



ASSESSMENT OF ANTIBIOTIC RESISTANCE GENES AND PATTERNS IN BACTERIAL ISOLATES OF SELECTED BOREHOLE WATER AROUND OPEN DUMPSITES IN ONITSHA SOUTH, ANAMBRA STATE, NIGERIA

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Abstract: The global increase in resistance to critically important antibiotics poses a serious public health threat, particularly due to mobile antibiotic resistance genes (ARGs) that facilitate rapid spread among bacteria through horizontal gene transfer. This study was conducted to assess antibiotic resistance genes and antibiotic resistance patterns in bacterial isolates from selected borehole water around open dump sites in the Onitsha South metropolis. Ten (10) borehole water samples were aseptically collected from ten (10) different boreholes 50 to 100m distance from the open dump sites and transported to Nnamdi Azikiwe University microbiology laboratory for microbial analysis. The membrane filtration method was used for the enumeration of total coliforms and fecal coliforms in the water samples, while serial dilution and pour plate methods were used for total heterotrophic bacterial counts. Bacterial isolates were identified based on cultural, morphological, and biochemical characteristics. Antibiotic susceptibility testing was carried out on the bacterial isolates using the Kirby-Bauer method, and results were interpreted in accordance with standard guidelines. The multiple antibiotic resistance (MAR) index was calculated for each isolate to assess antibiotic exposure levels. Antibiotic-resistant genes present in the MAR isolates were assessed by polymerase chain reaction (PCR) amplification of resistance genes (*bla*NDM, *bla*CTX-M, *aac*(6')-Ib-cr) followed by visualization by agarose gel electrophoresis. The isolates suspected in the water samples include *Staphylococcus aureus* (37.5%), *Bacillus* specie (25.0%), *Vibrio* specie, *Staphylococcus* specie, and *Escherichia coli* are 8.3% respectively, while *Shigella* specie, *Klebsiella* specie, and *Salmonella* specie are 4.3% respectively. The results showed widespread bacterial contamination, with a high prevalence of multidrug resistance among isolates. Ceftazidime and Cefuroxime were resistant to 100% of the Gram-positive isolates while Levofloxacin was resistant to 63.2% of the Gram-positive isolates. All the antibiotics were resistant to 100% of the Gram-negative isolates except streptomycin which was resistant to 60% of the Gram-negative isolates. MAR index values ranged from 0.5 to 1.0, with 100% of the isolates showing values > 0.2, an indication of exposure to high-risk sources of contamination. PCR showed 100% detection of *bla*NDM in all the selected isolates, *bla*CTX-M was detected in 33.3% of the isolates selected, while *aac*(6')-Ib-cr was not detected in any of the isolate. This study calls attention to routine water quality monitoring, improved sanitation practices, and strict regulation of antibiotic use in Nigeria.

Keywords: antibiotic resistance, antibiotic resistant genes, multiple resistance index, PCR.

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Introduction

Antibiotics remain among the most important medical discoveries in human history because of their central role in the treatment and control of bacterial infections. Their introduction has markedly reduced morbidity and mortality from infectious diseases and has facilitated major advances in modern medicine, including surgery, cancer chemotherapy, organ transplantation and neonatal care (Hutchings *et al.*, 2019). Commonly used antibacterial agents include β -lactams, fluoroquinolones and aminoglycosides, which are frequently prescribed for the management of both community-acquired and hospital-acquired infections. The continuing effectiveness of these agents is therefore essential to modern healthcare.

Despite their enormous benefits, the effectiveness of antibiotics is increasingly threatened by the emergence and spread of antimicrobial resistance. Antibiotic resistance occurs when bacteria acquire or develop mechanisms that enable them to survive exposure to antimicrobial agents to which they were previously susceptible. The development and dissemination of resistance are accelerated by inappropriate antibiotic use, self-medication, sub-therapeutic dosing, indiscriminate antimicrobial exposure and the improper disposal of pharmaceutical, domestic and agricultural wastes into the environment (Laxminarayan *et al.*, 2016). The problem is not restricted to clinical settings, as resistant organisms have also been recovered from food, environmental and community sources. In Anambra State, for example, Umeoduagu *et al.* (2023) documented antibiotic-resistant bacteria in fresh meat sold at Afor Oba Market, while Awari *et al.* (2024) reported resistance among pathogenic *Pseudomonas aeruginosa* isolated from a hospital environment. These findings further illustrate the circulation of resistant bacteria across different ecological niches.

Globally, resistance to critically important antibiotics has reached alarming levels. Of particular concern is resistance mediated by mobile antibiotic resistance genes (ARGs), which can be transferred between bacterial cells through horizontal gene transfer. Genes such as *bla*NDM and *bla*CTX-M encode β -lactamases capable of hydrolysing important β -lactam antibiotics, including carbapenems and extended-spectrum cephalosporins, whereas *aac*(6')-Ib-cr confers resistance to aminoglycosides and reduces susceptibility to fluoroquinolones (Munita and Arias, 2016; Bush and Bradford, 2019). Many of these resistance determinants are carried on plasmids and other mobile genetic elements, facilitating their rapid dissemination between bacterial species and across human, animal and environmental interfaces.

Although antibiotic resistance was traditionally regarded primarily as a clinical problem, increasing evidence

demonstrates that the environment constitutes an important reservoir and transmission pathway for resistant bacteria and ARGs. Aquatic environments are particularly important because they receive microorganisms and antimicrobial residues from domestic sewage, hospital effluents, agricultural runoff, abattoir wastewater, municipal waste and other anthropogenic sources (Berendonk *et al.*, 2015). The widespread occurrence of microorganisms in environmental matrices and their implications for both environmental and public health have also been emphasised by Awari *et al.* (2023). Similarly, Ezeokoli *et al.* (2023) demonstrated substantial bacterial contamination in abattoir wastewater in Awka, highlighting the potential of inadequately managed waste streams to introduce microbial contaminants into surrounding environments.

Groundwater, which was previously considered relatively protected from surface contamination, is now recognised as vulnerable, particularly in densely populated urban environments with poor sanitation, inappropriate waste disposal and shallow or poorly protected aquifers (Lapworth *et al.*, 2018). One of the major pathways through which groundwater contamination may occur is the percolation of leachate generated from open solid-waste dumps. Such leachate may contain high concentrations of organic matter, heavy metals, pathogenic microorganisms, antimicrobial residues and potentially antibiotic-resistant bacteria and their associated resistance genes. In Awka, Anambra State, Agu *et al.* (2014) demonstrated that leachate from solid-waste dumps contributed to both bacteriological and heavy-metal contamination of groundwater. More recently, Egurefa *et al.* (2024) reported bacteriological and heavy-metal contamination of drinking water obtained from boreholes in Enugu metropolis. These findings provide important evidence that groundwater sources in rapidly urbanising communities within south-eastern Nigeria may be vulnerable to contamination from surrounding human activities.

The microbiological quality of water used for domestic purposes is of particular public-health importance because contaminated water can serve as a vehicle for the dissemination of pathogenic and potentially antibiotic-resistant microorganisms. Victor-Aduloju *et al.* (2023), in a comparative assessment of water from selected hostels in Ifite, Awka, demonstrated microbial contamination of water sources used within the community. Similarly, Agu *et al.* (2023), while investigating fish-pond microbiota in Awka, demonstrated the diversity of microorganisms present in aquatic environments within Anambra State. Collectively, these studies reinforce the need for greater attention to the microbiological safety of water sources and to environmental reservoirs of microorganisms in densely populated communities.

