International Journal of Research Studies in Microbiology and Biotechnology (IJRSMB)
Volume 10, Issue 1, 2025, PP 1-9
ISSN 2454-9428 (Online)
https://doi.org/10.20431/2454-9428.1001001
www.arcjournals.org

Wastewater, A Reservoir of Antibiotic-Resistant Bacteria

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Abstract: *The widespread emergence of antibiotic resistance among bacterial pathogens has become one of the most serious challenges worldwide. This study aimed to evaluate the antibacterial resistance profiles of bacterial pathogens isolated from hospital and public wastewater discharges in the city of Lubumbashi. A total of 99 composite samples were collected, transported and aseptically processed for the detection of indicator organisms (Escherichia coli, Pseudomonas spp and Staphylococcus spp), bacteriological identification and susceptibility testing according to standard procedure. The data obtained were entered and analyzed using SPSS version 20 software. Among the total samples, 632 bacterial isolates were detected including 232 Escherichia coli , 211 Staphylococcus sp p and 189 Pseudomonas spp . Overall, resistance was observed to be greater than 35% and evolving in a crossing manner through quinolones, aminoglycosides, cephalosporins and greater than 60% for beta-lactams. The Multiple Resistance Index was greater than 0.2 for all samples overall and even for each wastewater origin (hospital or public network). It was concluded that hospital wastewater contains a large number of antibiotic-resistant bacteria, are untreated at the outlet and mixes with those in the public network, rich in trace metal elements and other factors favoring the occurrence of resistance with consequences on public health.*

Keywords: *wastewater, bacteria, resistant, antibiotics.*

1. INTRODUCTION

Wastewater serves as a reservoir for pathogenic microorganisms. This wastewater often contains antibiotic-resistant bacteria.[1] As such, wastewater plays an important role as a habitat for bacteria and as an environment conducive to gene transfer.[2] This situation poses a significant and urgent threat to global health[3-5] because the spread of antibiotic-resistant bacteria and antibiotic resistance genes in the environment is leading to the re-emergence of previously controlled pathogenic bacteria in new, more resistant forms, rendering traditional antibiotic therapies ineffective[6,7].

To date, several antibiotics are used in clinics, whose origins are either natural (e.g., amoxicillin is a semi-synthetic derivative of penicillin), or from microbial isolates (e.g., erythromycin from Saccharopolyspora erythraea), or synthetic (e.g., ciprofloxacins) [8]. Each of these has specific classes, including penicillins, macrolides, cephalosporins, fluoroquinolones, carbapenems, tetracyclines, sulfonamides, aminoglycosides, phenicols, lincosamides, glycopeptides, oxazolidinones, and rifampicin. [9] For each of these classes of antimicrobials, most antibiotic resistance is known to be readily transmitted between bacterial pathogens by specific genetic mechanisms of horizontal gene transfer.[10] Common mechanisms of antibiotic resistance include: inactivation of the antibiotic, alteration of the antibiotic target, alteration of cellular permeability to the antibiotic, active efflux of the antibiotic from the cell, and bypass function of a target inhibited by the drug [11].

The triggering of the possibility of transmission of antibiotic resistance genes among bacteria is presided over by several situations including: the use of antibiotics for several decades in humans and animals, for the therapeutic treatment of infectious diseases in humans and to treat and protect the health of animals [12] causes a selection pressure on environmental microorganisms, contributing to the proliferation of antibiotic resistance in microorganisms [13] and this resistance can spread between humans, animals and the environment by several routes [14]. The environment acting either as a bridge between the different interacting elements: from animals to compost, from soil to water, from sediments to wastewater, or as a reservoir, or simultaneously to mix the mobile genetic elements of resistance that interact and spread to other parts or to human and animal hosts [15]. And host-to-host transmission occurs either through direct contact during social behaviors or indirectly through excretion and acquisition of host microbes in a shared environment due to the intimacy of social interaction.[16] Given the complexity of the situation described, the present work aims to evaluate the resistance profile of some flagship bacteria isolated from wastewater.

2. METHODOLOGY

2.1. Study Framework and Sampling Locations

Antibiotic resistance surveillance was carried out in selected wastewater systems (communal drainage system and septic tanks) in the city of Lubumbashi in the Democratic Republic of Congo from January to October 2024. Wastewater from dormitories, restaurants, animal farms, and public and private hospitals was collected as well as from septic tanks receiving wastewater from hospitals, clinics, and hotels.

