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ORIGINAL PAPER

Multidrug-resistant bacteria in the wastewater of the hospitals in Port Harcourt metropolis: Implications for environmental health

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ABSTRACT

Dissemination of antibiotic resistance via aquatic systems is considered to be an important environmental health concern. The present study aimed to assess the levels of multidrug-resistant bacteria in the raw wastewater of two hospitals in Port Harcourt metropolis using standard microbiological techniques. Among 64 bacterial isolates, seven bacterial groups were identified, including *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter*, *Staphylococcus aureus*, *Citrobacter*, *Shigella*, and *Bacillus*. The bacterial counts were within the ranges of 7.8 x 10^4-4.8 x 10^6 and 6.9 x 10^4-1.09 x 10^5 CFU/mL in hospitals A and B, respectively. The obtained results indicated high resistance to quinolones/fluoroquinolones (83.3-90%) and penicillins (50-70%). In addition, 86.9% of the isolates showed multidrug resistance. The multiple antibiotic resistance (MAR) index was within the range of 0.1-0.8 in the gram-positive bacteria and 0.1-0.6 in the gram-negative bacteria. The findings confirmed the presence of bacteria with high MAR indices in the untreated hospital wastewater.

Keywords: Antibiotic resistance, Bacteria, Environmental health, Pollution, Wastewater, Water quality

Introduction

Antibiotics have extensive application in modern healthcare, while their use is currently associated with the resistance of microorganisms. A wide array of multifaceted factors lead to the emergence and spread of antimicrobial resistance.[1] The US Centers for Disease Control and Prevention (CDC) have associated more than two million infections and 23,000 annual deaths with multidrug bacterial resistance in the United States, the direct costs of which have been estimated at 20 billion dollars regardless of 35 billion dollars as the additional productivity losses.[2] In the past few decades, the occurrence and spectrum of antibiotic-resistant infections have surged across the world.

Some of the contributing factors to this increment are the selective pressure of antimicrobial use, evolution of the observed microbial properties, and anthropogenic activities that facilitate the transmission of these resistant microbes.[3]

Water plays a pivotal role in infection transmission to humans.[4] According to a study by Emmanuel *et al*.,[5] hospitals generate 750 L of wastewater per bed daily on average. The discharged water is laden with microorganisms, partly metabolized pharmaceuticals, radioactive elements, and other hazardous chemicals. The presence of micropollutants (especially antibiotic residues and heavy metals) even at low concentrations leads to the rapid proliferation of antibiotic-resistant bacteria.[6] Therefore, hospital wastewater (whether treated or untreated) is considered to be a key source of resistance

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propagation in the environment, acting as a reservoir of antibiotic-resistant bacteria and resistance genes, disseminating them into the ecosystem and further intensifying resistance in the bacterial communities in these systems through selective pressure.[7,8] In many developing countries, wastewater often remains untreated and enters waterbodies and waterways. Such wastewaters pose a potential risk to the surrounding farmlands and rural dwellings in the proximity of water bodies, which commonly receive these contaminated waters. Moreover, the water bodies contaminated with hospital wastewater effluents may frequently be used for drinking, recreation or irrigation. Regarding the development and propagation of resistant bacteria, some researchers have concluded that the release of poorly treated/untreated wastewater into aquatic systems causes numerous health concerns.[9,10]

The traces of the antibiotics and bacteria that have developed resistance to antibiotics are often found in the hospital wastewaters that are discharged into aquatic environments, leading to the hazard of re-contaminating humans and animals mainly through food or drinking water.[8,9,11] In developing countries, the spread of antibiotic resistance through the disposal of untreated hospital wastewater into aquatic systems is a major environmental health concern.

The present study aimed to provide insight into the possible environmental and public health hazards associated with untreated hospital wastewater through assessing the presence of multidrug-resistant bacteria in the untreated wastewater of two hospitals in Port Harcourt metropolis and determining their antibiotic resistance patterns.

