2023

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Elsevier

http://dx.doi.org/10.1016/j.enceco.2022.10.003

Environmental Chemistry and Ecotoxicology 5 (2023) 1–8

Contents lists available at ScienceDirect

Environmental Chemistry and Ecotoxicology

journal homepage: www.keaipublishing.com/en/journals/environmental-chemistry-and-ecotoxicology/

Antibiotic-resistant microbial populations in urban receiving waters and wastewaters from Tanzania

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ARTICLE INFO

Keywords:
Antimicrobial resistance (AMR)
WWTPs
Urban water bodies
Wastewaters
Enterobacteriaceae pathogens
Antibiotic-resistant bacteria (ARB)
Antibiotic resistant genes (ARGs)

ABSTRACT

Antimicrobial resistance against certain medications in the pathogenic microbial community is globally increasing due to the continual discharge and disposal of pharmaceuticals in the environment. The phenomenon resulted in significant antibiotic resistance among several exposed Enterobacteriaceae species, with wastewater treatment plants (WWTPs) and rivers serving as significant reservoirs. Despite antibiotic resistance being a tragedy, particularly in treating diseases by using antibiotics, local and regional studies indicating the severity, resistant species and the molecular level insight into these pathogens are scarce, thus requiring immediate intervention. This study, therefore, investigated wastewater from wastewater treatment ponds and receiving waters for the presence of resistant pathogens through phenotypical and molecular approach screening of their genes. Among the 57 analyzed samples, 18 (67%, $n = 27$) isolates of *Klebsiella* spp.., 4 (80%, $n = 5$) isolates of *Proteus* spp., 1 (100%, $n = 1$) of isolated *Pseudomonas aeruginosa* and 6 (18%, $n = 34$) of *E. coli* found were resistant to at least 1 among the tested antibiotics. *E. coli* had an 83% higher proportion of multi-drug resistance (MDR) than *Klebsiella* spp.., which had 68.5%, and no MDR was shown by *P. aeruginosa* isolates. Among the 20 bacterial isolates of antibiotic-resistant genes, showed that *E. coli* harboured 39%, followed by 22% of *Klebsiella* spp. Eleven (11) isolates of these 20 (55%) contained sulphonamides resistant genes: *Sul 1* ($n = 4$) and *Sul 2* ($n = 7$). Ten (10) isolates (50%) contained the tetracycline-resistant genes in which 4 isolates showed the *Tet A, Tet B – 1* and 5 isolates contained *Tet D*. β-lactamases (*bla CTX-M* and *bla SHV*) were found in 7 isolates (35%). The existence of these antibiotic-resistant species in the urban receiving and wastewater presents a threat of transmission of diseases to humans and animals that are not cured by the existing medications, jeopardizing public health safety.

1. Introduction

The increased need to combat communicable and non-communicable diseases has resulted in a global increase in the consumption of pharmaceuticals and their products for diagnosis, vaccination, treatment and recreation [1–5]. Partial metabolisms, accidental or intentional disposal of out-of-use drugs, and rampant misuse of antibiotics (under dose, not warranted use, uncontrolled availability of antibiotics), have been reported as potential sources of aquatic pollution [6–9]. Apart from these sources, wastewater treatment plants (WWTPs), clinical facilities [10–12], and treatment schemes release active chemicals (ACs) into the environment that may lead to the distribution of pharmaceuticals to various environmental compartments [13–16], leading to injury of ecosystems. Most contaminated sites were found in low to middle-income countries due to deteriorating waste management infrastructure and relying on wastewater treatment ponds that are not designed to

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Production and hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.enceco.2022.10.003
Received 21 July 2022; Received in revised form 13 October 2022; Accepted 13 October 2022
Available online 18 October 2022

eliminate pharmaceutical contaminants [17]. The presence of abundant pharmaceutical contaminants, particularly antibiotics, in the water exposes a non-targeted microbial community [18,19]. As antibiotics are active chemicals at minute concentration, either they may kill the exposed organism or those which will survive their genetic makeup may be altered for adaptation of resistance [20,21]. Antibiotics are essential in treating humans and other animals by direct killing the responsible bacteria or blocking its growth and multiplication. However, the surviving exposed microbes develop antimicrobial resistance (AMR) [20,21]. Pathogenic microbes acquire and disseminate resistance through various reported mechanisms leading to ecosystem injury [22–24]. The WHO has reported AMR as a severe public health issue, expected to cost approximately 10 million lives annually by 2050. When bacteria, viruses, fungi, and parasites adapt and fail to respond to antibiotic therapy, infections become more difficult to treat and therefore, a high risk of disease spread, severe sickness, and death increases [17,25–32]. Antibiotic resistance has become a global agenda, especially in developing countries such as Tanzania. It is reported that antibiotic accessibility, self-prescription, inadequate antibiotic treatment, poor waste management and water quality challenges contribute to antibiotic resistance's rapid spread and development in pathogenic and environmental microorganisms [17,25–33]. This suggests that effluents from WWTPs could be a major cause of antibiotic resistance to some aquatic microbial species.