In Nigeria, borehole water constitutes a major source of drinking and domestic water, particularly in urban and peri-urban communities where access to consistently treated municipal water is inadequate. Boreholes are commonly perceived by users as safer than surface-water sources; however, their microbiological quality depends strongly on borehole depth, construction, surrounding geology, sanitation conditions and proximity to potential sources of contamination. Where boreholes are located close to open refuse dumps, septic systems, drains, abattoirs or other pollution sources, contaminants may infiltrate the soil and eventually reach groundwater. The findings of Agu *et al.* (2014), Victor-Aduloju *et al.* (2023) and Eigurefa *et al.* (2024) are particularly relevant in this regard, as they demonstrate that groundwater and other domestic water sources within south-eastern Nigeria can harbour substantial bacterial contamination and may also be affected by nearby anthropogenic activities.

Open dumpsites pose an additional concern because they frequently receive heterogeneous wastes, including discarded medicines, household refuse, food waste, healthcare-related materials and other organic and inorganic substances. The resulting leachate creates conditions that may support the persistence, selection and dissemination of resistant bacterial populations. Studies from Anambra State have already demonstrated antibiotic-resistant bacteria in environmental and food-associated sources, including fresh meat (Umeoduagu *et al.*, 2023), hospital environments (Awari *et al.*, 2024), wastewater (Ezeokoli *et al.*, 2023) and other microbial ecosystems within Awka (Agu *et al.*, 2023). Although some local studies have focused on the antimicrobial susceptibility of bacteria or antimicrobial activity of natural products (Agu *et al.*, 2013; Adindu *et al.*, 2016; Awah *et al.*, 2016; Awah *et al.*, 2017; Umeoduagu *et al.*, 2023), comparatively little attention has been directed towards the molecular characterisation of resistance determinants in groundwater exposed to solid-waste pollution.

Onitsha South Local Government Area of Anambra State is a highly urbanised and intensely commercial area characterised by dense human settlement, considerable commercial activity, substantial generation of solid waste and challenges with organised waste disposal. The widespread occurrence of open dumpsites within or close to residential areas, together with heavy dependence on borehole water for drinking and domestic purposes, creates a plausible pathway for groundwater contamination. Under such conditions, leachate from poorly managed dumpsites may introduce faecal bacteria, opportunistic pathogens, antimicrobial-resistant organisms and mobile resistance determinants into groundwater systems, thereby creating potential risks for both individual and community health.

Despite these concerns, routine assessment of borehole water quality in the area has largely focused on physicochemical characteristics and conventional bacteriological indices. Molecular surveillance for clinically important antibiotic resistance genes such as *bla*NDM, *bla*CTX-M and *aac*(6')-Ib-cr remains limited. Furthermore, although previous studies in south-eastern Nigeria have demonstrated contamination of groundwater by waste-dump leachate (Agu *et al.*, 2014), microbial contamination of borehole water (Eigurefa *et al.*, 2024), microbial contamination of domestic water sources (Victor-Aduloju *et al.*, 2023) and the occurrence of antibiotic-resistant bacteria in environmental and food-associated samples (Umeoduagu *et al.*, 2023; Awari *et al.*, 2024), there remains a paucity of data specifically characterising antibiotic resistance genes in borehole water located around open dumpsites in Onitsha South Local Government Area. Given the public health importance of *bla*NDM, *bla*CTX-M, and *aac*(6')-Ib-cr, and their increasing detection in environmental matrices worldwide, there is a critical need to assess their presence in bacterial isolates from borehole water around open dump sites in Onitsha South (Munita and Arias, 2016; Bush and Bradford, 2019). Understanding both the antibiotic resistance patterns of bacterial isolates and the molecular distribution of these resistance genes is essential for evaluating environmental exposure risks and informing antimicrobial resistance control strategies.

Therefore, investigating the bacteriological quality, antibiotic susceptibility profiles and molecular occurrence of selected resistance genes in borehole water situated around open dumpsites in Onitsha South is important. Such data will help to determine whether these water sources constitute environmental reservoirs and potential transmission pathways for antimicrobial resistance, provide locally relevant evidence for groundwater surveillance and public-health interventions, and contribute to the broader understanding of antimicrobial resistance from a One Health perspective.

Materials and Methods

Sample Collection Site

The study was conducted in Onitsha South Local Government Area, Anambra State, Nigeria. Onitsha South is a densely populated urban area characterized by intense commercial activities, rapid urbanization, and inadequate waste management systems as shown in figure 1. Open dump sites are commonly used for solid waste disposal within residential and commercial zones, increasing the risk of environmental contamination. Borehole water serves as a major source of drinking and domestic water for residents due to limited access to treated municipal water supplies.

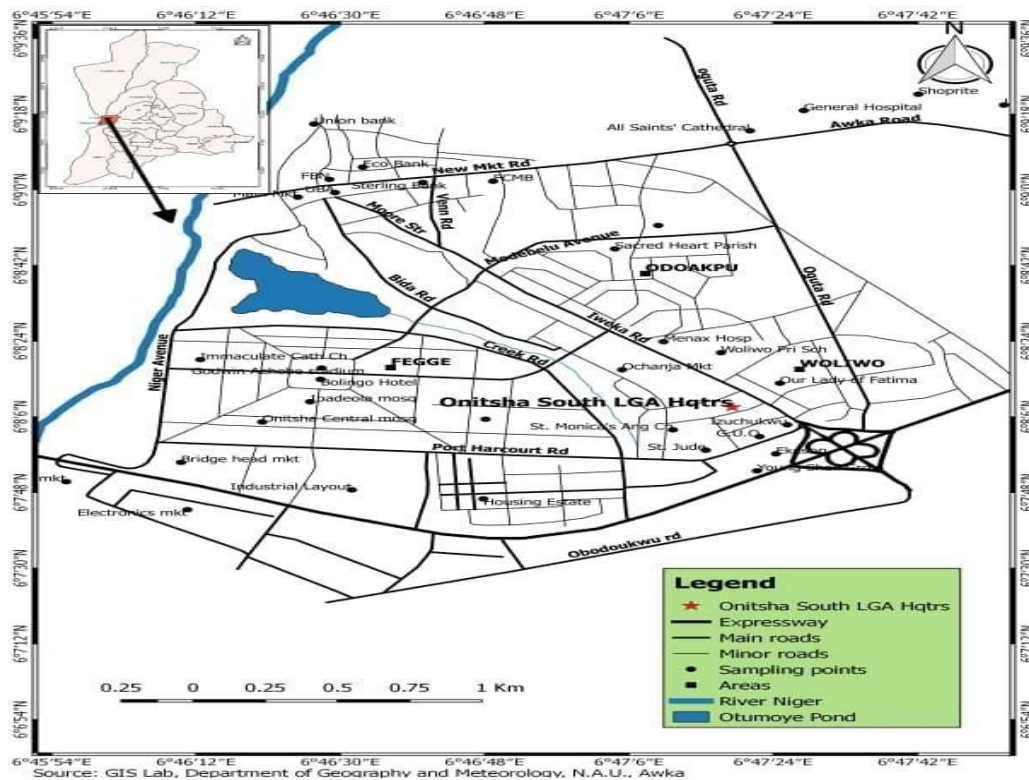


Figure 1: Map of Onitsha South (GIS Lab, Department of Geography and Meteorology, N.A.U., Awka)

Sample Size

A total of 10 borehole water samples were collected from selected boreholes situated at about a 50-100 meter distance from identified open dump sites within the Onitsha South Local Government Area. Samples were collected aseptically from the collection tap after allowing the water to run for 2 minutes before collecting with a sterile bottle, properly labeling, and transporting in an ice-packed container to the laboratory for immediate analysis within 2 hours.

