

## Research Article

# Assessment of Antibiotic-resistant Bacteria in Hospital Waste Water Effluents from a Tertiary Hospital in Abakaliki, Nigeria

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## Abstract

The study was carried out with an aim to isolate, characterize and determine the antibiotics resistance pattern of bacteria isolated from a tertiary hospital in Abakaliki. Waste water effluents were collected from various units of the Alex Ekwueme University Teaching Hospital, Abakaliki and analysed at the Applied Microbiology Laboratory unit of the Ebonyi State University Abakaliki using standard microbiology techniques. Susceptibility of the bacterial isolates to various classes of antibiotics was determined by disc diffusion method. Production of Extended spectrum beta lactamase was determined by the double disc synergy method and the presence methicillin resistant *Staphylococcus aureus* was determined using Cefoxitin and methicillin disc diffusion method. Our findings showed that *Escherichia coli* had the highest frequency of bacterial occurrence (43.24%) followed by *S. aureus* (40.54%), *Klebsiella* (8.11%) and *Campylobacter* (8.11%). Waste water effluents from the ward complex had the highest distribution of bacteria isolates (40.54%) followed by effluents from the laboratory complex. Effluents from the theatre had the least distribution (2.70%). Out of 15 *S. aureus* isolates obtained from this study, 46.66% (7) were MRSA positive while out of 16 *E. coli* isolated from this study, 31.25% (5) were positive for ESBL production. Multi-antibiotic resistance index of *S. aureus* showed the highest values to be from the ward complex (1.00) and laboratory complex (0.29). Multi-antibiotic resistance index of *Campylobacter* species showed the highest values to be from the ward complex (0.75) and the least from laboratory complex (0.50) and that of *Klebsiella* species showed the highest values to be from accident/emergency (0.88) and the least from the ward complex (0.63). Multi-antibiotic resistance index of *E. coli* showed the highest values to be from the ward complex and laboratory complex (1.00). The presence of antimicrobial resistant bacteria from hospital wastewaters showed the spreading of AMR bacteria into the environmental through wastewater. The presence of high MDR bacteria in hospital wastewater may impose public health challenges because they can transmit resistance traits to other enteric pathogenic bacteria in the community.

## Keywords

Hospital, Waste, Water, Antibiotics, Resistant, Abakaliki

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## 1. Introduction

Hospital waste is categorized into pathological waste; body fluids from surgery, infectious waste from laboratories, pharmaceutical waste; out-of-date pharmaceutical products, and chemical wastes; used solvents, disinfectants, pesticides, and diagnostic chemicals, aerosol containers and gas, and open sources used in *in vitro* diagnosis or nuclear medical therapy [4]. Sewage from hospitals, usually referred to as hospital waste water, is defined as a special category of waste which comprises all wastes, biological or non-biological, that are discarded from hospitals/healthcare centers and not intended for further use [11, 12].

The important usage of water in hospitals gives significant volumes of waste loaded with microorganisms (the majority of which being pathogenic), heavy metals, toxic chemicals, disinfectant and radioactive elements [10].

However, many non-metabolized drugs excreted from patients and residual disinfectants enter into wastewater, which finally interacts with the microflora of hospital sewage. Hospital wastewater are considered and best described as hot spots for the dissemination of antibiotic and disinfectant resistance bacteria that could threaten public health upon water reuse [9, 16].

Hospitals accumulate large amounts of antibiotics and disinfectant resistant human-related pathogens. For example, *Acinetobacter baumannii*, *Citrobacter freundii*, *Klebsiella*, *Aeromonas*, *Pseudomonas*, *S. aureus* (*E. coli*, *P. aeruginosa*, *Enterobacter* spp, coagulase-negative *Staphylococcus*, *Salmonella* spp, *Shigella* spp., which cause serious hospital-associated infections, have shown resistance to multiple antibiotics [15].

The abundance of antibiotic resistance in the hospital waste water has become a major global public health concern as a result of the widespread use of antibiotics in healthcare systems. If the resistant bacteria are carrying a transmissible gene, they transfer resistant genes to other community bacteria so that infection caused by these bacteria are usually difficult to treat, and it also decreases the antibiotic pool for the treatment of bacterial infections.