The major threat of AMR relies on gram-negative bacteria (GNB) due to their high susceptibility to antibiotic resistance. GNBs are particularly significant in hospitals because they put patients in danger and cause a high morbidity and mortality rate, implying that antibiotic resistance magnifies treatment complications, thus necessitating measures [11,12,18–21]. There are few local and regional reports of antibiotic-resistant microbes and antibiotic-resistance genes, especially in Tanzania's urban receiving and wastewater [34–37]. Yet, a major global health threat, antibiotic resistance is least addressed in developing countries [35]. In this case, the current study is designed to investigate the occurrence of aquatic antibiotic-resistant microbes through isolation and assessment of their ARGs from selected isolates to establish informed public health.

2. Materials and methods

2.1. Study area

A total of 57 samples were collected, whereby 47 were from wastewater, and 10 were from receiving waters among selected points of the Themi waste stabilization pond and Themi river receiving effluents from the treatment scheme in Arusha, Tanzania, as presented in Fig. 1.

Themi is an administrative ward located in the Arusha region in Tanzania. It has an area of 6.2 km^2 and a population of 9458 people, according to the 2012 Tanzania census [38]. In the western part of the ward borders with Themi river whole length is 46.41 km [38]. Themi, wastewater stabilization pond is the oldest stabilization pond receiving wastewater brought by sewage tracks while taking advantage of Themi river. However, due to rapid city growth, new wastewater treatment ponds are constructed at Terrat ward in Arusha Region, located along Themi river. Being the only operational, oldest pond in Arusha city and discharging its effluents in the Themi river, the studied area had sufficient potential to reflect the city's hygienic well-being and probable risks due to the transportation of contaminants at a long distance.

2.2. Microbiological analysis

2.2.1. Culture, isolation and identification and their biochemical confirmation

Wastewater samples were collected at a depth of 15 cm to 35 cm to avoid surface-suspended materials that may induce cross-contamination. Each sample was collected in a 20 mL sterile autoclavable polystyrene bottle with a screw cap that was rinsed three times with a water sample. At each sampling point, triplicate samples were collected and placed in a cool ice jar, followed by stored at 4 °C at the Nelson Mandela Institution of Science and Technology. Immediately, these samples were transported to the Sokoine University of Agriculture, College of Veterinary Medicine and Biomedical Sciences, Department of Microbiology Parasitology and Biotechnology laboratories for microbiological studies while maintained at 4 °C, as procedures adopted from [39–41].

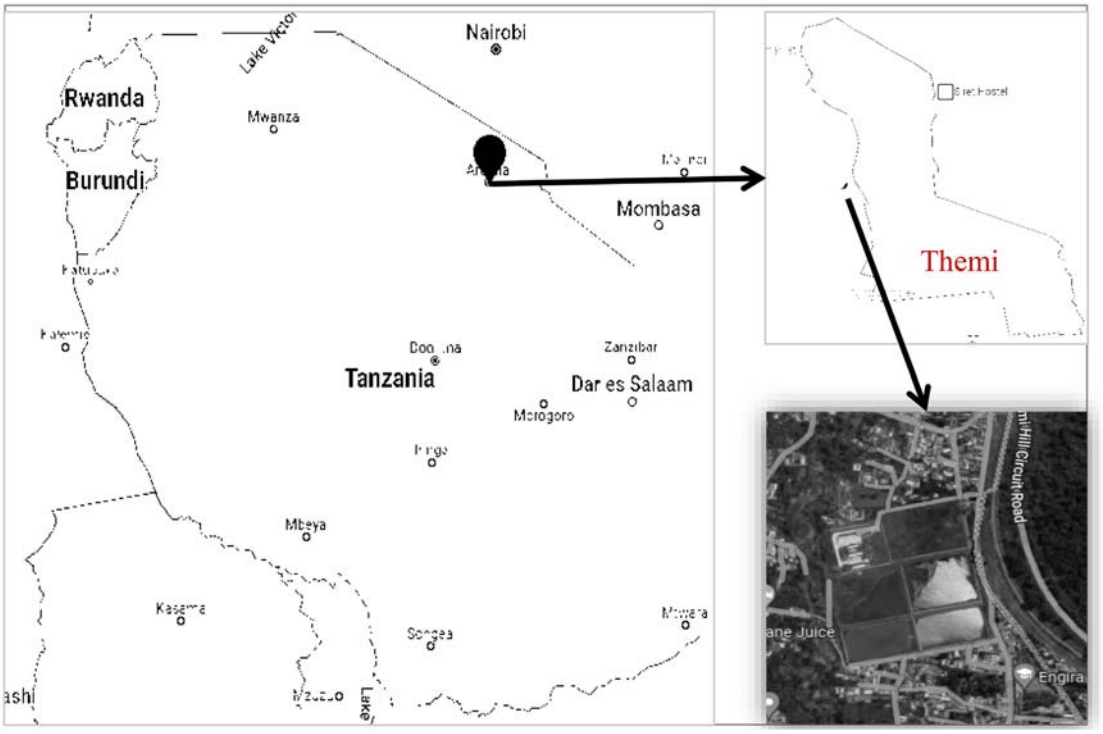

Fig. 1. Map of Tanzania showing the study area (Themi: −3.392481, 36.695334).

