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Original Article

Phenotypic Characterization and Plasmid DNA Profiling of Multidrug-Resistant *Escherichia coli* and *Staphylococcus aureus* in Wastewater Effluents From Healthcare Environments in Lafia, Nigeria

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Abstract

Background: Multidrug-resistant *Escherichia coli* and *Staphylococcus aureus* are frequent culprits of severe healthcare-associated infections and have been identified as significant pollutants in hospital settings. The research into plasmids as potential carriers for transferring new resistance genes among clinical pathogens has been quite constrained. This study was conducted to determine the extent of multidrug resistance and the presence of plasmids in *E. coli* and *S. aureus* isolates derived from wastewater effluents at healthcare institutions in Lafia, Nigeria.

Methods: A total of 231 effluent samples were collected from different units within the healthcare facilities and bacterial identification performed using standard CLSI identification techniques. Phenotypic multidrug resistance was analyzed using the Kirby-Bauer disc diffusion method while plasmid DNA was extracted by alkaline lysis and separated using 0.8% agarose gel electrophoresis.

Results: A total of 167 (72.3%) and 175 (75.6%) samples were positive for *E. coli* and *S. aureus*, respectively. Both *E. coli* and *S. aureus* exhibited the greatest resistance to amoxicillin, with resistance rates of 79.0% and 66.3%, respectively. Conversely, the lowest resistance was observed for levofloxacin (26.3%) and cotrimoxazole (25.1%) in *E. coli* and S. aureus, respectively. The study did not find any significant correlation between the phenotypic antibiotic resistance profiles of the isolates and different wastewater discharge points ($P>0.05$). Out of the total isolates, 77 (46.1%) of *E. coli* and 51 (29.1%) of *S. aureus* were resistant to all tested antibiotics. A majority of these isolates exhibited multiple antibiotic resistance index (MARI) values greater than 0.5, with 87.4% of *E. coli* and 80.6% of S. aureus demonstrating multidrug resistance. Plasmid analysis for *E. coli* indicated that the largest proportion of the selected isolates (46.7%) carried double plasmids with sizes ranging from 1500 to 6000 base pairs (bp), and 6.7% had no plasmids. In the case of S. aureus, 53.3% of the isolates harbored a single plasmid with a size of 7500 bp, while 46.7% had no plasmids.

Conclusion: The wastewater discharged from healthcare facilities in the examined community was found to be significantly contaminated with multidrug-resistant organisms carrying plasmids with resistance genes.

Keywords: Multidrug resistance, Plasmid profiling, Hospital effluents, *Escherichia coli*, *Staphylococcus aureus*, Nigeria

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Introduction

Microorganisms in hospital effluents consist of both saprophytic and pathogenic bacteria originating from various sources in the hospital environment, such as soil, patient waste, laboratory waste, medical equipment, and water used in different hospital activities. Bacterial pathogens are primarily introduced into the environment through excrement and contaminated fomites.[1]

Drugs have also been found to be contaminated with microorganisms, which can be released into the hospital environment through wastewater.[2]

Staphylococcus aureus and *Escherichia coli* are commonly implicated in nosocomial infections and are significant components of hospital effluents. *E. coli* O157:H7, an enterohaemorrhagic pathogen associated with human diarrhoea, can potentially be released into the healthcare environment through wastewater effluents.[3]

