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Multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolated from hospital sewage flowing through community sewage system and discharging into the Indian Ocean

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Abstract

Background Hospital sewage is a significant reservoir of antimicrobial-resistant pathogens and genes that pose a huge public health threat. In this study, we determined the occurrence of multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* in sewage flowing from a referral hospital through the urban sewage system to the point of discharge in the Indian Ocean.

Results A total of 400 sewage samples were collected, yielding 517 isolates. Of these, 32.3% (167/517) were from hospital sewage, while 67.7% (350/517) were from the community. *E. coli* was the most common isolate (44.5% (230/517)), followed by *K. pneumoniae* at 27.3% (141/517), and other gram-negative bacteria constituted 28.2% (146/517) of the isolates. Multidrug resistance (MDR) was seen in 80.9% (186/230) *E. coli* and 71.6% (101/141) *K. pneumoniae*. Of the MDR isolates, 27.2% (78/287) were resistant to four different classes of antibiotics, while 6.9% (20/287) exhibited resistance to eight classes. The most frequent MDR pattern was PEN/CEP/TET/QNL/SUL, seen in 14.2% (38/287) of the isolates. The isolation frequency of MDR *E. coli* and *K. pneumoniae* at different sampling sites was high, being 47.6% in hospital chambers, 62.0% in hospital ponds, 58.1% in the treated hospital wastewater, and 55.6% in the community stream draining into the Indian Ocean. Extended spectrum beta-lactamase production was observed in 40% (92/230) of *E. coli* and 36.2% (51/141) of *K. pneumoniae* isolates. Resistance to quinolones among *E. coli* was 54.8% (126/230) and was 39.7% in *K. pneumoniae* (56/141). Carbapenem resistance in *E. coli* was 39.6% (91/230), while among *K. pneumoniae* isolates was 32.6% (46/141).

Conclusions We found high proportions of multidrug-resistant *E. coli* and *K. pneumoniae* in the wastewater flowing from the hospital through the community sewage system to the point where it enters the Indian Ocean. Biological treatment did not significantly reduce the proportion of resistant bacteria, posing a very serious public health threat. The release of these highly resistant pathogens into the Indian Ocean is of international concern.

Keywords Hospital sewage, Urban wastewater, Wastewater treatment plant, Multidrug-resistant *E. coli*, Multidrug-resistant *K. pneumoniae*, Hospital effluent

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Background

Antimicrobial resistance (AMR) is a global health threat and has remarkable effects on public health and the global economy (Prestinaci et al. 2015; World Health Organization 2014). Globally, it is estimated that at least 700,000 people die annually from infections that are resistant to currently available antibiotics (O'Neill 2016). Bacterial AMR is at least as large as major diseases such as HIV and malaria, with the highest burden encountered in sub-Saharan Africa (AMR Collaborators 2022). In 2019, predictive statistical models found that the highest death rates to have occurred in western sub-Saharan Africa, at 27.3 deaths per 100,000 (20.9–35.3) due to drug-resistant bacterial infections. In most African countries, AMR situation is made worse by weak regulation in antimicrobial use (AMU), tendency for animal owners to stock drugs in their houses and engaging unskilled people in treating animals (Frumence et al. 2021), limited laboratory capacity to collect and analyse data on AMR and AMU (Matee 2023), and irrational use of antibiotics in human and animal sectors, with detrimental consequences to the environmental (Fletcher 2015; Kimera et al. 2020a, b). In addition, most countries in this region do have weak health systems for AMR and AMU surveillance, crucial for production of evidence-based data needed for quantifying risks, planning, prioritization, investment of resources, inform policy development and assess the impact of intervention (Frost et al. 2021). Some sectors such as the environment are particularly underprivileged as compared to humans, animals and food of animal origin, making it difficult in implementing international guidelines at the national level (Matee et al. 2023). Indeed, a recent study suggests that sub-Saharan African countries need to fully involve clinical, veterinary and environmental departments if they are to build a robust One Health AMR preparedness response (Elton et al. 2020).

One area that deserves more attention is the role of sewage as a driver of AMR and antimicrobial resistance genes (ARGs) (Hendriksen et al. 2019). Sewage from the hospital settings serves as hotspots for AMR, where antimicrobial agent metabolites from consumed antibiotics as well as the drug-resistant bacterial pathogens in patients' faeces and urine may be passed into the hospital sewage system (Verburg et al. 2019). As a consequence, hospital sewage often contains MDR bacteria (Zagui et al. 2020; Auguet et al. 2017) that spread rapidly into the environment by horizontal gene transfer through plasmids and transposons (Korzeniewska and Harnisz 2013). Moreover, the problem worsens as untreated or inadequately treated hospital wastewater is dumped into local sewage systems (Hocquet et al. 2016; Pärnänen et al. 2019). The effect of hospital wastewater treatment on AMR varies between studies, probably reflecting the heterogeneity of approaches used (Buelow et al. 2018).

It has been shown that AMR rates in the bacteria isolated from wastewater correlate positively with the frequency of the antibiotic resistance in the corresponding human population (Reinthaler et al. 2013). In one study, a possible transmission route for ampicillin- and ciprofloxacin-resistant *Enterococcus faecium* was traced from patients in hospital to urban sewage, further through wastewater treatment plants to surface water and back to humans (Iversen et al. 2004).

Gram-negative bacteria that are extended spectrum β-lactamase (ESBL) producers are of particular significance, causing infections that are particularly difficult to treat (Holmes et al. 2016). These bacteria produce a group of β-lactamases, which share the ability to hydrolyse penicillins, first-, second-, and third-generation cephalosporins, aztreonam and carry genes encoding resistance to other drug classes such as aminoglycosides (Holmes et al. 2016; Castanheira et al. 2021). *E. coli* and *K. pneumoniae*, which are also indicator bacteria in AMR surveillance in the environment (Anjum et al. 2021), represent the commonest multidrug-resistant (MDR) pathogens that exhibit ESBL, carbapenems and quinolone resistance (Castanheira et al. 2021; Cheng et al. 2018; Harmon et al. 2019; Klein et al. 2018).