The bacteria were isolated by inoculating 3 mL of water samples in 5 mL sterilized peptone water buffer tubes and incubated at 37 °C for 24 h to enrich the bacteria. Then, one microliter of each bacterial culture was inoculated in the blood agar (Oxoid Ltd) and MacConkey agar (Oxoid Ltd) plates and incubated overnight at 37 °C, followed by subsequent sub-culturing to obtain pure colonies. All bacterial colonies were studied macro-morphologically and suspected of *Proteus* spp.., *Escherichia coli*, *Klebsiella* spp. and *Pseudomonas* spp. were subjected to microscopic observation and later biochemical profiling. Among others, the biochemical tests included; the oxidase test, Urease test, TSI test and IMViC test, as similarly reported [42,43].

2.3. Phenotypic antibiotic susceptibility testing

The resistance testing was performed by the disc diffusion method on Muller Hinton (MH) agar (Oxoid Ltd). The isolates were tested against quinolones, namely Ciprofloxacin (CIP), aminoglycoside, Gentamicin (CN) and tetracycline (TE). Other antibiotics were Penicillins, Penicillin (P) and Co–Trimoxazole Sulphamethoxazole/Trimethoprim (COT). All antibiotics were supplied by Sigma-Aldrich (St. Louis, MO, USA). Bacterial suspensions were made and adjusted to an opacity equivalent to 0.5 red from McFarland standard densitometer then the inoculum was transferred onto well-dried Mueller Hinton agar plates. The test organisms were uniformly seeded on the Mueller-Hinton agar surface and exposed to a concentration gradient of antibiotic diffusing from antibiotic-impregnated paper disk into the agar medium. Afterwards, the plates were incubated at 37 °C for 24 h (overnight). After 24 h of incubation, the diameter of inhibition zones was measured using a ruler, and the findings were classified as either sensitive, intermediate sensitive or resistant according to CLSI guideline of 2020 [44,45].

2.4. Genotypic analysis

2.4.1. DNA isolation

The genomic DNA was isolated using the thermal extraction method described by Carriero et al. (2016) [46]. Briefly, 1.0 mL of the Tryptic broth culture was pelleted, washed, and resuspended by vortexing in nuclease-free water (Sourced from Inqaba biotech, Hatfield, South Africa), placed in a water bath at 95 °C for 5 min and immediately transferred to the ice for 5 min. This procedure was repeated, and the suspension was centrifuged at 10,000 rpm for 10 min. The concentration and quality of the extracted DNA were checked by electrophoresis (1% agarose gel) and spectrophotometrically quantified using NanoDropTM Lite Spectrophotometer (Thermo Scientific, Waltham, U.S.A) and stored at −20 °C until further use.

2.5. Molecular identification of bacterial species

All isolates presumptively identified based on biochemical and phenotypic characteristics were subjected to molecular identification, as reported by [47–49]. The universal primers designed to give a product of approximately 1500 base pairs and are complementary to conserved regions of 16S rRNA genes were used for PCR amplification. PCR was performed using a master mix (Bioneers premix-Korea), and the amplification was done as follows; Initial denaturation steps at 95 °C for 3 min and followed by 35 cycles of denaturation at 95 °C for the 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 1 min followed by terminal extension at 72 °C for 3 min. The agarose gel (1%) stained with ethidium bromide was used to analyse PCR products by electrophoresis. Positive bands were visualized by ultraviolet trans-illumination.

2.6. Identification of resistance genes

All isolates that expressed phenotypical resistance were screened by PCR for the presence of various recognised resistance genes to different antibiotics according to [50–52]. Positive and negative controls were used for resistance genes. However, it was impossible to source positive controls for some screened genes. Without positive controls, optimized and previously published primers and PCR protocols were used. The prevalence of the *Sul1* and *Sul 2* genes in the genomic DNA via PCR with gene-specific primers is presented in Table 1. The amplification conditions for the *Sul 1* and *Sul 2* genes were as follows: 94 °C for 5 min; 30 cycles of 94 °C for 30 s, 69 °C for 30 s and 72 °C for 45 s; and one cycle of 72 °C for 7 min. To detect bla_{SHV} and $bla_{CTX\text{-}M}$. PCR amplification conditions were as follows: initial denaturation step at 95 °C for 5 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s. Extension at 72 °C for 2 min, followed by a final extension step at 72 °C for 10 min. Gel electrophoresis was performed on 1.5% agarose gels. Table 1 provides details of the primers used to detect Cephalosporins, Tetracyclines, Sulfonamides and 16SrRNA used in the present study.

2.7. Ethics statement

Permission was obtained from Arusha Urban Water and Sanitation Authority (AUWASA) for entry and collection at all sample locations. This study did not involve endangered or protected areas and species.