Previous studies have reported an increase in the number of multidrug-resistant pathogens within the hospital environment due to the routine discharge of antibiotics into the environment, either in metabolized or unmetabolized forms. Microorganisms in hospital effluents tend to develop resistance to the antibiotics released alongside them in order to survive in such environments.[4] The development of such multidrug-resistant bacterial strains appears to depend on the nature of the hospital environment and selective survival mechanisms.[5] Pathogenic multidrug-resistant bacteria may persist in hospital effluents even after treatment, posing significant threats within the hospital environment.[6] Wastewater from hospitals, with its numerous multidrug-resistant enteric pathogens, may pose severe public health risks and the potential for transferring antibiotic-resistant plasmids to nonresistant bacterial populations.[7] Some strains of *E. coli* and *S. aureus* have shown resistance to multiple antimicrobial drugs. Plasmid DNA, along with conjugative transposons, plays a crucial role in horizontal gene transfer, which significantly influences bacterial growth and interactions. The effectiveness of antibiotic therapy in treating infectious diseases caused by clinical bacterial isolates has decreased due to the increasing resistance to antibiotics. Most antimicrobial resistance genes have been reported to be acquired through plasmid-mediated mechanisms.[8] The spread of multiple antibiotic-resistant bacteria in the environment is a major problem in developing countries, primarily due to improper antibiotic usage, ineffective infection control programs, and inadequate management of hospital wastewater.[9] Pharmaceutical compounds and antibiotic-resistant bacteria may also enter wastewater systems through the discharge of hospital, industrial, and residential wastewater, eventually finding their way into the environment.[10,11] Hospital wastewater has increasingly come under suspicion as a significant reservoir of multidrug-resistant bacteria that carry antibiotic-resistant genes, which can be released into the environment through effluents.[12] Comprehensive knowledge of the sources of multiple antibiotic resistance (MAR) in pathogenic bacteria and their distribution is crucial for effectively controlling and regulating the spread of antibiotic-resistant genes.[13] While problems associated with antibiotic administration and the spread of antibiotic resistance in healthcare environments have been widely reported, their role as environmental pollutants has not received sufficient attention. Consequently, the increasing incidence of multidrug resistance in pathogenic microorganisms has become a significant public health concern. However, our current understanding of the occurrence, spread, and distribution of multidrug resistance and associated genes in many environments remains limited.[13,14] This study aimed to assess the levels of multidrug resistance and plasmid profiling in resistant *E. coli* and *S. aureus* isolates in wastewater collected from various healthcare facilities in Lafia, Nigeria.

Materials and Methods

Study Area

The study area was Lafia, a semi-urban town located in North Central Nigeria and the capital city of Nasarawa State (Figure 1). It has a total population of 330,712 inhabitants who are mainly farmers, businessmen and artisans. Most settlements are usually congested with poor hygienic conditions. The healthcare environments used for this study were Primary Healthcare Centre (PHC) Mararaba- Akunza, Medical and Diagnostic Hospital (MDH) and Dalhatu Araf Specialist Hospital (DASH), representing primary, secondary and tertiary facilities respectively, all located within the area studied.

Sample Collection

The sampling was conducted three times a week from May to July 2021, between 8 a.m. and 12 noon. A total of 231 wastewater samples were collected, with 69 from PHC and 81 each from MDH and DASH, discharged from various units within the healthcare facilities. Approximately 15 mL of each sample was collected aseptically using a sterile syringe and placed in a 20 mL sterile universal container. All collected samples were transported in ice-cold packs to the Microbiology Laboratory at the Federal University of Lafia within 6 h for analysis.

Sample Analysis

Each wastewater sample was thoroughly shaken, and then 10 mL was mixed with 90 mL of sterile phosphate buffer solution (PBS) with a pH of 7.2."

Isolation and Identification of Escherichia coli

Ten 10.0 mL from the 10^{-1} diluent of each sample was transferred into 100 mL of MacConkey broth and incubated at 42 ˚C for 24 hours. After thoroughly shaken, a loopful of the broth culture was streaked on MacConkey agar and incubated at 37 ˚C for 24 hours. Growth of purple colonies indicated the presence of *E. coli* in the sample.

The isolates were identified using Gram stain and biochemical tests and confirmed with the API 20E identification system. The biochemical tests used for the identification of the isolates included indole, urease, oxidase, citrate utilization and Triple Sugar Iron agar tests.[15]

Isolation and identification of Staphylococcus aureus

Aliquots of 0.1 mL from 10^{-1} wastewater dilutions were spread on mannitol salt agar (MSA) plates and incubated

