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Antibiogram and molecular characterization of biofilm producing bacteria isolated from service water in tertiary hospitals in Rivers State

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ABSTRACT

Hospital service water can harbor biofilm-producing bacteria, posing significant health risks, especially in tertiary healthcare settings where patients are vulnerable to waterborne infections. These bacteria contribute to biofilm formation, persistent infections, and antibiotic resistance. In Rivers State, Nigeria, maintaining water quality in tertiary hospitals is challenging, necessitating the investigation of biofilm-producing bacteria to improve water management and reduce infections. This study aimed to identify biofilm-producing bacteria in hospital water, assess their antibiotic resistance, and explore the genetic mechanisms underlying their resistance. Over three months, 135 water samples were collected from strategic locations, including the water source, operating theaters, wards, and laboratories. Samples were aseptically collected, preserved at 4°C, and analyzed using biochemical tests, antibiotic susceptibility profiling, biofilm formation assays, and molecular characterization. Twelve bacterial genera were identified, including three Gram-positive species (*Staphylococcus*, *Bacillus*, and *Enterococcus*) and nine Gram-negative genera (*Escherichia*, *Proteus*, *Salmonella*, *Enterobacter*, *Pseudomonas*, *Serratia*, *Shigella*, *Klebsiella*, and *Providencia*). Biofilm production was highest in *Shigella*, *Staphylococcus*, *Bacillus*, *Escherichia coli*, *Pseudomonas*, and *Salmonella* (100%), while *Providencia* (90%) and *Klebsiella* (37.5%) had lower rates. Molecular analysis revealed ten isolates with 96–100% genetic similarity, including *Shigella sonnei*, *Escherichia coli* (ETEC), *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Salmonella enterica*. Antibiotic susceptibility varied, with resistance to multiple antibiotic groups. Ofloxacin and Nitrofurantoin showed effectiveness against some isolates. This study confirms the presence of biofilm-producing, antibiotic-resistant bacteria in hospital water systems. It emphasizes the need for improved aseptic practices, plumbing maintenance, and regular water treatment to enhance water quality and prevent waterborne infections in tertiary hospitals.

Keywords: Service water, biofilm producing bacteria, resistance, antibiotics.

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INTRODUCTION

The formation of biofilms is a common strategy employed by bacteria to survive in various environments, including healthcare settings, where it contributes to antibiotic resistance and chronic infections (Douterelo et al., 2018). Tertiary hospitals are particularly vulnerable to biofilm-producing bacteria due to the constant presence of potential sources, such as service water (Nwadike et al., 2024). This presents a significant challenge for infection control and treatment outcomes, underscoring the need for effective measures to prevent and manage biofilm-related infections (WHO, 2023). Understanding the antibiogram and molecular characteristics of biofilm-producing bacteria in tertiary hospitals is essential for developing targeted treatment strategies and improving

patient safety (Salam et al., 2023).

Water distribution systems have been implicated in several disease outbreaks, highlighting the importance of maintaining water quality (Williams et al., 2021). Hospital water sources can act as reservoirs of infection, and the World Health Organization (WHO, 2023) emphasizes that drinking water should be free from contaminants that may endanger human health. However, water quality can deteriorate during distribution, and disinfection processes may not completely eliminate microbial loads (Raimi et al., 2021). This allows surviving bacteria to grow and form biofilms on piping materials, subsequently releasing cells into the water flow (Sauer et al., 2022). Notably, most bacteria in drinking water systems exist within biofilms rather than in a free-floating state (Douterelo et al., 2018).

The presence of *E. coli* in drinking water indicates recent fecal contamination (WHO, 2023), and its antimicrobial resistance serves as a marker for the therapeutic and nontherapeutic use of antimicrobial drugs (Bennani et al., 2020). The emergence of antimicrobial resistance is a common phenomenon in areas where antimicrobials are extensively used (Popoola et al., 2024; Sartorius et al., 2024). Enteric bacteria in humans and livestock treated with antibiotics can develop resistance to these substances, with human and animal feces serving as direct sources of this resistance (Serwecińska, 2020; Nadeem et al., 2020).

The growing prevalence of antimicrobial resistance poses a significant threat to human health (Salam et al., 2023). Of particular concern is the presence and persistence of antimicrobial-resistant (AR) bacteria, especially multidrug-resistant (MDR) strains (Kusi et al., 2022). Resistance determinants can be transferred to clinically significant bacteria, exacerbating the issue (Mancuso et al., 2021). The emergence of resistance within the *Enterobacteriaceae* family (Naidu, 2023; Felis et al., 2020) and the occurrence of AR bacteria in aquatic environments (Harris, 2020) are often attributed to anthropogenic activities, nonhuman applications of antibiotics, and improper waste disposal. These factors create environmental reservoirs of resistance and virulence genes (Endale et al., Abdeta, 2023).

MATERIALS AND METHODS

Description of study area

The research was conducted in three healthcare facilities within the Port Harcourt metropolitan area of Rivers State, Nigeria. The selected hospitals were:

1. Rivers State University Teaching Hospital (RSUTH), located in Port Harcourt Local Government Area;
2. University of Port Harcourt Teaching Hospital (UPTH), situated in Obio-Akpor Local Government Area; and
3. Rivers State University Medical Centre (RSUMC), also located in Port Harcourt Local Government Area.

Before commencing the study, ethical clearance was obtained from the Ethics Committees of RSUTH, UPTH, and RSUMC to ensure compliance with established ethical guidelines and principles.

Collection of Water Samples

A total of 135 water samples, each measuring 100 milliliters (100 mL), were aseptically collected from five distinct sampling points within the three hospitals over a three-month period (June 2023 to August 2023). The sampling points included:

- The Theater,
- Gynae Ward,
- Emergency Department,
- Laboratory, and
- The storage tank receiving water from the borehole (source).

Samples were collected on three separate occasions from each hospital and were properly labeled with details of the date, time, and location using sterile specimen containers. The samples were preserved at 4°C and transported to the Department of Microbiology Laboratory at Rivers State University, Port Harcourt, for further analysis.

Microbiological analysis

Sample preparation followed the guidelines of the Clinical Laboratory Standards Institute (2011). A variety of culture media were utilized for bacterial growth, including:

- Nutrient Agar,
- Cetrimide Agar,
- Salmonella and Shigella Agar (SSA),
- Eosin Methylene Blue (EMB), and
- MacConkey Agar.

Each culture medium was prepared according to the manufacturer's instructions to ensure optimal growth conditions for the targeted microorganisms.

Serial Dilution and Inoculation

The method described by Prescott et al. (2011) was used for sample analysis. Briefly, 1 mL of each sample was mixed with 9 mL of normal saline (diluent) to achieve a 1:10 dilution. The mixture was gently swirled to ensure homogeneity. Subsequent tenfold serial dilutions were

performed aseptically using a sterile pipette.

After dilution, a 0.1 mL aliquot of each sample was cultured on various media, including Nutrient Agar, Cetrimide Agar, Salmonella and Shigella Agar (SSA), Eosin Methylene Blue (EMB), and MacConkey Agar, using a sterile bent rod. The cultured plates were incubated aerobically at 37.2°C for 24 hours, except for EMB agar plates, which were incubated at 44.5°C.