3. Results

3.1. Microbiological analysis

3.1.1. Culture, isolation, identification and biochemical confirmation

Macro-morphological characteristics of different bacteria were identified and analyzed, as presented in Fig. 2. The observed greenish colonies (Fig. 2C), which were medium to large with cattle eye appearance, were non-lactose fermenters when sub-cultured in MacConkey agar. Gram staining revealed gram-negative short rod bacteria arranged in singles. These

Table 1
Primers used to detect Cephalosporins, Tetracyclines, Sulfonamides and 16SrRNA.

Antibiotic	Gene	Primer (5′ -3′)	Size	A/temp	References
Cephalosporins	*bla* $_{SHV}$	F-ATG CGT TAT ATT CGC CTG TG R-AGC GTT GCC AGT GCT CGA TC	862	58	[53]
	Universal bla $_{CTX\text{-}M}$	F-5′-SCS ATG TGC AGY ACC AGT AA R-5′-CCG CRA TAT GRT TGG TGG TG	554	58	[54]
Tetracyclines	*Tet(A)*	(F) GGTTCACTCGAACGACGTCA (R) CTGTCCGACAAGTTGCATGA	577	57	[55]
	Tet(B)	(F) CCTCAGCTTCTCAACGCGTG (R) GCACCTTGCTGATGACTCTT	634	56	
	Tet D	AAA CCA TTA CGG CAT TCT GC GAC CGG ATA CAC CAT CCA TC	787	56	[54]
Sulfonamides	*Sul1*	(F) CGGCGTGGGCTACCTGAACG (R) GCCGATCGCGTGAAGTTCCG	433	69	[56]
	Sul2	(F) GCGCTCAAGGCAGATGGCATT (R) GCGTTTGATACCGGCACCCGT	293	69	
Universal Primer	*16 s rRNA*	27F AGAGTTTGATCATGGCTCAG 1492R TACGGYTACCTTGTTACGACTT	1500	58	

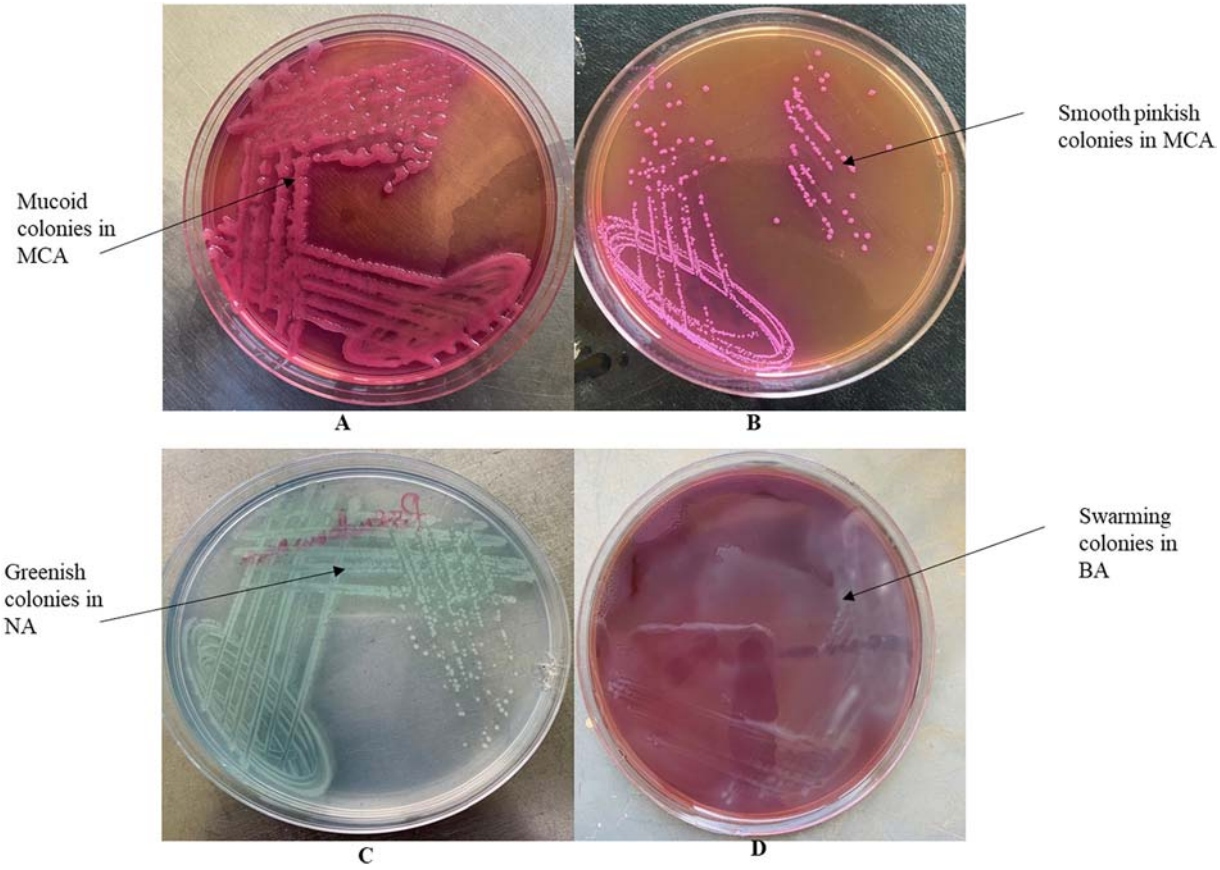

Fig. 2. Macro-morphological characteristics of different bacteria; *Klebsiella* spp. (Fig. 2A), *E. coli* (Fig. 2B), *Pseudomonas aeruginosa* (Fig. 2C) and *Proteus* spp. (Fig. 2D).