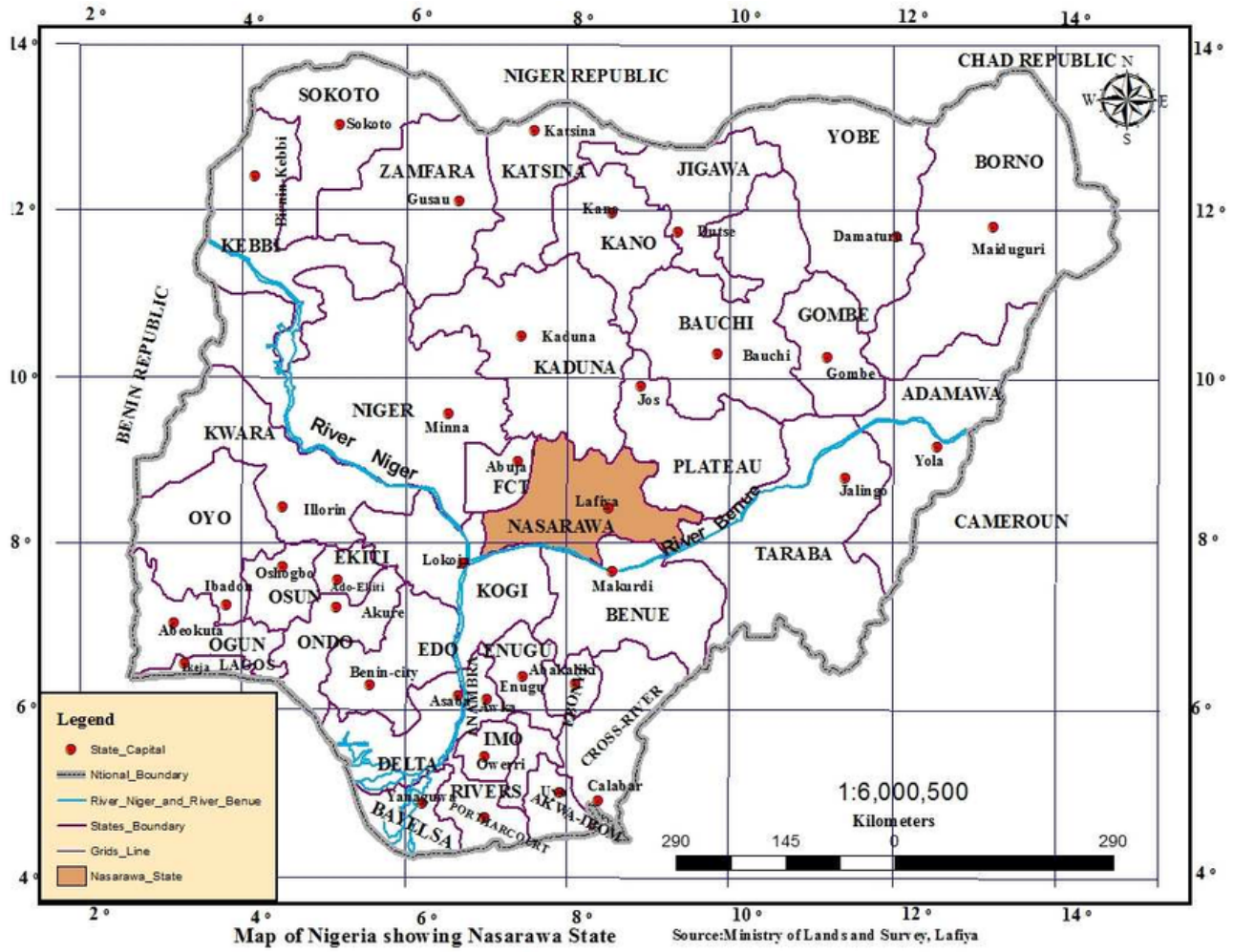

Figure 1. Map of Nigeria showing Nasarawa State and the Study area (Lafia)

for 48 hours at 37 ˚C. Yellow presumptive colonies were further screened using Gram staining, catalase test with 3% H_2O_2 and coagulase test using rabbit plasma. The pure cultures were maintained on Luria broth (LB) agar plates and stored at 4 ˚C.[15]

Antibiotics susceptibility testing

The antibiotic susceptibility of the identified bacteria was determined using the Kirby-Bauer disk diffusion method, as described by Gosden et al,[16] We selected ten commonly used antibiotics based on the National Committee for Clinical Laboratory Standard Assessment Criteria of 2012: Amoxicillin (10 µg), cefuroxime (30 µg), cefixime (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), cotrimoxazole (25 µg), doxycycline (30 µg), gentamicin (10 µg), and amikacin (30 µg). The pure cultures of the identified bacteria were inoculated in sterile nutrient broth and incubated at 37 °C for 24 hours. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 isolates served as standard control markers. Two loopfuls (0.08 mL) of each bacterial suspension (standardized by matching with a 0.5 x 10-8 McFarland standard) were inoculated into 20 mL of sterile molten Mueller-Hinton agar plates in triplicates. Antibiotic-impregnated disks were carefully placed on the agar surfaces using sterile forceps, ensuring proper spacing to prevent overlapping inhibition zones. The mean diameter of the inhibition zones around each triplicate disk was measured to the nearest millimeter, and the results were interpreted as either 'Sensitive' (S) or 'Resistant' (R) based on standard susceptibility breakpoints as specified by CLSI.[17]

Determination of Multiple Antibiotic Resistance Index (MARI)

MAR strains were determined based on isolates that exhibited resistance to at least three different classes of antibiotics.[18]

MARI value was determined using the formulae: $MARI = x/y$,

where x = number of antibiotics to which the test isolate displayed resistance and y = total number of antibiotics to which the test isolate was exposed.

