Antimicrobial Profiling of Resistant Genes in Bacteria Isolated from Drinking Water Sources in Bonny Island, Rivers State, Nigeria

ABSTRACT

The global health crisis of antimicrobial resistance (AMR) necessitates a profound understanding of its dynamics, particularly in environmental reservoirs such as drinking water sources. The aim of the study was to identify the presence and variety of antibiotic-resistant genes (ARGs) within bacteria isolated from drinking water sources in Bonny Island, Rivers State, Nigeria – an area characterized by unique ecological and human-induced factors. Eighty water samples were collected from drinking water distribution systems within Bonny Island for a period of six month. Bacteria isolated from drinking water samples within Bonny Island underwent antimicrobial profiling, including susceptibility tests against a range of clinically relevant antibiotics. Molecular techniques were employed to identify the isolated bacteria and characterize specific ARGs, illuminating the genetic basis of resistance in the isolated bacteria. The bacterial isolates displayed varying resistance patterns, with the highest resistance observed against cefuroxime (59.6%), cefotaxime (48.9%), ceftazidime (44.7%), ceftriaxone (36.3%), chloramphenicol, and Augmentin (29.8%), while resistance to cotrimoxazole and gentamicin was lower (12.8%). Among the bacterial isolates, the tet gene, conferring resistance to tetracycline antibiotics, was present in 46.7% of cases, while 100% exhibited the oxa gene and 93.3% possessed the aac(6')-ib gene. Preliminary findings revealed a diverse array of bacteria with varying resistance profiles, including both common and emerging pathogens. The identified ARGs covered genes providing resistance to a broad spectrum of antibiotics, including beta-lactams, fluoroquinolones, and tetracyclines. These results underscore the potential risks associated with the spread of ARGs in drinking water sources and their potential transfer to pathogenic bacteria.

Keywords: Bonny Island, Drinking water; antibiotics; antibiotic resistant genes

1 INTRODUCTION

A crucial class of therapeutic agents used to treat bacterial infections are antibiotics. However, due to their extensive and frequent usage, antibiotics are constantly released into the environment [13]. Antibiotic-resistant bacteria has increased as a result of antibiotic abuse in recent years, endangering the health of many people worldwide. The majority of research on antibiotic resistance takes place in the medical field, but there is rising concern over the spread of antibiotics into the environment [4]. The most difficult issues facing the modern world are the emergence and spread of antibiotic resistance among pathogens that threaten human health. The genetic elements that confer resistance are frequently carried in self-transmitting mobile elements like conjugative plasmids, gene cassettes in integrons, and transposons. These elements are transferred between bacterial species, passing on the resistance to other species [20]. The development of resistance to a specific antimicrobial compound is influenced by several environmental factors and it has been reported that the organisms isolated from the environment with high faecal contamination can easily acquire resistance to the common antimicrobial drugs [25]. The beta-lactam group of antibiotics is the most frequently used class in the therapeutic treatment of infections, surpassing all other antibiotic classes in therapeutic use. [5].

Antibiotic resistance genes (ARGs) represent a growing category of environmental contaminants [19]. The excessive use and abuse of antibiotics in medicine and agriculture has contributed to the proliferation, spread, and buildup of ARGs across different environmental matrices [12], involving both natural and man-impacted environments, such as soil [7], surface water [26], wastewater [21], groundwater [24], drinking water [22], and tap water [14]. The presence of antibiotic resistant bacteria (ARB) in drinking water and the assessment of ARB's effects on human health have both been the subject of some literature. However, the majority of studies have only looked at a single bacterial species or a collection of resistant bacteria, such as *Escherichia coli, Salmonella* spp*., Pseudomonas aeruginosa* strains, and aminoglycoside-resistant bacteria, as well as the presence of Gram-negative bacteria that produce -lactamases [9]. There is paucity of data on the profiling of antibiotics resistant genes in bacterial isolates of drinking water and water treatment facility. This study therefore seeks to profile antibiotics resistant genes in bacterial isolates from drinking water distribution systems within Bonny Island, Rivers State, Nigeria.