colonies were oxidase-positive when tested with oxidase strips. The conventional identification characteristics revealed that the bacterium was *P. aeruginosa,* as reported by [57–59]. Some bacteria in MacConkey agar had pinkish, smooth, shiny mucoid colonies ranging from medium to large, as indicated in Fig. 2-B. Blood agar displayed no haemolysis to these bacteria; however, some bacterial isolates showed swarming characteristics. All bacterial isolates in this group were gram-negative of varying cell sizes and arranged in singles. All isolates suspected of *Pseudomonas aeruginosa* were oxidase-positive, *Proteus* spp. were urease positive, and those suspected of *E. coli* or *Klebsiella* spp. responded positively according to their IMViC and TSI tests, as reported by [57–64]. The prevalence of these bacteria in the collected wastewater samples is given in Table 2.

3.2. Phenotypic antibiotic susceptibility testing

The findings revealed that 18 (67%, $n = 27$) isolates of *Klebsiella* spp.., 4 (80%, $n = 5$) isolates of *Proteus* spp.., 1 (100%, $n = 1$) isolating *Pseudomonas aeruginosa* and 6 (18%, $n = 34$) were resistant to at least 1 antibiotic out of 5 antibiotics that were used against them. All four bacterial species highly resisted penicillin (P), while the bacterial species showed less resistance to Gentamycin (CN), as shown in Table 3. Resistant isolates in their respective species were also assessed for phenotypic multidrug resistance (MDR), which is the resistance to at least three antibiotic classes among the 5 used drugs [65,66]. *E. coli* had an 83% higher proportion than *Klebsiella* spp., which had 68.5% and *P. aeruginosa,* which had 20%.

Table 2
Percentage of bacterial isolates from wastewater samples.

Bacteria species	Number of isolates ($n = 57$)	Percentage (%)
E. coli	34	59.65
Klebsiella spp.	27	47.37
Proteus spp.	5	8.77
Pseudomonas aeruginosa	1	1.75

3.3. Genotypic analysis

3.3.1. Molecular identification of bacterial species

A total of 20 Gram-negative bacterial isolates were amplified using universal primers targeting the 16SrRNA gene. Results showed that all positive isolates (Amplicons) appeared at 1500 bp, as shown in Fig. 3.

Whereby M is a 100 bp marker, lane 1–13 are samples, lane 1–13 are positives, and lane 14 and 15 are negative and positive controls. Lane 1 is *Pseudomonas* spp., lanes 2, 3, 4, 5, 6, 7, 8, and 9 are *Klebsiella species,* and lanes 10, 11, 12, and 13 are *Escherichia coli* spp. Positive products are located at 1500 bp.

3.4. Identification of resistance genes

The detection of antibiotic-resistant genes on 20 bacterial isolates showed that *E. coli* harboured more resistance genes (39%), followed by

Table 3
Phenotypic antibiotic resistance of bacterial species.

Antibiotic & Breakpoints	TE	CN	COT	CIP	P
	≤11	≤12	≤2	≤21	≤13
Microorganism					
Klebsiella spp. (n = 27)	17(63)	1(4)	12(44)	6(22)	18(67)
Pseudomonas aeruginosa (n = 1)	0(0)	0(0)	0(0)	0(0)	1(100)
E. coli (n = 34)	4(12)	1(3)	3(9)	1(3)	6(18)
Proteus spp. (n = 5)	5(100)	0(0)	1(20)	0(0)	4(80)

TE: Tetracyclines, CN: Gentamycin, COT: Co-Trimoxazole Sulphomethoxazole/ Trimethoprim CIP: Ciprofloxacin, P: Penicillin.

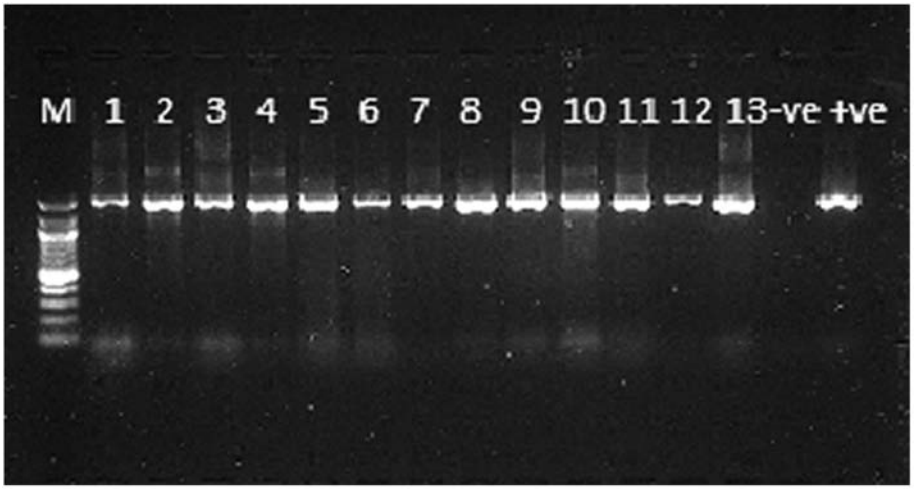

Fig. 3. PCR amplification of 16S rRNA.