Isolation and Identification of Bacterial Isolates

Isolates from overnight cultures of the water samples were further subcultured by streaking on nutrient agar to obtain pure isolates and incubated at 37°C for 18-24 hours. Pure isolates were identified based on their colony morphology, Gram staining characteristics, and standard biochemical tests according to established microbiological procedures.

Antibacterial Susceptibility Testing

Antimicrobial susceptibility testing of the bacterial isolates was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. Pure colonies from freshly cultured isolates (24 hour old) were emulsified in sterile normal saline to obtain a bacterial suspension equivalent to 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL). Turbidity was adjusted visually against the McFarland standard to ensure uniform inoculum density. A sterile cotton swab was dipped into the

standardized bacterial suspension, excess fluid was removed by pressing against the wall of the tube, and the entire surface of a sterile Mueller-Hinton agar plate was evenly inoculated by spreading in all directions to ensure uniform growth. The inoculated plates were allowed to stand for 3-5 minutes to permit surface drying.

Antibiotics impregnated discs (Gram-positive and Gram-negative) were aseptically placed on the surface of the inoculated agar using sterile forceps and gently pressed to ensure complete contact with the medium. The following antibiotics were used; Rifampicin (RD - 20µg), Ceftazidime (CTZ - 30µg), Streptomycin (S - 30µg), Azithromycin (AZM - 10µg), Amoxil (AMX - 20µg), Ciprofloxacin (CPX - 10µg), Erythromycin (E - 30µg), Levofloxacin (LEV - 20µg), Gentamycin (CN - 10µg), Cefuroxime (CEF - 30µg), Ofloxacin (OFX - 10µg), Augmentin (AU - 30µg), Peflaxine (PEF - 10µg), Ceporex (CEP - 10µg), Ceftriaxone (TRX - 30µg). The plates were incubated at 30°C for 24 hours. After incubation, the diameters of zones of inhibition were measured in millimeters using a calibrated ruler. Diameters were interpreted following the guideline of CLSI (2020) thus; ≥ 20 mm as Susceptible (S), 15-19 mm as Intermediate (I), and ≤ 14 mm as Resistant (R).

DNA Extraction and PCR

Genomic DNA from selected bacterial isolates was extracted using the boiling method as described by Chen and Kuo (1993). A loopful of a pure bacterial colony was suspended in 200µl of sterile distilled water in an eppendorf

tube and vortexed thoroughly using the vortex mixer to ensure homogenization of the bacterial cells. The suspension was incubated in a heating block at 95°C for 10 minutes, to break down the bacterial cells and release intracellular DNA into the solution. The mixture was then centrifuged at 12,000rpm for 1 minute. The supernatant containing the released DNA was carefully transferred into a new sterile micro-centrifuge tube using a micropipette. The recovered supernatant served as the final DNA for the PCR amplification of the antibiotic resistance genes.

PCR amplification was carried out to detect selected antibiotic resistance genes, *bla*CTX-M, *bla*NDM and *aac*(6')-Ib-cr, using gene-specific primers. Each PCR

reaction was prepared in a total reaction volume of 25 µl, containing: 12.5µl of One Taq Quick-Load 2X Master Mix with Standard Buffer, 0.5µl each of forward and reverse primers for each gene amplified, 8.5µl of Nuclease free water, and 3µl Extracted DNA template. The reaction mixture was gently mixed and transferred to an Eppendorf Gradient Mastercycler (New England Biolabs, 2024). PCR thermocycler condition and primer sequences for each ARG are summarized in Table 1. The presence or absence of a given target gene was assessed through observation of the expected amplicon length (determined using a DNA ladder) through gel electrophoresis.

Table 1. Primer sequences, PCR conditions, amplicon lengths for each ARG

	bla_NDM	bla_CTX-M	aac(6')-Ib-cr
Forward primer	5'-GGTTGCATCCCGGTGAAATC-3'	5'-CGCTTTGCGATGTGCAG-3'	TTGCGATGCTCTATGAGTGGCTA
Reverse primer	5'-ATGCTGGCCTTGGGGAACG-3'	5'-ACCGCGATATCGTTGGT-3'	CTCGAATGCCTGGCGTGTT
Initial denaturation	5 minutes at 94°C for 1 cycle	5 minutes at 94°C for 1 cycle	5 minutes at 94°C for 1 cycle
Cycles of denaturation	35 cycles at 94°C for 30 seconds	35 cycles at 94°C for 30 seconds	35 cycles at 94°C for 40 seconds
Primer annealing	55°C for 1 minute	55°C for 1 minute	54°C for 1 minute
Strand extension	72°C for 1 minute	72°C for 1 minute	72°C for 45 seconds
Final extension	72°C for 5 minutes for 1 cycle	72°C for 5 minutes for 1 cycle	72°C for 5 minutes for 1 cycle
Expected amplicon size	630 bp	875 bp	552 bp

2.6. Statistical Analysis

Data obtained from the study were analyzed using the Statistical Package for the Social Sciences (SPSS). Descriptive statistics were used to summarize the data. Antibiotic Susceptibility results obtained from replicate measurements were expressed as mean $\pm$ standard deviation (SD), while frequencies and percentages were used to summarize the distribution of bacterial isolates and their resistance patterns.

Results

Bacterial growth was observed in all the ten borehole water samples. The bacteriological analysis of the borehole water samples is presented in Table 2. The total heterotrophic bacterial count (THBC) ranged from 3.9×10^5 to 1.4×10^6 CFU/ml. The total coliform count (TCC) ranged from 0 to 20 CFU/100ml, while the fecal coliform count (FCC) ranged from 0 to 5 CFU/100ml.

Table 2: Bacteriological Characteristics of the Borehole Water Samples

Samples	Total Heterotrophic Bacteria Count (CFU/ML)	Total Coliform Count (CFU/100ML)	Fecal Coliform Count (CFU/100ML)
A	1.2×10^6	20	2
B	7.0×10^5	15	0
C	1.0×10^6	10	0
D	8.0×10^5	12	4
E	7.8×10^5	12	0
F	1.4×10^6	10	5
G	1.3×10^6	6	0
H	1.2×10^6	12	6
I	6.6×10^5	7	1
J	3.9×10^5	14	0

CFU = Colony Forming Unit.

A total of 24 bacterial isolates were recovered from the water samples. The morphological characteristics of bacterial isolates are presented in Table 3, showing the colony shape, elevation, margin, colour and surface of all

the isolates, while the biochemical characteristics and probable names of the isolates are shown in Table 4. The 24 isolates belong to the genera, *Staphylococcus*, *Klebsiella*, *Bacillus*, *Shigella*, *Escherichia*, *Salmonella* and *Vibrio* (Table 3).