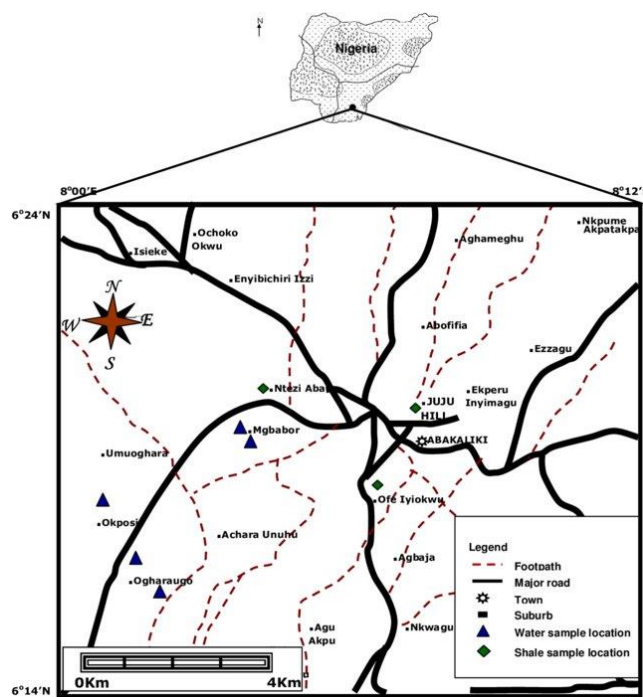
Without suitable treatment, the discharge of antibiotic-resistant bacteria (ARB) in hospital wastewater poses ecological and ARB evolution risks to aquatic environments and humans. This study was designed to isolate, characterize and determine the pattern of resistance of bacteria from hospital waste water effluents to various antibiotics.

## 2. Material and Methods

### 2.1. Study Area

The study was carried out at Alex Ekwueme Federal Teaching Hospital Abakaliki, Ebonyi State Abakaliki. Abakaliki is the capital city of Ebonyi State, located 64 kilometers

southeast of Enugu in southeastern Nigeria. The majority of the residents are Igbo rice and salt producers. The metropolis of Abakaliki is located in the eastern section of the country at latitude 6.3°E and longitude 8.1°N [2].



**Figure 1.** Map showing Abakaliki in Ebonyi State the study area [2].

### 2.2. Ethical Clearance

Ethical clearance (NHREC/16/05/22/361) was collected from the research and ethics committee of Alex Ekwueme Federal Teaching Hospital, Abakaliki, Ebonyi State, before the commencement of sample collection. Every fundamental study was done in accordance with the ARRIVE guidelines.

### 2.3. Sample Collection

A total of Two hundred and fifty (250) wastewater samples was collected from AEFUTHA at different intervals as follows:

- 1) From the untreated wastewater outlet pipe of the hospital before it enters the sewer system,
- 2) Sewage treatment plant (using activated sludge), and
- 3) Treated water (effluent) before being discharged.

A volume of 250ml wastewater was collected from each site using plastic bottles (sterilized with 70% ethanol for 3 min followed by three times rinsing with sterile distilled water). The collected samples were preserved on ice, and transported to the microbiology laboratory unit of Ebonyi State University for microbiological analysis.

## 2.4. Bacterial Isolation from Treated Hospital Wastewater

The HWW samples were grouped and pooled then thoroughly shaken to get a homogeneous mixture before a portion was taken for culture. Serial 10-fold dilutions of wastewater samples were prepared in 9ml volume of sterile water. The bacteria were cultivated by streak plating 0.5 ml of each of the desired serial dilutions of suspensions, 7th and 8th (10<sup>-7</sup> and 10<sup>-8</sup>) dilutions of HWW. Duplicate samples were plated onto MacConkey (MAC) agar, Mannitol Salt agar (MSA), Salmonella/Shigella agar and Ceftrimide agar and then incubated at 37°C for 24 hours. After obtaining pure colonies and recording important features, isolated bacteria were further identified at the species level by biochemical tests. The obtained pure cultures of the isolates were maintained at 4°C as agar slants and as glycerol stocks at -20°C in the same media broth containing 25% glycerol [8].

## 2.5. Morphological Identification and Biochemical Identification

Morphological and biochemical identification of Bacteria were carried out using Gram stain, Motility test (Hanging drop), Catalase, Coagulase and Carbohydrate fermentation tests.