Table 4
Occurrence of antibiotic-resistant genes detected on four bacterial species.

Bacterial species	*n*	*Sul1*	*Sul2*	*Tet A*	*Tet B*	*Tet D*	CTXM	SHV	MDR genes (%)
Pseudomonas spp.	1	0	0	0	0	0	0	0	0
Klebsiella spp.	11	3	4	4	1	2	1	2	22
E.coli spp.	4	1	3	0	0	3	2	2	39
Proteus spp.	4	0	0	0	0	0	0	0	0
%Resistant genes		20	35	20	5	25	15	20	

Klebsiella spp., 22%. Other bacterial species (*Pseudomonas and Proteus*) contained no resistant genes analyzed in this study, as shown in Table 4. Eleven isolates out of 20 contained sulphonamide-resistant genes as follows: *Sul 1* ($n = 4$) and *Sul 2* ($n = 7$), making up 55% of the total resistant gene analyzed in this study (Fig. 4 and Table 4). Ten (10) isolates (50%) contained the tetracycline-resistant genes, in which 4 isolates showed *Tet A, Tet B – 1*, and 5 isolates contained *Tet D* (Fig. 5 and Table 4). β-lactamases ($bla_{CTX\text{-}M}$ and bla_{SHV}) were found in 7 isolates (35%), with *E. coli* harbouring more resistance genes than *Klebsiella* species, as shown in Table 4.

4. Discussion

Fluorescent pseudomonads isolates of *P. aeruginosa* were categorized as mucoid or non-mucoid morphotypes [29]. Their pinkish, smooth, shiny, and mucoid colonies in Fig. 2 ranged in size from medium to large on MacConkey agar for several bacteria. These bacteria showed no haemolysis on blood agar, confirming its identification [42–45,47]. On the other hand, some bacterial isolates exhibited swarming behavior [43,51,61,62]. All the bacteria were gram-negative with different cell sizes and were grouped in singles [43,51,61,62]. All isolates suspected of *Pseudomonas aeruginosa* and *Proteus* spp.. were urease positive and those suspected of *E. coli* or *Klebsiella* spp. responded favourably to their IMViC, and TSI tests were oxidase-positive, confirming their presence [43,51,61,62]. The prevalence of these bacteria in urban receiving and wastewater samples collected from Themi is detailed in Table 2. *P. aeruginosa* is an aerobic pathogen facultative bacterium affecting both humans and plants. It causes soft rot in lettuce and cress plants [67], affecting nematodes, fruit flies and the moth [67]. Human

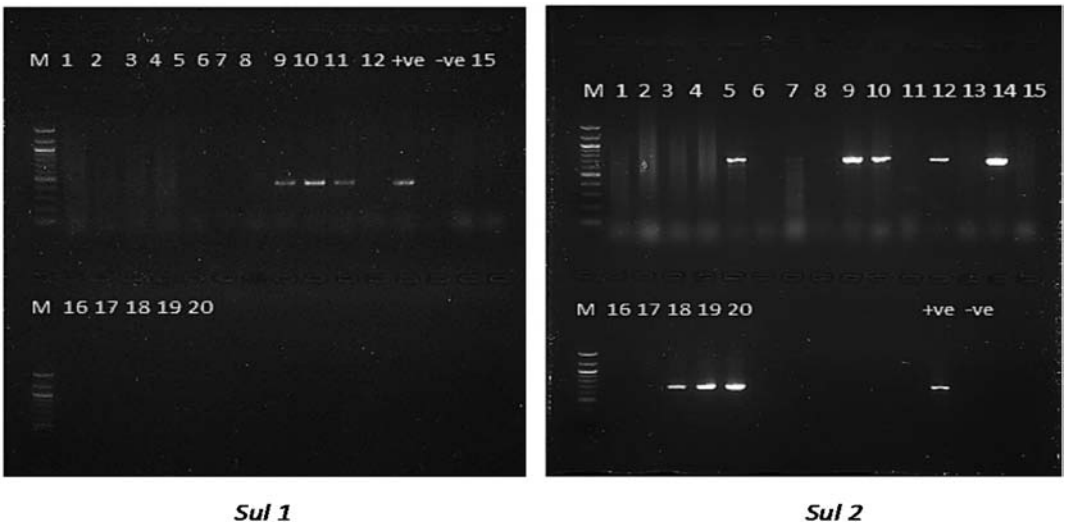

Fig. 4. PCR amplification of sulfonamide resistance gene (*Sul 1*). M is a 100 bp marker, and lane 1–12 and 15–20 are samples, where lane 9, 10 and 11 are positives, and lane 13 and 14 are positive and negative controls, respectively. *Sul 1* and lanes 5, 9, 10, 12, 14, 18, 19, and 20 are positives, and lanes 27 and 28 are positive and negative controls, respectively, for *Sul 2*.