Table 3: Morphological Characteristics of Bacterial Colonies

Isolates	Colony Shape	Elevation	Margin	Colour	Surface
A1	Filamentous	Raised	Filamentous	Milky	Dry
A2	Filamentous	Raised	Filamentous	Milky	Dry
A3	Irregular	Flat	Undulate	Milky	Smooth
A4	Circular	Raised	Entire	Milky	Smooth
B5	Irregular	Flat	Undulate	Milky	Dry
B6	Circular	Convex	Entire	Milky	Dry
C7	Filamentous	Raised	Filamentous	Milky	Dry
C8	Rhizoid	Flat	Lobate	Milky	Dry
C9	Circular	Convex	Entire	Milky	Smooth
D10	Irregular	Raised	Lobate	Milky	Smooth
D11	Circular	Convex	Entire	Milky	Dry
D12	Filamentous	Raised	Filamentous	Milky	Smooth
D13	Punctiform	Flat	Curled	Milky	Dry
D14	Filamentous	Raised	Filamentous	Milky	Rough
E15	Circular	Flat	Entire	Milky	Dry
E16	Punctiform	Flat	Entire	Milky	Dry
F17	Rhizoid	Raised	Lobate	Milky	Dry
G18	Circular	Pulvinate	Entire	Milky	Smooth
G19	Irregular	Raised	Lobate	Milky	Rough
G20	Irregular	Convex	Undulate	Milky	Dry
H21	Punctiform	Convex	Curled	Milky	Smooth
I22	Circular	Flat	Entire	Milky	Smooth
I23	Punctiform	Raised	Entire	Milky	Smooth
J24	Filamentous	Raised	Filamentous	Milky	Dry

Table 4: Biochemical Characteristics and identification of the Bacterial isolates

Isolates	Gram Reaction	CAT	IND	MR	OX	COA	VP	MO	CIT	GLU	SUC	LAC	MAL	GAL	Probably Organisms
A1	-ve rod	+	+	+	+	+	+	-	+	+	+	+	+	+	<i>Escherichia coli</i>
A2	+ve cocci	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Staphylococcus aureus</i>
A3	-ve rod	+	-	+	-	+	+	-	-	+	-	-	+	+	<i>Shigella</i> specie
A4	+ve cocci	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Staphylococcus aureus</i>
B5	-ve rod	+	-	-	+	+	+	-	+	+	+	+	+	+	<i>Klebsiella</i> specie
B6	+ve cocci	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Staphylococcus aureus</i>
C7	+ve cocci	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Staphylococcus aureus</i>
C8	-ve rod	+	-	+	-	+	+	+	+	+	-	-	+	+	<i>Salmonella</i> specie
C9	+ve cocci	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Staphylococcus aureus</i>
D10	+ve rod	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Bacillus</i> specie
D11	+ve rod	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Bacillus</i> specie
D12	+ve cocci	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Staphylococcus aureus</i>
D13	+ve cocci	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Staphylococcus aureus</i>
D14	+ve rod	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Bacillus</i> specie
E15	+ve cocci	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Staphylococcus aureus</i>
E16	+ve rod	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Bacillus</i> specie
F17	+ve rod	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Bacillus</i> specie
G18	-ve rod	+	-	-	+	+	+	+	+	+	+	-	+	+	<i>Vibrio</i> specie
G19	+ve cocci	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Staphylococcus aureus</i>
G20	+ve rod	+	-	+	+	+	+	-	+	+	+	+	+	+	<i>Bacillus</i> specie
H21	-ve rod	+	-	-	+	+	+	+	+	+	+	-	+	+	<i>Vibrio</i> specie
I22	-ve rod	+	+	+	+	+	+	-	+	+	+	+	+	+	<i>Escherichia coli</i>
I23	+ve cocci	+	-	+	+	-	+	-	+	+	+	+	+	+	<i>Staphylococcus</i> specie
J24	+ve cocci	+	-	+	+	-	+	-	+	+	+	+	+	+	<i>Staphylococcus</i> specie

Key: CAT = Catalase; IND = Indole; MR = Methyl Red; OX = Oxidase; COA = Coagulase; VP = Voges Proskauer; MO = Motility; CIT = Citrate Utilization; GLU = Glucose; SUC = Sucrose; LAC = Lactose; MAL = Maltose; GAL = Galactose. + = Positive Reaction, - = Negative Reaction.

The Frequency of distribution of bacterial isolates in the borehole water samples, showing the percentage occurrence of each genus, is shown in Table 5. *Staphylococcus aureus* was the most occurring, with 9 isolates (37.5%) of the total isolates while *Klebsiella*, *Shigella* and *Salmonella* had 4.2% each which were the least occurrences.

Table 5: Frequency of Occurrence of Bacterial Isolates

Isolates	Number of isolates	Frequency (%)
<i>Staphylococcus aureus</i>	9	37.5
<i>Bacillus</i> specie	6	25.0
<i>Staphylococcus</i> specie	2	8.3
<i>Escherichia coli</i>	2	8.3
<i>Vibrio</i> specie	2	8.3
<i>Klebsiella</i> specie	1	4.2
<i>Salmonella</i> specie	1	4.2
<i>Shigella</i> specie	1	4.2
Total	24	100

The result of the antibiotics susceptibility test, showing the zone of inhibition is presented in Table 6 and 7. The zone of inhibition ranged from 0 to 20 mm for the Gram-positive isolates and 0 to 17 mm for the Gram-negative isolates. The antibiogram was interpreted using the Clinical and Laboratory Standard Institute interpretive criteria breakpoints as shown in Table 8 and 9. The antibiogram for the Gram-positive isolates shows that out of 17 Gram-positive isolates, only two isolates (A2 and G20) showed susceptibility to gentamicin and levofloxacin respectively. The isolates showed varying levels of resistance to the antibiotics, while all isolates were resistant to ceftazidime and cefuroxime. For the Gram-negative isolates, there was 100% resistance to all the antibiotics except streptomycin.

Table 6: Zone of inhibition of Gram-positive isolates recorded in Mean $\pm$ SD (mm)

Isolate	LEV	CN	S	CTZ	AMX	CPX	AZM	CEF	E	RD
A2	16.00 $\pm$ 1.00	20.33 $\pm$ 0.58	0.67 $\pm$ 1.15	0.00 $\pm$ 0.00	0.33 $\pm$ 0.58	14.33 $\pm$ 0.58	14.00 $\pm$ 1.00	0.67 $\pm$ 0.58	13.33 $\pm$ 0.58	10.33 $\pm$ 0.58
A4	16.00 $\pm$ 1.00	10.67 $\pm$ 0.58	0.00 $\pm$ 0.00	0.67 $\pm$ 0.58	1.00 $\pm$ 0.58	14.33 $\pm$ 0.58	0.33 $\pm$ 0.58	0.00 $\pm$ 0.00	14.33 $\pm$ 0.58	10.33 $\pm$ 0.58
B6	11.67 $\pm$ 1.15	18.33 $\pm$ 0.58	0.33 $\pm$ 0.58	0.00 $\pm$ 0.00	0.67 $\pm$ 0.58	13.67 $\pm$ 0.58	13.33 $\pm$ 1.15	0.33 $\pm$ 0.58	12.00 $\pm$ 1.00	10.33 $\pm$ 0.58
C7	11.33 $\pm$ 0.58	11.67 $\pm$ 1.15	10.33 $\pm$ 0.58	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	11.00 $\pm$ 0.00	0.33 $\pm$ 0.58	0.67 $\pm$ 0.58	1.00 $\pm$ 1.00	10.67 $\pm$ 0.58
C9	0.33 $\pm$ 0.58	0.00 $\pm$ 0.00	0.67 $\pm$ 0.58	13.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.67 $\pm$ 0.58	0.67 $\pm$ 0.58	0.00 $\pm$ 0.00	0.33 $\pm$ 0.58	0.67 $\pm$ 0.58

