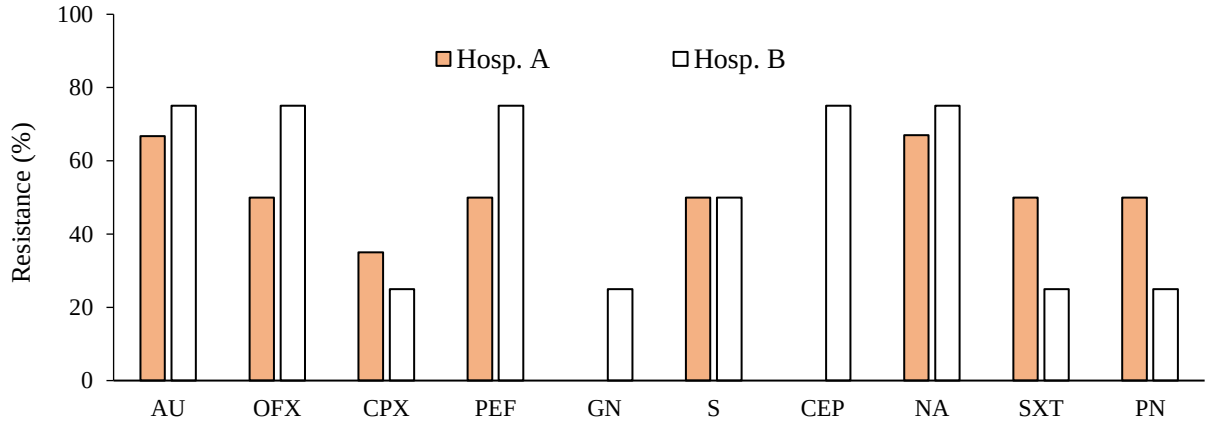

Fig. 3. Observed resistance in gram-negative bacterial isolates (AU: augmentin-amoxicillin/clavulanic acid, 10 µg; OFX: ofloxacin, 20 µg; CPX: ciprofloxacin, 10 µg; PEF: pefloxacin, 10 µg; GN: gentamicin, 10 µg; S: streptomycin, 30 µg; CEP: cephalexin, 10 µg; SXT: septrin-sulfamethoxazole/trimethoprim,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

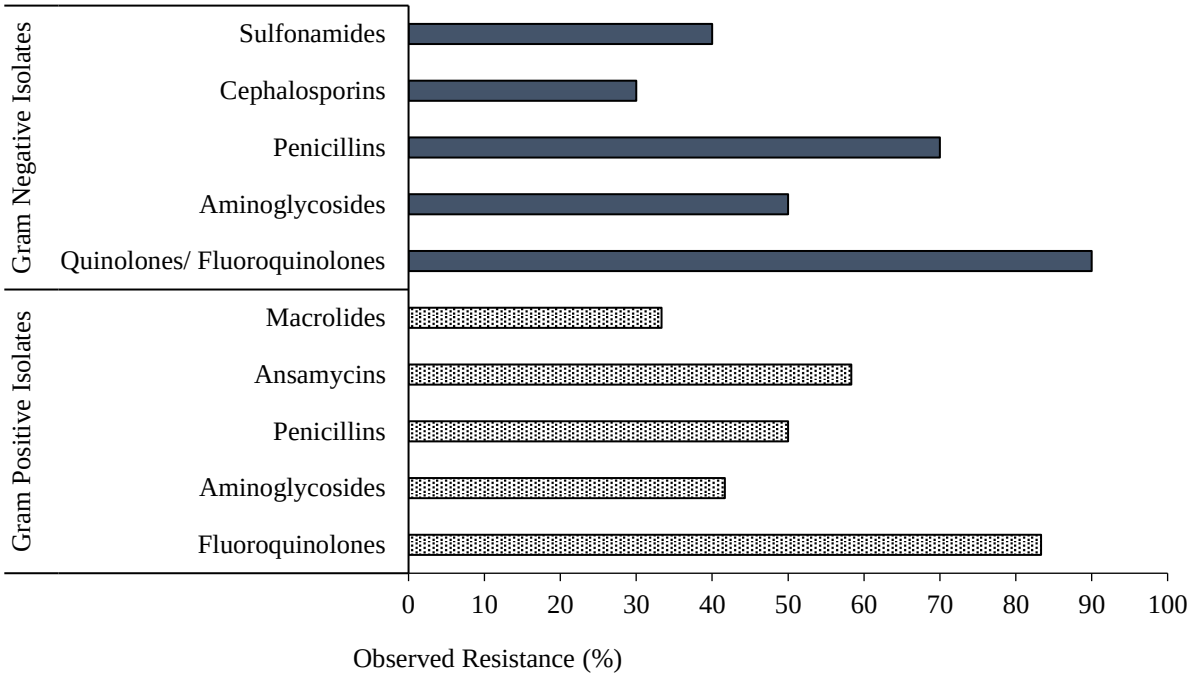

Fig. 4. Resistance pattern to applied antimicrobial classes

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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