Antimicrobial susceptibility testing results of the *E. coli* isolated from the different water sources (tank, tap and well).

in macrolide resistance in pneumococci (Jenkins & Farrell 2009) and streptococci (Gonzales *et al.* 2022) has been observed in recent years. However, azithromycin appears to be used rather infrequently in clinical practice in Uganda (Kiggundu *et al.* 2022; Mambula *et al.* 2023).

Ciprofloxacin, a fluoroquinolone antibiotic was included in the investigations. Ciprofloxacin is used in the treatment of malaria. In addition, there is a study showing that the heavy use of chloroquine for malaria treatment and prophylaxis is likely to result in ciprofloxacin resistance in *E. coli* (Davidson *et al.* 2008). Although malaria is a nationwide problem in Uganda, only 4% of tested *E. coli* isolates were resistant to ciprofloxacin. This may be due to the introduction of artemisinin combination therapies as the first-line treatment for malaria in Uganda since 2004 (Nanyunja *et al.* 2011). The low use of ciprofloxacin is reflected in different studies on antibiotic use in Uganda (Nayiga *et al.* 2020; Kiggundu *et al.* 2022; Mambula *et al.* 2023).

In this study, none of the isolates was resistant to imipenem and gentamicin, using imipenem as a proxy for a carbapenem antibiotic and gentamicin as a proxy for an aminoglycoside antibiotic. In a point prevalence survey of antibiotics used across 13 hospitals in Uganda, gentamicin was with 7% the third most prescribed antibiotic (Kiggundu *et al.* 2022). A similar percentage of prescriptions was found in a study of prescribing in paediatric care in hospitals and health centres (Mambula *et al.* 2023), while the evaluation of health management information from two hospitals in eastern Uganda found a proportion of only 1.7% for gentamicin. In addition, there is no evidence of increased use of carbapenems in Uganda (Kiggundu *et al.* 2022; Mambula *et al.* 2023).

However, other studies have found imipenem-resistant (Mahmoud *et al.* 2020; Nzima *et al.* 2020) and gentamicin-resistant *E. coli* (Mahmoud *et al.* 2020; Aabed *et al.* 2021) in different types of water, although the proportion of gentamicin-resistant isolates was rather low compared to other antibiotics. In a cross-sectional study at Mulago National Referral Hospital in Kampala, carbapenem-resistant *E. coli* were also recovered from wastewater (Bagaya *et al.* 2023). Nonetheless, both carbapenems and aminoglycosides play a very minor role in antibiotic use for human and animal treatment in Uganda (Nayiga *et al.* 2020).

Overall, it can be assumed that the *E. coli* isolates studied are of faecal origin. This means that these resistant isolates were excreted by humans or animals and introduced into the investigated water sources.

With regard to the spread of antimicrobial resistance, it is important to consider the possible transmission of resistant bacteria between animals, humans and the environment. Humans can acquire antibiotic-resistant pathogens through contact with animal excreta, via the food chain, or even by consuming contaminated drinking water (Gómez-Gómez *et al.* 2019). The use of antimicrobials in food animal production has been documented as a potential source of human AMR, either through horizontal transfer of ARGs to human pathogens or through direct transfer of ARB (Jibril *et al.* 2020). In Uganda in particular, farmers use large amounts of antibiotics in their livestock, which is known to accelerate the emergence of ARB (Iramiot *et al.* 2020). With regard to *E. coli*, it is also important to consider that this bacterium is one of the most important reservoirs of antibiotic resistance. In recent decades, an increasing number of antibiotic resistances has been observed in *E. coli* isolates (Salyers *et al.* 2004). *E. coli* acts as both a donor and a recipient of ARGs, and can thus acquire resistance genes from other bacteria, as well as pass on its resistance genes to other bacteria (Li *et al.* 2019).