Isolation and enumeration of bacterial isolates

Following incubation, the Total Heterotrophic Bacterial Count (THBC), Total Coliform Count (TCC), *Pseudomonas* count, and *Salmonella and Shigella* counts were determined by counting the colonies on the cultured plates. The Colony-Forming Unit per milliliter (CFU/mL) was calculated using the following formula:

$$\text{CFU/ml} = \frac{\text{number of colonies}}{\text{Dilution x volume plated}}$$

Identification of bacterial isolates

Cultural and biochemical tests

Pure bacterial isolates were identified using the method described by Cheesbrough (2006). The isolates were subjected to various biochemical tests, including oxidase, catalase, indole, coagulase, methyl red, Voges-Proskauer, starch hydrolysis, citrate utilization, sugar fermentation, and triple sugar iron agar tests. The identities of the bacterial isolates were confirmed by referencing the results of these tests against *Bergey's Manual of Determinative Bacteriology*.

Test for Biofilm Production

The biofilm formation assay was performed using Congo Red Agar (CRA) medium. The base medium was prepared by combining 37 g/L of brain heart infusion (BHI) broth, 50 g/L of sucrose, and 10 g/L of agar. Congo red dye was added at a final concentration of 0.8 g/L. The dye was sterilized by autoclaving at 121°C and 15 psi for 15 minutes and was incorporated into the base medium after cooling to 50°C to prevent thermal degradation.

The prepared medium was poured into sterile Petri dishes to a uniform thickness of 4 mm (approximately 20 mL per plate) and allowed to solidify under aseptic conditions. Test organisms were standardized to a concentration of 1×10^8 CFU/mL using a spectrophotometer (OD_{600} = 0.1) and inoculated onto the CRA plates by spot inoculation (10 µL per spot). Plates were incubated aerobically at 37°C for 24–48 hours in a standard incubator.

Biofilm formation was assessed by visual observation of colony morphology. Positive biofilm formation was indicated by black colonies with a dry, crystalline appearance, while non-biofilm-forming colonies appeared red or pink and lacked the characteristic dry, crystalline texture.

Antibiotic susceptibility testing

The antibiotic susceptibility of the isolates was evaluated using the Kirby-Bauer disc diffusion technique, following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) and the Clinical and Laboratory Standards Institute (CLSI). A 0.5 McFarland inoculum suspension was prepared from discrete colonies of each isolate and incubated in 0.1% peptone water diluents at 35°C for 4–6 hours. The suspension density was adjusted to match the 0.5 McFarland standard before inoculating each isolate onto separate Mueller-Hinton agar plates using the spread plate method.

Antibiotic sensitivity testing was performed using commercially available discs on Mueller-Hinton agar plates, which were incubated at 37°C for 24 hours. Results were interpreted based on the sizes of zones of inhibition. The following antibiotics were tested: amoxicillin-clavulanate, cefotaxime, imipenem/cilastatin, nitrofurantoin, cefuroxime, ceftriaxone-sulbactam, ampiclox, cefixime, levofloxacin, ofloxacin, gentamicin, and nalidixic acid.

Molecular identification

The following Gram-negative, biofilm-producing organisms were selected for molecular studies: *Escherichia*, *Proteus*, *Salmonella*, *Enterobacter*, *Pseudomonas*, *Providencia*, *Serratia*, *Shigella*, and *Klebsiella*.

Extraction of DNA

DNA extraction involves isolating DNA from cells and separating it from proteins, membranes, and other cellular components (Malkin and Bratman, 2020; Zhang et al., 2024). In this study, the boiling method was employed. A 24-hour-old pure culture of the isolates was incubated in Luria-Bertani (LB) broth at 37°C. Subsequently, 0.5 mL of the overnight broth culture of the *Salmonella* isolate was transferred to labeled Eppendorf tubes, filled with normal saline, and centrifuged at 14,000 rpm for 3 minutes. The supernatant was discarded, leaving the DNA at the base of the tube.

DNA quantification

The concentration and purity of the extracted DNA were determined using a Nanodrop 1000 spectrophotometer, which applies Beer-Lambert's principle. Before measurement, the instrument was calibrated using 2 µL of sterile distilled water and blanked with 2 µL of normal saline. A 2 µL sample of extracted DNA was loaded onto the lower pedestal, and the upper pedestal was lowered to establish contact with the DNA. The "measure" button was then clicked to obtain the DNA concentration reading.

16S rRNA amplification

The amplification of the 16S rRNA gene region from *Salmonella* isolates was conducted using an ABI 9700 Applied Biosystems Thermal Cycler, following the protocol outlined by Srinivasan et al. (2015). A 30 µL reaction mixture was prepared, consisting of X2 Dream Taq Master Mix, forward and reverse primers at 0.4 µM, the extracted DNA template, Buffer 1X, and water. The mixture underwent 35 cycles of thermal cycling to amplify the target 16S rRNA region. The resulting amplified DNA fragments served as a basis for subsequent molecular analysis.

DNA sequencing

Sequencing of the amplified DNA fragment was performed at Inqaba Biotechnological in Pretoria, South Africa, using the BigDye Terminator kit on a 3510 ABI sequencer. Primers used included 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'), targeting the 16S rRNA gene region. A 10 µL sequencing reaction consisted of 0.2 µL BigDye terminator v1.1/v3.1, 2.25 µL 5× BigDye sequencing buffer, 10 µM PCR primer, and 2–10 ng of PCR template per 100 bp. Sequencing included 32 cycles of denaturation (96°C for 10 seconds), primer annealing (55°C for 5 seconds), and extension (60°C for 4 minutes). This process enabled accurate determination of the nucleotide sequence of the amplified gene, providing valuable genetic data.

Phylogenetic analysis

Phylogenetic analysis was conducted by downloading similar sequences from the NCBI database using BLASTN and editing the sequences with the TraceEdit bioinformatics algorithm. Sequences were aligned using ClustalX, and evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). A bootstrap consensus tree with 500 replicates was generated to represent the evolutionary relationships among taxa. Evolutionary distances were computed using the Jukes-Cantor method.

Amplification of SHV and TEM genes

Amplification of SHV and TEM genes was conducted using specific primers on an ABI 9700 Applied Biosystems thermal cycler. The PCR mixture contained X2 Dream Taq Master Mix, primers at 0.4 µM concentration, and 50 ng of extracted DNA as the template. The amplification process involved 35 cycles, enabling the detection and analysis of these specific genes in the isolates.

RESULTS

The study identified 12 bacterial species from hospital service water, including three Gram-positive species (*Staphylococcus*, *Bacillus*, and *Enterococcus*) and nine Gram-negative species (*Escherichia coli*, *Proteus*, *Salmonella*, *Enterobacter*, *Pseudomonas*, *Providencia*, *Serratia*, *Shigella*, and *Klebsiella*). The ability of these isolates to produce biofilms was assessed, and the results are presented in Table 1. Notably, five species (*Shigella*, *Staphylococcus*, *Bacillus*, *Escherichia coli*, and *Salmonella*) demonstrated 100% biofilm production capability. Other species showed varying levels of biofilm production, including *Providencia* (90%), *Serratia* and *Proteus* (80%), *Enterococcus* (70%), *Enterobacter* (60%), and *Klebsiella* (40%). These findings highlight the prevalence of biofilm-producing bacteria in hospital service water, which could have implications for healthcare-associated infections.