## 2.6. Preparation of 0.5 McFarland Turbidity Equivalent Standards

Turbidity standard equivalent of 0.5 McFarland standards was prepared by adding 1.0 ml of concentrated tetraoxosulphate (VI) acid (H<sub>2</sub>SO<sub>4</sub>) to 99 ml of distilled water; then 0.5 g of dehydrated barium chloride (BaCl<sub>2</sub>. H<sub>2</sub>O) was dissolved in 50ml of distilled water in a separate flask. A volume of 0.6ml of barium chloride solution was introduced into tetraoxosulphate (vi) acid solution (99.4 ml) in a separate conical flask. It was mixed well to obtain 0.5 MacFarland turbidity equivalent standards. Some portions of the mixed solutions were transferred to test tube and stored at room temperature (28°C). This was used to compare the turbidity of the test organisms prior to susceptibility test [7].

## 2.7. Standardization of Test Bacteria

All the test bacteria were standardized before use by inoculating a loopful of a 24 hrs culture of the test organism from a nutrient agar slant into test tubes with 5ml sterile water. It was diluted using loopful of the test organism and sterile water in order to obtain microbial population of 1 x 10<sup>6</sup> colony forming unit per milliliter (cfu/ml) by comparing it with 0.5 McFarland turbidity standards.

## 2.8. Antibiotic Susceptibility Testing

This was aseptically carried out using Kirby-Bauer disk diffusion method, and in conformity to the recommended standard of Clinical and Laboratory Standard Institute (CLSI, 2019). A suspension of the test bacteria was made from a 24 hours growth of the test organisms in sterile water to match the 0.5 McFarland turbidity standard. This was seeded on the entire surface of solidified Mueller-Hinton agar (Sigma-Aldrich, U-S-A) plate. The following antibiotic discs with their potencies were used: Oxacillin (OX-30ug), Ofloxacin (OFX-5ug), Linezolid (L-15ug), Clindamycin (DA-2ug), Vancomycin (VA-10ug), Imipenem (IPM-10ug), Erythromycin (E-5ug), Meropenem (MEM-10ug), Cefoxitin (FOX-30ug), Azithromycin (ATM-30ug), Ciprofloxacin (CIP-5ug), Tetracycline (TE-30ug), Ceftriazone (CRO-30ug), Trimethoprim/Sulphamethoxazole (SXT-25ug), Nalidixic acid (NA-30ug), Nitrofurantoin (F-100ug), Amoxicillin/Clavulanic acid (AMC-30ug), Ceftazidime (CAZ-30ug), Gentamycin (CN-30ug), Ceftriazone (CRO-30ug), Ceftazidime (CAZ-30ug).

The Mueller-Hinton agar (Sigma-Aldrich, U-S-A) plates was incubated at (35°C -37°C) in an aerobic atmosphere for 18-24 hours, after which the percentage susceptibility and resistance were interpreted from the inhibition zone diameters (IZD) produced by the antibiotic discs against the test isolates [3].

## 2.9. Determination of Multi-Drug Resistant Index (MDRI)

Multi-drug resistant index (MDRI) was determined to ascertain the resistance level of the isolates which is the number of antibiotics the test isolates are resistant to. The MDRI formula used is:

$$\text{MDRI} = a/b$$

Where, a = number of antibiotics to which the isolate is resistant to.

b = total number of antibiotics to which the isolates are subjected to.

*Test for MRSA (Brilliance MRSA Chromagar):* A colony of an organism suspected to be MRSA was smeared and streaked out on this media, along with a known MRSA colony and incubated at 35°C for 24 hours. Thereafter the culture plate was observed and MRSA colonies displayed deep blue coloration (Brilliance blue).

## 2.10. ESBL Confirmation Using Double Disc Synergy Test (DDST)

Using a double disc synergy test, the *E. coli* isolates resistant to the 2<sup>nd</sup> and 3<sup>rd</sup> generation cephalosporin were further investigated for extended-spectrum beta-lactamase. This was performed on a Mueller Hinton agar with 3 major

antibiotics to detect extended-spectrum beta-lactamase-producing organisms. The antibiotics used were ceftazidime (CAZ) 30 µg, cefotaxime (CTX) 30 µg, and amoxicillin-clavulanic acid (AMC) 30 µg, which were placed in a parallel form at a distance of 15 mm from each other with amoxicillin clavulanic acid being at the center of the Petri dish. The culture plates containing the suspected ESBL-producing *E. coli* were impregnated with the ceftazidime (CAZ-30ug), Cefotaxime (CTX-30ug) and Amoxicillin-Clavulanic acid (AMC-30ug) and incubated at 37°C for 18-24 hrs. The isolates were considered to be ESBL producers when there was a synergy between the center disc and the two discs with an increase in zone diameter of  $\geq 5$  mm for any of the antibiotics tested in combination with the clavulanic acid than when tested alone [5].