Due to its high affinity for horizontal gene transfer, multi-drug resistance is more common in *E. coli*. In this study, 17% of *E. coli* showed multi-drug resistance, with isolates resistant to two, three and five antibiotics. A systematic review of drug-resistant urinary tract infections in pregnant women in developing countries in Africa and Asia between 2005 and 2016 documented high levels of multi-drug resistance to ampicillin (67.2%), ciprofloxacin (71.2%) and ceftriaxone (74.1%) (Belete & Saravanan 2020). It is also interesting to note that antibiotics have been used as contraceptives in some parts of Africa (Iheanacho 2022) for curative and prophylactic purposes in livestock farming (Samuel *et al.* 2023). High rates of multi-drug-resistant bacterial isolates from poultry farms and from pregnant mothers suspected of having a urinary tract infection have been documented in Uganda ((Johnson *et al.* 2021; Samuel *et al.* 2023).

3.3. Occurrence of antibiotic resistance genes in the *E. coli* isolates

In addition to detecting phenotypic resistance, the isolates obtained were tested for the presence of genotypic resistance determinants. The focus was on the detection of β-lactamases especially ESBLs and carbapenemases. With regard to ESBLs, the TEM and SHV-type genes as well as the gene $bla_{\text{CTX-M-32}}$ and $bla_{\text{CMY-2}}$ were included in the investigations. While bla_{TEM} and bla_{SHV} were detected in only 2% of isolates, the percentage of $bla_{\text{CTX-M-32}}$ and $bla_{\text{CMY-2}}$ positive isolates was significantly higher (64 and 36%, respectively, see Figure 3). In the past, TEM- and SHV-type ESBLs were the predominant families of

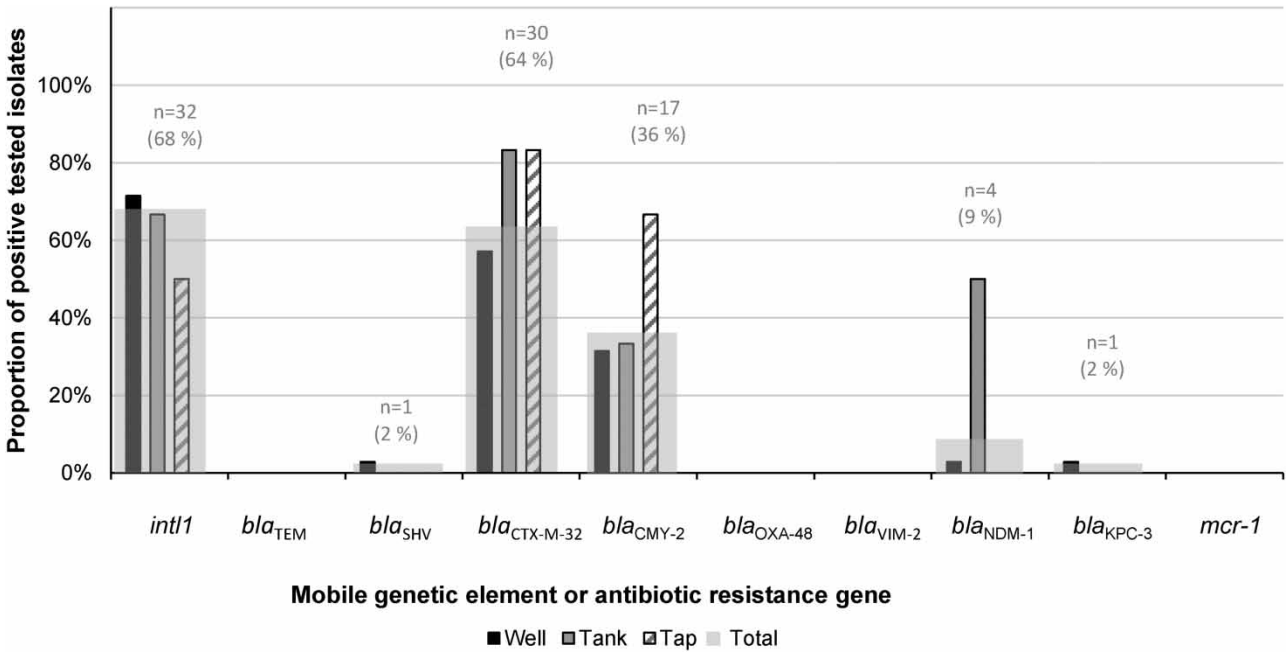


Figure 3 | Detected antibiotic resistance gene and integrons in the *E. coli* isolates. Data and column in grey indicate the results for all isolates regardless of water type.