Escherichia coli was susceptible to Nitrofurantoin, Ceftriaxone-Sulbactam, Ofloxacin, and Levofloxacin, but resistant to Amoxicillin-Clavulanate, Cefotaxime, Imipenem/Cilastatin, Cefuroxime, Ampiclox, and Nalidixic Acid (Table 2). *Salmonella* species were resistant to multiple antibiotics but susceptible to Nitrofurantoin, Ceftriaxone-Sulbactam, Cefixime, Levofloxacin, Ofloxacin, and Nalidixic Acid. *Shigella* species were highly susceptible to Nitrofurantoin, Ceftriaxone-Sulbactam, and Levofloxacin but resistant to Amoxicillin-Clavulanate and Ampiclox (Table 3). *Providencia* species were highly susceptible to Ceftriaxone-Sulbactam, Cefixime, Levofloxacin, Ofloxacin, and Nalidixic Acid but resistant to Amoxicillin-Clavulanate and Cefotaxime (Table 4). *Pseudomonas* species were susceptible to Nitrofurantoin and Ofloxacin but resistant to many antibiotics, including Amoxicillin-Clavulanate and Imipenem/Cilastatin (Table 5). *Serratia* species were highly susceptible to Ceftriaxone-Sulbactam,

Table 1. Percentage frequency of biofilm production.

Isolates	Biofilm producing potential	
	% Positive	% Negative
Serratia sp.	21(84)	4(16)
Shigella sp.	21(100)	0(0)
Enterobacter sp.	36(74.4)	11(25.6)
Enterococcus sp.	16(72.7)	6(27.3)
Staphylococcus sp.	40(100)	0(0)
Providencia sp.	18(90)	2(10)
Bacillus sp.	38(100)	0(0)
Klebsiella sp.	12(37.5)	20(62.5)
Escherichia coli	56(100)	0(0)
Pseudomonas sp.	16(100)	0(0)
Salmonella sp	16(100)	0(0)
Proteus sp.	12(80)	3(20)

Table 2. Antibiotic sensitivity pattern of *Escherichia coli* Isolated from service water sources in three hospitals in Port Harcourt.

Code	Antibiotic	Conc.	RSUTH N=18			RSUMC N=16			UPTH N=20		
			R	I	S	R	I	S	R	I	S
AUG	Amoxicillin Clavulanate	30µg	18(100)	0(0)	0(0)	16(100)	0(0)	0(0)	20(100)	0(0)	0(0)
CTX	Cefotaxime	25 µg	18(100)	0(0)	0(0)	16(100)	0(0)	0(0)	20(100)	0(0)	0(0)
IMP	Imipenem/Cilastatin	10 µg	18(100)	0(0)	0(0)	16(100)	0(0)	0(0)	20(100)	0(0)	0(0)
NF	Nitrofurantoin	300 µg	0(0)	0(0)	18(100)	0(0)	0(0)	16(100)	0(0)	0(0)	20(100)
CXM	Cefuroxime	30 µg	18(100)	0(0)	0(0)	12(75)	3(18.8)	1(6.2)	16(80)	4(20)	0(0)
CRO	Ceftriaxone Sulbactam	45 µg	0(0)	0(0)	18(100)	0(0)	2(12.5)	14(87.5)	0(0)	0(0)	20(100)
ACX	Ampiclox	10 µg	18(100)	0(0)	0(0)	16(100)	0(0)	0(0)	20(100)	0(0)	0(0)
ZEM	Cefexime	5 µg	9(50)	9(50)	0(0)	6(37.5)	2(12.5)	8(50)	6(30)	0(0)	14(70)
LBC	Levofloxacin	5 µg	4(22.2)	5(27.8)	9((50)	4(25)	3(18.8)	9((56.3)	0(0)	0(0)	20(100)
OFX	Ofloxacin	5 µg	0(0)	0(0)	18(100)	0(0)	0(0)	16(100)	0(0)	0(0)	20(100)
GN	Gentamycin	10 µg	9(50)	0(0)	9(9)	8(50)	6(37.5)	2(12.5)	7(35)	4(20)	9(45)
NA	Nalidixic Acid	30 µg	10(55.6)	0(0)	8(44.4)	7(43.8)	1(6.2)	8(50)	6(30)	4(20)	10(50)

Levofloxacin, Ofloxacin, and Nitrofurantoin but resistant to Amoxicillin-Clavulanate and Cefuroxime (Table 6). *Enterobacter* species were highly susceptible to Ofloxacin and Ceftriaxone-Sulbactam but resistant to Amoxicillin-Clavulanate and Ampiclox (Table 7). *Proteus* species were susceptible to Nitrofurantoin, Ceftriaxone-Sulbactam, and Levofloxacin but resistant to Amoxicillin-Clavulanate and Cefotaxime (Table 8). *Klebsiella* species were susceptible to Ofloxacin and Nalidixic Acid but resistant to Amoxicillin-Clavulanate and Ampiclox (Table 9). *Staphylococcus* species were susceptible to Levofloxacin and Gentamicin but resistant to Cefotaxime and Erythromycin (Table 10).

Table 3. Antibiotic sensitivity pattern of *Salmonella* sp. isolated from service water sources in three hospitals in Port Harcourt.

Code	Antibiotic	Conc.	RSUTH N=6			RSUMC N=4			UPTH N=6		
			R	I	S	R	I	S	R	I	S
AUG	Amoxicillin Clavulanate	30µg	6(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)
CTX	Cefotaxime	25 µg	6(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)
IMP	Imipenem/Cilastatin	10 µg	6(100)	0(0)	0(0)	4(100)	0(0)	0(0)	5(83.3)	1(16.7)	0(0)
NF	Nitrofurantoin	300 µg	0(0)	0(0)	6(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)
CXM	Cefuroxime	30 µg	6(100)	0(0)	0(0)	2(50)	2(50)	0(0)	6(100)	0(0)	0(0)
CRO	Ceftriaxone Sulbactam	45 µg	0(0)	0(0)	6(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)
ACX	Ampiclox	10 µg	6(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)
ZEM	Cefexime	5 µg	2(33.3)	0(0)	4(66.7)	0(0)	0(0)	4(100)	2(33.3)	4(66.7)	0(0)
LBC	Levofloxacin	5 µg	0(0)	0(0)	6(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)
OFX	Ofloxacin	5 µg	0(0)	0(0)	6(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)
GN	Gentamycin	10 µg	6(100)	0(0)	0(0)	3(75)	0(0)	1(25)	2(33.3)	0(0)	4(66.7)
NA	Nalidixic Acid	30 µg	0(0)	0(0)	6(100)	0(0)	0(0)	4(100)	0(0)	3(50)	3(50)

Table 4. Antibiotic sensitivity pattern of *Shigella* sp. isolated from service water sources in three hospitals in Port Harcourt.