In the present study, we analysed AMR patterns of *E. coli* and *K. pneumoniae* in wastewater samples collected from a regional referral hospital and along the community sewage system of Temeke District, Tanzania, up to the point of discharge in the Indian Ocean. The aim of this study was to compare the antibiotic resistance levels at the different sites of the sewage system, as well as before and after the treatment plant. This was done in order to improve our knowledge on the potential role of hospital sewage as a driver of AMR spread in the community and to determine the extent to which the treatment plan reduces antibiotic resistant bacteria. We recognize that wastewater-based epidemiology (WBE) of AMR is an important epidemiological approach to generate information on potential risk to human populations on a community scale (Choi et al. 2018). Conducting WBE on AMR by simultaneously sampling both healthcare- and community-associated sewage is gaining traction and is important in addressing the burden of AMR (Fahrenfeld and Bisceglia 2016).

Methods

Study setting

This cross-sectional study was conducted between October 2021 and January 2022 in Temeke municipality, one of the five districts in Dar es Salaam, Tanzania. The study involved microbiological examination of sewage starting

from the Temeke Regional Referral Hospital (which has a bed capacity of 304, and serves more than 1,368,881 people), through various points along the community sewage system in Temeke municipality till the discharging point into the Indian Ocean (see Fig. 1).

Sampling frame and strategy

The sampling frame consisted of wastewater samples collected from wards, clinics and other administrative blocks in the hospital, as well as households, industries, markets and government institutions in the community. The samples were purposively collected from 33 sampling sites (13 from the hospital settings, 7 from the community stream and 13 from the four community ponds at Kurasini). A proportional probability-to-size sampling technique was applied to determine the number of samples to be collected from each site. A total of 400 samples were collected as follows: 109 from the hospital chambers, 48 from the hospital ponds, 85 from the community stream and 158 from the community ponds.

Sample collection and processing

At each sampling site, 3 mL of wastewater was collected using a sterile 50-mL falcon tube (BD, Nairobi, Kenya), twice a week (Monday and Thursday) for a period of 6 weeks. At the hospital, the samples were directly drawn from each inspection chamber and the general collecting chamber, whereas pond samples were taken from inlets and outlets, and samples from open streams were collected at the beginning, midpoint and end of the stream. All the samples were collected in the morning between 8:00 am and 10:00 a.m., placed in a zip-top bag, transported in a cool box at 2–8 °C, and processed in the Microbiology teaching laboratory of the Muhimbili University of Health and Allied Sciences (MUHAS) within 6 h of collection.

Isolation and identification of enteric bacteria

In the laboratory, the wastewater samples were mixed with sterile 0.9% normal saline at a ratio of 1:1 to reduce bacterial density as described by Moremi et al (2016). Then, a loopful of the diluted sample was inoculated onto MacConkey agar (Oxoid, Basingtoke, UK) and incubated aerobically at 37 °C for 18–24 h. Lactose fermenters were further examined using Gram stain and biochemical tests (Oxidase, Indole, Methyl red, Voges–Proskauer, Citrate utilization tests, and Kligler's iron agar) for the identification of *E. coli* and *K. pneumoniae* isolates.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed using the Kirby Bauer disc diffusion method as per Clinical Laboratory Standard Institute (CLSI) guideline of 2019 (Clinical Laboratory Standards Institute 2019). In brief, a bacterial suspension from pure culture was adjusted to 0.5 McFarland turbidity standard, and by

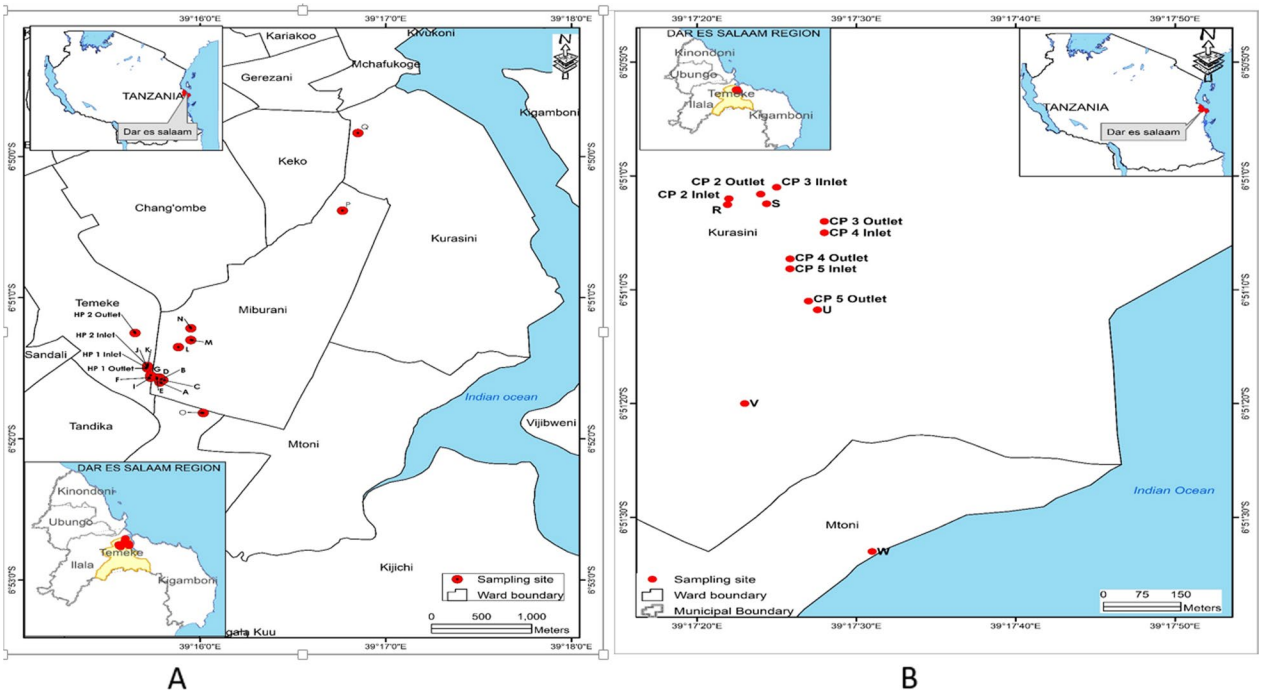

Fig. 1 Map of Temeke Municipal showing sampling sites. **A** Hospital sewage system, **B** Community sewage system

using a sterile cotton swab the inoculum was evenly distributed into the Mueller–Hinton agar (MHA; HiMedia Mumbai, India) and then incubated aerobically at 37 °C for 16 to 18 h. The tested antibiotic discs were ampicillin (AMP 10 µg; Oxoid, UK), tetracycline (TET 30 µg; Oxoid, UK), nalidixic acid (NAL 30 µg; Oxoid, UK), ciprofloxacin (CIP 5 µg; Oxoid, UK), imipenem (IMI 10 µg; Oxoid, UK), trimethoprim-sulfamethoxazole (SXT 1.25/23.5 µg; Oxoid, UK), gentamicin (GEN 10 µg; Oxoid, UK), cefotaxime (CTX 30 µg; Oxoid, UK) and chloramphenicol (CHL 30 µg; Oxoid, UK). An isolate was considered to be MDR if it showed resistance to at least three or more different classes of antibiotics (Magiorakos et al. 2012).