2 MATERIALS AND METHODS

2.1 Study Area

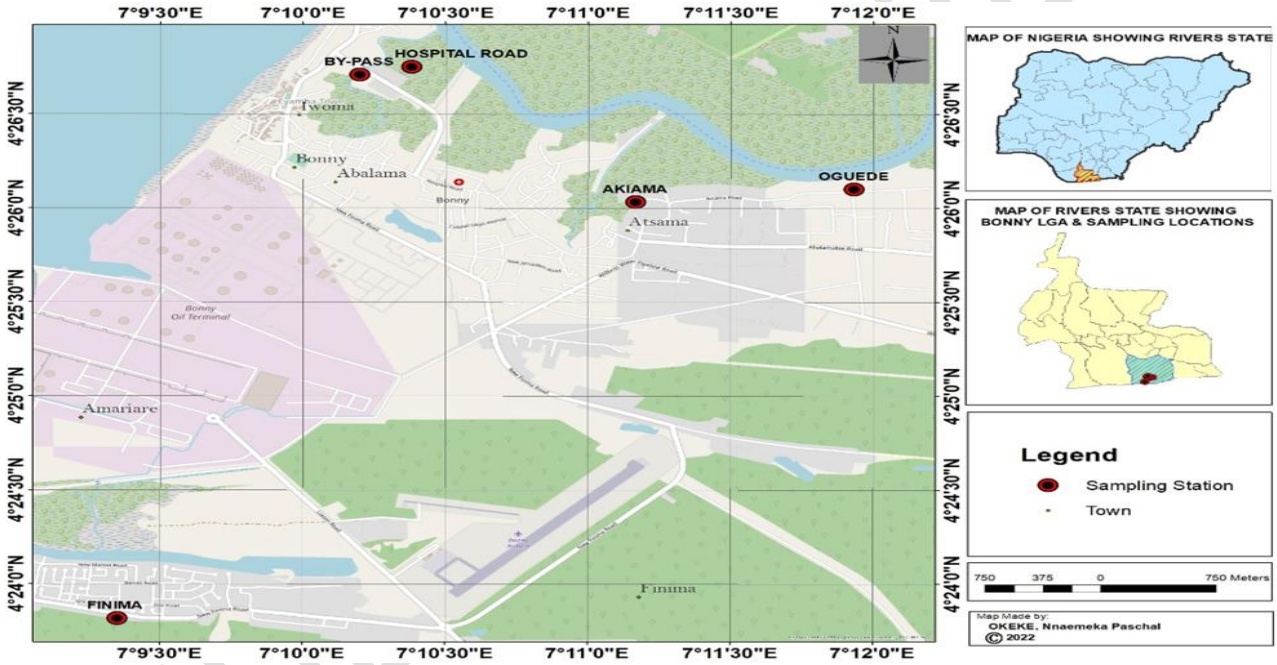


Fig 1. Map of Bonny Island showing sampling locations

2.2 Sample Collection

A total of eighty (80) water samples were collected for a period of six months from drinking water distribution systems within Bonny Island. The samples were collected aseptically into sterile red capped sample bottles. These samples were transported from the Island of collection to the Microbiology Laboratory, Department of Microbiology, Rivers State University, Port Harcourt, Nigeria in an ice packed box at a temperature of 10 to 15°C [8].

2.3 Microbiological Analysis of Water Samples

2.3.1 Isolation of Bacteria

Isolation of the bacterial isolates from the water sources was done according to standard method [23]. In this method, ten-fold serial dilution was carried out by transferring 1ml of the samples using sterile pipette into a test tube containing 9ml sterile normal saline. Subsequent dilution was carried out serially to obtain dilutions of 1:100000 and aliquots (0.1ml) from the 10^{-2} dilution was transferred into dried surfaces of prepared Nutrient agar, Eosin methylene blue agar, MacConkey agar and Thio citrate bile sucrose agar plates in triplicates. The plates were incubated for 24-48 hours at 37℃ after plating [3]. This was done for all the water samples. Colonies resulting from the plates after incubation were streaked onto the surface of freshly prepared nutrient agar. These subcultured plates were incubated at 37℃ for 24 hours before they were preserved in the refrigerator in sterile nutrient agar slants. These isolates were identified based on their morphological and biochemical characterization [15]. The preserved isolates were subjected to antibiotics susceptibility tests and isolates which showed multi-drug resistance were further profiled for the presence of resistant genes.

2.3.1.1 Agar Disk Diffusion Method (Kirby Bauer Disk Diffusion)

The Kirby-Bauer disk diffusion method, which is a standardized technique for antimicrobial susceptibility testing of bacteria, involves the use of filter paper disks impregnated with different antimicrobial agents, these disks are then positioned onto the surface of an agar plate inoculated with the test organism. To carry out Kirby-Bauer disk diffusion assay, Mueller-Hinton agar was first prepared according to the manufacturer's instructions and sterilized by autoclaving at 121^{o}C for 15 minutes at 15PSI. The agar was poured into sterile petri dishes and allowed to solidify. Test organism suspension was inoculated onto the Mueller-Hinton agar plate using a sterile swab to streak the surface uniformly. Subsequently, sterile forceps was used to place the antimicrobial disks onto the surface of the inoculated agar plate, which was then incubated at 37°C for 18 to 24 hours. The distinct antibiotics used for screening includes; Ampicillin (10µg), Meropenem (10µg), Erythromycin (5µg), Tetracycline (30µg), Cotrimoxazole (25µg), Cefuroxime (10µg), Gentamicin (10µg), Ciprofloxacin (5µg), Augmentin (30µg), Vancomycin (30µg), Ceftazidime (10µg), Cephalexin (1.5µg), Chloramphenicol (10µg), Ceftriaxone (30µg), Cefotaxime (30µg) and Amikacin (30µg). After incubation, the diameter of the zone of inhibition around each disk was measured and interpreted based on the Clinical and Laboratory Standards Institute guidelines [10]. Based on the size of the zone of inhibition, the organism was classified as either susceptible, intermediate, or resistant to the particular antimicrobial agent tested.

2.4 **Molecular Characterization**

Extraction of DNA from bacterial cells as described by Ducarmon et al. [6], in the procedure bacterial cells were mixed with isotonic buffer and then placed in a ZR BashingTM Lysis tube. Lysis Solution was added, and the tube was processed in a bead beater for at least 5 minutes. After centrifugation, the supernatant was filtered and mixed with DNA Binding Buffer. The mixture was filtered again, washed with DNA Pre-Wash Buffer and DNA Wash Buffer, and finally, the DNA was eluted using DNA Elution Buffer.

Agarose powder was mixed with Tris-Acetate-EDTA (1xTAE) solution and microwaved until fully dissolved, avoiding boiling to prevent buffer loss. The solution was then cooled to approximately 50°C. EZ vision DNA stain was added, and the mixture was poured into a gel tray with a well comb. The gel was left to solidify either by refrigerating at 4°C for 10-15 minutes or at room temperature for 20-30 minutes. This prepared gel allows DNA analysis under ultraviolet light.