Materials and Methods

Sample collection

Samples were collected using the composite method every 48 h over three weeks from two hospitals in Port Harcourt, Nigeria. Neither hospital treated their wastewater. In total, 11 composite samples of untreated wastewater per hospital were collected in sterile glass bottles for bacteriological analysis and antibiotic susceptibility testing. Sodium thiosulfate was used to neutralize the possible disinfectants in the samples.

Determination of total heterotrophic count

After the 10-fold serial dilution of the samples in physiological saline, one milliliter of aliquots was placed on plate count agar in duplicate using the spread plate technique. The plates were incubated at the temperature of 37 °C for 48 h, and the number of the colonies on the duplicate plates was determined.[12]

Bacterial isolation from the samples

Bacteriological analysis was performed using nutrient agar, Eosin-Methylene blue agar, Mannitol salt agar, MacConkey agar, Salmonella Shigella agar, and thiosulfate-citrate-bile salts agar, which were prepared in accordance with the instructions of the manufacturer. The aliquots of approximately 1 mL of the appropriate dilutions were inoculated onto the relevant agar plates using the spread plate technique with incubation at the temperature of 37 °C for 48 h.[12]

The isolated bacteria were characterized based on the morphological, microscopic, and biochemical properties as proposed by Cheesbrough.[13] The experiments included the sugar fermentation test, oxidase test, hydrogen sulfide (H_2S) production, citrate utilization, motility, indole synthesis, urea hydrolysis, catalase and coagulase tests, lysine decarboxylase and lysine deaminase production, methyl red test, Voges-Proskauer test, and Gram staining. Finally, discrete colonies were sub-cultured onto fresh agar plates, and the pure isolates were obtained and preserved on slants.

Antimicrobial sensitivity testing

Antibiotic susceptibility tests were performed using the Kirby-Bauer disk-diffusion method on Mueller-Hinton agar using 0.5 McFarland standard.[14] In total,

10antibiotics were used in each assay for the obtained Gram-positive and Gram-negative bacterial isolates.

In addition, the commercially prepared paper antibiotic disks (n=10) at fixed concentrations were applied onto the agar surface, which was smeared with the test isolate using flame-sterilized forceps. Following that, the agar plates were incubated at the temperature of 37 °C for 24 h. The inhibition zones of the isolated bacteria were measured in millimeters using calipers. Sensitivity and resistance were interpreted based on the zone size interpretative chart, and the zone diameter limits of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were considered as the quality controls.[15-17] The organisms were tested against five classes of antibiotics each; the Gram-positive isolates were tested against fluoroquinolones, aminoglycosides, penicillins, ansamycins and macrolides and the Gram-negative isolates were tested against quinolones/fluoroquinolones, aminoglycosides penicillins, cephalosporins and sulfonamides.

The multiple antibiotic resistance (MAR) index was ascertained by determining the ratio between the number of antibiotics to which the bacterium showed resistance and the total number of the tested antibiotics.[18]

Results and Discussion

According to the obtained results, hospital B had lower bacterial counts compared to hospital A. The mean total viable counts were within the ranges of 7.8×10^4-4.8×10^6 and 6.9×10^4-1.09×10^5 CFU/mL in the sample sites of hospitals A and B, respectively. Among 64bacterial isolates in the two test sites, seven organisms were identified, including *Klebsiella pneumoniae*, *E. coli*, *Enterobacter* spp., *S. aureus*, *Citrobacter* spp., *Shigella* spp., and *Bacillus*spp. The distribution of the isolates is depicted in Fig.1. Table 1 shows the resistance profile of the bacterial isolates. It is notable that the *Shigella* isolates (n=3) had to be discarded due to contamination and were not used for antibiotic testing.

Figs. 2 and 3 depict the differences in the resistance profiles based on the size of the observed inhibition zones in the two hospitals for Gram-positive and Gram-negative bacterial isolates, respectively. In total, 80% of the gram-positive isolates were resistant to ciprofloxacin and rifampicin, while 60% showed resistance to amoxicillin, and 50% were resistant to norfloxacin. In hospital A, the gram-positive bacterial isolates were most susceptible to gentamicin, erythromycin, and ampiclox (ampicillin/cloxacillin), while the highest resistance was observed against ciprofloxacin, norfloxacin and rifampicin. On the other hand, the gram-negative bacterial isolates showed greatest resistance to Augmentin and nalidixic acid, while they had no resistance against cephalexin and gentamicin.