### 3. Results

Results from our study show that four bacteria were isolated. These were confirmed by various morphological and biochemical tests as shown in Table 1. Bacteria isolated include *S. aureus*, and *E.coli*. Some samples yielded no growth. *Escherichia coli* presented with the highest frequency (43.24%) of Bacteria isolated followed by *S. aureus* (40.54%), *Klebsiella* (8.11%) and *Campylobacter* (8.11%) as shown in Table 2. Waste water effluents from the ward complex had the highest distribution of bacteria isolated from this study (40.54%) followed by effluents from the laboratory complex. Effluents from the theatre had the least distribution (2.70%) as shown in

### Table 3.

Imipenem was the most resisted antibiotics by *S. aureus* (100%) and Ofloxacin was the most effective with distribution of 73.34% each as shown in Table 4. Nalidixic acid, ceftriazone and Trimethoprim were all completely resisted by *Campylobacter* and Gentamicin was the most effective Table 5. Ceftriazone was the most resisted antibiotics by *Klebsiella* (100%). Aztreomicin and Gentamicin were highly effective as shown in Table 6. Nalidixic acid was the most resisted antibiotics by *E. coli* (81.25%). Ceftriazone was the most effective (68.75%) in Table 7. Multi-antibiotic resistance index of *S. aureus* showed the highest values to be from the ward complex and laboratory complex. The least MARI was recorded with *S. aureus* isolates from Accident and Emergency as shown in Table 8. Multi-antibiotic resistance index of *Campylobacter* species showed the highest values to be from the ward complex and the least from laboratory complex as shown in Table 9. Multi-antibiotic resistance index of *Klebsiella* species showed the highest values to be from accident/emergency followed by the laboratory complex. The least MARI was recorded with *Klebsiella* isolated from the ward complex as shown in Table 10. Multi-antibiotic resistance index of *E. coli* showed the highest values to be from the ward complex and laboratory complex. The least MARI was recorded with *E. coli* isolates from the Theatre as shown in Table 11. Of 15 isolates obtained from this study, 46.66% were MRSA positive and 53.33% were MRSA negative as shown in Table 12. Out of 16 *E. coli* isolated from this study, 31.25% were positive for ESBL production while 68.75% were negative for ESBL production as shown in Table 13.

Table 1. Showing Morphological and Biochemical characteristics of bacteria isolated.

Morphological Characteristics				Microscopic Characteristics	Biochemical Test									Probable Organism
S/N	Cell Shape	Cell arrangement	Colour	Gram Reaction	Motility test	Catalase test	Citrate Test	Coagulase test	Indole Test	Oxidase Test	Methyl red	Nitrate reduction test	Vp test	
1	Rod	Single	Pink	-	+	+	-	-	+	-	+	+	-	<i>Escherichia coli</i>
2	Rod	Single	Shiny and dark pink	-	-	-	+	-	+	-	-	+	+	<i>Klebsiella sp</i>
3	Cocci	Clusters	Golden	+	-	+	+	+	-	-	-	-	-	<i>S. aureus</i>
4	Rod	Single	Cream	-	+	+	+	-	+	-	+	+	-	<i>Campylobacter sp</i>

**Table 2.** Percentage Distribution of Bacteria Isolated from Waste Water in *Ae-Futha*.

Bacteria Isolated	No Isolated (%)
Klebsiella sp	3 (8.11)
<i>Staphylococcus aureus</i>	15 (40.54)
<i>Campylobacter</i> species	3 (8.11)
<i>Escherichia coli</i>	16 (43.24)
Total	37 (100)

**Table 3.** Percentage Distribution of Bacteria from Various Locations of *Ae-Futha*.

Locations	No of Bacteria Isolated (%)
Wards	15 (40.54)
Laboratory	13 (35.14)
Theatre	1 (2.70)
Accident and Emergency	3 (8.11)
GOPD	5 (13.51)
Total	37 (100)