A loading buffer was added to DNA samples or PCR products. The agarose gel, once solidified, was placed in the gel box and covered with 1xTAE solution. A molecular weight ladder was loaded into the first lane, and samples were added to other wells. The gel was electrophoresed at 80-150 V for 1-1.5 hours. Afterward, the power was turned off, electrodes were disconnected, and the gel was carefully removed. DNA fragments or PCR products were visualized under a UV transilluminator.

The PCR mix is made up of 12.5µl of Taq 2X Master Mix from New England Biolabs (M0270); 1µl each of 10µM forward (27F: AGAGTTTGATCCTGGCTCAG) and reverse (1429R: GGTTACCTTGTTACGACTT) primer; 2µl of DNA template and then made up with 8.5µl Nuclease free water.

Initial denaturation at 94˚C for 5mins, followed by 36 cycles of denaturation at 94˚C for 30sec, annealing at 55˚C for 30secs and elongation at 72˚C for 45sec. Followed by a final elongation step at 72˚C for 7 minutes and hold temperature at 10 ˚C.

2.5 Plasmid Profiling

Plasmid profiling was performed using the Zyppy™ Plasmid Miniprep Kit (Catalog Nos. D4019). A 600µl aliquot of bacterial culture grown in Luria Bertani broth medium was collected and centrifuged at 14,000rpm for 30seconds. The resulting supernatant was discarded. Subsequently, 100µl of 7X Lysis Buffer 1 (Blue) was added to the microcentrifuge tube containing the pellet, and the tube was gently inverted 4-6 times to ensure proper mixing. The solution transitioned from opaque to clear blue, indicating complete lysis. To neutralize the sample, 350µl of cold Neutralization Buffer (Yellow) was added and thoroughly mixed. The sample turned yellow, and a yellowish precipitate formed, signified successful neutralization. To ensure complete neutralization, the tube was inverted an additional 2-3 times. The tube was then centrifuged at 11,000 - 16,000 x g for 2-4 minutes, and the resulting supernatant (~900µl) was carefully transferred to the provided Zymo-Spin™ IIN column. Placing the column into a collection tube, it was centrifuged for 15seconds to remove the flow-through, which was discarded. The column was returned to the collection tube, and 200µl of Endo-Wash Buffer was added and centrifuged for 30seconds. Following that, 400µl of Zyppy™ Wash Buffer was added to the column and centrifuged for 1minute. The column was then transferred to a clean 1.5ml microcentrifuge tube, and 30µl of Zyppy™ Elution Buffer2 was directly added to the column matrix. After standing for one minute at room temperature, the tube was centrifuged for 30seconds to elute the plasmid DNA.

To prepare the agarose gel, 1g of agarose powder was measured and mixed with 100ml of 1xTAE in a microwavable flask. The mixture was microwaved for 1-3minutes until the agarose was completely dissolved, ensuring that the solution was not overboiled. The agarose solution was allowed to cool to approximately 50°C, which took approximately 5minutes. Next, 10µL of EZ vision DNA stain was added to the agarose solution, and the DNA was visualized under ultraviolet (UV) light. The agarose solution was then poured into a gel tray with the well comb in place and placed at 4°C for 10-15minutes until it solidified completely. Loading buffer was added to each purified plasmid DNA sample. Once the gel had solidified, it was placed into the gel box (electrophoresis unit) and covered with 1xTAE solution. The molecular weight ladder was carefully loaded into the first lane of the gel, and the purified plasmid DNA samples were carefully loaded into the additional wells. The gel was electrophoresed at 80-150 V for approximately 1-1.5hours. Afterward, the power was turned off, the electrodes were disconnected from the power source, and the gel was carefully removed from the gel box. The purified plasmid fragments were visualized under a UV transilluminator.

3 RESULTS AND DISCUSSION

Figure 2 illustrates the proportional distribution of each bacterial species in the sample. *Bacillus* spp. and *Enterococcus* spp. each represent 8.5% of the total bacterial isolates. *Erythrobacter* spp. constitutes 17.0% of the bacterial isolates. *Pseudomonas* spp. and *Shigella* spp. both make up 19.1% of the isolates, indicating they are the most prevalent species. *Serratia* spp. comprises 12.8% of the isolates, and *Staphylococcus* spp. accounts for 14.9% of the bacterial isolates.