2.2. Sample Collection

Wastewater samples were collected between 9:00 and 12:00 every quarter from January to September 2024 at the specified sampling points in the sewerage system. Sterile plastic falcon containers were used to collect the samples. After collection, the samples were protected from direct sunlight and transported in a cooler containing ice packs to the laboratory for analysis. All samples were stored at 4 ◦ C and analyzed within 24 h of sample collection.

2.3. Enumeration, Isolation and Identification of Bacteria

Water samples were analyzed for the target bacteria using standard methods for the examination of water and wastewater. Samples were thoroughly mixed to evenly distribute the bacteria prior to analysis. Serial dilutions (10^2-10^6) of samples were prepared in sterile distilled water. Fifty milliliters of dilution replicates of each sample were filtered using a 0.45 µm, 47 mm diameter white cellulosic mesh filter placed on the filter holder. Approximately 25 mL of distilled water was first added to wet the filter paper. Media were selected according to the manufacturer's recommended procedure and sterilized by autoclaving at 15 lb (121 °C) pressure for 15 minutes. Membrane filters were aseptically transferred to 45 mm Petri dishes with the appropriate selective media.

Table 1. *Distribution of bacteria to be isolated according to culture media*

Bacteria	Media	Incubation conditions
Total number of heterotrophs	Agar R	37°C; 24 h
Escherichia coli	m-TEC Agar	35–37°C for 2 hours and 44.5 ± 0.5°C for 22 hours
Staphylococcus spp	MSA Agar	37°C; 24 h
Pseudomonas aeruginosa	Cetrimide Agar	35°C for 18 hours

2.4. Antimicrobial Susceptibility Testing

All sample isolates of each bacterial species were collected for antibiotic susceptibility testing. The standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the isolates [17]. Bacterial inocula were prepared by suspending freshly grown bacteria in 4–5 mL of normal saline, and the turbidity was adjusted to that of a McFarland standard of 0.5. Then, this suspension was spread over the entire surface of Mueller-Hinton agar using a cotton swab to produce confluent growth.

Susceptibility testing was performed by the diffusion method, which involves placing paper disks impregnated with specific amounts of antibiotics on a bed of bacteria grown on agar and incubated aerobically at 35 ± 1 °C for 18 to 24 hours. After an incubation period, the diameter of the zone of inhibition, the area around the disk without bacterial growth, was measured.

3. MULTIPLE ANTIBIOTIC RESISTANCE INDEX (MARI)

The IRMA was determined for each isolate using the formula **IRMA= a / b** , where **a** represents the number of antibiotics to which the tested isolate showed resistance and **b** represents the total number of antibiotics to which the tested isolate was evaluated for susceptibility [18]. An IRMA value of **0.2** indicates a high-risk environment in which antibiotics are often used [19,20].

4. ANALYSIS

Data analysis was performed using descriptive and inferential statistical tools in the R programming environment. A p-value ≤ 0.05 was considered a statistically significant difference. Box-and-whisker plots were chosen to illustrate the distribution of MERI values using mean values. In order to decide which statistical test should be used to determine significance, the data were first analyzed for normal distribution using the Shapiro-Wilk test. The data were not normally distributed and the Kruskal-Wallis test, a nonparametric version of the classical one-way analysis of variance (ANOVA), was used to determine variations in the level of antibiotic resistance (measured by MERI) among the bacterial groups studied. The result was used to assert whether the level of antibiotic resistance is significantly different between the three monitored systems and whether the level of antibiotic resistance varies during the progress of wastewater treatment .

5. RESULTS

After analysis, our results are presented in the form of tables and figures followed by a commentary and discussion of the results.

Table 2. *Number of samples and bacterial isolates obtained by surveillance sites.*

Site	Number of sampling points	Number of samples	*Escherichia coli*	*Staphylococcus spp*	*Pseudomonas spp*
Dormitory	7	21	23	12	9
Restaurant	5	15	11	45	23
Farms	4	12	67	49	51
Hotel	7	21	69	32	39
Hospital	10	30	62	73	67
Total	**33**	**99**	**232**	**211**	**189**

It follows from this table that *Escherichia coli* was the most isolated bacterium followed by *Staphylococcus spp* and finally *Pseudomonas spp* ; *Escherichia coli* was more isolated from community wastewater (hotels, and farms) as well as from water from health facilities.