Code	Antibiotic	Conc.	RSUTH N= 7			RSUMC N=5			UPTH N=10		
			R	I	S	R	I	S	R	I	S
AUG	Amoxicillin Clavulanate	30µg	6(100)	0(0)	0(0)	5(100)	0(0)	0(0)	10(100)	0(0)	0(0)
CTX	Cefotaxime	25 µg	6(100)	0(0)	0(0)	3(60)	2(40)	0(0)	10(100)	0(0)	0(0)
IMP	Imipenem/Cilastatin	10 µg	5(83.3)	1(16.7)	0(0)	5(100)	0(0)	0(0)	5(50)	5(50)	0(0)
NF	Nitrofurantoin	300 µg	0(0)	0(0)	6(100)	0(0)	0(0)	5(100)	0(0)	0(0)	10(100)
CXM	Cefuroxime	30 µg	6(100)	0(0)	0(0)	0(0)	3(60)	2(40)	10(100)	0(0)	0(0)
CRO	Ceftriaxone Sulbactam	45 µg	0(0)	0(0)	6(100)	0(0)	0(0)	5(100)	0(0)	0(0)	10(100)
ACX	Ampiclox	10 µg	6(100)	0(0)	0(0)	5(100)	0(0)	0(0)	10(100)	0(0)	0(0)
ZEM	Cefexime	5 µg	2(33.3)	4(66.7)	0(0)	0(0)	0(0)	5(100)	4(40)	6(60)	0(0)
LBC	Levofloxacin	5 µg	0(0)	0(0)	6(100)	0(0)	0(0)	5(100)	0(0)	0(0)	10(100)
OFX	Ofloxacin	5 µg	0(0)	0(0)	6(100)	0(0)	3(60)	2(40)	0(0)	0(0)	10(100)
GN	Gentamycin	10 µg	1(16.7)	0(0)	5(83.3)	2(40)	0(0)	3(60)	3(30)	0(0)	7(70)
NA	Nalidixic Acid	30 µg	0(0)	0(0)	6(100)	0(0)	1(20)	4(80)	1(10)	1(10)	8(80)

Enterococcus species were susceptible to Levofloxacin and Gentamicin but resistant to Cefuroxime and Erythromycin (Table 11). *Bacillus* species were susceptible to Levofloxacin and Gentamicin but resistant to Amoxicillin-Clavulanate and Erythromycin (Table 12).

The antibiotic resistance patterns varied among the different bacterial species (Table 13), with some species showing high susceptibility to certain antibiotics and others exhibiting significant resistance. Tables 14 and 15 provide insights into the levels of antibiotic resistance among the

Table 5. Antibiotic sensitivity pattern of *Providencia* sp. isolated from service water sources in three hospitals in Port Harcourt.

Code	Antibiotic	Conc.	RSUTH N=4			RSUMC N=7			UPTH N=9		
			R	I	S	R	I	S	R	I	S
AUG	Amoxicilin Clavulanate	30µg	4(100)	0(0)	0(0)	7(100)	0(0)	0(0)	9(100)	0(0)	0(0)
CTX	Cefotaxime	25 µg	4(100)	0(0)	0(0)	7(100)	0(0)	0(0)	9(100)	0(0)	0(0)
IMP	Imipenem/Cilastatin	10 µg	4(100)	0(0)	0(0)	7(100)	0(0)	0(0)	6(66.7)	3(33.3)	0(0)
NF	Nitrofurantoin	300 µg	0(0)	0(0)	4(100)	0(0)	0(0)	4(100)	0(0)	0(0)	8(88.9)
CXM	Cefuroxime	30 µg	2(50)	2(50)	0(0)	7(100)	0(0)	0(0)	9(100)	0(0)	0(0)
CRO	Ceftriaxone Sulbactam	45 µg	0(0)	0(0)	4(100)	0(0)	0(0)	7(100)	0(0)	0(0)	9(100)
ACX	Ampiclox	10 µg	4(100)	0(0)	0(0)	4(100)	0(0)	0(0)	9(100)	0(0)	0(0)
ZEM	Cefexime	5 µg	0(0)	0(0)	4(100)	0(0)	0(0)	7(100)	0(0)	0(0)	9(100)
LBC	Levofloxacin	5 µg	0(0)	0(0)	4(100)	0(0)	0(0)	7(100)	0(0)	0(0)	9(100)
OFX	Ofloxacin	5 µg	0(0)	0(0)	4(100)	0(0)	0(0)	7(100)	0(0)	0(0)	9(100)
GN	Gentamycin	10 µg	3(75)	0(0)	1(25)	0(0)	2(28.6)	5(71.4)	7(77.8)	0(0)	2(25.2)
NA	Nalidixic Acid	30 µg	0(0)	0(0)	4(100)	0(0)	0(0)	7(100)	0(0)	1(11.1)	8(88.9)

Table 6. Antibiotic sensitivity pattern of *Pseudomonas* sp. isolated from service water sources in three hospitals in Port Harcourt.

Code	Antibiotic	Conc.	RSUTH N=5			RSUMC N=4			UPTH N=7		
			R	I	S	R	I	S	R	I	S
AUG	Amoxicillin Clavulanate	30µg	5(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)
CTX	Cefotaxime	25 µg	3(60)	2(40)	0(0)	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)
IMP	Imipenem/Cilastatin	10 µg	5(100)	0(0)	0(0)	4(100)	0(0)	0(0)	5(83.3)	1(16.7)	0(0)
NF	Nitrofurantoin	300 µg	0(0)	0(0)	5(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)
CXM	Cefuroxime	30 µg	0(0)	3(60)	2(40)	2(50)	2(50)	0(0)	6(100)	0(0)	0(0)
CRO	Ceftriaxone Sulbactam	45 µg	0(0)	0(0)	5(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)
ACX	Ampiclox	10 µg	5(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)
ZEM	Cefexime	5 µg	0(0)	0(0)	5(100)	0(0)	0(0)	4(100)	2(33.3)	4(66.7)	0(0)
LBC	Levofloxacin	5 µg	0(0)	0(0)	5(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)
OFX	Ofloxacin	5 µg	0(0)	3(60)	2(40)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)
GN	Gentamycin	10 µg	2(40)	0(0)	3(60)	3(75)	0(0)	1(25)	1(16.7)	0(0)	5(83.3)
NA	Nalidixic Acid	30 µg	0(0)	1(20)	4(80)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)

tested bacterial isolates based on multiple antibiotic resistance (MAR) index levels. These tables reveal the extent of resistance among isolates, highlighting the potential spread of antibiotic resistance in these bacterial populations. Table 16 displays the molecular identification of the isolated bacteria, showing that two isolates had 100% similarity to bacteria stored in the NCBI GenBank, while five had 99% similarity. The remaining isolates had 98%, 97%, and 96% similarity, respectively. The agarose gel electrophoresis image in Figure 1 shows the

Table 8. Antibiotic sensitivity pattern of *Enterobacter* sp. Isolated from Service water sources in three hospitals in Port Harcourt.

Code	Antibiotic	Conc.	RSUTH N=16			RSUMC N=9			UPTH N=18		
			R	I	S	R	I	S	R	I	S
AUG	Amoxicillin Clavulanate	30µg	16(100)	0(0)	0(0)	9(100)	0(0)	0(0)	18(100)	0(0)	0(0)
CTX	Cefotaxime	25 µg	16(100)	0(0)	0(0)	9(100)	0(0)	0(0)	18(100)	0(0)	0(0)
IMP	Imipenem/Cilastatin	10 µg	0(0)	0(0)	16(100)	6(66.7)	3(33.3)	0(0)	18(100)	0(0)	0(0)
NF	Nitrofurantoin	300 µg	0(0)	0(0)	16(100)	0(0)	0(0)	8(88.9)	0(0)	0(0)	18(100)
CXM	Cefuroxime	30 µg	12(75)	3(18.8)	1(6.2)	9(100)	0(0)	0(0)	18(100)	0(0)	0(0)
CRO	Ceftriaxone Sulbactam	45 µg	0(0)	2(12.5)	14(87.5)	0(0)	0(0)	9(100)	0(0)	0(0)	18(100)
ACX	Ampiclox	10 µg	16(100)	0(0)	0(0)	9(100)	0(0)	0(0)	18(100)	0(0)	0(0)
ZEM	Cefexime	5 µg	6(37.5)	2(12.5)	8(50)	0(0)	0(0)	9(100)	9(50)	9(50)	0(0)
LBC	Levofloxacin	5 µg	4(25)	3(18.8)	9((56.3)	0(0)	0(0)	9(100)	4(22.2)	5(27.8)	9((50)
OFX	Ofloxacin	5 µg	0(0)	0(0)	16(100)	0(0)	0(0)	9(100)	0(0)	0(0)	18(100)
GN	Gentamycin	10 µg	8(50)	6(37.5)	2(12.5)	7(77.8)	0(0)	2(25.2)	9(50)	0(0)	9(9)
NA	Nalidixic Acid	30 µg	7(43.8)	1(6.2)	8(50)	0(0)	1(11.1)	8(88.9)	10(55.6)	0(0)	8(44.4)