**Table 4.** Percentage Frequency of Antibigram of *S. Aureus* Isolates from Waste Water.

Antibi-otics	No of Iso-lates tested	No Resistant (%)	No Susceptible (%)
OFX	15	4 (26.66)	11 (73.34)
L	15	12 (80.00)	3 (20.00)
DA	15	11 (73.34)	4 (26.66)
VA	15	8 (53.33)	7 (46.67)
IPM	15	15 (100.00)	0 (0.00)
E	15	5 (33.33)	10 (66.67)
MEM	15	7 (53.33)	8 (46.67)

Key: OFX: Ofloxacin, L; Lincomycin, DA: Clindamycin, VA: Vancomycin, IPM: Imipenem, E: Erythromycin, MEM: Meropenem

**Table 5.** Percentage Frequency of Antibigram of *Campylobacter Sp.* Isolates from Waste Water.

Antibi-otics	No of Iso-lates tested	No Resistant (%)	No Susceptible (%)
ATM	3	1 (33.33)	2 (66.67)

Antibi-otics	No of Iso-lates tested	No Resistant (%)	No Susceptible (%)
F	3	0 (0.00)	3 (100.00)
CN	3	0 (0.00)	3 (100.00)
TE	3	2 (33.33)	1 (66.67)
GR	3	3 (100.00)	0 (0.00)
CRO	3	3 (100.00)	0 (0.00)
SXT	3	3 (100.00)	0 (0.00)
NA	3	3 (100.00)	0 (0.00)

Key: ATM; Azithromycin, F: Nitrofurantoin, CN; Gentamicin, TE: Tetracycline, CRO: Ceftriazone, SXT: Trimethoprim, NA: Nalidixic acid

**Table 6.** Percentage Frequency of Antibigram of *Klebsiella Sp.* Isolates from Waste Water.

Antibi-otics	No of Iso-lates tested	No Resistant (%)	No Susceptible (%)
ATM	3	0 (0.00)	3 (100.00)
F	3	0 (0.00)	3 (100.00)
CN	3	0 (0.00)	3 (100.00)
TE	3	1 (33.33)	2 (66.67)
GR	3	1 (33.33)	0 (0.00)
CRO	3	3 (100.00)	0 (0.00)
SXT	3	2 (66.67)	1 (33.33)
NA	3	2 (66.67)	1 (33.33)

Key: ATM; Azithromycin, F: Nitrofurantoin, CN; Gentamicin, TE: Tetracycline, CRO: Ceftriazone, SXT: Trimethoprim, NA: Nalidixic acid

**Table 7.** Percentage Frequency of Antibigram of *Escherichia Coli* Isolates from Waste Water.

Antibi-otics	No of Iso-lates tested	No Resistant (%)	No Susceptible (%)
ATM	16	10 (62.50)	6 (37.50)
F	16	7 (43.75)	9 (56.25)
CN	16	11 (68.75)	5 (31.25)
TE	16	8 (50.00)	8 (50.00)
GR	16	12 (75.00)	4 (25.00)
CRO	16	5 (31.25)	11 (68.75)

Antibi-otics	No of Iso-lates tested	No Resistant (%)	No Susceptible (%)
SXT	16	8 (50.00)	8 (50.00)
NA	16	13 (81.25)	3 (18.75)

Key: ATM; Azithromycin, F: Nitrofurantoin, CN; Gentamicin, TE: Tetracycline, CRO: Ceftriazone, SXT: Trimethoprim, NA: Nalidixic acid

**Table 8.** Multi-Antibiotic Resistance Index of *Staphylococcus aureus* Isolated from Waste Water Effluents from Various Units of Ae-Futha.

Location of isolate	MARI
Ward Complex	1.00
Laboratory Complex	1.00
Accident/ Emergency	0.29
Outpatient Department	0.71

**Table 9.** Multi-Antibiotic Resistance Index of *Campylobacter Species* Isolated from Waste Water Effluents from Various Units of Ae-Futha.

Location of isolate	MARI
Ward Complex	0.75
Laboratory Complex	0.50

**Table 10.** Multi-Antibiotic Resistance Index of *Klebsiella Species* Isolated from Waste Water Effluents from Various Units of Ae-Futha.

Location of isolate	MARI
Ward Complex	0.63
Laboratory Complex	0.75
Accident/ Emergency	0.88

**Table 11.** Multi-Antibiotic Resistance Index of *Escherichia Coli* Isolated from Waste Water Effluents from Various Units of Ae-Futha.