Screening and confirmation of ESBL production

All identified *E. coli* and *K. pneumoniae* isolates were inoculated onto MacConkey agar containing 2 µg /mL cefotaxime for screening and confirmed by a combination disc diffusion method, using cefotaxime (30 µg) alone and in combination with clavulanic acid (30 µg/10 µg) and ceftazidime (30 µg) alone and in combination with clavulanic acid (30 µg/10 µg). The zones of inhibition around the disc of cefotaxime alone and the disc of cefotaxime with clavulanic acid were measured. A difference of ≥5 mm between the two diameters indicated ESBL production as per 2019 CLSI guideline.

Screening for resistance to quinolones and carbapenems

The zones of inhibition for quinolones (nalidixic acid and ciprofloxacin) and carbapenems (imipenem) measured during susceptibility testing were used to determine the resistance to the two classes of antibiotics. For imipenem, a zone of <19 mm (resistance) and 20–22 mm (intermediate). For nalidixic acid, a zone of <13 mm (resistance), 14–18 mm (intermediate); and for ciprofloxacin, a zone of <21 mm (resistant), 22–25 mm (intermediate). All intermediate zones were considered resistant (Clinical Laboratory Standards Institute 2019).

Quality control procedures

Media were prepared according to the manufacturer's instructions, and sterility checks were performed. Standard *K. pneumoniae* (ATCC 700603) and *E. coli* (ATCC 25922) were used as positive and negative controls, respectively, during AST and when confirming ESBL-producing organisms.

Statistical analysis

Data were analysed using GraphPad Prism version 9. The proportion of MDR *E. coli* and *K. pneumoniae* was calculated by dividing the number of isolates that showed resistance to at least three different classes of antibiotics over the total number of tested isolates in a specific sewage source. Fisher's exact test was used to compare the isolation frequency of antibiotic-resistant *E. coli* and *K. pneumoniae* between the hospital and community sewage isolates. A p value of <0.05 was considered statistically significant.

Results

Distribution of *Enterobacteriaceae* in the hospital and community sewage

A total of 400 sewage samples were collected, yielding 517 isolates, of which 32.3% (167/517) were from the hospital sewage system and 67.7% (350/517) were from the community (Fig. 2A). Regarding bacterial species, *E. coli* was the most common 44.5%, (230/517), followed by *K. pneumoniae* at 27.3% (141/517) and 28.2% (146/517) of the isolates were other gram-negative bacteria (Fig. 2B).

Frequency of *E. coli* and *K. pneumoniae* isolates in the hospital and community sewage

The isolation frequency of *E. coli* was significantly higher in the hospital than in the community sewage, being

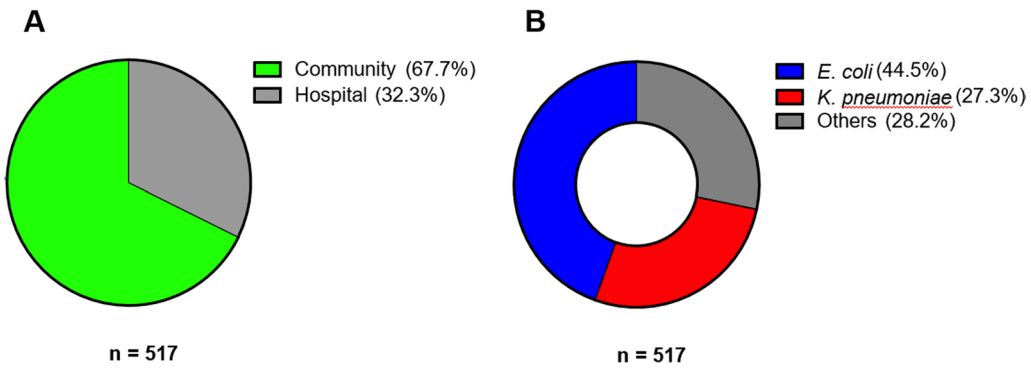

Fig. 2 **A** Isolates from hospital and community, **B** proportions of isolated organisms

55.1% (92/167) against 39.4% (138/350), respectively $p=0.0009$ (Fig. 3A). However, the isolation frequency of *K. pneumoniae* isolates from the hospital sewage (28.7% (48/167)) did not differ significantly from that of the community sewage 26.6% (93/350), $p=0.5994$ (Fig. 3B).

Resistance patterns of *E. coli* and *K. pneumoniae* isolates from the hospital and community sewage

E. coli isolated from hospital and community samples were similarly highly resistant to most antibiotics except chloramphenicol, with hospital *E. coli* 6.5% (6/92) isolates being significantly more susceptible to chloramphenicol compared to community isolates, 26.1% (36/138), $p=0.0001$. However, *K. pneumoniae* isolates from the hospital sewage were significantly more resistant to cefotaxime 31/48 (64.6%), against 45/93 (48.4%), gentamicin 39.6% (19/48) against 12.9% (12/93), imipenem 43.8% (21/48) against 26.9% (25/93) and sulfamethoxazole/trimethoprim 62.5% (30/48), against 36.6% (34/93) than those from the community, $p<0.05$ (Table 1).

The percentage of multidrug resistance of *E. coli* and *K. pneumoniae* to tested antibiotics at different sampling points

The isolation frequency of MDR enteric bacteria at different sampling sites is shown in Fig. 4. The percentages were 47.6% in hospital chambers, 62.0% in hospital ponds, 58.1% in the treated hospital