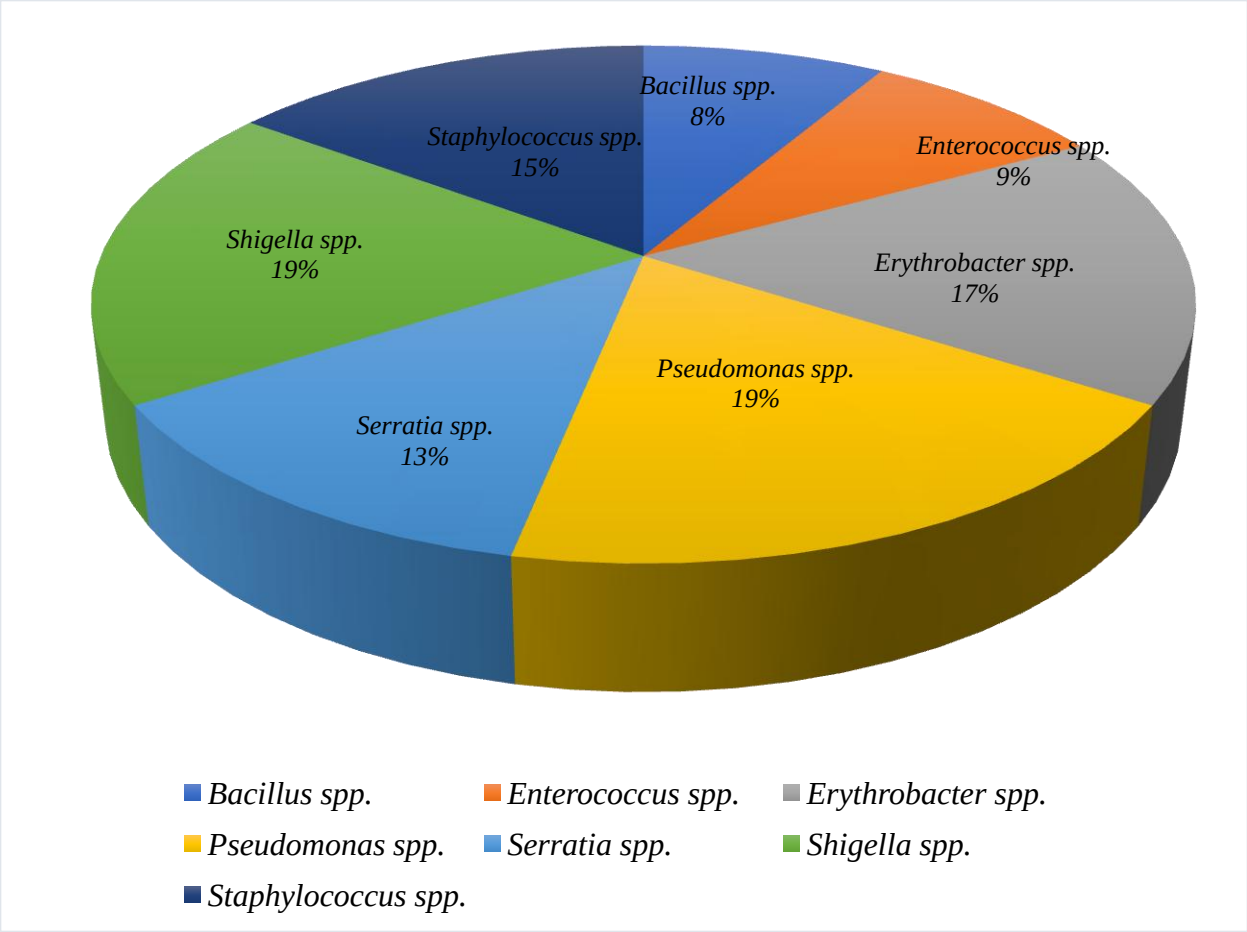


Fig 2: Percentage Distribution of Bacterial Isolates

Table 1 presents the prevalence of isolates with resistance to specific antibiotics within each drug class, offering insights into the prevalence of antibiotic resistance for different antibiotic classes. Among the 47 isolates tested: Aminoglycosides- 6 (12.8%) showed resistance to Amikacin, and 9 (19.1%) to Gentamicin. Carbapenems: 19 (40.4%) of the isolates exhibited resistance to Meropenem. Cephalosporins- Various cephalosporins were tested in this class. Notably, 28 (59.6%) isolates were resistant to Cefuroxime, 21 (44.7%) to Ceftazidime, 17 (36.2%) to Ceftriaxone, and 23 (48.9%) to Cefotaxime, with only 2 (4.3%) displaying resistance to Cephalexin. Chloramphenicols- 14 (29.7%) of the isolates were resistant to Chloramphenicol. Fluoroquinolones- Resistance to Ciprofloxacin was observed in 8 (17.0%) of the isolates. Glycopeptides- 10 (21.3%) of the isolates exhibited resistance to Vancomycin. Macrolides-Among the 47 isolates tested, 4 (8.5%) displayed resistance to Erythromycin. Penicillin- In this class, 5 (10.6%) were resistant to Ampicillin, and 14 (29.8%) exhibited resistance to Augmentin. Sulphonamides: Resistance to Cotrimoxazole was observed in 6 (12.8%) of the isolates. Tetracyclines- Out of the 47 isolates, 16 (34.0%) were resistant to Tetracycline.

Table 1: Prevalence of Antibiotic Resistance (n=47)

Class of antibiotics	Number	Percentage(%)
Aminoglycosides		
Amikacin	6	12.8
Gentamicin	9	19.1
Carbapenems		
Meropenem	19	40.4

Cephalosporins		
Cefuroxime	28	59.6
Ceftazidime	21	44.7
Ceftriaxone	17	36.2
Cefotaxime	23	48.9
Cephalexin	2	4.3
Chloramphenicols		
Chloramphenicol	14	29.7
Fluoroquinolones		
Ciprofloxacin	8	17.0
Glycopeptides		
Vancomycin	10	21.3
Macrolides		
Erythromycin	4	8.5
Penicillin		
Ampicillin	5	10.6
Augmentin	14	29.8
Sulphonamides		
Cotrimoxazole	6	12.8
Tetracyclines		
Tetracycline	16	34.0

Figure 3 shows a phylogenetic tree that illustrates the evolutionary relationships and genetic diversity among bacteria isolated from the Bonny drinking water supply. It provides a valuable tool for understanding the common ancestry and evolutionary history of these bacterial species