According to the observations in hospital B, none of the gram-positive bacterial isolates were resistant to gentamicin, levofloxacin, and chloramphenicol, while the gram-negative isolates were highly susceptible to ciprofloxacin, gentamicin, septrin (sulfamethoxazole/ trimethoprim), and ampicillin, with the observed resistance estimated to be less than 30%. However, the obtained results were contradictory regarding the resistance patterns of the gram-negative isolates against cephalexin in the studied hospitals, while high susceptibility to these antibiotics was denoted in hospital A. Furthermore, the gram-negative bacterial isolates obtained from hospital B showed relatively high antibiotic resistance.

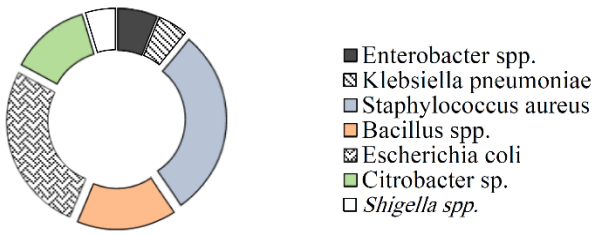

Fig. 1. Distribution of bacterial isolates

In general, the highest resistance was observed against quinolones/fluoroquinolones (83.3-90%) in both gram-positive and gram-

negative isolates. Fig. 4 shows the results regarding resistance to various classes of the tested antibiotics in the two groups.

According to the information in Table 2, multidrug resistance (MDR) was observed in all the tested genera (n=6). Approximately 86.9% of the bacterial isolates had MDR, showing resistance to more than two of the administered antibiotics. The MAR index was within the range of 0.1-0.8 for the gram-positive isolates and 0.1-0.6 for the gram-negative isolates. However, none of the bacterial isolates were resistant to all the antibiotics (n=10). The highest MAR indices were observed for *S. aureus* and *E. coli* in the gram-positive and gram-negative isolates, respectively. In addition, approximately 89.5% of the *S. aureus* isolates and 100% of the *E. coli* isolates had MDR.

Table 1. Antibiotic resistance profile of bacterial isolates

S/N	Organism		CPX	NB	CN	AML	S	RD	E	CH	APX	LEV
1.	*Staphylococcus aureus* (19)	R	19 (100%)	3 (15.8%)	0 (0%)	9 (47.4%)	7 (36.8%)	11 (57.9%)	3 (15.8%)	4 (21.1%)	4 (21.1%)	1 (5.3%)
		S	0 (0%)	16 (84.2%)	19 (100%)	10 (52.6%)	12 (63.2%)	8 (42.1%)	16 (84.2%)	15 (78.9%)	15 (78.9%)	18 (94.7%)
2.	*Bacillus* spp. (10)	R	4 (40%)	6 (60%)	0 (0%)	0 (0%)	2 (20%)	8 (80%)	6 (60%)	2 (20%)	0 (0%)	4 (40%)
		S	6 (60%)	4 (40%)	10 (100%)	10 (100%)	8 (80%)	2 (20%)	4 (40%)	8 (80%)	10 (100%)	6 (60%)
3.	*Enterobacter* spp. (4)	R	0 (0%)	3 (75%)	4 (100%)	4 (100%)	0 (0%)	1 (25%)	0 (0%)	1 (25%)	2 (50%)	2 (50%)
		S	4 (100%)	1 (25%)	0 (0%)	0 (0%)	4 (100%)	3 (75%)	4 (100%)	3 (75%)	2 (50%)	2 (50%)
			AU	OFX	CPX	PEF	GN	S	CEP	NA	SXT	PN
4.	*Escherichia coli* (17)	R	17 (100%)	17 (100%)	0 (0%)	17 (100%)	2 (11.8%)	6 (35.3%)	8 (47.1%)	17 (100%)	5 (29.4%)	7 (41.2%)
		S	0 (0%)	0 (0%)	17 (100%)	0 (0%)	15 (88.2%)	11 (64.7%)	9 (52.9%)	0 (0%)	12 (70.6%)	10 (58.8%)
5.	*Citrobacter* spp. (8)	R	0 (0%)	0 (0%)	8 (100%)	0 (0%)	0 (0%)	4 (50%)	3 (37.5%)	3 (37.5%)	3 (37.5%)	0 (0%)
		S	8 (100%)	8 (100%)	0 (0%)	8 (100%)	8 (100%)	4 (50%)	5 (62.5%)	5 (62.5%)	5 (62.5%)	8 (100%)
6.	*Klebsiella pneumoniae* (3)	R	2 (66.7%)	0 (0%)	1 (33.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (66.7%)	2 (66.7%)	2 (66.7%)
		S	1 (33.3%)	3 (100%)	2 (66.7%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	1 (33.3%)	1 (33.3%)	1 (33.3%)