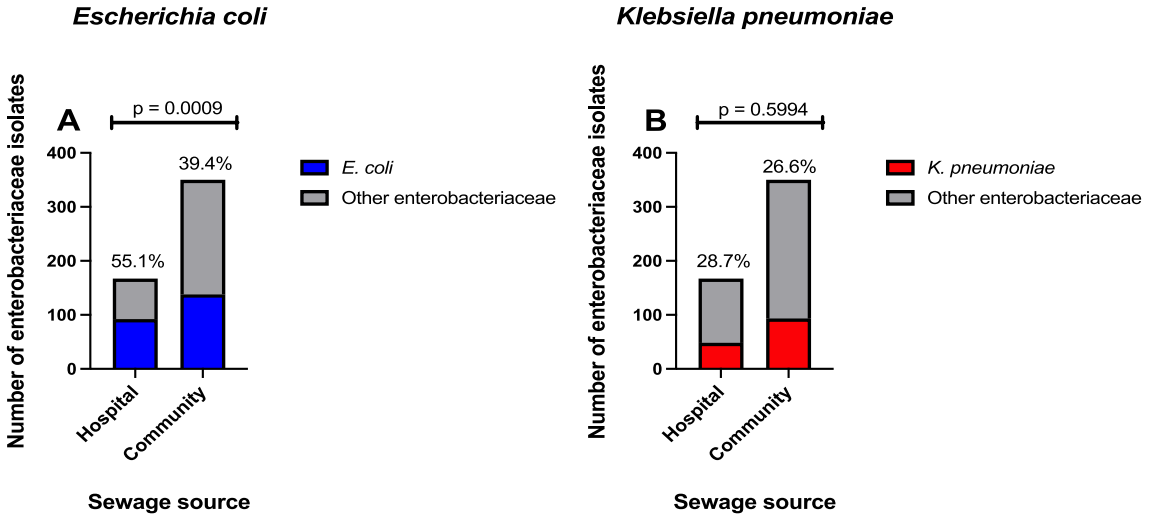

Fig. 3 Distribution of the *E. coli* and *K. pneumoniae* isolates among hospital and community sewage systems

Table 1 Antibiotic resistance pattern of *E. coli* and *K. pneumoniae* isolated from hospital and community sewage

Antibiotics	*E. coli*			*K. pneumoniae*		
	Resistance, *n* (%)			Resistance, *n* (%)		
	Hospital ($n=92$)	Community ($n=138$)	*p*-value	Hospital ($n=48$)	Community ($n=93$)	*p*-value
AMP	67 (72.8%)	101 (73.2%)	0.534	48 (100%)	87 (93.5%)	0.078
CTX	48 (52.2%)	82 (59.4%)	0.171	31 (64.6%)	45 (48.4%)	0.049
CHL	06 (6.5%)	36 (26.1%)	0.0001	8 (16.7%)	11 (11.8%)	0.291
GEN	38 (41.3%)	48 (34.8%)	0.194	19 (39.6%)	12 (12.9%)	0.0001
TET	61 (66.3%)	77 (55.8%)	0.072	11 (22.9%)	28 (30.1%)	0.242
NAL	47 (51.1%)	79 (57.2%)	0.216	23 (47.9%)	33 (35.5%)	0.106
CIP	72 (78.3%)	119 (86.2%)	0.082	36 (75.0%)	73 (78.5%)	0.394
IMI	37 (40.2%)	53 (38.4%)	0.380	21 (43.8%)	25 (26.9%)	0.034
SXT	58 (63.0%)	74 (53.6%)	0.100	30 (62.5%)	34 (36.6%)	0.003

n = Total number of isolates in the corresponding sewage system, *AMP* ampicillin, *CTX* cefotaxime, *CHL* chloramphenicol, *GEN* gentamicin, *TET* tetracycline, *NAL* nalidixic acid, *CIP* ciprofloxacin, *IMI* imipenem, *SXT* sulfamethoxazole/trimethoprim

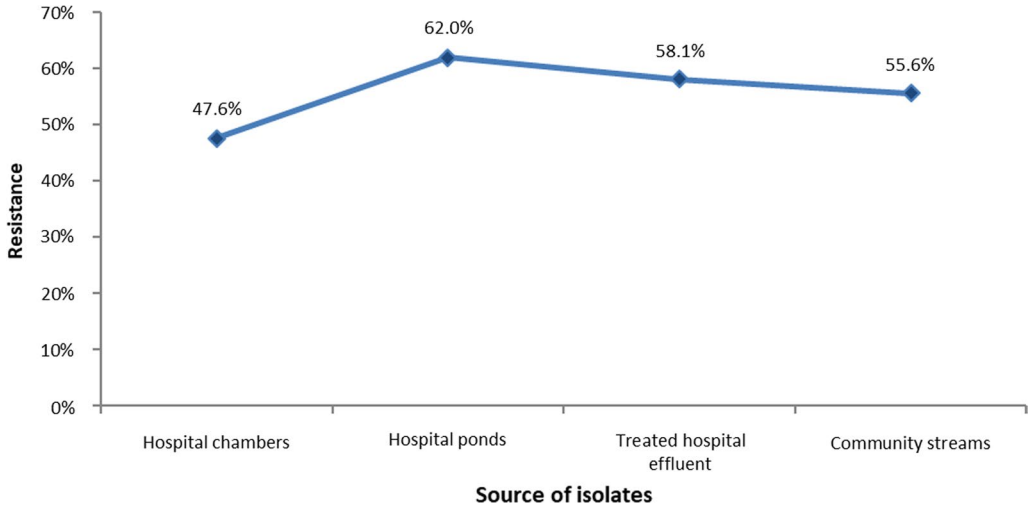

Fig. 4 Isolation frequency of resistant *E. coli* and *K. pneumoniae* to the tested antibiotics

wastewater and 55.6% in the community stream draining into the Indian Ocean.

Proportion of MDR *E. coli* and *K. pneumoniae* in the hospital and community sewage

Overall, the proportion of MDR isolates was high for both *E. coli* 80.9% (186/230) and *K. pneumoniae* 71.6% (101/141) (Fig. 5A). There was no significant difference between the two sewage sources, with the frequency of hospital and community MDR *E. coli* being 83.7% (77/92) and 79.0% (109/138), respectively ($p=0.3979$) (Fig. 5B). A similar pattern was observed among *K. pneumoniae* isolates, with the proportion of MDR in the hospital and community sewage isolates being 79.2% (38/48) and 67.7% (63/93), respectively, $p=0.1725$ (Fig. 5C).

The patterns of MDR isolates

Overall, most of the MDR isolates were resistant to four classes of antibiotics 27.2% (78/287), and 6.9% (20/287) of the isolates were resistant to eight classes of antibiotics. The most frequent MDR pattern was PEN/CEP/TET/QNL/SUL, exhibited by 14.2% (38/287) of the isolates (Table 2).