Staphylococcus spp was isolated more from wastewater from medical facilities and the same is true for Pseudomonas aeruginosa. Indeed, several studies have reported the isolation of *Escherichia coli* in wastewater; this is the case of Daisy S Addae-Nuku et al in the wastewater of the Korle Bu Teaching Hospital in Accra, Ghana [21], In Bangladesh, Md. Mijanur Rahman et al isolated it mainly in the same proportions as Staphylococcus aureus [22], same observation in Bulawayo in Zimbabwe [23] and Ethiopia [24,25]

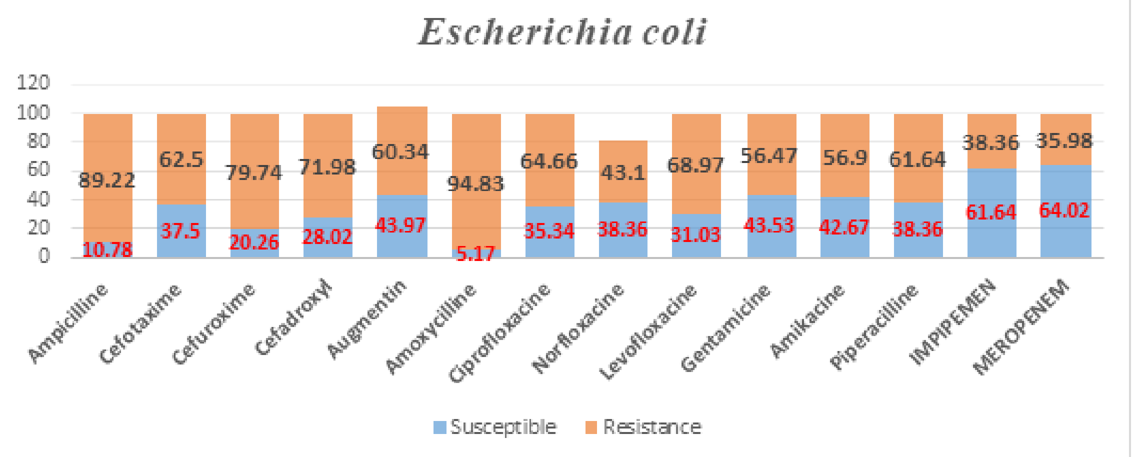

Figure 1. *Antibiotic resistance profile of Escherichia coli isolated from wastewater*

Escherichia ***coli*** isolated from wastewater exhibit resistance greater than 60% to all beta-lactam antibiotics (resistance ranging from 60.34% to 94.83% depending on whether one goes from amoxicillin + clavulanic acid to simple amoxicillin). Against quinolones, resistance varies between 43.1% and 68.97%, and from 56.47% to 61.65% for aminoglycosides and finally 35.98% to 38.36% for penems.

It should be noted that bacteria resistant to all β-lactams, including carbapenems and ciprofloxacin, were mainly disseminated by hospital wastewater [26] than in municipal wastewater.[27] Resistance to amoxicillin was highest (96.3%), cefixime (81.3%), ciprofloxacin (73.6%) [28] Isolates showed reduced susceptibility to β-lactams and cephalosporins. Ampicillin, recorded the highest frequency of resistance, estimated at 94.7% for E. coli isolated from hospital wastewater, followed by ceftazidime with a frequency of 86.8% for the same isolates. [29]. These *Escherichia coli* exhibiting resistance in hospital and municipal wastewater, constitute a potentially significant risk to public health [30] and could lead to the emergence of transmission of ESBL- and carbapenemase-producing E. coli with MDR profiles to the environment [31].

THE ***Pseudomonas spp*** from wastewater have resistance evaluated between 59.76% and 94.18% to beta-lactams, 64.55% to 79.37% to quinolones, 47.32% to 52.91% to aminoglycosides and 35.98% to 42.33% to penems.

Pseudomonas spp. is consistently isolated from domestic and hospital wastewater[32] and several Pseudomonas species are involved in the biodegradation and bioremediation of xenobiotics, suggesting a positive impact on wastewater treatment.[33] In *Pseudomonas spp* ., resistance to β-lactams and fluoroquinolones as well as multidrug resistance to antibiotics become more frequent after wastewater treatment[34]. Indeed, *Pseudomonas spp.* are equipped with concomitant mechanisms, such as low outer membrane permeability, synthesis of β-lactamases and efflux systems [35] and a remarkable genome plasticity that makes them capable of acquiring almost all known mechanisms of antimicrobial resistance [36,37]