R – RESISTANT; S – SUSCEPTIBLE

CPX (Ciprofloxacin, 10 µg), NB (Norfloxacin, 10 µg), CN (Gentamycin, 10 µg), AML (Amoxil – Amoxicillin, 20 µg), S (Streptomycin, 30 µg), RD (Rifampicin, 20 µg), E (Erythromycin, 30 µg), CH (Chloramphenicol, 30 µg), APX (Ampiclox – Ampicillin /Cloxacillin, 20 µg), LEV (Levofloxacin, 20 µg)

AU (Augmentin – Amoxicillin/clavulanic acid, 10 µg), OFX (Ofloxacin, 20 µg), CPX (Ciprofloxin, 10 µg), PEF (Pefloxacin, 10 µg), GN (Gentamycin, 10 µg), S (Streptomycin, 30 µg), CEP (Cephalexin, 10 µg), SXT (Septrin – Sulfamethoxazole/Trimethoprim, 30 µg), PN (Ampicillin, 30 µg), NA (Nalidixic Acid, 30 µg)

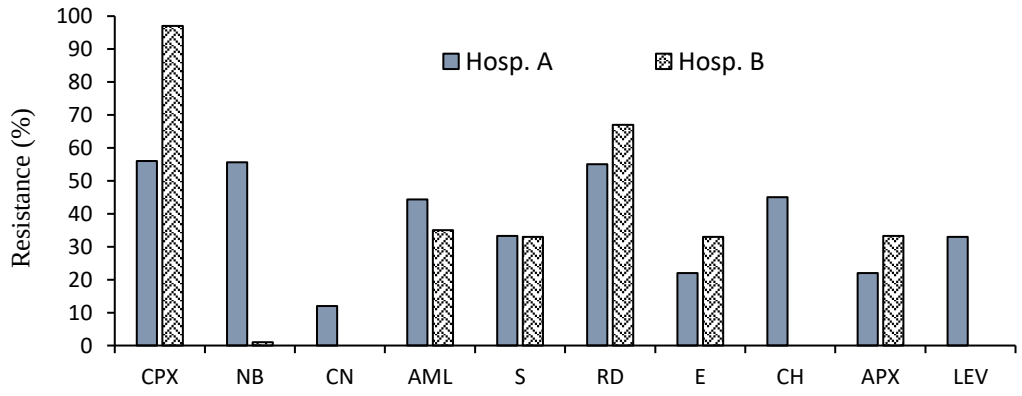

Fig. 2. Observed resistance in gram-positive bacterial isolates (CPX: ciprofloxacin, 10 µg; NB: norfloxacin, 10 µg; GN: gentamicin, 10 µg; AML: amoxil-amoxicillin, 20 µg; S: streptomycin, 30 µg; RD: rifampicin, 20 µg; E: erythromycin, 30 µg; CH: chloramphenicol, 30 µg; APX: ampiclox-ampicillin/cloxacillin, 20 µg; LEV: levofloxacin, 20 µg)