ESBLs, but there has been a shift in the distribution of different ESBL genes in Africa and Europe, with the CTX-M ESBL gene increasing dramatically over the TEM and SHV variants (Castanheira *et al.* 2021). Also CMY-2 is reported to be the very common plasmid-carried AmpC-type CMY in *E. coli* isolates from various global regions (Poirel *et al.* 2018). Overall, our results are consistent with previous studies on the occurrence of CMY-2 and CTX-M β-lactamases in clinical isolates in Uganda (Najjuka *et al.* 2020) and on the distribution of genes in African wastewater (Abia *et al.* 2023).

When considering the occurrence of ARGs in relation to the resistance exhibited by the isolates, resistance to third-generation cephalosporins is of particular interest. Of the ceftazidime- and/or ceftriaxone-resistant *E. coli* ($n = 7$), 71% carried $bla_{\text{CMY-2}}$ and 29% $bla_{\text{CTX-M-32}}$, with two isolates carrying both genes and two isolates carrying neither gene.

Interestingly, many of the isolates tested had the specific gene sequences for $bla_{\text{CTX-M-32}}$ and $bla_{\text{CMY-2}}$, but showed no resistance to ceftazidime and/or ceftriaxone in the antibiotic susceptibility test. The CTX-M β-lactamases hydrolyze ceftazidime with a very low catalytic efficiency but are effective against ceftriaxone (Poirel *et al.* 2001). CMY ESBL could confer resistance to both cephalosporins. This suggests that the expression level of the genes was too low in the sensitive isolates.

In addition to the ESBLs genes, the carbapenemase genes $bla_{\text{OXA-48}}$, $bla_{\text{VIM-2}}$, $bla_{\text{NDM-1}}$ and $bla_{\text{KPC-3}}$ were included in this study. The low level of detection is consistent with the data from the phenotypic study, where no isolate showed clear imipenem resistance, but two isolates showed intermediate behaviour. Nevertheless, $bla_{\text{NDM-1}}$ and $bla_{\text{KPC-3}}$ were detected in isolates that did not show resistance to imipenem, indicating that the genes were not highly expressed in these isolates. Worldwide, there is evidence of the spread of carbapenem-resistant *E. coli* in humans and livestock, caused by the acquisition of various carbapenemase genes, such as the genes under study (Tuhamize *et al.* 2023). This is due to the fact that carbapenemase-encoding genes usually occur in association with mobile genetic elements, which enable their spread by horizontal transfer (Nordmann & Poirel 2014). The detection of bla_{VIM}, $bla_{\text{OXA-48}}$, bla_{KPC}, and $bla_{\text{NDM-1}}$ β-lactamases genes in 196 Enterobacteriaceae isolated from patient samples in a hospital in Kampala provide evidence of the occurrence of these resistance determinants in Uganda (Okoche *et al.* 2015). In a similar study of multi-resistant Enterobacteriaceae isolated from diverse specimens obtained from patients attending Mbarara Regional Referral Hospital, southwestern Uganda only bla_{VIM} and $bla_{\text{OXA-48}}$ genes were detected (Ampaire *et al.* 2015). Studies on the occurrence of KPCs *in E. coli* isolated from humans and livestock also confirm their presence in Uganda (Tuhamize *et al.* 2023), although no assignment of KPC variants has been made. Based on the available environmental data, it could be speculated that KPC-2 rather than KPC-3 may be present in Uganda. In many countries, this carbapenemase has been detected in both wastewater and surface water (Kohler *et al.* 2020).