Plasmid DNA Extraction

Out of the 146 *E. coli* and 141 *S. aureus* isolates identified as multidrug-resistant, 15 isolates of each test organism were randomly selected for analysis (5 from each healthcare facility). Plasmid DNA extraction was performed using

the alkaline lysis method with slight modifications following Opera and Ojo.[19] Each isolate was cultured in an Eppendorf tube containing 5 mL of Muller-Hinton broth and centrifuged at 10 000 rpm for 2 minutes. The resulting pellets were inoculated in 150 µL of EDTA-Tris buffer and vortexed. Approximately 175 µL of both 2% SDS and 0.4N NaOH were added. After thorough mixing, 250 µL of cold 5 M potassium acetate was introduced, and the tube's contents were centrifuged at 12 000 rpm for 5 minutes. The supernatant was then transferred to a sterile 1.5 mL Eppendorf tube, and an equal volume of cold isopropanol was added. The mixture was gently inverted and immediately centrifuged at 12 000 rpm for 10 minutes. The resulting residue (DNA pellets) was washed with 650 µL of cold (4 °C) 70% ethanol by centrifuging at 12 000 rpm for 15 minutes. The supernatant was discarded, and the pellets were dried for 30 minutes before being resuspended in 40 µL of sterile deionized water. The samples were stored at 4 °C or frozen until needed.

Plasmid Profiling of DNA Extracts

The extracted DNA was separated using 0.8% agarose gel electrophoresis, following the procedure outlined by Coban et al,[20] The prepared agarose gel was placed in the electrophoresis tank and allowed to settle. For each DNA extract, a 15 µL aliquot was combined with 2 µL of dye and loaded into the respective wells. Electrophoresis was conducted at 120V for 1 hour, and the separated DNA was visualized and photographed using a digital UV trans-illuminator (Clinix, Japan). Plasmid sizes were estimated in base pairs (bp) using a super mix plasmid DNA marker (GeNei TM, Genei Laboratories Private Limited, India). Plasmid profiles for each test organism were created by grouping strains with the same number of plasmid bands, each associated with various molecular sizes.

Statistical Analyses

The results were presented as frequencies and percentages. Contingency chi-square tests were employed to investigate the association between antibiotic resistance in both bacterial species and their wastewater locations. Data analysis was carried out using the Statistical Package for Social Sciences (SPSS), version 26 (IBM SPSS Inc., Chicago, IL, USA) for Windows. Significance was determined with two-tailed *P* values, with values less than 0.05 considered significant.

Results and Discussion

Antibiotic Resistance of Escherichia coli and Staphylococcus aureus Isolates

The antibiotics resistance patterns of *E. coli* and *S. aureus* isolates from the various effluents have been shown in Figure 2. In this study, *E. coli* (72.23%) and *S. aureus* (75.76%) isolated from the wastewater samples had

Figure 2. Antibiotics resistance pattern of *Escherichia coli* and *Staphylococcus aureus* isolates from healthcare effluent samples. Amx, Amoxicillin; Cef, Cefuroxime; Cfx, Cefixime; Ery, Erythromycin; Cpr, Ciprofloxacin; Lev, Levofloxacin; Cot, Cotrimoxazole ; Dox, Doxycycline; Gen, Gentamicin; Ami, Amikacin

highest resistance to amoxicillin with values of 79.0% and 66.3%, respectively, while the least resistance was obtained with levofloxacin (26.3%) and cotrimoxazole (25.1%), respectively. The detection of *E. coli* and *S. aureus* in the samples may be attributed to the fact that these pathogens are common contaminants in the hospital environment and are commonly found in the gastrointestinal tracts of both humans and animals.[3,5,21] Direct and indirect fecal contamination of wastewater from hospitals and healthcare centers is a common phenomenon, which may contribute to the development of antibiotic-resistant strains that can horizontally disseminate to other organisms. A similar study on untreated liquid hospital waste reported the highest resistance (75%) among *E. coli* isolates to amoxicillin, although its prevalence value of 24.1% was significantly lower than that obtained in this study.[22] High percentages of resistance to amoxicillin in these organisms were also reported for *E. coli* from hospital wastewater[23] and aquafarms,[24] while *S. aureus* isolates with similar potentials were reported in hospital wastewater.[25] *E. coli* and *S. aureus* were found to be prevalent in different hospital environments in Umuahia, Nigeria, and exhibited resistance to 50%–100% of the antibiotics used.[5] However, a study by Odonkor and Addo[26] on *E. coli* isolates from drinking water sources reported low resistance to amoxicillin and relatively high resistance to ceftriaxone. The observed deviation from the findings of this study may be attributed to the differences in the dynamics of the two environments. The seemingly statistically non-significant difference in the resistance ($P = 0.897$) exhibited by both bacterial species to most of the antibiotics in this study reflects the fact that these organisms are equally exposed to antimicrobial agents in various healthcare environments.