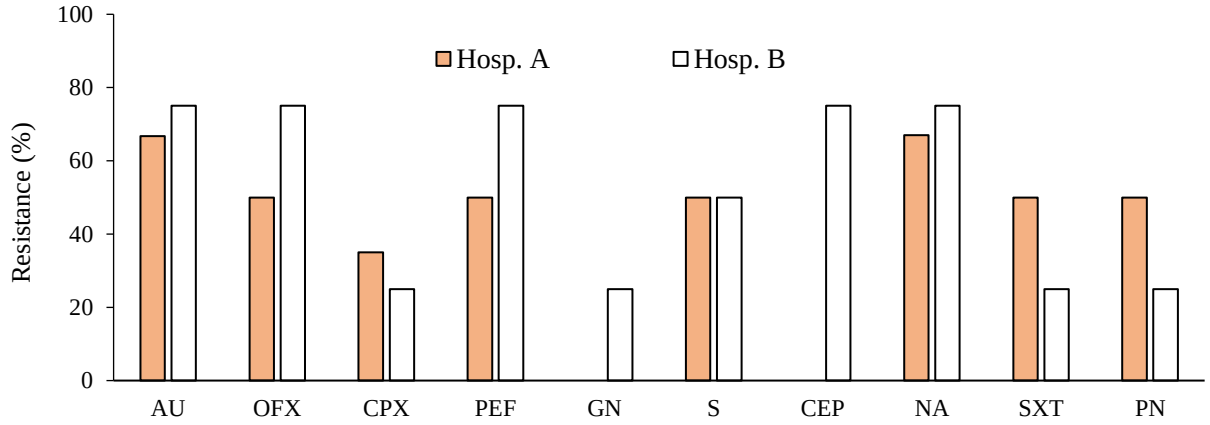

Fig. 3. Observed resistance in gram-negative bacterial isolates (AU: augmentin-amoxicillin/clavulanic acid, 10 µg; OFX: ofloxacin, 20 µg; CPX: ciprofloxacin, 10 µg; PEF: pefloxacin, 10 µg; GN: gentamicin, 10 µg; S: streptomycin, 30 µg; CEP: cephalexin, 10 µg; SXT: septrin-sulfamethoxazole/trimethoprim, 30 µg; PN: ampicillin, 30 µg; NA: nalidixic acid, 30 µg)