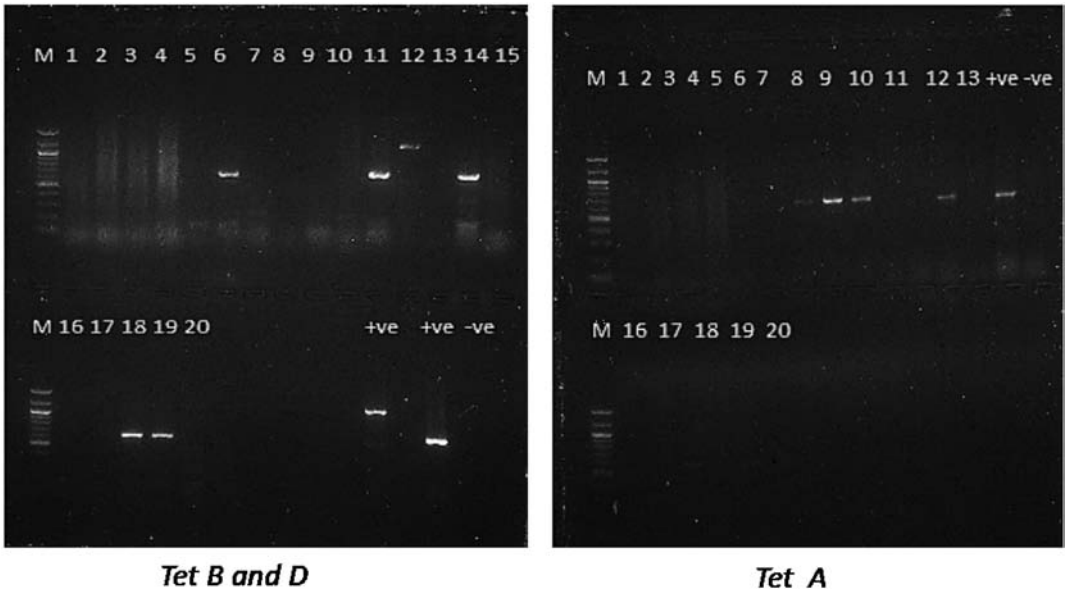

Fig. 5. PCR amplification of tetracycline resistance genes (*Tet B and Tet D*) and *Tet A*. M is a 100 bp marker, and lane 1–20 are samples, where lane 12 is a positive sample of the resistance gene for *Tet B* and lane 6,11,14, 18, and 19 are positives sample for the resistance gene for *Tet D*, and first positive control is for *Tet B* and the second for *Tet D*. In Tet A; M is a 100 bp marker, and lane 1–13,16–20 are samples, where lane 8,9,10,12, are positives and lane 14 and 15 are positive and negative controls respectively.

beings get the effect in their urinary tract, airway, blood infections, wounds and burns [67,68]. As wastewater after treatment is released into urban water bodies, the presence of *P. aeruginosa* may impair ecosystem health.

Among the identified microbes, *Escherichia coli* was dominant at 59.65%, followed by *Klebsiella* spp. by 47.37%. A similar study by Kayode and colleagues revealed that *E. coli* was the predominant enteric bacteria isolates by 39.3%, followed by *Klebsiella* spp. by 6.6%, *Proteus* spp. was 13.1% from teaching hospitals in Southwest Nigeria [30]. During this study, these isolates were resistant to at least 1 antibiotic out of 5 used against them. Penicillin (P) was highly resisted among all four bacterial species, while the bacterial species showed less resistance to gentamycin (CN), as indicated in Table 3. Most species of *E. coli,* including [69], have no effect in humans and contribute to the human gut's healthful bacterial flora. However, species, including [70], are reported to cause abdominal pain, fever, diarrhoea and even vomiting [70]. *K. pneumonia* is among bacterial species residing in the intestine and faeces of humans without causing harmful effects. However, their occurrence in other parts of the human body affects the liver, lungs, brain, eyes, bladder and blood [12,21].