D10	13.67±1.15	1.00±1.00	12.67±1.15	0.00±0.00	0.33±0.58	12.00±0.00	0.67±0.58	11.33±0.58	0.00±0.00	10.33±0.58
D11	14.00±0.00	10.33±0.58	15.67±0.58	0.67±0.58	13.33±0.58	14.67±1.15	12.00±0.00	0.33±0.58	13.00±0.00	16.00±1.00
D12	15.00±1.00	0.00±0.00	0.33±0.58	0.33±0.58	0.67±0.58	12.33±0.58	12.67±1.15	0.00±0.00	0.67±0.58	10.00±0.00
D13	13.33±0.58	14.00±0.00	0.33±0.58	0.67±0.58	0.00±0.00	13.33±0.58	0.67±0.58	0.33±0.58	13.67±1.15	12.33±0.58
D14	18.33±0.58	0.67±0.58	0.67±0.58	0.00±0.00	0.33±0.58	0.00±0.00	13.67±0.58	0.00±0.00	0.33±0.58	10.67±0.58
E15	15.33±0.58	0.33±0.58	14.00±0.00	0.67±0.58	16.00±1.00	16.67±0.58	12.33±0.58	0.67±1.15	19.33±0.58	17.00±0.00
E16	11.67±1.15	11.33±0.58	11.00±0.00	0.33±0.58	0.00±0.00	11.33±0.58	0.67±0.58	0.33±0.58	0.00±0.00	10.67±0.58
F17	11.33±0.58	14.67±1.15	14.33±0.58	13.00±0.00	12.67±0.58	0.00±0.00	12.67±1.15	0.33±0.58	12.67±0.58	10.00±0.00
G19	13.67±0.58	0.00±0.00	13.33±0.58	0.67±1.15	0.00±0.00	12.33±0.58	0.67±0.58	11.00±0.00	0.33±0.58	10.00±0.00
G20	20.33±0.58	16.00±1.00	14.67±0.58	0.00±0.00	12.33±0.58	16.00±1.00	15.00±0.00	0.00±0.00	13.33±0.58	12.67±0.58
I23	15.67±0.58	12.33±0.58	0.67±0.58	0.33±0.58	1.00±1.00	12.33±0.58	0.00±0.00	0.33±0.58	0.67±0.58	0.00±0.00
J24	16.33±0.58	0.00±0.00	11.33±0.58	0.67±0.58	0.00±0.00	14.00±0.00	13.67±0.58	0.33±0.58	1.00±1.00	10.33±0.58

Key: LEV = Levofloxacin; CN = Gentamycin; S = Streptomycin; CTZ = Ceftazidime; AMX = Amoxil; CPX = Ciprofloxacin; AZM = Azithromycin; CEF = Cefuroxime; E = Erythromycin; RD = Rifampicin.

Table 7: Zone of Inhibition Gram-negative isolates recorded in Mean ± SD(mm)

Isolates	OFX	AU	PEF	CTZ	CN	CPX	CEP	TRX	S	CEF
A1	0.00±0.00	0.33±0.58	0.33±0.58	1.00±1.00	0.67±0.58	0.00±0.00	0.67±0.58	10.33±0.58	17.67±0.58	10.00±0.00
A3	3.67±0.58	0.00±0.00	4.67±1.15	7.33±0.58	0.67±0.58	0.00±0.00	10.33±0.58	9.33±0.58	13.33±0.58	11.33±0.58
B5	0.33±0.58	1.00±1.00	0.00±0.00	0.67±0.58	0.67±0.58	0.33±0.58	0.00±0.00	10.67±1.15	17.33±0.5	10.33±0.58
C8	9.67±1.15	0.33±0.58	4.00±0.09	4.33±0.58	1.00±1.00	0.67±0.58	0.00±0.00	6.67±0.58	10.00±0.00	9.67±0.58
G18	13.00±1.00	0.67±0.58	0.67±0.58	0.00±0.00	1.00±1.00	13.67±1.15	0.33±0.58	0.67±0.58	0.00±0.00	10.33±0.58
H21	11.67±0.58	14.33±0.58	14.00±0.00	13.67±1.15	12.67±0.58	0.33±0.58	12.33±0.58	0.00±0.00	13.00±0.00	10.67±1.15
I22	9.33±0.58	0.67±0.58	1.00±1.00	0.33±0.58	0.33±0.58	0.00±0.00	8.67±0.58	6.00±0.00	5.33±0.58	13.00±0.00

Key: OFX = Ofloxacin; AU = Augmentin; PEF = Peflacin; S = Streptomycin; CTZ = Ceftazidime; CN = Gentamycin; CEP = Ceporex; CEF = Cefuroxime; TRX = Ceftriaxone; CPX = Ciprofloxacin. SD = Standard Deviation.

Table 8: Antibigram of Gram-positive Isolates

Isolates	LEV	CN	S	CTZ	AMX	CPX	AZM	CEF	E	RD
A2	I	S	R	R	R	R	R	R	R	R
A4	I	R	R	R	R	R	R	R	R	R
B6	R	I	R	R	R	R	R	R	R	R
C7	R	R	R	R	R	R	R	R	R	R
C9	R	R	R	R	R	R	R	R	R	
D10	R	R	R	R	R	R	R	R	R	
D11	I	R	I	R	R	R	R	R	R	I
D12	R	R	R	R	R	R	R	R	R	R
D13	R	R	R	R	R	R	R	R	R	I
D14	I	R	R	R	R	R	R	R	R	R
E15	I	R	R	R	I	I	R	R	I	I
E16	R	R	R	R	R	R	R	R	R	R
F17	R	R	R	R	R	I	R	R	I	I
G19	R	R	R	R	R	R	R	R	R	R
G20	S	R	R	R	R	I	I	R	R	R
I23	I	R	R	R	R	R	R	R	R	R
J24	I	R	R	R	R	R	R	R	R	R
% Resistance	63.2	89.5	94.7	100	94.7	84.2	94.7	100	89.5	78.9

Key: LEV = Levofloxacin; CN = Gentamycin; S = Streptomycin; CTZ = Ceftazidime; AMX = Amoxil; CPX = Ciprofloxacin; AZM = Azithromycin; CEF = Cefuroxime; E = Erythromycin; RD = Rifampicin. Sensitive (S) = ≥ 20 mm; Intermediate (I) = 15-19mm and Resistant = ≤ 14 mm.