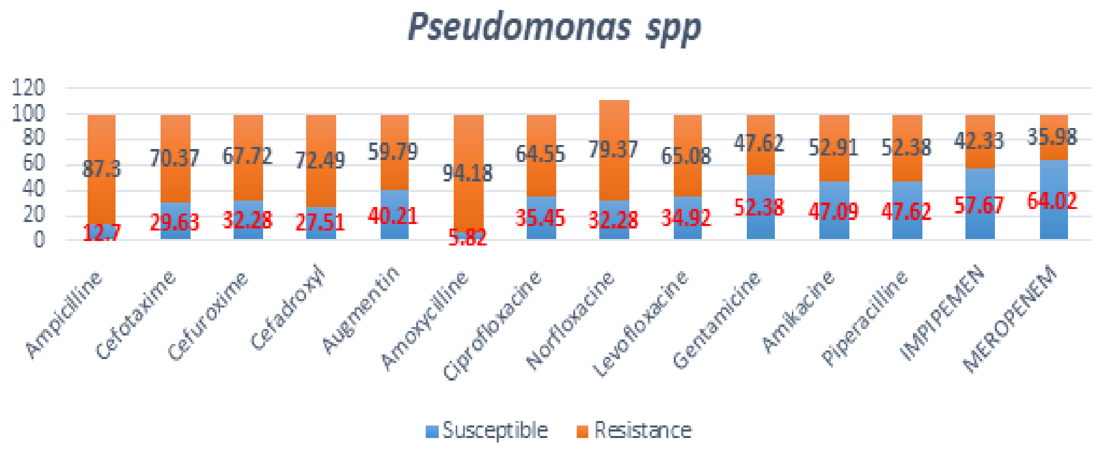

Figure 2. *Antibiotic resistance profile of Pseudomonas spp isolated from wastewater*

THE families of antibiotics tested against ***Staphylococcus aureus*** isolated from wastewater, showed ineffectiveness ranging from 52.13% to 91% for beta-lactams; 58.29% to 72.04% for quinolones, 48.34% to 57.77% for aminosides and 32.23% to 41.71% for penems.

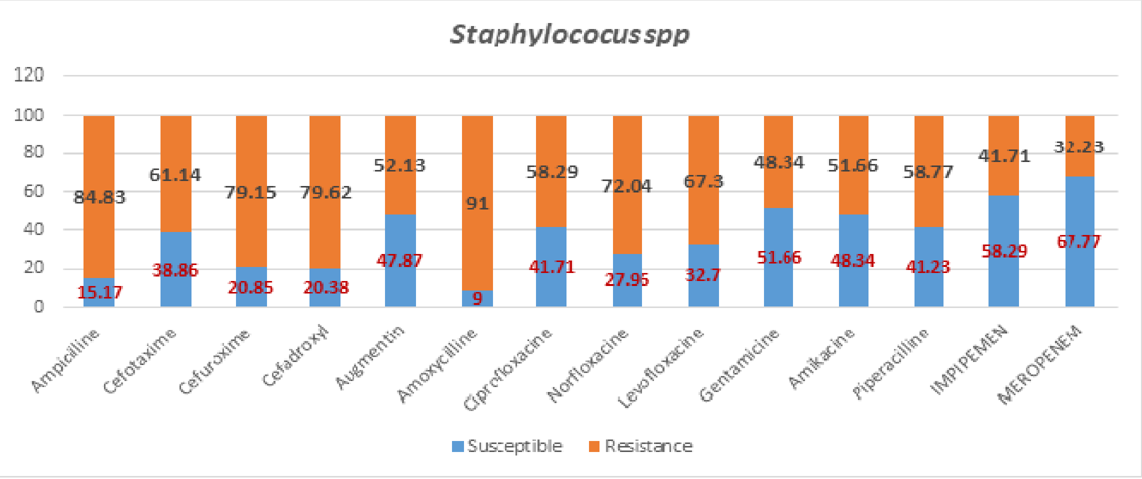

Figure 3. *Antibiotic resistance profile of Sataphylocossus spp isolated from wastewater*

Staphylococcus spp are a normal flora, present in the mucous membranes and skin of humans and other mammals [38] capable of colonizing wastewater from hospitals, municipalities, livestock and poultry and consequently frequently isolated from wastewater from various sources [39] and Most strains of *Staphylococcus spp* isolated from wastewater have shown, as in our study, resistance to ampicillin, cefoxitin, ciprofloxacin, erythromycin, gentamicin, chloramphenicol and vancomycin [40,41] and isolates of *Staphylococcus spp* from hospital settings have shown resistance to the majority of antimicrobials commonly used for the treatment of staphylococcal infections [42]