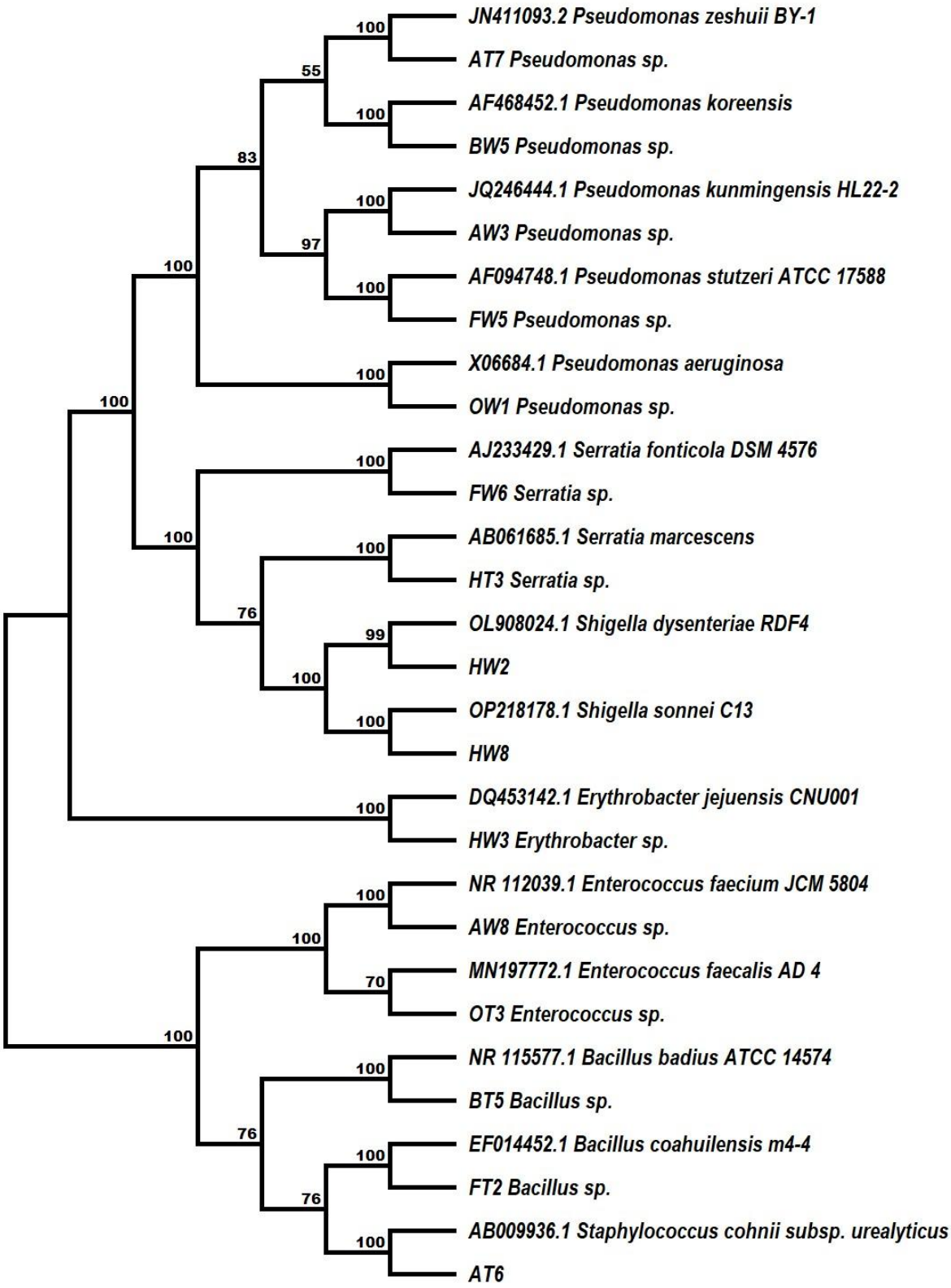


Fig 3: Phylogenetic Tree showing Evolutionary Relationship between Bacterial Isolates

Table 2 presents the antibiotic resistance patterns amongst identified bacterial species. Showing specific bacterial species, detailing the classes of drugs tested, the antibiotics within those classes to which the bacterial species exhibited resistance, and the frequency and percentage of occurrence of each resistance pattern within total isolates identified (15 isolates).

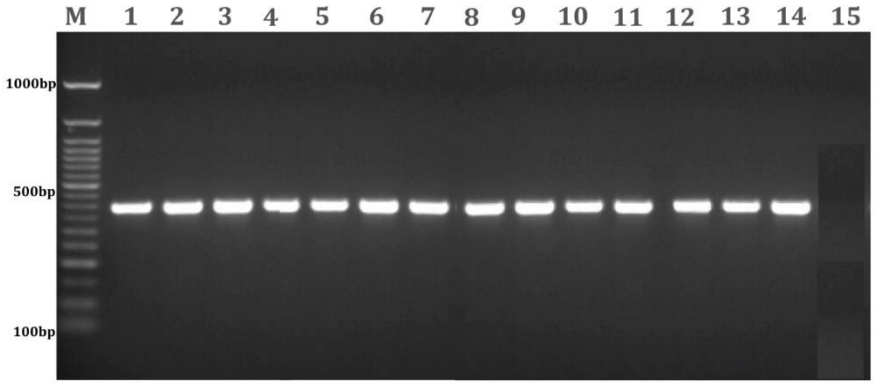
Table 2: Antibiotic Resistance Patterns of Identified Bacterial Species