Overall Antibiotic Resistance Pattern of Isolates From Wastewater of Different Healthcare Environments

Figure 3 shows the overall antibiotics resistance profile of all the isolates in the wastewater from the various healthcare facilities. There was no significant difference in the phenotypic antibiogram among the wastewater isolates from the healthcare facilities ($P > 0.05$). This may suggest a similar antibiotics exposure within different wastewater environments. The behavior of patients and healthcare personnel regarding hygiene and drug handling was observed to be similar across various healthcare facilities, which could lead to the generation of wastewater with similar properties. Comparable findings were reported by researchers working on wastewater from different hospital environments in Ethiopia[25] and Umuahia, Nigeria.[5] However, the results of this study contradict those of Moges et al,[27] who reported differences in the antibiotic resistance patterns of bacterial isolates from hospital and non-hospital environments. The antibiogram obtained in this study also differs from those obtained from drinking water isolates.[26]

MAR Profile of Escherichia coli and Staphylococcus aureus

The multiple antibiotics resistance (MAR) profile of *E. coli* and *S. aureus* isolates from various wastewater outlets has been presented in Table 1. Results showed that (46.1%) of the *E. coli* and (29.1%) of *S. aureus* isolates were resistant to all the antibiotics tested (MARI = 1.0). Most isolates of the test organisms had MARI values greater than 0.5, with 87.4% of *E. coli* and 80.6% of *S. aureus* isolates exhibiting

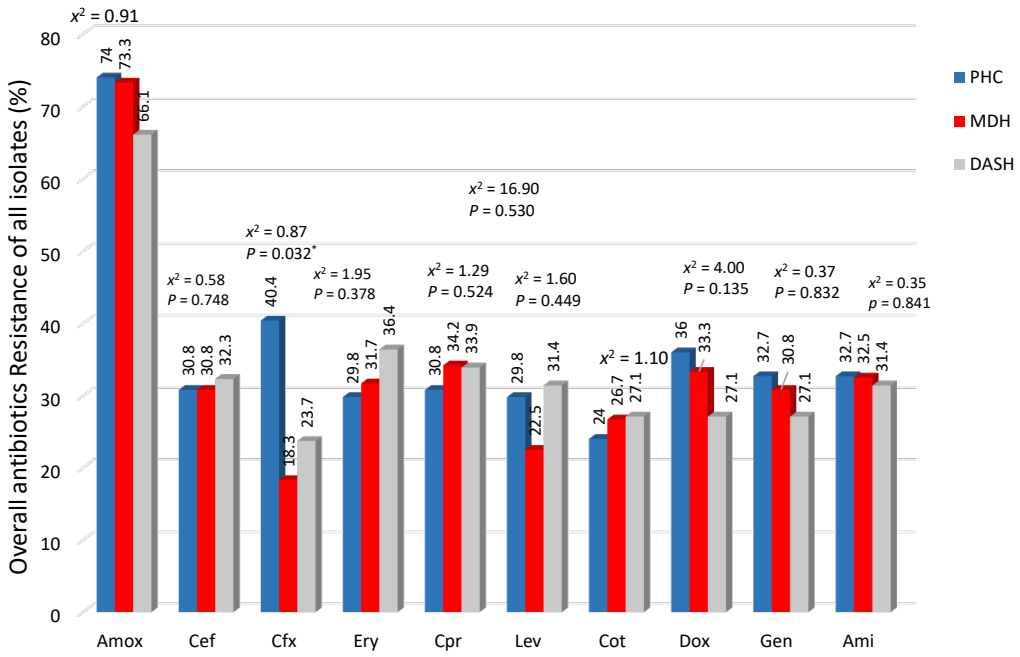

Figure 3. Overall antibiotics resistance pattern of all the isolates in the wastewater from various healthcare environments. Amx, Amoxicillin; Cef, Cefuroxime; Cfx, Cefixime; Ery, Erythromycin; Cpr, Ciprofloxacin; Lev, Levofloxacin; Cot, Cotrimoxazole; Dox, Doxycycline; Gen, Gentamicin; Ami, Amikacin; PHC, primary health center; MDH, medical and diagnostic hospital; DASH, Dalhatu Araf Specialist Hospital; *P value with significant difference.