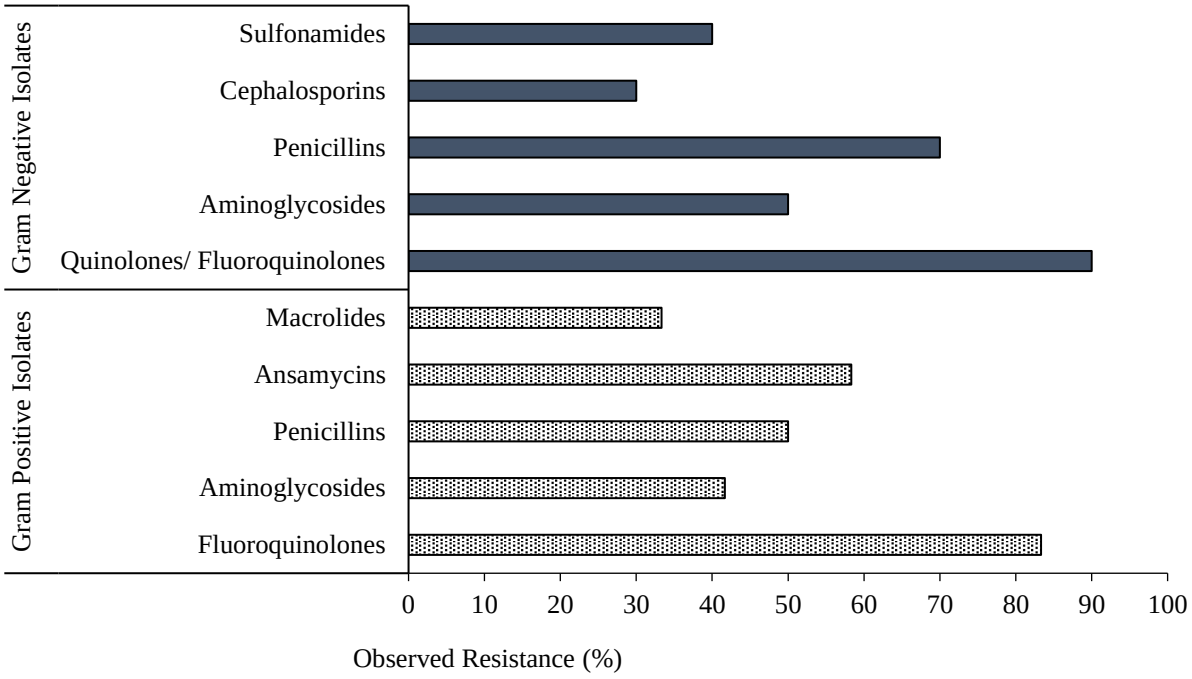

Fig. 4. Resistance pattern to applied antimicrobial classes

Table 2. Multiple antibiotic resistance index of bacterial isolates

S/N	Organism	Antibiotic resistance pattern	MAR index
GRAM POSITIVE ISOLATES			
1.	*Staphylococcus aureus*	CPX, NB, AML, S, RD, CH, APX, LEV	0.8
2.	*Staphylococcus aureus* (3)	CPX, AML, S, CH	0.4
3.	*Staphylococcus aureus* (2)	CPX	0.1
4.	*Bacillus* sp. (4)	RD, E	0.2
5.	*Bacillus* sp. (2)	NB, S, E, CH	0.4
6.	*Staphylococcus aureus* (2)	CPX, NB, RD	0.3
7.	*Bacillus* sp. (4)	CPX, NB, RD, LEV	0.4
8.	*Staphylococcus aureus* (3)	CPX, S, E,	0.3
9.	*Staphylococcus aureus* (3)	CPX, RD, APX	0.3

10.	*Staphylococcus aureus* (5)	CPX, AML, RD	0.3
11.	*Enterobacter* sp. (2)	NB, CN, AML, LEV	0.4
12.	*Enterobacter* sp.	CN, AML, RD, CH, APX	0.5
13.	*Enterobacter* sp.	NB, CN, AML, APX	0.4
GRAM NEGATIVE ISOLATES			
14.	*Escherichia coli* (3)	AU, OFX, PEF, NA, SXT, PN	0.6
15.	*Citrobacter* sp. (2)	CPX	0.1
16.	*Klebsiella pneumoniae*	CPX, PN, NA	0.3
17.	*Klebsiella pneumoniae*	AU, SXT, NA	0.3
18.	*Klebsiella pneumoniae*	AU, SXT, PN	0.3
19.	*Escherichia coli* (2)	AU, OFX, PEF, S, NA, SXT	0.6
20.	*Citrobacter* sp. (3)	CPX, S, NA	0.3
21.	*Escherichia coli*	AU, OFX, PEF, S, NA, PN	0.6
22.	*Escherichia coli* (3)	AU, OFX, PEF, NA, PN	0.5
23.	*Citrobacter* sp. (2)	CPX, CEP, SXT	0.3
24.	*Citrobacter* sp.	CPX, S, CEP, SXT	0.4
25.	*Escherichia coli* (3)	AU, OFX, PEF, S, CEP, NA	0.6
26.	*Escherichia coli* (3)	AU, OFX, PEF, CEP, NA	0.5
27.	*Escherichia coli* (2)	AU, OFX, PEF, CN, CEP, NA	0.6

CPX (Ciprofloxacin), NB (Norfloxacin), CN (Gentamycin), AML (Amoxicillin), S (Streptomycin) RD (Rifampicin), E (Erythromycin), CH (Chloramphenicol), APX (Ampicillin /Cloxacillin), LEV (Levofloxacin). AU (Augmentin – Amoxicillin/clavulanic acid), OFX (Ofloxacin), CPX (Ciprofloxin), PEF (Pefloxacin), CN (Gentamycin), S (Streptomycin), CEP (Cephalexin), SXT (Septrin – Sulfamethoxazole/Trimethoprim), PN (Ampicillin), NA (Nalidixic Acid).