The proportion of ESBL-producing *E. coli* and *K. pneumoniae* in the sewage

ESBL production was observed in 40% (92/230) of the *E. coli* isolates and 36.2% (51/141) of the isolated *K. pneumoniae* (Fig. 6A). The proportion of ESBL-producing *E. coli* was significantly higher in the community 46.4% (64/138) than in the hospital sewage 30.4% (28/92), $p=0.0194$ (Fig. 6B). However, for *K. pneumoniae,* isolates from the hospital sewage had a significantly higher proportion of ESBL producers than those from the

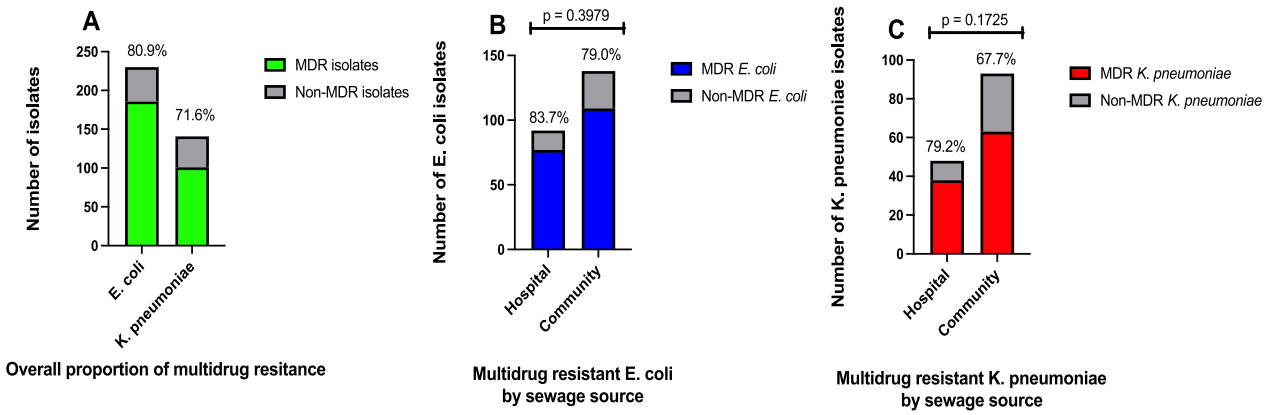

Fig. 5 Proportion of multidrug resistance *E. coli* and *K. pneumoniae* isolates from hospital and community sewage

Table 2 Resistance patterns of MDR *E.coli* and *K. pneumoniae* isolates

No. of antibiotics classes	Resistance pattern	No. of isolates	Percentage (%)
3	AMI/QNL/CAR	4	1.48
	AMI/TET/QNL	3	1.11
	PEN/AMN/QNL	2	0.74
	PEN/AMN/SUL	2	0.74
	PEN/CEP/QNL	23	8.49
	PEN/PHE/QNL	8	2.95
	PEN/QNL/CAR	1	0.37
	PEN/QNL/SUL	3	1.11
	PEN/TET/QNL	2	0.74
	PHE/QNL/SUL	2	0.74
	TET/CAR/SUL	3	1.11
4	CEP/AMN/QNL/CAR	3	1.11
	CEP/QNL/CAR/SUL	6	2.21
	PEN/AMN/QNL/CAR	4	1.48
	PEN/AMN/QNL/SUL	2	0.74
	PEN/CEP/AMN/QNL	5	1.85
	PEN/CEP/AMN/SUL	2	0.74
	PEN/CEP/PHE/QNL	2	0.74
	PEN/CEP/QNL/CAR	11	4.06
	PEN/CEP/QNL/SUL	6	2.21
	PEN/CEP/TET/SUL	8	2.95
	PEN/QNL/CAR/SUL	6	2.21
	PEN/TET/QNL/CAR	2	0.74
	PEN/TET/QNL/SUL	21	7.75
5	CEP/TET/QNL/CAR/SUL	4	1.48
	PEN/AMN/QNL/CAR/SUL	2	0.74
	PEN/CEP/AMN/QNL/CAR	7	2.58
	PEN/CEP/AMN/TET/QNL	2	0.74
	PEN/CEP/QNL/CAR/SUL	6	2.21
	PEN/CEP/TET/QNL/CAR	2	0.74
	PEN/CEP/TET/QNL/SUL	38	14.02
	PEN/PHE/QNL/CAR/SUL	2	0.74
	PEN/PHE/TET/QNL/CAR	1	0.37
	PEN/PHE/TET/QNL/SUL	4	1.48
	PEN/TET/QNL/CAR/SUL	2	0.74
6	PEN/CEP/AMN/QNL/CAR/SUL	17	6.27
	PEN/CEP/AMN/TET/QNL/CAR	3	1.11
	PEN/CEP/AMN/TET/QNL/SUL	7	2.58
	PEN/CEP/PHE/TET/QNL/SUL	2	0.74
	PEN/CEP/TET/CAR/QNL/SUL	5	1.85
	PEN/CEP/TET/QNL/CAR/SUL	4	1.48
	PEN/PHE/AMN/TET/QNL/SUL	3	1.11
7	PEN/CEP/AMN/TET/QNL/CAR/SUL	12	4.43
	PEN/CEP/PHE/AMN/TET/QNL/CAR	4	1.48
	PEN/CEP/PHE/TET/QNL/CAR/SUL	7	2.58
	PEN/PHE/AMN/TET/QNL/CAR/SUL	2	0.74
8	PEN/CEP/PHE/AMN/TET/QNL/CAR/SUL	20	7.38
		287	77.35

KEY: *PEN* phenolics, *CEP* cephalosporins, *PHE* phenolics, *AMN* aminoglycosides, *TET* tetracyclines, *QNL* quinolones, *CAR* carbapenems, *SUL* sulphonamides

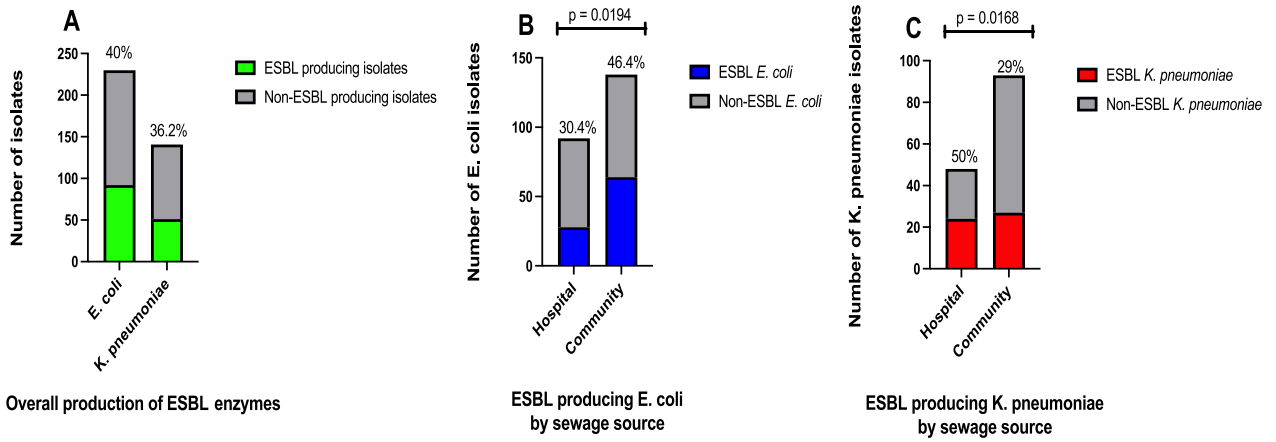

Fig. 6 Proportion of ESBL-producing *E. coli* and *K. pneumoniae* isolates from hospital and community sewage