S/N	Isolates	Resistance Pattern	Classes of Drugs	Frequency	Percentage (%)
1	*Pseudomonas zeshuii BY-1*	TET-COT-CTX-CHL-CRX-CIP-VAN-CPZ	TET-SULPH-CEPH-FLUO-GLYCO-CHL	**1**	**6.7**
2	*Pseudomonas Koreensis*	TET-COT-GEN-CRX-CHL-CTR-CTX-AMK-VAN-CPZ-MEM	TET-SULPH-CEPH-CARB-FLUO-AMINO	**3**	**20**
3	*Pseudomonas kunmingensis HL22-2*				
4	*Pseudomonas aeruginosa*				
5	*Pseudomonas stutzeri ATCC 17588*	COT-GEN-CRX-CHL-CTR-CTX-MEM	SULPH-AMINO-CEPH	**1**	**6.7**
6	*Serratia fonticola DSM 4576*	TET-COT-CRX-CHL-CTX-AMK-CPZ-MEM	TET-FLUO-CEPH-CHL-AMINO-CARB	**1**	**6.7**
7	*Serratia marcescens*	COT-CRX-CHL-CTR-CTX-CPZ-MEM	FLUO-CEPH-CHL-CARB	**1**	**6.7**
8	*Shigella dysenteriae RDF4*	TET-GEN-CHL-AMK-VAN-CPZ-MEM	TET-AMINO-CHL-AMINO-GLYCO-CARB	**1**	**6.7**
9	*Shigella sonnei C13*	TET-COT-GEN-CHL-CRX-CTX-CIP-AMK-VAN-CEF-MEM	TET-SULPH-AMINO-CEPH-FLUO-CARB	**1**	**6.7**
10	*Erythrobacter jejuensis CNU001*	TET-COT-GEN-CRX-CHL-CTR-CTX-CIP-AMK-VAN-MEM	TET-SULPH-AMINO-CHL-FLUO-CARB-CEPH	**1**	**6.7**
11	*Enterococcus faecium JCM 5804*	AMP-MEM-ERY-CRX-GEN-AUG	PEN-CARB-MACR-AMINO	**1**	**6.7**
12	*Enterococcus faecalis*	MEM-ERY-	CARB-MACRO-TET-SULPH-	**1**	**6.7**

	AD4	TET-COT-CRX-GEN-AUG-CPZ	AMINO-PEN-CEPHA		
13	*Bacillus badius ATCC 14574*	AMP-TET-CRX-GEN-AUG-VAN-CPZ-CP	PEN-TET-CEPH-GLY-AMINO	**1**	**6.7**
14	*Bacillus coahuilensis M4-4*	CRX-AUG-CPZ	CEPH-PEN	**1**	**6.7**
15	*Staphylococcus cohnii subsp. Urealyticus*	AMP-CRX-AUG-VAN-CPZ-CP	PEN-GLYCO-CEPHA	**1**	**6.7**

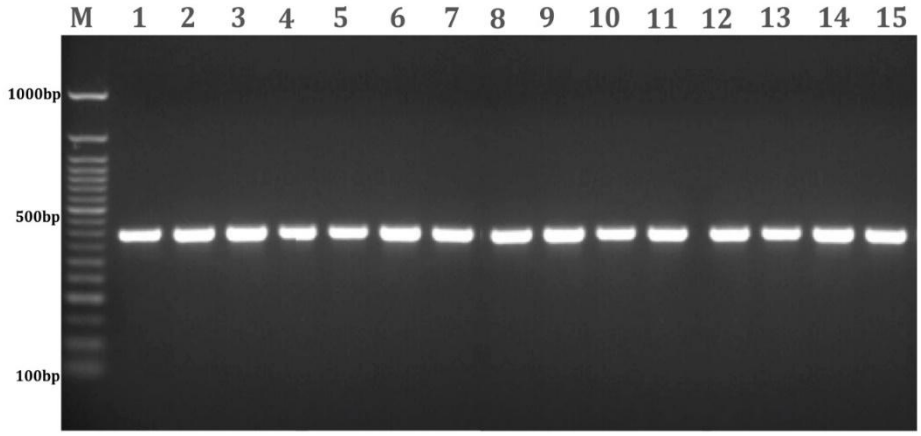
AMINO-Aminoglycosides, **CARB**–Carbapenems, **CEPH**–Cephalosporins, **CHL**– Chloramphenicols, **GLYCO**–Glycopeptides, **FLUO** – Fluoroquinolones, **MACRO** – Macrolides, **PEN** – Penicillin, **SULPH** – Sulphonamides, **TET** – Tetracyclines, AMP-Ampicillin, MEM-Meropenem, ERY-Erythromycin, TET-Tetracycline, COT- Cotrimoxazole, CRX- Cefuroxime, GEN-Gentamicin, CIP- Ciprofloxacin, AUG-Augmentin, VAN-Vancomycin, CPZ-Ceftazidime, CP- Cephalexin CHL-Chloramphenicol, AMK- Amikacin, COT- Cotrimoxazole, CRX- Cefuroxime, CTR- Ceftriaxone, CTX -Cefotaxime