The obtained results of the present study confirmed that hospital wastewater had greater bacterial diversity and higher levels of antibiotic-resistant bacteria compared to other sources of wastewater.[19, 20] Previous studies have also recorded relatively similar bacterial counts, and the values were within the ranges of 1.1 x 10^4-2.2 x 10^6 and 1.2 x 10^4-2.2 x 10^8 CFU/mL on average in the wastewater of hospitals and pharmaceutical facilities, respectively.[21,22] On the other hand, Mustapha and Imir[23] observed lower counts (2.73 x 10^3-4.21 x 10^5 CFU/mL) in the sewage of the hospitals in Maiduguri (Nigeria).

In another research, Eze and Onwurah[24] reported higher mean values (13.7 x 10^7-22.8 x 10^{10} CFU/mL), while the findings of Fekadu *et al*.[25] indicated the bacterial counts of 2.1 x 10^6 and 5.2 x 10^6 CFU/mL in the wastewater of two hospitals in south Ethiopia. The isolates obtained in the present study are comparable to those observed by Fekadu *et al*.,[25] who isolated *Staphylococcus* spp., *Klebsiella* spp., *E. coli*, *Bacillus* spp., *Proteus* spp., *Enterococcus* spp., *Salmonella* spp., *Shigella* spp., and *Citrobacter* spp. from the hospital wastewater in south Ethiopia. However, Eze and Onwurah[24] and Asfaw *et al*.[21] detected *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella* spp., *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella* spp., *Enterobacter* sp., and *Bacteroides* sp. in the wastewater of the hospitals in Nigeria and north Ethiopia, respectively. In line with the current research, the mentioned studies demonstrated that *S. aureus* was the most frequently isolated organism from hospital wastewater samples.

According to the results of the present study, the gram-negative bacterial isolates had greater resistance compared to the gram-positive isolates, which could be due to the differences in their cell wall structure (mode of action of the tested antibiotics).In addition, approximately 86.9% of the isolates had MDR, showing resistance to more than two of the administered antibiotics. Based on the modified definition of MDR by the CDC regarding the organisms that are resistant to at least one agent in three or more antibacterial classes,[2, 26] the observed MDR in the current research was estimated at 68.2%, which is still significantly high.

In a study on the antibiotic susceptibility of *Pseudomonas* isolated from wastewater treatment facilities, intermediate resistance

was observed against chloramphenicol (50%), minocycline (60%), nalidixic acid (70%), vancomycin (60%), and ampicillin-sulbactam (50%). In addition, 90-100% resistance was reported against penicillins, rifampicin, and sulfamethoxazole, while high resistance (70%) was observed against cephems (cephalothin, cefotaxime, and cefepime).[27]

The high resistance of bacteria to fluoroquinolone within communities has been well documented. This widespread resistance has been attributed to the chemical stability of fluoroquinolones, which enables them to persist in the environment longer than other antimicrobials. Such persistence results in the increased exposure of microorganisms to fluoroquinolones in the environment.[28] Moreover, it has been reported that this antimicrobial group is no longer the first treatment choice for the hospital-acquired *E. coli* infections and *E. coli* urinary tract infections in Europe. In a study conducted in China, approximately 60% of the *E.coli* isolated from nosocomial infections and 50% of community-isolated *E.coli* strains exhibited ciprofloxacinresistance.[29, 30] Similar findings have also demonstrated that 86% of *S. aureus* and 92% of *E. coli* isolates have MDR, with the highest resistance values recorded in the case of *S. aureus* isolates.[31, 32] The mentioned observations validate the well-established MDR propensity of *S. aureus*.