community, 50% (24/48) against 29% (27/93), respectively, $p=0.0168$ (Fig. 6C).

Resistance patterns of ESBL-producing *E. coli* and *K. pneumoniae* from the sewage

ESBL-producing *E. coli* from both the hospital and community wastewater were highly resistant to most antibiotics, with hospital isolates being significantly more resistant to tetracycline than community isolates, $p=0.022$. All hospital isolates of ESBL-producing *E. coli* were susceptible to chloramphenicol. ESBL-producing *K. pneumoniae* were also highly resistant to the tested antibiotics, with no significant difference seen between isolates from the two sources of wastewater (Table 3).

The proportion of quinolone-resistant *E. coli* and *K. pneumoniae* in the hospital and community sewage

Resistance to quinolones among *E. coli* and *K. pneumoniae* isolates was 54.8% (126/230) and 39.7% (56/141), respectively (Fig. 7A). There was no significant difference between community and hospital *E. coli* isolates, 57.2% (79/138) against 51.1% (47/92), respectively $p=0.4174$ (Fig. 7B). Similarly, for *K. pneumoniae*, no significant differences in isolation frequency were observed between hospital sewage isolates 47.9% (23/48) and those from the community, 35.5% (33/93), $p=0.2034$ (Fig. 7C).

Resistance patterns of quinolone-resistant *E. coli* and *K. pneumoniae* in hospital and community sewage

Quinolone-resistant *E. coli* from the hospital wastewater were more susceptible to chloramphenicol than those from the community, $p=0.0001$. All quinolone-resistant *K. pneumoniae* from the community wastewater were susceptible to gentamycin (Table 4).

The proportion of carbapenemase-producing *E. coli* and *K. pneumoniae* in the hospital and community sewage

Carbapenem resistance among *E. coli* was 39.6% (91/230), while in *K. pneumoniae* was 32.6% (46/141) (Fig. 8A). There was no significant difference in carbapenem resistance between *E. coli* isolates from the hospital 41.3% (38/92) and those from the community 38.4% (53/138), $p=0.6815$ (Fig. 8B). Similarly, the proportion of hospital *K. pneumoniae* isolates with carbapenem resistance

Table 3 Resistance patterns of ESBL-producing *E. coli* and *K. pneumoniae* in the hospital and community sewage

Antibiotics	ESBL-producing *E. coli* Resistance, *n* (%)			ESBL-producing *K. pneumoniae* Resistance, *n* (%)		
	Hospital ($n=28$)	Community ($n=64$)	*p*-value	Hospital ($n=24$)	Community ($n=27$)	*p*-value
CHL	0 (0.0%)	18 (28.1)	0.001	04 (16.7%)	04 (14.8%)	0.578
GEN	12 (42.9%)	25 (39.1%)	0.454	14 (58.3%)	08 (29.6%)	0.051
TET	24 (85.7%)	42 (65.6%)	0.022	11 (45.8%)	16 (59.3%)	0.249
NAL	22 (78.6%)	53 (82.8%)	0.416	15 (62.5%)	16 (59.3%)	0.521
CIP	26 (92.9%)	62 (96.9%)	0.365	24 (100%)	25 (92.6%)	0.491
SXT	22(78.6%)	39 (60.9%)	0.999	22 (91.7%)	25 (92.6%)	0.999

n total number of isolates in the corresponding sewage source *CHL* chloramphenicol, *GEN* gentamicin, *TET* tetracycline, *NAL* nalidixic acid, *CIP* ciprofloxacin, *SXT* sulfamethoxazole /trimethoprim

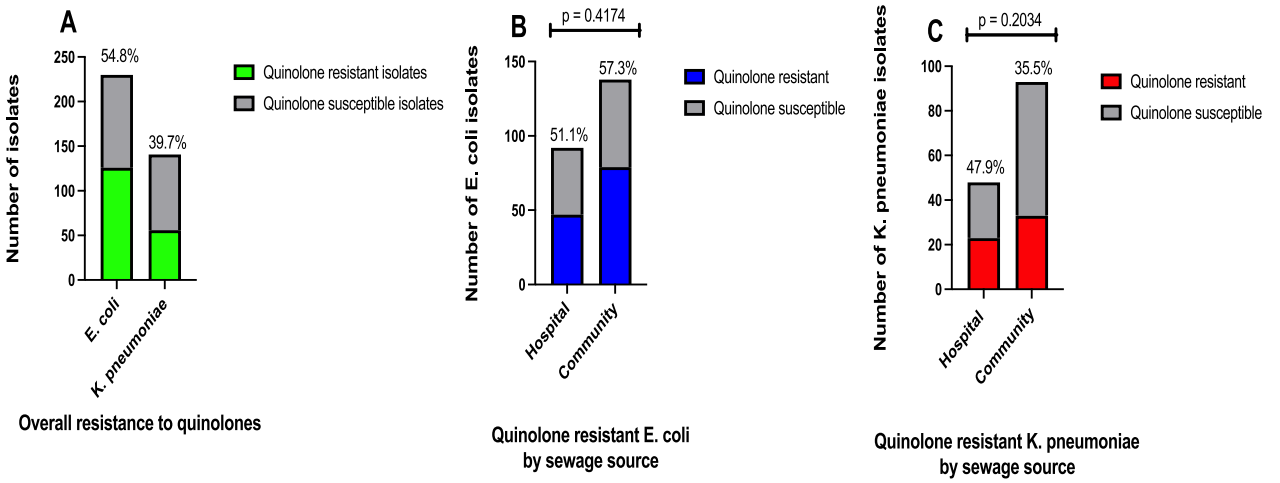

Fig. 7 Proportion of quinolone-resistant *E. coli* and *K. pneumoniae* isolates from hospital and community sewage systems