Agarose gel image presented in Plate 1 demonstrates the aac(6')-Ib (482bp) gene amplification. Notably, *Pseudomonas zeshuii BY-1*, *Pseudomonas Koreensis*, *Pseudomonas kunmingensis HL22-2*, *Pseudomonas stutzeri ATCC 17588*, *Pseudomonas aeruginosa*, *Serratia fonticola DSM 4576*, *Serratia marcescens*, *Shigella dysenteriae RDF4*, *Shigella sonnei C13*, *Erythrobacter jejuensis CNU001*, *Enterococcus faecium JCM 5804*, *Enterococcus faecalis AD4*, *Bacillus badius ATCC 14574* and *Bacillus coahuilensis M4-4* (isolates 1-14) exhibited positive amplification, indicating the presence of the gene in these 14 isolates. Conversely, isolate 15 displayed a negative result, suggesting the absence of the aac(6')-Ib gene in that particular isolate. Furthermore, Plate 3 reveals the amplification of the Oxa gene (438bp). *Pseudomonas zeshuii BY-1*, *Pseudomonas Koreensis, Pseudomonas kunmingensis HL22-2*, *Pseudomonas stutzeri ATCC 17588*, *Pseudomonas aeruginosa*, *Serratia fonticola DSM 4576*, *Serratia marcescens*, *Shigella dysenteriae RDF4*, *Shigella sonnei C13*, *Erythrobacter jejuensis CNU001*, *Enterococcus faecium JCM 5804*, *Enterococcus faecalis AD4*, *Bacillus badius ATCC 14574*, *Bacillus coahuilensis M4-4* and *Staphylococcus cohnii subsp. Urealyticus* (all 15 isolates), exhibited positive amplification, indicating the presence of the Oxa gene across the entire sample set. Regarding the tetB gene(636bp), it was detected in a subset of isolates. Specifically, *Pseudomonas zeshuii BY-1*, *Pseudomonas Koreensis*, *Pseudomonas kunmingensis HL22-2*, *Serratia marcescens*, *Shigella sonnei C13*, *Erythrobacter jejuensis CNU001* and *Bacillus coahuilensis M4-4* (isolates 1, 2, 3, 7, 9, 10, and 14) displayed positive amplification, signifying the presence of the tetB gene in these particular isolates as shown in Plate 3. Fig 2 shows prevalence of tet, Oxa and aac(6')- 1b antibiotic resistant genes.



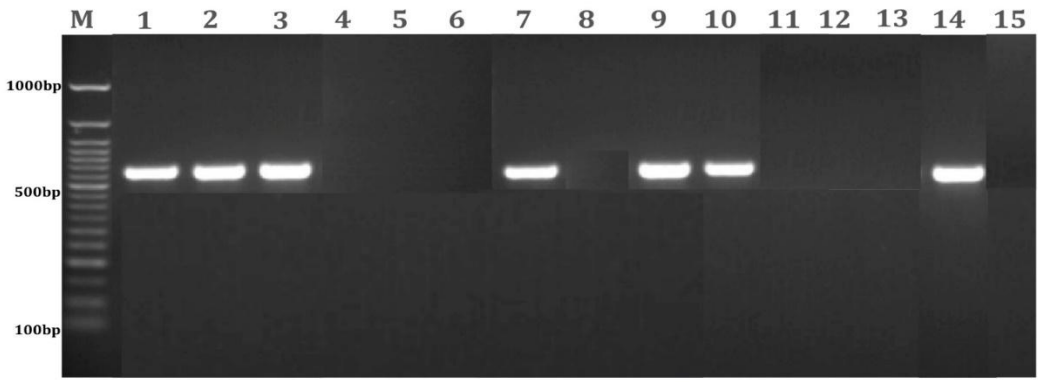
Gel image showing amplification of ESBL aac(6')-1b (482bp) isolates 1-14 showed positive amplification meaning that the gene is present in all 14 isolates while isolate 15 was negative.M is a 100bp DNA leader.

Plate 1: Agarose Gel Electrophoresis Analysis of Amplified aac (6')-Ib Gene Bands in Bacterial Isolates (482 base pairs)



Gel image showing amplification of ESBL Oxa (438bp) isolates 1-15 showed positive amplification meaning that the gene is present in all 15 isolates. M is a 100bp DNA lader

Plate 2: Agarose Gel Electrophoresis Analysis of Amplified Oxa Gene Bands in Bacterial Isolates (438 base pairs)



Gel image showing amplification of ESBL tetB (636bp) isolates 1,2,3,7,9,10,14 showed positive amplification meaning that the gene is present in these isolates while isolates 4,5,6,8,11,12,13,15 were negative. M is a 100bp DNA leader.

Plate 3: Agarose Gel Electrophoresis Analysis of Amplified tetB Gene Bands in Bacterial Isolates (636 base pairs)

Figure 4 displays the distribution of antibiotic-resistant genes among the bacterial isolates. Specifically, within the tested bacterial isolates: Tetracycline resistance genes (Tet) were found in 46.7% of the isolates, conferring resistance to tetracycline antibiotics. All of the bacterial isolates (100%) were found to carry the Oxa gene, which provides resistance against penicillin, cephalosporins, and carbapenems. The aac(6')-Ib gene, which imparts resistance to aminoglycoside antibiotics, was present in 93.3% of the bacterial isolates.