Location of isolate	MARI
Ward Complex	1.00
Laboratory Complex	1.00
Accident/ Emergency	0.63
Outpatient Department	0.88

Location of isolate	MARI
Theatre	0.13

**Table 12.** Distribution Of MRSA.

MRSA	No of Isolates (%)
MRSA Positive	7 (46.66)
MRSA Negative	8 (53.33)
TOTAL	15 (100)

**Table 13.** Distribution of ESBL producing *E. coli*.

ESBL	No of Isolates (%)
ESBL Positive	5 (31.25)
ESBL Negative	11 (68.75)
TOTAL	16 (100)

## 4. Discussion

Hospital wastewater, a critical yet often overlooked component in the spread of AMR, serves as a significant reservoir and conduit for antimicrobial-resistant bacteria (ARB). Hospital wastewater is a hotspot for ARB due to its high concentration of antibiotics and other pharmaceuticals, which significantly contribute to the development of antimicrobial resistant pathogenic bacteria, and spreading infections globally.

Findings from our study showed heterotrophic bacterial count that generally ranged from between  $1.12 \times 10^2$  cfu to  $5.6 \times 10^2$  cfu. The highest ranges of heterotrophic counts were recorded with our findings from waste water effluents collected from the ward complex and the Laboratory complex. Effluents from the ward complex recorded a range between  $3.2 \times 10^2$  cfu to  $4.2 \times 10^2$  cfu and effluents from the Laboratory complex recorded a range between  $2.0 \times 10^2$  cfu to  $5.6 \times 10^2$  cfu. Effluents from the accident and emergency complex recorded a range between  $1.48 \times 10^2$  cfu to  $4.2 \times 10^2$  cfu. Effluents from the General outpatient department recorded a range between  $1.12 \times 10^2$  cfu to  $2.2 \times 10^2$  cfu. Only one heterotrophic count ( $1.72 \times 10^2$ ) was recorded for the effluents from the theatre complex. Our results disagree with studies by Usman *et al* [14] who examined waste water from various hospitals in Offa; Kwara State. In their study the mean bacterial count population of wet season samples ranged between  $7 \pm 4.00 \times 10^5$  and  $150 \pm 43.59 \times (10^5 \text{ cfu/ml})$ , while that of dry season samples ranged between  $10 \pm 2.00 \times 10^5$  and  $225 \pm 67.27 \times 10^5 \text{ cfu/ml}$ . These variations might be due to differences in study period, strategies of the wastewater disposal system and differences

in approaches to infection prevention and control measures.

Findings from our study also show that various bacterial species were isolated from waste water effluents emanating from various sections of the hospital. The bacteria isolated include *Staphylococcus aureus*, *Campylobacter* species, *Klebsiella* and *Escherichia coli*.

*Escherichia coli* had the highest distribution (43.24%), followed by *Staphylococcus aureus* (40.54%), *Klebsiella* (8.11%) and *Campylobacter* (8.11%).

Findings from our study are consistent with previous studies from Pakistan [13]. Our findings did not agree with findings by [10]. Their study assessed resistance profile of Bacteria isolated in waste water in a tertiary hospital in Maiduguri. In addition to *E. coli*, *Klebsiella* and *S. aureus*, they also isolated *Enterobacter* species, *Pseudomonas*, *Salmonella*, *Proteus* and *Bacillus*. Another study in Akure reported the isolation of *E. coli*, *S. aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Salmonella typhi* and *Aeromonas hydrophila* [6].

With respect to location, waste water effluents from the ward complex had the highest distribution of bacterial isolates (40.54%) followed by effluents from the Lab complex (35.14%), General outpatients department (13.51%), Accident and emergency complex (8.11%) and the Theatre complex (2.70%).

Antibiogram results from this study showed that Imipenem was the most resisted of antibiotics by *S. aureus* (100.00%) and Ofloxacin was the most effective with distribution of 73.34%. Nalidixic acid, ceftriazone and Trimethoprim were all completely resisted by *Campylobacter* and Gentamicin was the most effective. Ceftriazone was the most resisted antibiotic by *Klebsiella* (100%) while Aztreomycin and Gentamicin were highly effective. Nalidixic acid was the most resisted antibiotics by *E. coli* (81.25%) while Ceftriazone was the most effective (68.75%).