Table 4 Resistance patterns of quinolone-resistant *E. coli* and *K. pneumoniae* isolates

Antibiotics	Quinolone resistance *E. coli* Resistance, *n* (%)			Quinolone resistance *K. pneumoniae* Resistance, *n* (%)		
	Hospital (*n* = 47)	Community (*n* = 79)	*p*-value	Hospital (*n* = 23)	Community (*n* = 33)	*p*-value
CHL	06 (12.8%)	36 (45.6%)	0.0001	04 (17.4%)	07 (21.2%)	0.500
GEN	20 (42.6%)	37 (46.8%)	0.390	07 (30.4%)	00 (0.0%)	0.001
TET	34 (72.3%)	51 (64.6%)	0.241	7 (30.4%)	15 (45.5%)	0.197
SXT	28 (59.6%)	52 (65.8%)	0.303	15 (65.2%)	16 (48.5%)	0.167

n number of isolates in corresponding sewage source, *CHL* chloramphenicol, *GEN* gentamicin, *TE* tetracycline, *SXT* sulfamethoxazole/trimethoprim

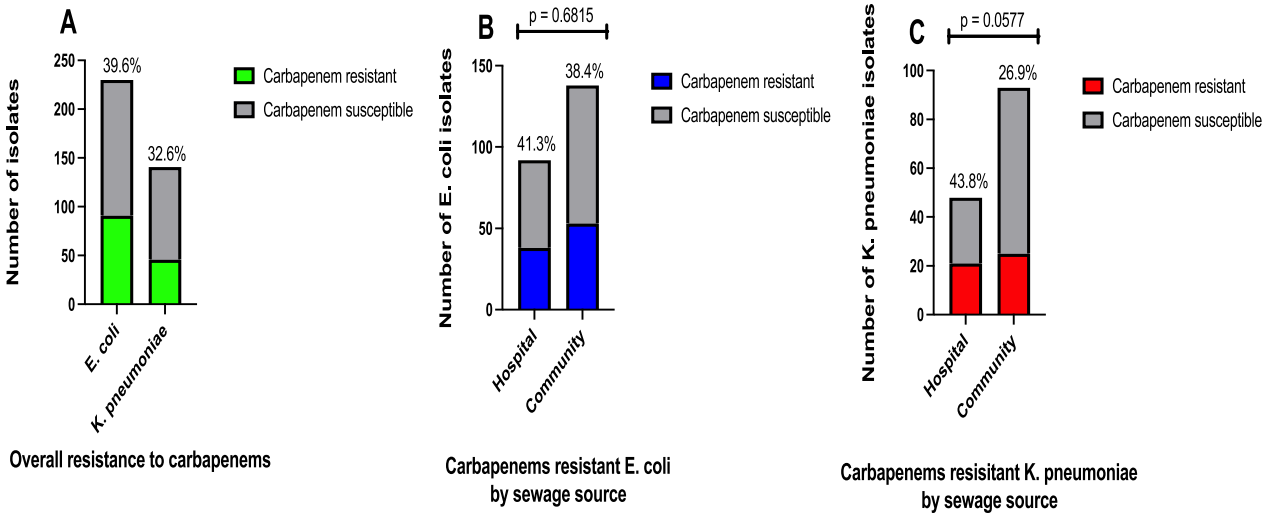

Fig. 8 Proportion of carbapenem-resistant *E. coli* and *K. pneumoniae* in sewage, overall and by source

43.8% (21/48) did not significantly differ from that of the community sewage 26.9% (25/93), $p = 0.0577$ (Fig. 8C).

Resistance patterns of carbapenemase-producing *E. coli* and *K. pneumoniae* in the hospital and community sewage

Carbapenem-resistant *E. coli* from the hospital sewage were significantly less resistant to chloramphenicol compared to community isolates. Additionally, they were highly resistant to gentamycin and sulfamethoxazole/trimethoprim like their community counterparts. Carbapenem-resistant *K. pneumoniae* from the hospital sewage were significantly more resistant to gentamicin and sulfamethoxazole/ trimethoprim than the community isolates (Table 5).

Discussion

In this study, we found high proportions of drug-resistant, including MDR *E. coli* and *K. pneumoniae*, in the wastewater samples from all sampled sites from the hospital through the community sewage system to the point where it enters the Indian Ocean. A quarter of the isolates showed resistance to four antimicrobial classes, while some of the isolates resisted all eight classes of antimicrobial drugs. The most frequent MDR pattern was PEN/CEP/TET/QNL/SUL, exhibited by most of the isolates. In addition, biological treatment did not significantly reduce the proportion of resistant bacteria isolated from the hospital ponds compared to those isolated from hospital effluents. We found some variations in the proportion of quinolone and carbapenem-resistant *E. coli* and *K. pneumoniae* isolated from the hospital and community sewage wastewater. The isolation frequency of quinolone-resistant *E. coli was* higher in the community wastewater (57.3%) than among the hospital isolates (51.7%). Comparable findings were reported in Romania, whereby the proportions of MDR *E. coli* from the hospital and community wastewater were 85.11% and 73.53%, respectively (Gaşpar et al. 2021). Another study conducted in Nigeria found that hospital sewage harboured a high proportion (86.9%) of MDR *E. coli* and *K. pneumoniae*, with the majority showing resistance to more than two classes of tested antibiotics (Osadebe and Okounim 2020). We found co-occurrence of ESBL and carbapenemase resistance in many MDR *E. coli* and *K. pneumoniae* isolates, due to shared transfer mechanisms, implying that infections with such bacteria often result in high morbidity and mortality rates (Mazzariol et al. 2017).

Our results have several implications: (i) the persistence of a high proportion of MDR bacteria certainly indicates that the sewage system in the studied area is a major driver of AMR in the community, (ii) the lack of significant difference in reduction in the trend of isolation frequencies of MDR bacteria from the hospital pond (62.0%) to 58.1% from the treated effluent released to the community streams and 58.6% in the community sewage wastewater before being released to the Indian ocean signifies ineffective biological treatment of sewage wastewater, at the treatment plants, and (iii) the loads of highly resistant bacteria being discharged into Indian Ocean poses public health issues of international concern. This is highly significant given the fact the World Health Organization has classified *Enterobacteriaceae*, carbapenem-resistant, and ESBL-producing bacteria as critical pathogens that can pass along genetic material that allows other bacteria to become resistant to the best available antibiotics for treating MDR bacteria (WHO 2017). The classification was based on how deadly the infections they cause are, specifically (i) duration of hospital stays, (ii) frequency of potential occurrence of resistance to existing antibiotics, (iii) the extent of spread between animals, from animals to humans, and (iv) whether new antibiotics to treat them are already in the research and development (R&D) pipeline.