Table 9. Antibiotic sensitivity pattern *Proteus* sp. isolated from service water sources in three hospitals in Port Harcourt.

Code	Antibiotic	Conc.	RSUTH N=5			RSUMC N=4			UPTH N=6		
			R	I	S	R	I	S	R	I	S
AUG	Amoxicillin Clavulanate	30µg	5(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)
CTX	Cefotaxime	25 µg	3(60)	2(40)	0(0)	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)
IMP	Imipenem/Cilastatin	10 µg	5(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)
NF	Nitrofurantoin	300 µg	0(0)	0(0)	5(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)
CXM	Cefuroxime	30 µg	0(0)	3(60)	2(40)	2(50)	2(50)	0(0)	6(100)	0(0)	0(0)
CRO	Ceftriaxone Sulbactam	45 µg	0(0)	0(0)	5(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)
ACX	Ampiclox	10 µg	5(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)
ZEM	Cefexime	5 µg	0(0)	0(0)	5(100)	0(0)	0(0)	4(100)	2(33.3)	4(66.7)	0(0)
LBC	Levofloxacin	5 µg	0(0)	0(0)	5(100)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)
OFX	Ofloxacin	5 µg	0(0)	3(60)	2(40)	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)
GN	Gentamycin	10 µg	2(40)	0(0)	3(60)	3(75)	0(0)	1(25)	2(33.3)	0(0)	4(66.7)
NA	Nalidixic Acid	30 µg	0(0)	1(20)	4(80)	0(0)	0(0)	4(100)	0(0)	0(0)	6(50)

amplification of the 16S rRNA gene of the ten bacterial isolates identified in this study, along with a phylogenetic tree illustrating the evolutionary distances between the bacterial isolates and their close relatives in the GenBank. As shown in Table 16, molecular identification revealed varying degrees of similarity to known bacteria in the NCBI GenBank.

The agarose gel electrophoresis image in Figure 2 shows the amplification of the *bla*_{TEM} gene, with all isolates except W1 (*Salmonella enterica*) showing positive

Table 10. Antibiotic sensitivity pattern *Klebsiella* sp. isolated from service water sources in three hospitals in Port Harcourt.

Code	Antibiotic	Conc.	RSUTH N=5			RSUMC N=4			UPTH N=6		
			R	I	S	R	I	S	R	I	S
AUG	Amoxicillin Clavulanate	30µg	16(100)	0(0)	0(0)	6(100)	0(0)	0(0)	10(100)	0(0)	0(0)
CTX	Cefotaxime	25 µg	16(100)	0(0)	0(0)	6(100)	0(0)	0(0)	4(40)	6(60)	0(0)
IMP	Imipenem/Cilastatin	10 µg	0(0)	0(0)	16(100)	5(83.3)	1(16.7)	0(0)	5(50)	5(50)	0(0)
NF	Nitrofurantoin	300 µg	0(0)	0(0)	16(100)	0(0)	0(0)	6(100)	0(0)	0(0)	10(100)
CXM	Cefuroxime	30 µg	12(75)	3(18.8)	1(6.2)	6(100)	0(0)	0(0)	10(100)	0(0)	0(0)
CRO	Ceftriaxone Sulbactam	45 µg	0(0)	2(12.5)	14(87.5)	0(0)	0(0)	6(100)	0(0)	0(0)	10(100)
ACX	Ampiclox	10 µg	16(100)	0(0)	0(0)	6(100)	0(0)	0(0)	10(100)	0(0)	0(0)
ZEM	Cefexime	5 µg	6(37.5)	2(12.5)	8(50)	2(33.3)	4(66.7)	0(0)	5(50)	5(50)	0(0)
LBC	Levofloxacin	5 µg	4(25)	3(18.8)	9((56.3)	0(0)	0(0)	6(100)	0(0)	0(0)	10(100)
OFX	Ofloxacin	5 µg	0(0)	0(0)	16(100)	0(0)	0(0)	6(100)	0(0)	0(0)	10(100)
GN	Gentamycin	10 µg	8(50)	6(37.5)	2(12.5)	1(16.7)	0(0)	5(83.3)	3(30)	0(0)	7(70)
NA	Nalidixic Acid	30 µg	7(43.8)	1(6.2)	8(50)	0(0)	0(0)	6(100)	0(0)	0(0)	10(100)

Table 11. Antibiotic sensitivity pattern *Staphylococcus* sp. isolated from service water sources in three hospitals in Port Harcourt.

Code	Antibiotic	Conc.	RSUTH N=4			RSUMC N=6			UPTH N=4		
			R	I	S	R	I	S	R	I	S
AUG	Amoxicillin Clavulanate	30µg	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)	4(100)	0(0)	0(0)
CTX	Cefotaxime	25 µg	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)	4(100)	0(0)	0(0)
CXM	Cefuroxime	30 µg	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)	4(100)	0(0)	0(0)
LEV	Levofloxacine	5 µg	0(0)	0(0)	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)	4(100)
OFX	Ofloxacine	5 µg	4(100)	0(0)	0(0)	4(75)	1(18.8)	1(6.2)	2(80)	2(20)	0(0)
GN	Gentamicine	10 µg	0(0)	0(0)	4(100)	0(0)	2(12.5)	4(87.5)	0(0)	0(0)	4(100)
ERY	Erythromycine	15 µg	4(100)	0(0)	0(0)	6(100)	0(0)	0(0)	4(100)	0(0)	0(0)
CXC	Cloxacillines	5 µg	2(50)	2(50)	0(0)	2(37.5)	2(12.5)	2(50)	2(30)	0(0)	2(70)

Table 12. Antibiotic sensitivity pattern *Enterococcus* sp. isolated from service water sources in three hospitals in Port Harcourt.

Code	Antibiotic	Conc.	RSUTH N=2			RSUMC N=2			UPTH N=2		
			R	I	S	R	I	S	R	I	S
AUG	Amoxicillin Clavulanate	30µg	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)
CTX	Cefotaxime	25 µg	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)
CXM	Cefuroxime	30 µg	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)
LEV	Levofloxacine	5 µg	0(0)	0(0)	2(100)	0(0)	0(0)	6(100)	0(0)	0(0)	2(100)

Table 12. Continues.