Multi-antibiotic resistance index (MARI) of *S. aureus* showed the highest values to be from the ward complex and laboratory complex. The least MARI was recorded with *S. aureus* isolates from accident and emergency. Multi-antibiotic resistance index of *Campylobacter* species showed the highest values to be from the ward complex and the least from laboratory complex. Multi-antibiotic resistance index of *Klebsiella* species showed the highest values to be from Accident/Emergency followed by the laboratory complex. The least MARI was recorded with *Klebsiella* isolated from the ward complex. Multi-antibiotic resistance index of *E. coli* showed the highest values to be from the ward complex and laboratory complex. The least MARI was recorded with *E. coli* isolates from the Theatre.

Similarly, all bacterial isolates from hospital wastewater collected were found to be 100% resistant to some of the antibiotics tested. Similar observation was reported by Iweriebor *et al.* (2015) from Alice, Eastern Cape province of South Africa and European countries. More so, presence of high percentage of drug resistant isolates from hospital wastewater

suggests that, hospital wastewater could have contributed massively to the resistances observed among the isolates from the final effluent. Of 15 *Staphylococcus aureus* isolates obtained from this study, 46.66% were methicillin resistant and 53.33% were not methicillin resistant. A study in Akure reported that 6 out of 13 *Staphylococcus* isolates obtained from hospital waste water were methicillin resistant [6].

Out of 16 *E. coli* isolated from this study, 31.25% were positive for ESBL production while 68.75% were negative for ESBL production. Our study disagrees with findings from a study in Ibadan were 13.23% of bacteria isolated were ESBL producers [1]. The presence of antimicrobial resistant bacteria from hospital wastewaters showed the spreading of AMR bacteria into the environment through wastewater. Additionally, the results the present study highlight the importance of infection control measures within hospitals and the prudent antibiotic use to curb the spread of antibacterial resistant bacteria. This finding advocates for the implementation of disinfection of wastewater before discharging to the disposal systems.

The high prevalence of drug-resistant isolates from hospital wastewater samples analyzed suggests their persistence in the hospital environment, and their ability to spread antibiotic resistance due to selection pressure and horizontal gene transfer. Therefore, patients are advised to adhere strictly to the directives of the physician in administration of drugs so as to reduce the cases of antibiotics resistance. Also, adequate liquid waste treatment system should be developed to disinfect pathogens in hospital wastewater effluent before discharging into municipal water supply, so as to prevent diseases associated with hospital wastewater effluent microbes.

The presence of high MDR bacteria in hospital wastewater may impose public health challenges because they can transmit resistance traits to other enteric pathogenic bacteria in the community. Thus, wastewater effluents must be disinfected to minimize the microbial burden at each respective ward or unit. A further study is needed to assess factors that could be associated with bacterial contamination of the sewage system.

## Abbreviations

ESBL	Extended Spectrum Beta-lactamase
AMR	Antimicrobial Resistance
MDR	Multidrug Resistance
MDRI	Multidrug Resistance Index
ARB	Antibiotic Resistance Index
HWW	Hospital Waste Water

## Author Contributions

**Elom Ugochukwu Okpo:** Conceptualization, Funding acquisition

**Edeson Lucy Ogayi:** Data curation, Methodology

**Amaechi-Nnaji Victoria Obumneme:** Formal Analysis

**Odo Ikechukwu Ituma:** Investigation

**Agumah Nnabuife Bernard:** Writing – original draft, Writing – review & editing

**Iroha Ifeanyi Romanus:** Validation, Supervision

## Conflicts of Interest

There was no conflict of interest in this study.