Our findings conform to other studies that have shown hospital wastewater to be the hotspot for ARB and ARG, especially high proportion of MDR-*E. coli* with the potential of being transmitted to the community (Gumede et al. 2021). The lack of appreciable reduction in ARB and ARG in the wastewater treatment plant effluents has also been shown by others (Leclercq et al. 2013; Okoh and Igbinosa 2010) leading to large amounts of resistant bacteria, of hospital origin being released into the recipient waters (Rizzo et al. 2013a, b). In Temeke, waster is treated biologically by aerobic digestion technique, which does

Table 5 Resistance patterns of carbapenemase-producing *E. coli* and *K. pneumoniae* in the hospital and community sewage

Antibiotics	Carbapenemase-producing *E. coli* Resistance, *n* (%)			Carbapenemase-producing *K. pneumoniae* Resistance, *n* (%)		
	Hospital ($n = 38$)	Community ($n = 53$)	*p*-value	Hospital ($n = 21$)	Community ($n = 25$)	*p*-value
CHL	03 (7.9%)	24 (45.3%)	0.0001	03 (14.3%)	03 (12.0%)	0.578
GEN	19 (50.0%)	34 (64.2%)	0.128	18 (85.7%)	06 (24.0%)	0.0001
TET	25 (65.8%)	33 (62.3%)	0.452	5 (23.8%)	07 (28.0%)	0.508
SXT	30 (78.9%)	29 (54.7%)	0.025	17 (81.0%)	12 (48.0%)	0.032

n number of isolates in corresponding sewage source, *CHL* chloramphenicol, *GEN* gentamicin, *TET* tetracycline, *SXT* sulfamethoxazole/trimethoprim

not seem to be effective. During the study, we observed the following constraints in sewage management practices; low priority accorded to sanitation and hygiene improvement, inadequate investment financial resources, fragmented planning, limited participation of beneficiaries and other stakeholders, inadequate availability of effective sewerage and sanitation systems, lack of attention on selecting the most appropriate technology and general low public awareness. This calls for significant allocation of resources and modification in wastewater treatment protocols and continuous monitoring for AMR and antimicrobial groups (AMG). The study of resistant microbes in sewage should cover a range of factors, including the evolution of resistance at the molecular level within a given organism, transmission mechanisms and pathways between organisms, and dissemination to humans and animal hosts and across the wider environment including soil and water. Indeed, hospital and community wastewater are now known to be the source of AMR transmission within the environment (Daoud et al. 2017), and therefore, curbing of AMR needs to involve a One Health approach since all three compartments need to be considered (O'neill 2016). We advocate that future research on AMR and sewage should focus on identifying the influence of various interventional activities such as (i) antibiotic stewardship in hospital settings, environmental sanitation (effective disposal of waste), transmission pathways-resistant bacteria and cost-effective sustainable technological, social and economic initiatives for the mitigation of environmental antibiotic resistance.

Although this study has provided valuable information to the international community, we do acknowledge some limitations. First, the study was conducted in dry season, the pattern of AMR could have seasonal variations (Ramsey et al. 2019). Secondly, due to logistical issues we could not perform whole genome sequencing (WGS) of the MDR *E. coli* and *K. pneumoniae*, which could have provided an insight into the AMR transmission dynamics of the wastewater from the hospital sewage down the stream to the Indian Ocean. Future studies should use advanced technologies such as WGS and metagenomics and involve several compartments (sewage and the surrounding community) to decipher dynamics and transmission patterns and pathways of resistomes among the various compartments. This approach is important given the immense diversity of antibiotic resistance genes (ARGs), the complexity of ARG transfer, and the broad range of omnipresent factors contributing to AMR.

Conclusions

The high proportions of drug-resistant, including multidrug-resistant, *E. coli* and *K. pneumoniae* in the wastewater samples from all sampled sites from the hospital through the community sewage system to the point where it enters the Indian Ocean are a major threat to public and the environment. The lack of significant difference in reduction in the trend of isolation frequencies of MDR bacteria from the hospital sewage system to the Indian Ocean signifies ineffective biological treatment of sewage wastewater, at the treatment plants. The loads of highly resistant bacteria being discharged into Indian Ocean possess public health issues of international concern. Strict implementation of appropriate disinfection technologies for hospital sewage would reduce the bacterial load in the sewage that will reach urban wastewater treatment plants, minimizing the spread of the resistance pathogens in the environment.

Abbreviations

AMP	Ampicillin
AMR	Antimicrobial resistance
AMU	Antimicrobial use
ARB	Antibiotic-resistant bacteria
ARG	Antibiotic resistance genes
ATCC	American Type Culture Collection
CHL	Chloramphenicol
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standards Institute
CTX	Cefotaxime
DMDP	Dar es Salaam Metropolitan Development Project
E. coli	*Escherichia coli*
ESBL	Extended spectrum beta lactamase
GEN	Gentamicin
IMI	Imipenem
IPC	Infection prevention and control
K. pneumoniae	*Klebsiella pneumoniae*
KCMC	Kilimanjaro Christian Medical College
KIA	Kligler Iron Agar
MDR	Multidrug-resistant
MHA	Mueller Hinton Agar
MUHAS	Muhimbili University of Health and Allied Sciences
NAL	Nalidixic acid
SPSS	Statistical Package for Social Sciences
SXT	Sulphamethoxazole/trimethoprim
TET	Tetracycline
TRRH	Temeke Regional Referral Hospital
UK	United Kingdom

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Author contributions

NZS, MIM and FM contributed to conception and study design; NZS, ZK and FXM contributed to data collection and laboratory investigations; NZS, MIM and FM analysed the data; AM, NZS, FM and MIM drafted the initial manuscript; AM, NZS, ZK, FM, AJ and MIM reviewed the final manuscript. All the authors read and approved the final version.

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Availability of data and materials

All data generated and/ or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

The Research and Publications Committee of the Muhimbili University of Health and Allied Sciences (MUHAS) approved the study reference number DA.282/298/01.C/. Permission to conduct the study was granted by Temeke regional referral hospital and the Dar es Salaam water supply and sanitation authority (DAWASA).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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