Resistant isolates in their respective species were also assessed for phenotypic multidrug resistance (MDR), which is the resistance to at least three antibiotic classes. *E. coli* had a higher proportion (83%) of MDR than the rest of the bacterial spp., and no MDR phenomenon was shown *by the P. aeruginosa* isolate. In a similar study, multidrug resistance (resistance ≥ three antibiotic classes) was observed in all the 75 ESBL-positive isolates, dominated by resistance to trimethoprim-sulfamethoxazole and ciprofloxacin and gentamicin [71]. This indicates that these drugs' potential for managing diseases caused by these microbes will be impaired, therefore challenging the clinical settings [72,73]. This investigation showed that phenotypic resistance to tetracyclines and macrolides encoded by *tet(M)* and *erm(B)* was common among *Enterococcus* spp. Independent of the source. Table 3 shows the phenotypic antibiotic resistance of the bacteria studied in this investigation *E. faecium* from cattle demonstrated resistance to lactams and quinolones in 3% and 8% of isolates, respectively, compared to 76% and 70% of human clinical isolates [74]. Results of the amplification of 20 g-negative bacterial isolates using universal primers targeting the 16SrRNA gene showed that all positive isolates (Amplicons) appeared at 1500 bp, as shown in Fig. 3. This further supports the presence of identified microbes [21,50–52]. Pathogens and multi-resistant bacteria, which may be released into the environment via effluent from wastewater treatment plants (WWTPs), are collected from several sources that can impact ecosystem health [16,28,75–80]. This implies that wastewater effluents may be a source of resistant pathogenic microorganisms that may deteriorate ecosystem health.

The antibiotic-resistant genes technically occur due to poor healthcare settings and sanitation that expose antibiotics to non-targeted organisms. Neither faecal indicator bacteria (*E. coli* or *Enterococci*) nor sul1, sul2, or erm(F) were substantially linked with ARG concentrations of 25 absolute or normalized to 16SrRNA levels. Both *E. coli* and *Enterococci* were linked to 26 tetracycline resistance genes, *tet(O), tet(W)* and *tet(S)*, respectively. It demonstrates that small urban streams and bathing waters with the least hygienic practices may be potential reservoirs of ARGs and may pose a previously unrecognized public health risk as they can transmit uncured pathogenic diseases. As presented in Table 4 and Fig. 4, the detection of antibiotic-resistant genes means that they can be transferred between bacteria through numerous mechanisms, such as bacteriophages, which are viruses that attack and reproduce within bacterial cells, can also transfer antibiotic-resistance genes between bacteria; the acquisition of naked DNA from their environment [81], and conjugation between bacteria, which may transfer antibiotics resistance genes occurring on plasmids and transposons [81]. Microbes can develop resistance to multiple families of antibiotics due to their ability to acquire multiple antimicrobial resistance traits. Once within the microbial population, antimicrobial resistance can be transferred vertically when the next generation inherits the ARGs and horizontally when bacteria share or exchange sections of genetic material with other bacteria, which may even occur among different bacterial species. The dissemination or transfer of antimicrobial resistance in the environment occurs as the microbes are transported among the various environmental compartments such as soil, air, water, humans and animals [12,14,20,21,76,78]. Since wastewater systems, including urban wastewater collection and treatment plants, receive diverse wastewater from households, pharmaceutical industries, and medical facilities, they are potential distribution roots for antibiotic-resistant microbes, which later compromise treatment. An increasing body of literature shows that wastewaters contain a complex mixture of emerging contaminants, including illicit and prescription drugs, pesticides, and pharmaceutical and personal care products [81–87].

Hendriksen and colleagues reported that, in over 79 locations in 60 nations [88], in the observed AMR variation, no evidence was obtained for cross-selection between antibiotics classes or the effect of air travel between sites. Nevertheless, AMR gene abundance strongly correlates

with environmental, global health, and socioeconomic influences used to predict AMR gene abundance [88]. These findings suggest that global AMR gene diversity and richness vary by region and that improving sanitation and health might limit AMR's global burden [88]. Therefore, metagenomic sewage analysis as an ethically acceptable and economically feasible approach should be used for continuous global surveillance and prediction of AMR to ensure public health safety.

5. Conclusions

Wastewater and receiving bodies are observed as storage and transportation media of antibiotic-resistant microbes due to poor hygienic practices or wastewater treatment schemes not designed to remove antibiotics. Antibiotic-resistant microorganisms and resistant genes in the urban receiving and wastewater threaten public health because they are transmittable between and among microbial communities and transmit incurable diseases to the entire ecosystem. Practices such as reusing wastewater effluents for irrigation and aquaculture must adhere to effluent quality guidelines. On the other hand, policymakers need to revisit the current water and wastewater guidelines to include emerging contaminants such as antibiotics in routinely quality assessment and monitoring to ensure entire ecosystem safety. These findings imply a need to re-assess the existence of antibiotic-resistant microbes and establish ineffective antibiotics for the prospective of curing some diseases. Again, maintenance and expansion of wastewater management facilities' efficiency and capacity are inevitable.

Ethics approval and consent to participate

NA

Consent for publication

NA

Availability of data and material

Data for this paper are included within.

Competing interests

The authors declare no conflict of interest.

Authors' contributions

Ideation and concept writing A.R, H.M and M.J.R; methodology and initial manuscript draft A.R, A.M and E.M; analysis and data evaluation (N.L.B., A.M. and E.M); data validation and manuscript text editing (E.C.N., and K.N.N.); final draft scripting and reference management (S.H.V.); English language, coherence, and grammar check (R.L.M). All authors have read and consented to the published version of the manuscript text.

Funding statement

This study received no external funding.

Concert for submission

All authors have agreed to the submission version of the proposal.

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