## References

- [1] Adenike, O and Ayansina, D (2022). Occurrence of Extended Spectrum Beta Lactamase (ESBL) Producing Gram-Negative Bacteria in Wastewaters from Selected Hospitals in Ibadan, Oyo State, Nigeria. *Tropical Journal of Natural Product Research (TJNPR)*, 6(5), 826-830.
- [2] Adibe-Nwafor, J. O., Uduku, N. D., Iroha, C. S., Ibiam, F. A., Onuora, A. L., Nwafor, K. A., Peter, I. U., Iroha, I. R (2023). Distribution and Antibiotic Resistance Profile of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* from Fish Farms within Abakaliki Metropolis. *Advance in Research*, 24(5): 175-184.
- [3] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-eighth edition (M100). Wayne, PA: Clinical and Laboratory Standards Institute; 2019.
- [4] Dadi, B. R., Girma, E., Tesfaye, M and Seid, M (2021). Assessment of the Bacteriological Profile and Antibiotic Susceptibility Patterns of Wastewater in Health Facilities of Ethiopia. *International Journal of Microbiology*, 9(6): 45-50.
- [5] Ekuma, P. U., Ibiam, F. A., Ekuma, M I., Iroha, C. S., Peter, I. U and Iroha, I. R (2023). Evaluating the Bacteria Profile and Drug Susceptibility Patterns of Urinary Tract Infectious Pathogens in Pregnant Women in Abakaliki Metropolis, Nigeria. *International Journal of Pathogen Research*, 12(5): 52-62.
- [6] Emoruwa, T., Omoya, F., Okewale, A and Ajayi, K. (2024). Occurrence of extended spectrum beta lactamase (ESBL) producing *Escherichia coli* in wastewater from two hospitals in Akure. *Microbes and Infectious Diseases*.
- [7] Ilang D. C., Peter I. U., and Iroha I. R (2023). Antibiotic Resistance Profile of Clinical Importance Biofilm forming Extended Spectrum Beta-lactamase and Carbapemase Phenotype in Gram-negative bacteria isolates. *International Journal of Pharmacognosy and Life Science*, 4(2): 120-127.
- [8] Iroha, I. R., Orji, J. O., Onwa, N. C., Nwuzo, A. C., Okonkwo, E. C., Ibiam, E. O., Nwachi, A. C., Afuikwa, F. N., Agah, V. M., Ejikeugwu, E. P. C., Agumah, N. B., Moses, I. B., Ugbo, E., Ukpai, E. G., Nwakaeze, E. A., Oke, B., Ogbu, L and Nwunna, E (2019). *Microbiology Practical Handbook*. (Editor; Ogbu. O), 1st Edition. Charliteximage Africa (CiAfrica Press), Pp: 344.
- [9] Iweriebor, B. C., Obi, L. C and Okoh, A. I. Virulence and antimicrobial resistance factors of *Enterococcus* spp. isolated from fecal samples from piggery farms in Eastern Cape, South Africa. *BMC Microbiol* 15, 136 (2015). <https://doi.org/10.1186/s12866-015-0468-7>
- [10] Kummerer, K (2004). Resistance in the environment. *Journal of Antimicrobial Chemotherapy*, 54(2): 311–320.
- [11] Odu, C. E., Egbere, J. O., Onyimba, I. A., Ghamba, P. E., Godiya, S., Isyaka, T. M., Collins- Odu, J. O., Idigo, M. A and Ngene, A. C. (2022). Resistance Profiles of Bacteria Isolated from Wastewater in the State Specialist Hospital Maiduguri. *European Journal of Biology and Biotechnology*, 3(3), 1–6.
- [12] Oyeleke, S., Istifanus, N and Manga, S (2008). The effects of hospital solid waste on the receiving environment. *International Journal of Integrative Biology*, 3(3): 191.
- [13] Shahzad, A., Mian, A. H., Ul haq, I., Khan, M. A and Ali, K (2021). The emergence of different bacterial pathogens in hospital wastewater samples and their antibiotic resistance pattern. *Mater Circular Econ.*; 3: 1–8.
- [14] Usman, K. M., Arotupin, D. J and Ekundayo, F. O. (2021). Antibiotic resistant pattern of bacteria in untreated hospital wastewaters from Offa Local Government Area, Kwara State, Nigeria. *African Journal of Microbiology Research*, 15(11), 572-582.
- [15] Uzoije U. N., Moses I. B., Nwakaeze E. A., Uzoeto H. O., Otu J. O., Egbuna N. R., Ngwu J. N., Chukwunwejim C. R., Mohammed D. I., Peter I. U., Oke B and Iroha I. R (2021). Prevalence of Multidrug-resistant Bacteria Isolates in Waste Water from Different Hospital Environment in Umuahia, Nigeria. *International Journal of Pharmaceutical Sciences Review and Research*, 69(2): 25-32.
- [16] Yuan, T and Pia, Y (2023). Hospital wastewater as hotspots for pathogenic microorganisms spread into aquatic environment: A Review. *Frontier in Environmental Science*, 10: 17-34.