Table 9: Antibigram of Gram-negative Isolates

Isolates	OFX	AU	PEF	CTZ	CN	CPX	CEP	TRX	S	CEF
A1	R	R	R	R	R	R	R	R	I	R
A3	R	R	R	R	R	R	R	R	R	R
B5	R	R	R	R	R	R	R	I	R	
C8	R	R	R	R	R	R	R	R	R	R
G18	R	R	R	R	R	R	R	R	R	R
H21	R	R	R	R	R	R	R	R	R	R
I22	R	R	R	R	R	R	R	R	R	R
% resistance	100	100	100	100	100	100	100	100	60	100

Key: OFX = Ofloxacin; AU = Augmentin; PEF = Peflacin; S = Streptomycin; CTZ = Ceftazidime; CN = Gentamycin; CEP = Ceporex; CEF = Cefuroxime; TRX = Ceftriaxone; CPX = Ciprofloxacin. Intermediate (I) = 15-19mm and Resistant = ≤ 14 mm.

Table 10 shows the Multiple Antibiotic Resistance (MAR) index of the bacterial isolates recovered from the borehole water samples. MAR index values ranged from 0.5 to 1.0.

Table 10: Multidrug Resistant Index (MRI) of the Bacterial Isolates

Isolates	Number of Antibiotics Tested	Number Resistant	MRI
A1	10	9	0.9
A2	10	8	0.8
A3	10	10	1.0
A4	10	9	0.9
B5	10	9	0.9
B6	10	9	0.9
C7	10	10	1.0
C8	10	10	1.0
C9	10	10	1.0
D10	10	10	1.0
D11	10	7	0.7
D12	10	10	1.0
D13	10	9	0.9
D14	10	9	0.9
E15	10	5	0.5
E16	10	10	1.0
F17	10	7	0.7
G18	10	10	1.0
G19	10	10	1.0
G20	10	8	0.8
H21	10	9	0.9
I22	10	10	1.0
I23	10	9	0.9
J24	10	9	0.9

Detection of antibiotics resistance genes is presented in Table 11. A total number of 6 (six) isolates were selected amongst the isolates with the highest MRI. Polymerase chain reaction (PCR) showed 100% detection of bla_{NDM} in all isolates. bla_{CTX-M} was detected in 33.3% of the isolates which were in G18 and G19, while aac (6')-Ib-cr was not detected in any of the Isolates.

Table 11: Detection of Antibiotic Resistance Genes in the Isolates

Isolates	<i>bla</i> NDM	<i>bla</i> CTX-M	<i>aac</i> (6')-Ib-cr
A3	+	-	-
C8	+	-	-
D10	+	-	-
G18	+	+	-
G19	+	+	-
I22	+	-	-

NDM = New Delhi Metallo-beta-lactamase; CTX-M = Cefotaximase-Munich; + = Gene detected, - = Gene not detected

4. Discussion

Natural and human activities induce changes in water quality that are mostly observed in its physical, chemical and microbiological parameters (Martinez-Santos *et al.*, 2017). The total heterotrophic bacterial (THB) counts obtained in this study revealed presence of heterotrophic bacteria in all the water samples. The total coliform and fecal coliform counts obtained indicate that a substantial proportion of the samples exceeded recommended limits for potable water. Similar findings have been reported by Gwimbi *et al.* (2019), who explained that borehole water in populated areas can easily become contaminated when proper hygiene is not maintained. The morphological characteristics of bacterial isolates obtained revealed variations in colony shape, elevation, margin, colour and surface.

Gram reaction and biochemical characteristics revealed a diverse bacterial population comprising both Gram-positive and Gram-negative organisms. The identification of *Staphylococcus aureus*, *Staphylococcus* specie and *Bacillus* specie suggests environmental and human-related contamination, while the presence of *Escherichia coli*, *Vibrio* specie, *Salmonella* specie, *Shigella* specie and *Klebsiella* specie indicate fecal pollution. As explained by Odonkor and Mahami (2020), *Escherichia coli* remains one of the most reliable indicators of fecal contamination in water, thereby confirming the sanitary risk associated with the borehole sources examined in this study. The bacterial profile obtained in this study was comparable with the findings of Adegoke (2022) who reported *Escherichia coli*, *Bacillus* specie, *Klebsiella* specie, *Staphylococcus* specie and *Salmonella* specie in borehole water from Kogi State University, Anyigba, Nigeria.

The high prevalence of *Staphylococcus aureus* and *Bacillus* specie in this study is consistent with findings of Obuekwe *et al.* (2021) who reported *Staphylococcus aureus* and *Bacillus* species among bacterial pathogens isolated from borehole water in selected areas of Benin City, Edo State. These findings support the occurrence of *staphylococci* and *bacilli* as common bacterial contaminants of borehole water. The relatively low occurrence of *Escherichia coli*, *Salmonella* specie and *Shigella* specie in this study differs from reports by Traoré *et al.* (2023), who observed higher levels of isolated environmental and enteric bacteria from groundwater sources in Burkina Faso, and attributed their occurrence to poor sanitary conditions and environmental pollution.

This study evaluated both phenotypic and genotypic antibiotic resistance among the borehole water isolates. Phenotypically, multiple antibiotic resistance was observed among both Gram-positive and Gram-negative isolates to commonly used antibiotics in the study area, including

levofloxacin, gentamycin, streptomycin, ceftazidime, amoxil, ciprofloxacin, azithromycin, cefuroxime, erythromycin, rifampicin, ofloxacin, augmentin, peflacin, ceporex and ceftriaxone. Notably, ciprofloxacin, ceftriaxone, and gentamicin, which were among the antibiotics tested in this study were also reported among the five most frequently prescribed antibiotics in a multi center hospital surveys in northern Nigeria accounting for 17.1%, 16.8%, and 11.8% of prescriptions respectively (Abubakar 2020). Among the Gram-positive isolates, only *Staphylococcus aureus* and *Bacillus* specie were susceptible to gentamicin and levofloxacin, respectively, whereas all the isolates showed complete (100%) resistance to ceftazidime and cefuroxime. Similarly, the Gram-negative isolates exhibited complete resistance to all the antibiotics tested except streptomycin. The high level of antimicrobial resistance observed in this study is consistent with the findings of Aromolaran *et al.* (2023) who reported that bacterial isolates recovered from groundwater sources in southwestern Nigeria exhibited resistance to several commonly used antibiotics, indicating the widespread occurrence of multidrug-resistant bacteria in environmental water sources. Comparable observations were also reported by Odonkor *et al.* (2022), who found that bacteria isolated from drinking water sources in Ghana exhibited high resistance to β -lactam antibiotics and concluded that environmental water bodies are important reservoirs of antimicrobial-resistant bacteria.

The Multiple Antibiotic Resistance (MAR) index values obtained in this study ranged from 0.5 to 1.0, with all the bacterial isolates showing MAR index values greater than 0.2. According to Krumperman (1983), MAR index values exceeding 0.2 indicate that bacterial isolates originate from high-risk environments where antibiotics are frequently used, thereby exposing microorganisms to continuous antibiotic selection pressure. The high MAR indices recorded in this study may be associated with the continuous introduction of antibiotic-resistant bacteria and antibiotic residues into the environment through indiscriminate waste disposal, leachate infiltration from open dumpsites and other human activities as well as antibiotics misuse.