Table 1. Mar profile of *Escherichia coli* and *Staphylococcus aureus* Isolates From Hospital Effluents

Bacterial Isolates	Total No. of Isolates	Phenotypic Resistance Pattern	No. of Isolates Showing Pattern No. (%)	No. of Antibiotics Class	Number of Antibiotics Resistant N=10	MARI
Escherichia coli	167	$Gen^{a}Ami^{a}Ery^{a}Cef^{c}Cfx^{c}Amx^{p}Cpr^{q}Lev^{q}Cot^{s}Dox^{t}$.	77 (46.1)	6	10	1.0
		$Gen^{a}Ery^{a}Ami^{a}Cfx^{c}Amx^{p}Cpr^{q}Cot^{s}Dox^{t}$.	29 (17.4)	6	8	0.8
		$Gen^{a}Ery^{a}Amx^{p}Cot^{s}Dox^{t}$.	18 (10.8)	4	5	0.5
		$Ami^{a}Cfx^{c}Cot^{s}Dox^{t}$,	20 (12.0)	4	4	0.4
		$Gen^{a}Amx^{p}Dox^{t}$	2 (1.2)	3	3	0.3
Total			146 (87.4)			
Staphylococcus aureus	175	$Gen^{a}Ami^{a}Ery^{a}Cef^{c}Cfx^{c}Amx^{p}$ $Cpr^{q}Lev^{q}Cot^{s}Dox^{t}$	51 (29.1)	6	10	1.00
		$Gen^{a}Ami^{a}Ery^{a}Cfx^{c}Amx^{p}Cpr^{q}Lev^{q}Cot^{s}Dox^{t}$,	43(24.6)	6	9	0.9
		$Gen^{a}Cfx^{c}Amx^{p}Cpr^{q}Lev^{q}Cot^{s}Dox^{t}$,	34 (19.4)	6	7	0.7
		$Ery^{a}Amx^{p}Cpr^{q}$ $Lev^{q}Cot^{s}Dox^{t}$	7 (4.0)	5	6	0.6
		$Gen^{a}Ami^{a}Lev^{q}$ Dox^{t}	2 (1.1)	3	4	0.4
		$Amx^{p}Cpr^{q}Dox^{t}$	4 (2.3)	3	3	0.4
Total			141(80.6)			

MARI, Multi-antibiotics resistant index; Amx, Amoxicillin; Cef, Cefuroxime; Cfx, Cefixime; Ery, Erythromycin; Cpr, Ciprofloxacin; Lev, Levofloxacin; Cot, Cotrimoxazole; Dox, Doxycycline; Gen, Gentamicin; Ami, Amikacin; [a] Aminoglycosides class; [c] Cephalosporins class; [p] Penicillins class; [q] Quinolones class; [s] Sulfonamides class; [t] Tetracyclines class.

resistance to at least three classes of antibiotics (multidrug resistance). The high MARI values obtained from most of the isolates indicate that the wastewater environments were indeed characterized by the presence of most of the tested antibiotics. These organisms likely acquired resistance due to prolonged exposure. This is further supported by the fact that the antibiotics used in this study were regularly administered either through prescription or over-the-counter, suggesting that they must have been introduced to the healthcare environment by the handlers. A similar study reported multidrug resistance in 85.11% of all the hospital wastewater isolates screened.[21] Additionally, Uzoije et al,[5] reported mean average MARI values of 0.5 and 0.8 for *E. coli* and *S. aureus* wastewater isolates, respectively. MARI values in the range of 0.8 to 1.0 for *E. coli* isolates from abattoir wastewater have also been reported.[28] A significantly lower value of 60.7% has been reported for multidrug resistance using hospital sewage isolates.[23] The findings of this study also contradict those of Lihan et al,[24] who reported MARI range values of 0.17 to 0.83 for *E. coli* isolates from aquafarms. They concluded that MAR in *E. coli* from rivers was significantly higher compared to aquaculture environments. The difference between their study and ours may be attributed to the distinct environmental characteristics between aquafarms and healthcare environments.