Table 3. *Multiple antibiotic resistance index (MIRA)*

	HAS	**B**	**IRMA**
Escherichia coli	1834	3248	0.56
Pseudomonas spp	1240	2646	0.47
Staphylococcus spp	1636	2954	0.55

Multiple resistance index is greater than 0.2; these values indicate a high-risk source of contamination where antibiotics are often used[43]. It is noted that the emergence of multiple antibiotic resistance has increased rapidly, that bacterial resistance has increased day by day due to environmental factors[44]. Indeed, environmental aspects should be considered when assessing the risks associated with antimicrobial resistance [45,46] because the transfer of resistance genes from environmental bacteria to human pathogens can pose a significant threat to a community[47]. Antibiotic-resistant bacteria present in wastewater complicate treatment and increase public health concerns.[48]. Fluctuating levels of antibiotics in wastewater have been shown to be associated with horizontal transfer of antibiotic resistance genes [49-52]. In addition, it has been demonstrated that stress factors such as pH, temperature, the presence of antibiotic residues, heavy metals, are likely to trigger and propagate a phenomenon of resistance to antimicrobials in bacteria living in wastewater [53-55]. This situation is characteristic of the city of Lubumbashi in particular and the Katanga region in the South of the DR Congo in general. In this region, trace metal elements are present in the environment suspended in the air or dissolved in runoff water, mixed with acidic water [56,57]

Table 4. *Comparison of Multiple Antibiotic Resistance Index (MARI) according to wastewater types and bacterial species*

	Escherichia coli		**Staphylococcus spp**		**Pseudomonas spp**	
	Hospital wastewater	**Wastewater Public Networks**	**Hospital wastewater**	**Wastewater Public Networks**	**Hospital wastewater**	**Wastewater Public Networks**
N	**62**	**170**	**73**	**138**	**67**	**122**
HAS	490	1344	564	1072	529	963
B	796	2115	915	1738	861	1562
IMRA	0.615578	0.635461	0.616393	0.616801	0.614402	0.616517
pValue	0.33		0.50		0.49	

Comparison of the Multiple Antibiotic Resistance Index (MAI) of bacteria isolated from hospital wastewater and public wastewater shows that there is no statistically significant difference between bacteria isolated from these two sites. As in our study, studies conducted in Pakistan[58] have also concluded that the multiple antibiotic resistance (MAR) index was calculated and the resistance phenotype was independent of the wastewater source. Management of solid and liquid biomedical waste is inadequate in African hospitals[59] Wastewater, sewage sludge, septic tank sludge or excreta containing about 95.5% water and 0.1% to 0.5% organic and inorganic matter are generated in different sections of the hospital [60] The management of these liquid healthcare wastes is an often neglected issue, which has negative consequences in terms of environmental damage and human health.[61] In most hospitals in developing countries, there are no guidelines, standards or committees for the management of these liquid healthcare wastes [62,63]. Thus, liquid hospital wastes carrying multi-resistant bacteria are discharged into the public wastewater network [64] , in this context that municipal and hospital wastewater play a key role in the dissemination of antibiotic-resistant bacteria [65]. Hence, there is no statistically significant difference in the antibacterial activity between bacteria isolated from different wastewater networks.

6. CONCLUSION

Wastewater is indeed a source of antimicrobials and antibiotic-resistant bacteria in both hospital and public wastewater systems. The use of antibiotic agents in the treatment and prevention of pathogenic infections in humans and animals is thought to be responsible for the accelerated spread of bacterial resistance in wastewater. This study highlights the importance of the role of wastewater in the dissemination of antimicrobial resistance and its effects on human health.

Our results highlight the need for urgent and coordinated interventions to limit antimicrobial use and limit the global spread of AMR. This intervention requires proper management of waste and chemical discharges from pharmaceutical plants, including industrial runoff, and the use of efficient technologies in the treatment of hospital wastewater before its discharge into urban and industrial public networks.

It is therefore essential to consider the entire chain of the problem in order to effectively study preventive measures and better implement solutions to protect environmental waters from antibiotic-resistant bacteria. And ensure good water quality before discharge in order to maintain a little or no pollution environment while protecting public health.

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Citation: *Kasamba Ilunga Eric. et al. "Wastewater, A Reservoir of Antibiotic-Resistant Bacteria" International Journal of Research Studies in Microbiology and Biotechnology (IJRSMB), vol 10, no. 1, 2025, pp. 1-9. DOI: https://doi.org/10.20431/2454- 9428.1001001.*