PCR was done to detect the presence/absence of three ARGs. Among the selected bacterial isolates, 100% of them harboured the *bla*NDM gene, while the *bla*CTX-M gene was detected in only two isolates (33.3%), namely *Vibrio* specie and *Staphylococcus aureus*. In contrast, the *aac*(6')-Ib-cr gene was not detected in any of the isolates analyzed. The detection of *bla*NDM agrees with Yusuf *et al.* (2023), who detected *bla*NDM-producing bacteria in wastewater, sewage and municipal solid wastes in Nigeria. Although *bla*NDM is predominantly associated with Gram-negative bacteria, its occurrence in Gram-positive organisms has occasionally been reported in public health studies.

The detection of the *bla*CTX-M gene in two of the six isolates supports the report of Banjo et al. (2020) who detected the β -lactamase genes *bla*CTX-M, *bla*SHV, and *bla*TEM among ESBL-producing bacteria recovered from hospital wastewater and nearby hand-dug well water in southwestern Nigeria. The lower occurrence of *bla*CTX-M compared with *bla*NDM in this study may indicate differences in the distribution of resistance genes among bacterial species or variations in local antibiotic selection pressure within the study area.

Unlike *bla*NDM and *bla*CTX-M, the *aac*(6')-Ib-cr gene was not detected in any of the isolates screened. The absence of this gene suggests that plasmid-mediated quinolone resistance associated with *aac*(6')-Ib-cr may not be the predominant mechanism of quinolone resistance among the bacterial isolates investigated. Furthermore, the relatively small number of isolates selected for molecular analysis may have reduced the likelihood of detecting less prevalent resistance genes. In contrast, Wilcox et al. (2023) detected the *aac*(6')-Ib-cr gene among bacteria isolated from drinking water sources in Rivers State, Nigeria, suggesting that the distribution of quinolone resistance genes may vary across geographical locations and environmental conditions.

5. Conclusion

This study provides compelling evidence of microbial contamination and alarming antimicrobial resistance patterns in borehole water sources in Onitsha South. The bacteriological analysis revealed the presence of organisms with high levels of resistance to commonly used antibiotics, which raises serious concerns about the quality of water consumed by residents in this area. The isolation of organisms such as *Staphylococcus aureus*, *Bacillus* specie, *Staphylococcus* specie, *Escherichia coli*, *Shigella* specie, *Vibrio* specie, *Salmonella* specie and *Klebsiella* specie confirms both environmental and fecal contamination of the water sources. The occurrence of multidrug-resistant isolates further emphasizes the potential health risk, especially in situations where infections arising from such organisms may become difficult to treat.

The detection of the NDM (New Delhi metallo- β -lactamase) gene in all the selected isolates is particularly alarming as NDM is a potent carbapenemase associated with resistance to last-resort antibiotics, indicating a severe level of antimicrobial resistance circulating within the environment. The detection of CTX-M genes in some isolates further confirms the presence of extended-spectrum β -lactamase (ESBL)-producing bacteria, reinforcing the role of borehole water as a reservoir for clinically relevant resistance determinants. Although the *aac*(6')-Ib-cr gene was not detected, the combination of other resistance genes identified is sufficient to indicate a serious public health concern.

The presence of these resistance determinants in borehole water highlights a critical intersection between environmental contamination and public health risk. It suggests possible infiltration of resistant bacteria from anthropogenic sources such as poor sanitation, sewage leakage, and indiscriminate disposal of antibiotics. Consequently, individuals relying on these water sources are at risk of continuous exposure to resistant pathogens, which may contribute to the silent spread of antimicrobial resistance within the community. Hence, the findings of this study highlight a silent but dangerous reality. What is assumed to be a safe source of water may, in fact, be contributing to the growing burden of antimicrobial resistance.

References

1. Abubakar, U. (2020). Antibiotic use among hospitalized patients in northern Nigeria: A multicenter point-prevalence survey. *BMC Infectious Diseases*, 20, 86.
2. Adegoke, A. A., Faleye, A. C., Singh, G., & Stenström, T. A. (2020). Antibiotic resistant bacteria and genes in hospital wastewaters, groundwater and surface waters in Nigeria. *Journal of Environmental Management*, 267, 110656.
3. Adegoke, S. A. (2022). Multi-drug resistant bacterial status of borehole water in Kogi State University, Anyigba, Nigeria. *Asian Journal of Biology*, 16(1), 20–30.
4. Adindu, C. S., Odili, L. C., Nwagu, E. E., Agu, K. C., Awah, N. S., Okeke, B. C., & Orjionwe, R. N. (2016). Phytochemical and antimicrobial screening of *Cola gigantea* leaves, stem and bark. *Universal Journal of Microbiology Research*, 4(2), 49–54.
5. Agu, K. C., Igweoha, C. A., & Umeh, C. N. (2013). Antimicrobial activity of the ethanolic and petroleum ether extracts of tangerine seeds on selected bacteria. *International Journal of Agriculture and Bioscience*, 2(1), 22–24.
6. Agu, K. C., Orji, M. U., Onuorah, S. C., Ejurefa, S. O., Anaukwu, C. G., Okafor, U. C., Awah, N. S., Okafor, O. I., Mbachu, A. E., & Anyaegbunam, B. C. (2014). Influence of solid waste dumps leachate on bacteriological and heavy metals contamination of groundwater in Awka. *American Journal of Life Science Researches*, 2(4), 450–457.
7. Agu, K. C., Umeoduagu, N. D., Ejurefa, S. O., Awari, V. G., Uwanta, L. I., Ikenwa, B. O., Udenweze, E., Nwiyi, I. U., Chidubem-Nwachinemere, N. O., Ozoh, C. N., Ohanazoeze, C. F., & Nwosu, J. C. (2023). Comparative study of the microbiota of fish ponds in Awka, Anambra, Nigeria. *Global Scientific Journal*,