The *E. coli* isolates were also screened for the polymyxin resistance gene *mcr-1*, which could not be detected in any isolate. The gene *mcr-1* confers resistance to colistin, a last-resort antibiotic for multi-drug-resistant infections. First reports of the gene were published in November 2015 (Liu *et al.* 2016). A recent study has highlighted the growing threat of *mcr-1* gene resistance to colistin, with documented examples of resistance in animals, humans, food and the environment (Elbediwi *et al.* 2019). In our study, colistin resistance was not assessed phenotypically, however, the lack of detection of *mcr-1* in the *E. coli* isolates did not provide evidence of colistin resistance. In the survey by Najjuka *et al.* (2020), some respondents reported frequent use of colistin. No studies have been conducted on the occurrence of colistin resistance in clinical isolates or even in waterborne bacteria. Further studies at a later date could provide important information.

An evaluation of the isolates according to the water source from which they were isolated showed only minor deviations in the ARG pattern compared to the total number of isolates. The carbapenemase gene $bla_{\text{NDM-1}}$ was found in a higher percentage in the isolates from tank water than in the other isolates. In contrast, the percentage of $bla_{\text{CMY-2}}$ was highest in the isolates from tap water. However, the number of isolates from the tank and tap water was very limited, so individual positive findings were strongly reflected in the percentages.

The horizontal transfer of ARGs is considered a major factor for the spread of AMR, also in water environments. Integrons, i.e. genetic elements containing a site-specific recombination system capable of integrating, expressing and exchanging specific DNA elements, are of particular importance in the various mechanisms of horizontal gene transfer. Class 1 integrons (*intl1*) are the most common type of integrons found in clinical isolates (Ye *et al.* 2020) and were therefore included in the PCR analyses. A sequence specific for *intl1* was detected in 68% of all isolates. These data show the high mobility of ARGs in the environment.

Worldwide, there is evidence of the spread of cephalosporin- and carbapenem-resistant *E. coli* in humans and livestock, caused by the acquisition of the according genes, such as the genes under study (Tuhamize *et al.* 2023). Overall, there is limited information on the extent of the spread of these resistant *E. coli* in the Ugandan environment. Overall, our studies show a

high prevalence of medically relevant ESBL genes. Carbapenemase genes are also present, albeit at low levels, in waterborne *E. coli* isolates. This suggests an additional health risk for people using this water for drinking.

4. CONCLUSION

AMR is a global problem that necessitates a 'One Health' approach in order to achieve significantly better public health outcomes for people. It is no longer possible to address the problem solely by studying it in healthcare settings because most ecosystems contribute to the emergence, acquisition, and spread of AMR. This is especially true in low-income countries, where a wide range of antibiotics are used and few studies on the emergence of medically relevant antibiotic resistance, such as resistance to third-generation cephalosporins, carbapenems, or polymyxins, are available. As a result, *E. coli* isolates from Ugandan waters that are also used for drinking water were isolated and analysed phenotypically and genotypically. The findings revealed a wide range of ampicillin and third-generation cephalosporin resistance, as well as a high prevalence of mobile genetic elements and ESBL genes in these isolates. This has increased awareness of the presence of ARGs in drinking water from Mbarara town sources.

As faecal contamination, including ARB, was found in the different types of drinking water, indicating the presence of other and possibly resistant pathogenic micro-organisms, measures should be taken to improve the microbiological quality of the water. This includes educating the population on the installation and use of sanitation facilities, but also on the use of antibiotics. In addition, regular monitoring of water quality by the government, maintenance of the water supply system to repair leaks, and the establishment of protection zones/fences around boreholes and wells to prevent the entry of animals should be implemented.

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DATA AVAILABILITY STATEMENT

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

CONFLICT OF INTEREST

The authors declare there is no conflict.

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