Plasmid Profile Analysis of Escherichia coli and Staphylococcus aureus Multidrug Resistant Isolates

The plasmid profile results for randomly selected multidrug resistant wastewater isolates of both organisms are shown in Table 2, Figures 4 and 5. The plasmid analysis indicated that *E. coli* isolates had single (20.0%), double (46.7%), and triple (26.7%) plasmids, while 6.1% had no plasmids. In contrast, 53.3% of *S. aureus* isolates had a single plasmid, and 46.7% had no plasmids. This study aligns with the work of Atuanya et al,[29] whose plasmid DNA analysis of *S. aureus* and *E. coli* showed the presence of plasmids in some isolates while others had none. The plasmid sizes of the *E. coli* isolates ranged between 1000 and 8000 bp, while a single plasmid size of 7500 bp was observed for S. aureus. These findings suggest that multiple drug resistance displayed by some test isolates may have been acquired through a plasmid-mediated process. This is consistent with the findings of Akter et al,[30] who reported plasmid sizes ranging from 1.5 to 15 kb for *E. coli* isolates from human sewage. However, their observation that the most common plasmids, with a size range of approximately 11 to 12 kb, were harbored by all *E. coli* strains, could not be confirmed by this study. Jaran's[31] plasmid analysis of clinical isolates in Saudi Arabia revealed the presence of 0 to 3 plasmids with a size range of 2 to 31 kb, which differs from the results obtained in this study. These differences may be attributed to variations in wastewater characteristics from different healthcare outlets. It is worth noting that this study had some limitations, including the restriction to healthcare environments within a specific location and the relatively smaller size of the test strains screened for plasmid analysis. Bacteria with the same number of plasmid DNA and base pair sizes imply that their plasmid DNA likely originated from the same environment, possibly transferred through horizontal gene transfer within that environment. It is worth noting that those with large plasmids tend to exhibit higher resistance to antibiotics, as reported by Zulkifli et al,[32] However, isolates showing a multiple drug resistance pattern with a high number of antibiotics but lacking plasmids may suggest that their acquisition of resistant genes may be either chromosome-mediated or from other genetic materials.[33] In addition to

Table 2. Plasmid DNA Analysis of *Escherichia coli* and *Staphylococcus aureus* Isolates in Effluents From Various Healthcare Environments

Bacterial isolates (N=15)	*Escherichia coli*					*Staphylococcus aureus*				
	Plasmid size (bp)	R_1	R_2	R_3	R'	Plasmid Size (bp)	R_1	R_2	R_3	R'
P1	1000, 3800, 8000	-	-	+	-	7500	+	-	-	-
P2	2700, 3600, 5300	-	-	+	-	7500	+	-	-	-
P3	4800, 5000	-	+	-	-	-	-	-	-	+
P4	5500, 6000	-	+	-	-	7500	+	-	-	-
P5	-	-	-	-	+	-	-	-	-	+
M1	3800, 4000	-	+	-	-	-	-	-	-	+
M2	4000, 4800	-	+	-	-	-	-	-	-	+
M3	5000, 5500	-	+	-	-	-	-	-	-	+
M4	3500, 4800, 7500	-	-	+	-	7500	+	-	-	-
M5	2000, 4800, 5300	-	-	+	-	7500	+	-	-	-
D1	1500, 3800	-	+	-	-		-	-	-	+
D2	3800	+	-	-	-	7500	+	-	-	-
D3	3700	+	-	-	-		-	-	-	+
D4	1300	+	-	-	-	7500	+	-	-	-
D5	1500, 4000	-	+	-	-	7500	+	-	-	-
Total (%)		3(20.0)	7(46.7)	4(26.7)	1(6.7)		8(53.3)	0(0.0)	0(0.0)	7(46.7)

P1-P5, Isolates from Primary Health Center; M1-M5, Isolates from Medical and Diagnostic Hospital; D1–D5, Isolates from Dalhatu Araf Specialist Hospital; R_1, Single plasmid; R_2, Double plasmid; R_3, Triple plasmid; R', no plasmid; +, Obtained; –, Not obtained.