- 11(6), 1625–1646.
http://www.globalscientificjournal.com/researchpaper/Comparative_Study_of_the_Microbiota_of_Fish_Ponds_in_Awka_Anambra_Nigeria.pdf
8. Aromolaran, O., Ayansina, A. D. V., Adegbami, A. A., Oshanisi, P. O., & Oladoyin, D. M. (2023). Prevalence of multidrug-resistant bacteria in groundwater supplies in Osogbo, Southwest Nigeria. *International Journal of Environmental Studies*, 80(6), 1526–1538.
9. Awah, N. S., Agu, K. C., Ikedinma, J. C., Uzoechi, A. N., Eneite, H. C., Victor-Aduloju, A. T., Umeoduagu, N. D., Onwuatiegwu, J. T. C., & Ilikannu, S. O. (2017). Antibacterial activities of the aqueous and ethanolic extracts of the male and female *Carica papaya* leaves on some pathogenic bacteria. *Bioengineering and Bioscience*, 5(2), 25–29.
<https://doi.org/10.13189/bb.2017.050201>
10. Awah, N. S., Agu, K. C., Okorie, C. C., Okeke, C. B., Iloanus, C. A., Ironi, C. R., Udemezue, O. I., Kyrian-Ogbonna, A. E., Anaukwu, C. G., Eneite, H. C., Ifediegwu, M. C., Umeoduagu, N. D., Abah, N. H., & Ekong, U. S. (2016). In-vitro assessment of the antibacterial quality of some commonly used herbal and non-herbal toothpastes on *Streptococcus mutans*. *Open Journal of Dentistry and Oral Medicine*, 4(2), 21–25.
<https://doi.org/10.13189/ojdom.2016.040201>
11. Awari, V. G., Umeoduagu, N. D., Agu, K. C., Obasi, C. J., Okonkwo, N. N., & Chidozie, C. P. (2024). Antibigram of pathogenic *Pseudomonas aeruginosa* isolated from hospital environment. *International Refereed Journal of Engineering and Science*, 13(2), 1–10.
12. Awari, V. G., Umeoduagu, N. D., Agu, K. C., Okonkwo, N. N., Ozuah, C. L., & Victor-Aduloju, A. T. (2023). The ubiquity, importance and harmful effects of microorganisms: An environmental and public health perspective. *International Journal of Progressive Research in Engineering Management and Science*, 3(12), 1–10.
13. Banjo, O. A., Adekanmbi, A. O., & Oyelade, A. A. (2020). Occurrence of CTX-M, SHV and TEM β -lactamase genes in extended spectrum beta-lactamase (ESBL)-producing bacteria recovered from wastewater of a privately owned hospital in Nigeria and a hand-dug well within its vicinity. *Gene Reports*, 21, 100970.
14. Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M. N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., & Martinez, J. L. (2015). Tackling antibiotic resistance: The environmental framework. *Nature Reviews Microbiology*, 13(5), 310–317.
15. Bush, K., & Bradford, P. A. (2019). Interplay between β -lactamases and new β -lactamase inhibitors. *Nature Reviews Microbiology*, 17(5), 295–306.
16. Chen, W. P., & Kuo, T. T. (1993). A simple and rapid method for the preparation of gram-negative bacterial genomic DNA. *Nucleic Acids Research*, 21(9), 2260.
17. Clinical and Laboratory Standards Institute. (2020). *Performance standards for antimicrobial susceptibility testing* (30th ed.). CLSI.
18. Egurefa, S. O., Okinedo, J. I., Awari, V. G., Ogbonna, U. S. A., Obianom, A. O., Victor-Aduloju, A. T., Abana, C. C., Umeoduagu, N. D., Uwanta, L. I., Agu, K. C., Nwosu, J. C., & Onoyima, L. C. (2024). Assessment of the bacteriological and heavy metal contamination in drinking water from borehole sites in Enugu metropolis. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, 10(5), 13–26.
19. Ezeokoli, C. M., Agu, K. C., Nwosu, J. C., Orji, M. U., Uwanta, L. I., Umeoduagu, N. D., Victor-Aduloju, A. T., & Ikenwa, B. O. (2023). Bacteriological evaluation of Kwata Abattoir wastewater, Awka, Nigeria. *Innovare Journal of Science*, 11, 1–4.
20. Gwimbi, P., George, M., & Ramphalile, M. (2019). Bacterial contamination of drinking water sources and associated health risks in rural communities. *[Journal title not provided]*, 45(1), 1–7.
21. Hutchings, M. I., Truman, A. W., & Wilkinson, B. (2019). Antibiotics: Past, present and future. *Current Opinion in Microbiology*, 51, 72–80.
22. Krumperman, P. H. (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Applied and Environmental Microbiology*, 46(1), 165–170.
23. Kumar, M., Jaiswal, S., Sodhi, K. K., Shree, P., Singh, D. K., Agrawal, P. K., & Shukla, P. (2021). Antibiotics bioremediation: Perspectives on its ecotoxicity and resistance. *Environment International*, 156, 106725.
24. Lapworth, D. J., Fogg, P., Ward, R., & Stuart, M. E. (2018). Antibiotic resistance genes in groundwater: A review of sources, fate and persistence. *Environmental Pollution*, 237, 567–578.
25. Laxminarayan, R., Sridhar, D., Blaser, M., Wang, M., & Woolhouse, M. (2016). Achieving global targets for antimicrobial resistance. *Science*, 353(6302), 874–875.
26. Martinez-Santos, P., Martin-Loeches, M., Garcia-Castro, N., Solera, D., Diaz-Alcaide, S., Montero, E., &

- Garcia-Rincon, J. (2017). A survey of domestic wells and pit latrines in rural settlements of Mali: Implications of on-site sanitation on the quality of water supplies. *International Journal of Hygiene and Environmental Health*, 220(7), 1179–1189.
27. Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology Spectrum*, 4(2).
28. Obuekwe, I. S., Oshoma, C. E., & Omorogbe, J. N. (2021). Evaluation of physicochemical and bacteriological properties of borehole water in selected areas of Benin City, Edo State. *Nigerian Journal of Life Sciences*, 11(1–2), 10–17.
29. Odonkor, S. T., & Mahami, T. (2020). *Escherichia coli* as a tool for disease risk assessment of drinking water sources. *International Journal of Microbiology*, 2020, Article 2534130.
30. Odonkor, S. T., Simpson, S. V., Morales Medina, W. R., & Fahrenfeld, N. L. (2022). Antibiotic-resistant bacteria and resistance genes in isolates from Ghanaian drinking water sources. *Journal of Environmental and Public Health*, 2022, Article 2850165.
31. Traoré, O., Kpoda, D. S., Dembélé, R., Saba, C. K. S., Cairns, J., Barro, N., & Haukka, K. (2023). Microbiological and physicochemical quality of groundwater and risk factors for its pollution in Ouagadougou, Burkina Faso. *Water*, 15(21), 3734.
32. Umeoduagu, N. D., Chidozie, C. P., Ifemeje, E. M., Anazodo, C. A., Okoli, F. A., Okonkwo, N. N., Uwanta, L. I., Agu, K. C., & Azuka, G. E. (2023). Halitosis: The antimicrobial susceptibility pattern of some commercially sold toothpaste on selected isolates. *International Journal of Scientific Research & Engineering Trends*, 9(5), 1309–1315.
33. Umeoduagu, N. D., Egunfa, S. O., Awari, V. G., Agu, K. C., Udenweze, E. C., & Uwanta, L. I. (2023). Bacteriological and antibiotic resistance evaluation of bacteria isolated from fresh meat sold at Afor Oba Market, Anambra State. *International Journal of Research Publication and Reviews*, 4(8), 554–560.
34. Victor-Aduoju, A. T., Okonkwo, N. N., Okoli, F. A., Agu, K. C., Okoye, C. W., Awari, V. G., & Umeoduagu, N. D. (2023). Comparative analysis of microbial load of water in selected hostels in Ifite, Awka. *International Journal of Progressive Research in Engineering Management and Science*, 3(9), 400–408.
35. Wilcox, I. M., Sokari, T. G., Akani, N. P., & Amadi, L. O. (2023). Antimicrobial profiling of resistant genes in bacteria isolated from drinking water sources in Bonny Island, Rivers State, Nigeria. *Journal of Advances in Microbiology*, 23(10), 146–157.
36. Yusuf, I., Muhammad, Z. D., Amin, B. M., Shuaibu, M. D., Hamza, N., Isah, H. D., Abdullahi, N. B., Ene, P. J., Shuaibu, S. S., Doguwa, N., Pedro, S. L., & Muhammad, M. A. (2023). Detection of clinically relevant antibiotic-resistant bacteria in shared fomites, wastewater and municipal solid wastes disposed near residential areas of a Nigerian city. *Access Microbiology*, 5(12), 000641.