OFX	**Ofloxacine**	**5 µg**	**2(100)**	**0(0)**	**0(0)**	**2(75)**	**1(18.8)**	**1(6.2)**	**1(80)**	**1(20)**	**0(0)**
GN	Gentamicine	10 µg	0(0)	0(0)	2(100)	0(0)	2(12.5)	4(87.5)	0(0)	0(0)	2(100)
ERY	Erythromycine	15 µg	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)
CXC	Cloxacillines	5 µg	1(50)	1(50)	0(0)	1(50)	0(0)	1(50)	1(30)	0(0)	1(70)

Table 13. Antibiotic sensitivity pattern *Bacillus* sp. isolated from service water sources in three hospitals in Port Harcourt.

Code	Antibiotic	Conc.	RSUTH N=2			RSUMC N=2			UPTH N=2		
			R	I	S	R	I	S	R	I	S
AUG	Amoxicillin Clavulanate	30µg	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)
CTX	Cefotaxime	25 µg	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)
CXM	Cefuroxime	30 µg	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)
LEV	Levofloxacine	5 µg	0(0)	0(0)	2(100)	0(0)	0(0)	6(100)	0(0)	0(0)	2(100)
OFX	Ofloxacine	5 µg	2(100)	0(0)	0(0)	2(75)	1(18.8)	1(6.2)	1(80)	1(20)	0(0)
GN	Gentamicine	10 µg	0(0)	0(0)	2(100)	0(0)	2(12.5)	4(87.5)	0(0)	0(0)	2(100)
ERY	Erythromycine	15 µg	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)
CXC	Cloxacillines	5 µg	0(0)	2(100)	0(0)	1(50)	0(0)	1(50)	1(30)	0(0)	1(70)

Table 14. Multiple antibiotic resistance of gram positive isolate.

MAR index	Organisms and frequency in parenthesis								
	Escherichia coli n=56	***Salmonella*** n=16	***Shigella*** n=22	***Providencia*** n=20	***Pseudomonas*** n=16	***Serratia* sp.** n=25	***Enterobacter*** n=43	***Proteus* sp.** n=15	***Klebsiella*** n=15
0.1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
0.2	0(0)	0(0)	5(22.7)	(0)	5(31.2)	10(40)	16(37.3)	5(33.3)	11(73.3)
0.3	36(66.3)	10(62.5)	17(77.3)	13(65)	11(68.8)	9(36)	9(20.9)	4(26.7)	4(26.7)
0.4	20(35.7)	6(37.5)	0(0)	7(35)	0(0)	6(24)	18(41.8)	6(40)	0(0)
0.5	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
0.6	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
0.7	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
0.8	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
0.9	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
1.0	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

Table 15. Multiple antibiotic resistance for gram positive isolate.

MAR indices	*Staphylococcus* sp. n=4	*Enterococcus* sp. n=2	*Bacillus* sp. n=2
0.1	3(75)	1(50)	0(0)
0.2	1(25)	0(0)	0(0)
0.3	0(0)	0(0)	0(0)
0.4	0(0)	0(0)	2(100)
0.5	0(0)	1(50)	0(0)
0.6	0(0)	0(0)	0(0)
0.7	0(0)	0(0)	0(0)
0.8	0(0)	0(0)	0(0)
0.9	0(0)	0(0)	0(0)
1.0	0(0)	0(0)	0(0)

Table 16. Identified bacterial isolates 16sRNA sequences relatedness and their assigned GeneBank accession numbers.

Isolate code	Tentative identity	Genotypic	NCBI GeneBank accession number	Accession (%)
W2	*Pseudomonas* sp.	*Pseudomonas oryzihabitans*	NZ_JAVSJB010000077.1.	100
T2	*Providencia* sp.	*Providencia rettgeri*	EU587038.1.	100
T1	*Shigella* sp.	*Shigella sonnei*	*NR_104826.1*	98
L1	*Escherichia coli*	*Escherichia coli ETEC*	MF919609.1.	97
W1	*Salmonella* sp.	*Salmonella enterica*	KU255189.1.	
L2	*Proteus* sp.	*Proteus mirabilis*	OU548749.1.	99
I1	*Pseudomonas* sp.	*Pseudomonas aeruginosa*	KF527574.1.	99
S2	*Enterococcus* sp.	*Enterococcus faecium*	MH976720.1.	99
S1	*Bacillus* sp.	*Bacillus subtilis*	KY296353.1.	99
S3	*Staphylococcus* sp.	*Staphylococcus aureus*	OR398657.1	99

amplification. Figure 3 shows the amplification of the *CTX-M* gene, with isolates T1 (*Shigella sonnei*) and S1 (*Bacillus subtilis*) showing negative amplification, indicating the absence of the gene. In contrast, all isolates showed positive amplification of the *SHV* gene, as depicted in Figure 4. The phylogenetic tree in Figure 1 further illustrates the evolutionary distances between the bacterial isolates and their close relatives in the GenBank, highlighting their genetic relatedness.

DISCUSSION

The isolation of bacterial species from hospital service water suggests that the water distribution system may be compromised, allowing for the growth and spread of microorganisms. This can result in a range of healthcare-associated infections, from mild to severe and even life-threatening conditions. The presence of antibiotic-resistant bacteria, as identified in this study, further complicates the treatment of these infections, emphasizing the need for effective infection control measures.

Moreover, the detection of bacterial species typically associated with environmental sources, such as *Pseudomonas* and *Serratia*, indicates that the hospital's water system may be vulnerable to external contamination. These findings underscore the importance of regular water quality monitoring and maintenance to ensure the safety of patients and healthcare workers.

According to Oludairo and Aiyedun (2016), pathogenic bacteria are frequently found in borehole water systems, particularly in underdeveloped countries. Many researchers (e.g., Amadi, 2022; Atobatele and Owoseni, 2023; Elijah, 2023; Eze et al., 2023; Nvene et al., 2024; Amadi et al., 2022; Onuorah et al., 2018; Akinola et al., 2018) have isolated coliforms and other Gram-negative bacteria from boreholes in various parts of Nigeria. Agbabiaka et al. (2014) highlighted that the presence of potentially harmful organisms, such as *Enterobacter* sp. and *Klebsiella* sp., in service water samples poses a significant public health concern.

Similarly, Eniola et al. (2007) identified coliform bacteria in borehole water samples, revealing that these organisms can induce gastroenteritis in humans and render potable water unsuitable for consumption (WHO, 2017). This study also isolated two coliform bacteria species, *Escherichia* and *Klebsiella*, both of which are known to cause gastroenteritis and render potable water unsuitable for ingestion.