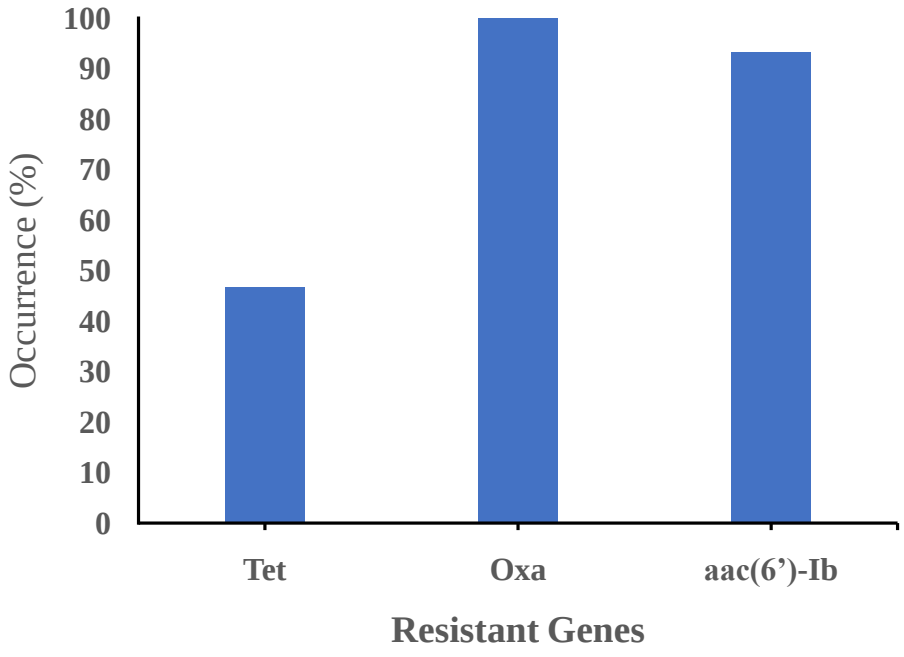


Figure 4: Prevalence of Antibiotic Resistance Genes

Antibiotic resistance genes (ARGs) have emerged as significant environmental contaminants and their prevalence is heightened by the excessive and inappropriate use of antibiotics [18]. This widespread misuse has facilitated the diffusion and escalation of ARGs across diverse environmental contexts, encompassing natural ecosystems and areas influenced by human activities such as soil [7], sediment [12], surface water [26], wastewater [21], groundwater [24], drinking water [22], and tap water [14]. Consequently, antimicrobial resistance has grown into a global crisis, eliciting grave concerns among both researchers and the general public.

Among these genes, the aac(6')-Ib gene, encoding aminoglycoside-modifying enzymes, stands out as the most prevalent, conferring resistance to tobramycin, kanamycin, and amikacin. Its variant, aac(6')-Ib-cr, induces resistance against both aminoglycosides and fluoroquinolones simultaneously. This gene produces the enzyme aminoglycoside acetyltransferase, modifying aminoglycoside antibiotics by adding an acetyl group. This alteration diminishes the antibiotics' affinity for their target sites, hindering their effective binding and inhibitory action. In this study, the presence of the aac(6')-Ib (482bp) antibiotic-resistant gene was identified in various bacterial strains, including *Pseudomonas zeshuii BY-1, Pseudomonas Koreensis, Pseudomonas kunmingensis HL22-2, Pseudomonas stutzeri ATCC 17588, Pseudomonas aeruginosa, Serratia fonticola DSM 4576, Serratia marcescens, Shigella dysenteriae RDF4, Shigella sonnei C13, Erythrobacter jejuensis CNU001, Enterococcus faecium JCM 5804, Enterococcus faecalis AD4, Bacillus badius ATCC 14574,* and *Bacillus coahuilensis M4-4*, underscoring the widespread presence of this gene in water sources within Bonny Island. Similarly, the Oxa gene cluster (438bp) was detected in several bacterial strains, including *Pseudomonas zeshuii BY-1, Pseudomonas Koreensis, Pseudomonas kunmingensis HL22-2, Pseudomonas stutzeri ATCC 17588, Pseudomonas aeruginosa, Serratia fonticola DSM 4576, Serratia marcescens, Shigella dysenteriae RDF4, Shigella sonnei C13, Erythrobacter jejuensis CNU001, Enterococcus faecium JCM 5804, Enterococcus faecalis AD4, Bacillus badius ATCC 14574, Bacillus coahuilensis M4-4, and Staphylococcus cohnii subsp. Urealyticus.* Previous studies conducted in Nigeria, notably by Adelowo et al. [2], Oluduro *et al.,* [18], and Okeke et al, [16], corroborate these findings, emphasizing the consistent presence of the Oxa gene in various water sources across the country.

Additionally, the tetB gene (636bp), belonging to the tetracycline resistance gene family, was identified in few of the isolates, including *Pseudomonas zeshuii BY-1, Pseudomonas Koreensis, Pseudomonas kunmingensis HL22-2, Serratia marcescens, Shigella sonnei C13, Erythrobacter jejuensis CNU001,* and *Bacillus coahuilensis M4-4.* This discovery aligns with prior research conducted globally, including studies by Ji et al., [11], Olawale et al., [17], and Adefisoye et al. [1], emphasizing the widespread occurrence of the tetB gene in bacterial communities found in drinking water sources.

These collective findings underscore the urgent need for comprehensive measures to address antibiotic resistance in Nigerian water sources, highlighting the critical importance of responsible antibiotic use and stringent monitoring practices to safeguard public health and environmental integrity.

4 CONCLUSIONS

This study plays a crucial role in unravelling the specific antimicrobial resistance (AMR) patterns within Bonny Island's drinking water distribution system, underscoring the necessity for meticulous monitoring and effective management approaches to counter the threats posed by antibiotic resistance genes (ARGs) in drinking water supplies. Additionally, this research adds valuable knowledge to the worldwide campaign against AMR, emphasizing the vital need to maintain the effectiveness of antimicrobial agents for public health. The findings of this study are particularly valuable for policymakers, public health officials, and researchers, offering important insights into the prevalence and genetic mechanisms underlying antimicrobial resistance in this essential environmental context.

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