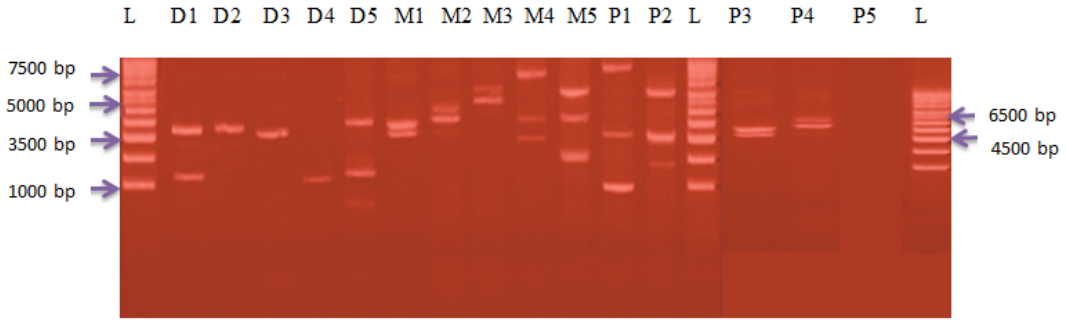

Figure 4. Agarose Gel Electrophoresis of Plasmid DNA From the *Escherichia coli* Isolates. Lane L: Supercoil DNA ladder composed of DNA fragments (in base pairs); Lane D1-D5: *Escherichia coli* isolates from Dalhatu Araf Specialist Hospital Lafia; Lane M1-M5: *Escherichia coli* isolates from M & D Hospital Lafia; Lane P1-P5: *Escherichia coli* isolates from Primary Healthcare Centre Mararaba, Lafia

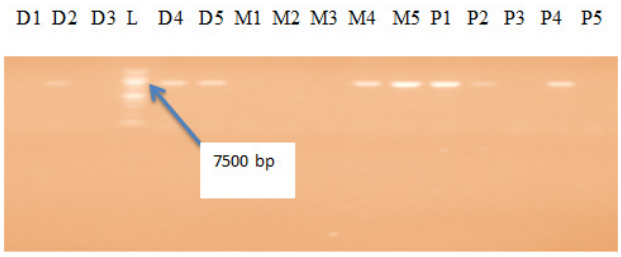

Figure 5. Agarose Gel Electrophoresis of Plasmid DNA From the *Staphylococcus aureus* Isolates. Lane L: Supercoil DNA ladder composed of DNA fragments (in base pairs); Lane D1-D5: *Staphylococcus aureus* isolates from Dalhatu Araf Specialist Hospital Lafia; Lane M1-M5: *Staphylococcus aureus* isolates from M & D Hospital Lafia; Lane P1-P5: *Staphylococcus aureus* isolates from Primary Healthcare Centre Mararaba, Lafia

plasmids, antibiotic resistance genes have also been found in transposons within mobile gene cassettes located at specific sites known as integrons.[34] For instance, clinical isolates of *Pseudomonas aeruginosa* have been shown to possess aac(6)II (in class I integrons) and dfrAI and aacA7 (both in class II integrons) as the most prevalent multidrug resistant genes.[35] They also observed that 27.5, 25.5, and 15% of the clinical isolates were positive for Class 1, 2, and 3 integrons, respectively, with genes that code for resistance to aminoglycosides being the most commonly harbored by the integrons. Integron markers have also been shown to be more prevalent in hospital wastewater environments where the use of antibiotics is more intensive.[36] Horizontal gene transfer through mechanisms such as transduction and natural transformation has been reported to play a significant role in the spread of antibiotic resistance integron genes in hospitals and other clinical settings.[37] It is important to note that while plasmids were investigated in this study, the possible involvement of integron genes in transposons for the acquisition of antibiotic resistance by the non-plasmid isolates cannot be discounted.

Conclusion

This study revealed that *E. coli* and *S. aureus* are prevalent

in wastewater from various healthcare facilities in the study area. These pathogens exhibited high resistance to amoxicillin and moderate resistance to most commonly administered antibiotics. The degree of resistance was not associated with the wastewater source. Among the screened isolates, 46.1% of *E. coli* and 29.1% of S. aureus showed resistance to all antibiotics (MARI = 1.0). Additionally, a significant proportion of the isolates exhibited resistance to more than half of the tested antibiotics. Specifically, 87.4% of *E. coli* and 80.6% of S. aureus isolates were classified as multidrug resistant. The test organisms were found to possess different sizes of plasmids, which may play a role in the dissemination of multidrug resistance within the microbial population. Controlled administration of antibiotics to patients and the proper treatment and management of wastewater from healthcare facilities in Lafia, Nigeria, could significantly mitigate the public health threat posed by these multidrug-resistant pathogens in wastewater effluents.

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Authors' Contribution

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Software: Dantani Dauda Odonye.
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Competing Interests

All items used in this research were obtained locally and mainly used in our area of research. There is therefore no conflict of interest between the authors and producers of such items.

Ethical Approval

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