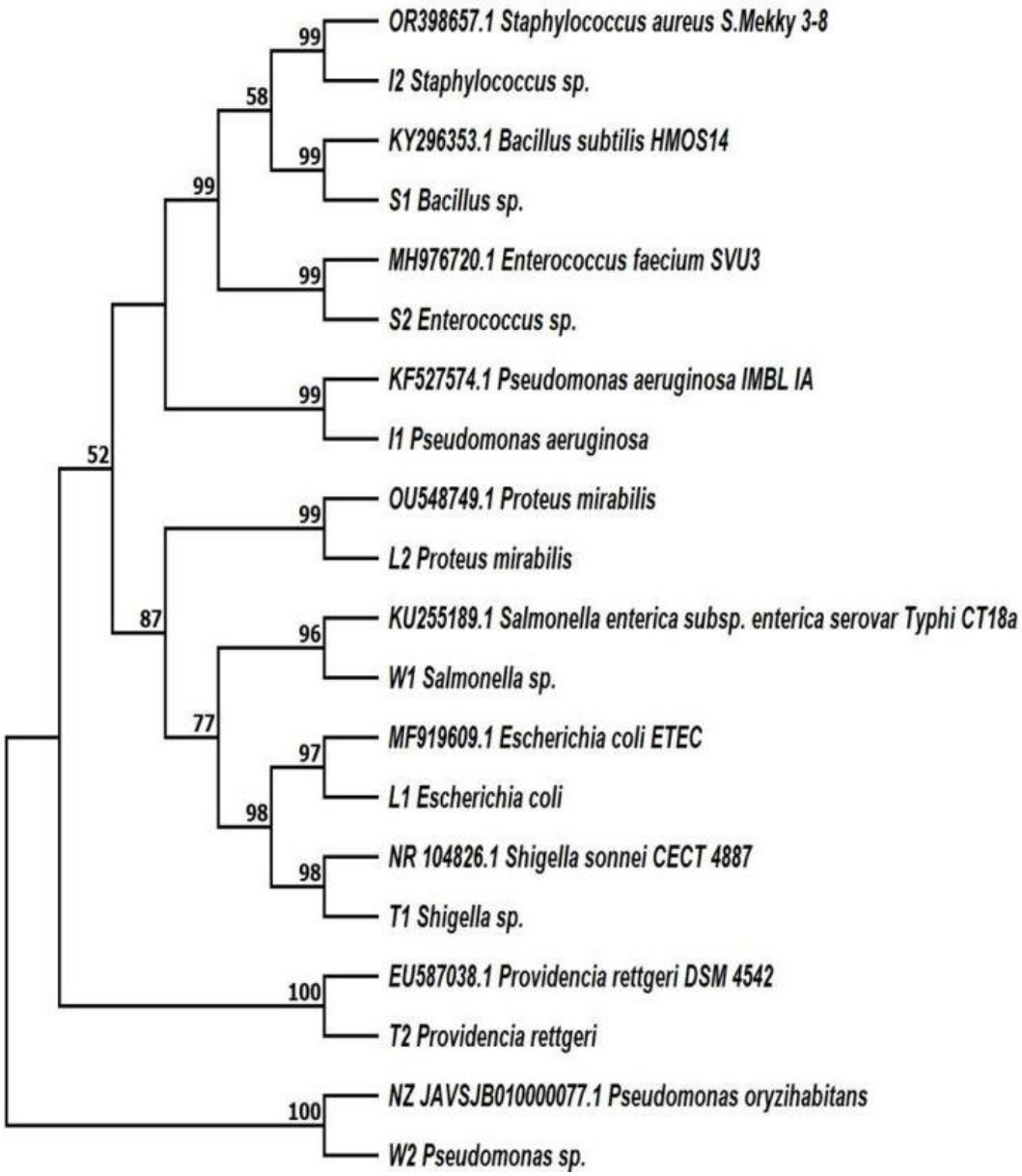

Figure 1. Phylogenetic tree showing evolutionary distance between bacterial isolates.

The identification of bacterial isolates from hospital service water plays a crucial role in ensuring the safety and hygiene of water systems. Understanding the microbial composition of service water enables hospitals to implement targeted interventions to reduce the risk of waterborne infections and protect the health of patients, staff, and visitors. Furthermore, the detection of coliforms in water samples suggests that WHO guidelines for water quality have not been met (Eniola et al., 2015).

This study found that organisms isolated from the three hospitals can produce biofilms. Biofilms are complex microbial communities encased within a self-produced extracellular polymeric substance (EPS) matrix that adheres to biotic or abiotic surfaces. This aligns with previous research by Allam et al. (2017), which documented a consistent pattern of biofilm production among bacterial isolates.

The World Health Organization (WHO) has reported that a significant percentage of bacterial species can form biofilms, posing a concern for healthcare settings where biofilms contribute to persistent infections and resistance to antibacterial agents. Many biofilm-producing bacteria are pathogenic and exhibit resistance to antibacterial treatments (Vestby et al., 2020). While most isolates in

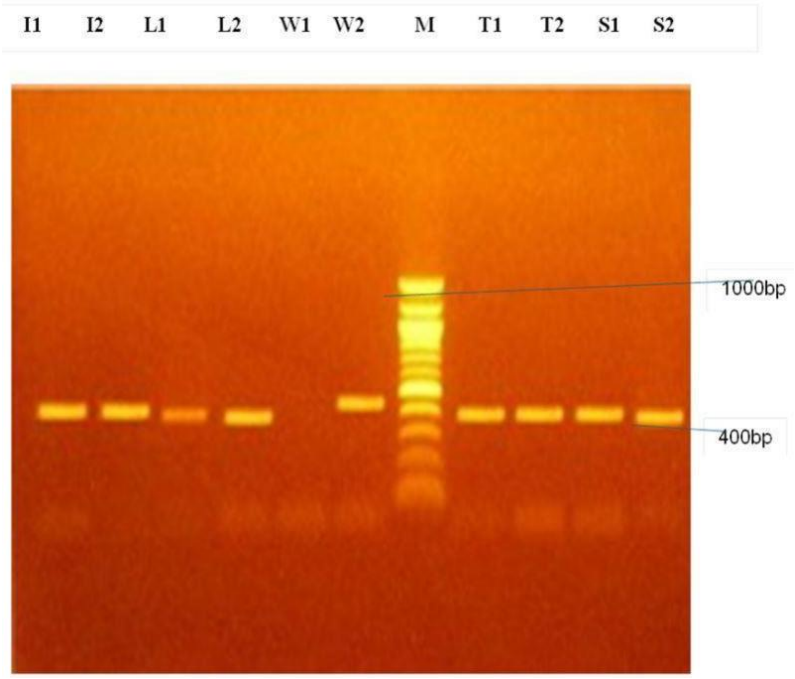

Figure 2. Agarose gel electrophoresis of *BlaTem* gene of some selected isolates. Lane 1-10 represents the *blaTem* gene bands (400bp). Lane M represents the 1000bp molecular ladder.

Key: M = Molecular ladder, **T1** = *Shigella sonnei,* **L1** = *Escherichia coli ETEC,* **W2** = *Pseudomonas oryzihabitans,* **I1** = *Pseudomonas aeruginosa,* **L2** = *Proteus mirabilis,* **W1** = *Salmonella enterica,* **I2** = *Staphylococcus aureus,* **T2** = *Providencia rettgeri,* **S1**= *Bacillus subtilis* , **S2** = *Enterococcus faecium.*

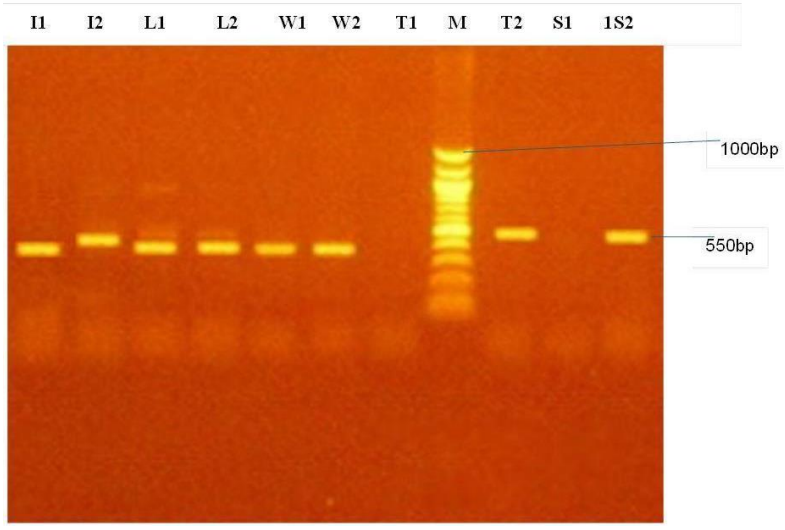

Figure 3. Agarose gel electrophoresis of CTX-M gene of the bacterial isolates. Lane 1-10 represents the CTX-M gene bands (550bp). Lane M represents the 1000bp molecular ladder.