In the present study, 86.9% of the isolates had MAR values of higher than 0.2, which was rather expected considering the source of the samples. The MAR index exceeding 0.2 characterizes high-risk organisms, which often originate from cases with high antibiotic use.[27] The growing rate of MDR has led to numerous ecological and environmental health concerns. MDR has been reported to be on the rise in community-acquired infections. Meanwhile, MDR *E. coli* and *S. aureus* are the foremost sources of infection in every clinical setting, accounting for 17.3-18.8% of the nosocomial infections requiring hospitalization; it is notable that *S. aureus* ranks higher in this regard.[33]

According to the literature, *E. coli* is responsible for hospital-acquired enterocolitis and urinary tract infections. In addition, *Klebsiella pneumoniae* is considered to be an opportunistic pathogen, with the propensity of hypervirulence. In places where poor sanitation is rife, *Klebsiella* spp. has been regarded as a causative agent of major nosocomial infections and epidemics.[34] Selective pressure has been reiterated as a key reason for high MDR in hospital wastewater.[7,35] In a study in this regard, MDR was reported in nine out of 17 (52.9%) bacterial isolates of hospital wastewater.[19] The studies focused on antibiotic resistance have often place more emphasis on hospital infections than the environment and its role in the development and dissemination of resistance. While the risk of direct human exposure to the antibiotic residues in environmental media remain poorly defined, the environmental health concerns mainly lie within the potential development of antibiotic resistance by bacteria, which may transmit antibiotic-resistant genes to humans and animals. Although it has been asserted that there are no differences between the observed antibiotic resistant bacterial loads in hospital and municipal wastewaters, some studies have highlighted significantly greater resistance in hospital effluents.[8, 9]

Use of inefficient waste disposal techniques and poor sanitation conditions often give rise to the persistent cycling of resistance genes and resistant bacteria in the environment, which in turn adversely affects community health; this is particularly true in the case of rural communities. The environmental impact as a result of the indiscriminate disposal of tainted wastewater has led to the noticeable increment in antibiotic-resistant bacteria in aquatic systems, which may be linked to wastewater effluents.[4, 36] This issue could be attributed to two factors, including the environmental composition of antibiotic-resistant bacteria and genes and levels of antibiotic-resistant bacteria in the gut. The interrelationship between animals, humans, and the environment through aquatic and edaphic

systems has been well documented in terms of the dissemination of antibiotic resistance.[11, 37]

With the release of antibiotic-resistant bacteria into receiving environments, transmissible resistance genes are transmitted to other bacterial groups within the community, thereby increasing the number of resistance gene vectors in the environment and making the treatment of the possible infections difficult. The challenges in the treatment and management of these bacterial infections increase the financial burden on the patients, government, and healthcare facilities, while also increasing the risk of hospital-acquired infections.[4, 11]

Proper waste management and sanitation measures are considered to be the primary means of mitigating the issue of MDR propagation through hospital effluents. In developing countries, specific laws are lacking to enforce the treatment of hospital wastewater before discharge to wastewater treatment facilities or release into aquatic ecosystems. Considering the associated environmental health risks, explicit regulations are required to define the thresholds for hospital effluents regarding antibiotic-resistant bacteria. Furthermore, regular environmental sampling is essential to the monitoring of the changes in the levels of antibiotic resistance genes and resistant bacteria in the ecosystem.

Conclusion

The results confirmed the environmental health risks posed by hospital wastewater. Accordingly, the presence of MDR bacteria with relatively high MAR indices was established in untreated wastewater. The water bodies surrounding Port Harcourt metropolis are regularly loaded with these high-risk microorganisms, thereby mediating the dissemination of antibiotic resistance within the region.

According to the results, the bacterial isolates were most resistant to fluoroquinolones/quinolones. In addition, *S. aureus* and *E. coli* showed the greatest distribution in the investigated hospitals. In such case, the recommended solution encompasses the proper monitoring and more regimented use of antibiotics in hospitals and healthcare facilities, as well as the proper treatment of wastewater prior to disposal. Furthermore, the regular testing of hospital effluents before disposal into aquatic systems is of utmost importance. The pre-treatment of hospital effluents should be enforced by healthcare authorities and policymakers as hospital wastewater is associated with dire environmental health hazards when improperly treated before discharge.

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