Key: M = Molecular Ladder, **T1**= *Shigella sonnei,* **L1** = *Escherichia coli ETEC,* **W2** = *Pseudomonas oryzihabitans,* **I1** = *Pseudomonas aeruginosa,* L2 = *Proteus mirabilis,* W1 = *Salmonella enterica,* I2 = *Staphylococcus aureus,* **T2** = *Providencia rettgeri,* **S1** = *Bacillus subtilis,* **S2** = *Enterococcus faecium.*

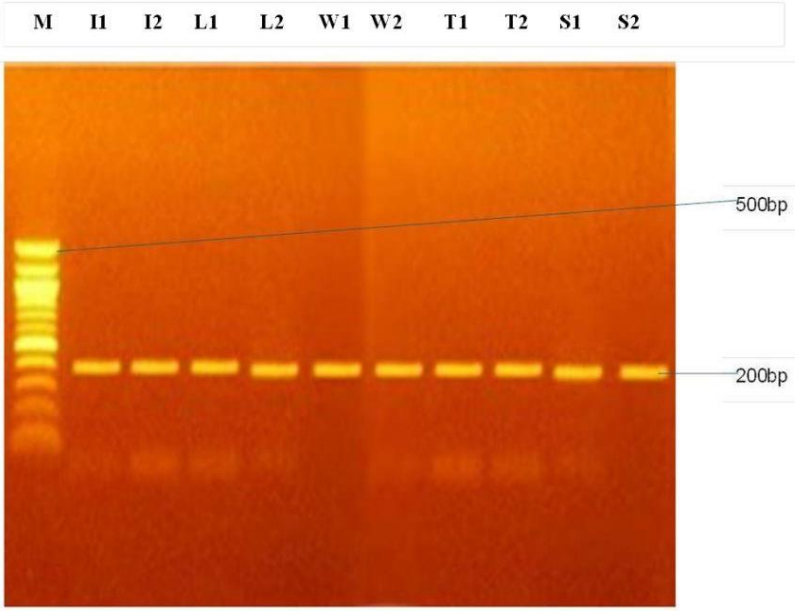

Figure 4. Agarose gel electrophoresis showing the amplified SHV genes. Lane 1-10 represents the SHV gene bands at 200bp, while Lane L represents to 100bp Molecular Ladder.

Key: M = Molecular Ladder, **T2** = *Shigella sonnei,* **L1** = *Escherichia coli ETEC,* **W2** = *Pseudomonas oryzihabitans,* **I1** = *Pseudomonas aeruginosa,* **L2** = *Proteus mirabilis,* **W1** = *Salmonella enterica,* **I2** = *Staphylococcus aureus,* **T2** = *Providencia rettgeri,* **S1** = *Bacillus subtilis,* **S2** = *Enterococcus faecium.*

this study were biofilm producers, some, such as *Serratia* sp., *Enterobacter* sp., *Enterococcus* sp., *Providencia* sp., *Klebsiella* sp., and *Proteus* sp., were identified as non-biofilm producers.

Variation in biofilm production among bacterial isolates within the same genera may be attributed to genetic differences and strain types. Genetic information exchange within biofilm communities can enhance biofilm formation potential in certain bacteria, illustrating the adaptability and complexity of microbial communities. Notably, some bacteria that cannot form biofilms are still contagious to humans, demonstrating that biofilm formation is not a prerequisite for bacterial transmission or infection.

Antibiotic sensitivity analysis

The antibiotic sensitivity analysis of microorganisms isolated from service water sources in three hospitals in Port Harcourt revealed significant trends. A majority of *Pseudomonas* sp. exhibited susceptibility to nitrofurantoin and ofloxacin but demonstrated resistance to amoxicillin-clavulanate, ampiclox, and imipenem/cilastatin. This resistance pattern contrasts with previous studies, suggesting a shift in susceptibility profiles (Williams et al., 2021).

The resistance of biofilm bacteria to beta-lactam antibiotics, carbapenems, and third-generation cephalosporins suggests the overuse of these antibiotics, leading to resistance mechanisms such as *blaCTX-M*, *blaSHV*, and *blaTEM* (Davis and Brown, 2016). Similarly, *Serratia* sp. showed susceptibility to ceftriaxone-sulbactam and levofloxacin, but resistance to amoxicillin-clavulanate and cefuroxime.

Enterobacter sp. demonstrated susceptibility to ofloxacin and ceftriaxone-sulbactam, while resistance to amoxicillin-clavulanate and ampiclox was prevalent. *Proteus* sp. exhibited notable susceptibility to nitrofurantoin, ceftriaxone-sulbactam, and levofloxacin but showed resistance to several antibiotics, including amoxicillin-clavulanate and imipenem/cilastatin (William et al., 2021).

In *Klebsiella* sp., susceptibility to ofloxacin was observed, while resistance to amoxicillin-clavulanate and ampiclox aligned with previous research (Wasfi et al., 2021). Due to the public health impact of biofilm producers and the rise of multidrug resistance (MDR), it is crucial to discover new antibacterial agents to inhibit biofilm formation and growth.

Ofloxacin and nitrofurantoin were the most effective drugs against biofilm-producing bacteria, including *Escherichia coli*, *Salmonella*, *Proteus*, *Serratia*, and *Pseudomonas* species. Molecular studies identified

various pathogenic bacterial species in service water samples, such as *Shigella sonnei*, *Escherichia coli* ETEC, *Pseudomonas oryzihabitans*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella enterica*, *Staphylococcus aureus*, *Providencia rettgeri*, *Bacillus subtilis*, and *Enterococcus faecium*.

The 16S rRNA sequences showed high similarity with those in the NCBI gene bank, indicating genetic relatedness among the isolates. Agarose gel electrophoresis amplified the 16S rRNA gene, elucidating the genetic makeup of the strains. Genetic analysis revealed key resistance genes, including *PapC*, *CTX-M*, *ICAD*, and *TET A* in *Pseudomonas aeruginosa* and *Proteus mirabilis*, explaining their resistance to certain antibiotics.

CONCLUSION

This study analyzed bacterial isolates from hospital service water, revealing a diverse range of Gram-positive and Gram-negative species, including pathogenic and non-pathogenic strains. The presence of biofilm-forming bacteria poses a risk of persistent infections and resistance to antibacterial treatments in healthcare facilities.

The study's examination of antibiotic sensitivity patterns highlights the need for judicious antibiotic use and exploration of alternative antibacterial solutions. Molecular investigations identified key resistance genes, underscoring the need for continuous surveillance and research to combat antibiotic resistance.

Ultimately, this study emphasizes the importance of ongoing water quality monitoring and assessment in hospitals to maintain public health standards and reduce the risk of waterborne infections.

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