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Bacteriological Quality, Molecular Identification and Antibiotic Resistance Profile of Bacteria Isolated from Water Sources in Isiala-Ngwa North and South Local Government Areas, Abia State, Nigeria

Otigbuo, C. P.*, Ugboma, C. J. and Nrior, R. R.
Department of Microbiology, Faculty of Science,
Rivers State University, Nkpolu-Oroworukwo,
Port Harcourt, Rivers State, Nigeria.
***Corresponding Author:** *otigbuochimere@gmail.com*

ABSTRACT

The presence of multidrug-resistant enteric bacteria in domestic water sources is a growing public health concern. This study evaluated the bacteriological quality, molecular identity and antibiotic resistance profile of bacteria isolated from tap (government treated potable water), rain, upstream and downstream river water sources in Isiala-Ngwa North and South Local Government Areas, Abia State, Nigeria. Twenty-four water samples were collected monthly over three months (May–July 2025) and analyzed using standard microbiological, biochemical, molecular (16S rRNA) and antimicrobial susceptibility (disc diffusion) methods. Total heterotrophic bacterial counts ranged from 1.3×10^3 to 9.2×10^3 CFU/mL and total coliform counts ranged from 1.2×10^1 to 6.0×10^1 CFU/mL, exceeding recommended safe limits of the WHO guidelines across all sources. Forty bacterial isolates belonging to eleven genera were recovered and identified biochemically, with *Citrobacter* sp. (30.0%) the most prevalent, followed by *Serratia* sp. (17.5%) and *Klebsiella* sp. (15.0%). Molecular identification by 16S rRNA sequencing confirmed *Citrobacter freundii, Proteus mirabilis, Klebsiella pneumoniae and Enterobacter cloacae* among the isolates. Hemolysis assays showed 47.5% β-hemolysis, 40.0% α-hemolysis and 12.5% γ-hemolysis. Antibiotic susceptibility testing revealed high resistance, with 100% of isolates resistant to streptomycin and augmentin, and multiple antibiotic resistance (MAR) indices ranging from 0.25 to 1.0 in all isolates; ciprofloxacin retained the highest activity (97.5% susceptibility). These findings indicate that the water sources examined harbors multidrug-resistant enteric bacteria; and are therefore bacteriologically unsafe for direct consumption. Therefore, routine bacteriological surveillance and appropriate treatment of these water sources before domestic use are recommended.

Keywords: Water Quality Bacteria, Multiple Antibiotic Resistance, 16S rRNA, Biofilm, Hemolysis, Isiala-Ngwa.

Introduction

Microbial risk assessment and management of water quality remains an important focus of governmental regulation and scientific inquiry, as microorganisms are ubiquitous in aquatic environments and some are capable of producing substances harmful to human health (Burgess & Pletschke, 2009). Contaminated water sources are important vehicles for the transmission of water-borne diseases such as cholera, shigellosis and typhoid fever, and coliform bacteria are widely used as indicators of faecal contamination because their presence suggests that disease-causing organisms may be present in a water system (Amyes, 2007). The bacteriological examination of water is undertaken to identify sources contaminated with potential disease-causing microorganisms, contamination that generally arises directly from human or animal faeces, or indirectly through inadequately treated sewage. Because it is impractical to test every water sample for every possible pathogen, non-pathogenic indicator organisms such as coliforms and *Escherichia coli* are used as proxies for the likely presence of faecal contamination (Croxen et al., 2013).

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Beyond simple contamination, the aquatic environment has increasingly been recognised as a reservoir of bacteria carrying antibiotic resistance determinants (Biyela et al., 2004). Multidrug-resistant *Enterobacteriaceae* recovered from environmental waters have been linked to clinical isolates implicated in community disease outbreaks (Akinyemi et al., 2011), highlighting the public health relevance of characterising the antibiotic resistance profile of bacteria recovered from domestic water sources, not only their presence.

Molecular techniques, particularly 16S rRNA gene sequencing, now allow bacterial isolates from environmental sources to be identified with greater precision than conventional biochemical methods alone, providing molecularly verified evidence of the specific organisms present in a given water source (Srinivasan et al., 2015). In addition, phenotypic virulence-associated traits such as haemolysin production and biofilm formation provide further insight into the potential pathogenicity of water-borne isolates (Flemming & Wingender, 2010).

In Isiala-Ngwa North and South Local Government Areas of Abia State, Nigeria, households rely on a mixture of tap water, harvested rainwater and stream water for domestic use, frequently without treatment. This study was therefore designed to determine the bacterial load, isolate and identify (by conventional and molecular methods) the bacteria present in these water sources, and to characterise their haemolytic pattern, biofilm-forming potential and antibiotic resistance profile, including their multiple antibiotic resistance (MAR) index.

Materials and Methods

Study Area and Sample Collection

The study was carried out in Isiala-Ngwa North and Isiala-Ngwa South Local Government Areas of Abia State, Nigeria. Twenty-four water samples were aseptically collected from tap, rain, upstream river and downstream river water sources at monthly intervals over three months (May–July 2025) — from Umuakwu stream, a tap source and a rain source at Eziamata (Isiala-Ngwa North), and from Owerrinta stream, a tap source (government treated potable water) and a rain source at Umunko (Isiala-Ngwa South). For the tap water source the tap was turned on and allowed to rush for three minutes before the water samples were collected, Rain water was collected from a concrete reservoir by using a sterile pipette. The same method was also used for the upstream and downstream samples. Samples were collected in sterile bottles without touching the inside of the cap or bottle. Then it was transported on ice to the Department of Microbiology Laboratory, Rivers State University, Port Harcourt, within 8 hours, and processed immediately on arrival.

Cultivation and Enumeration of Bacterial Population

Ten-fold serial dilutions of each water sample were prepared in sterile normal saline. Aliquots (0.1 mL) of appropriate dilutions. The tap, rain and stream samples were inoculated in duplicate onto Nutrient agar, Salmonella–Shigella agar and Eosin Methylene Blue (EMB) agar by the spread plate method and incubated at 37 °C for 24 hours. Colonies on Nutrient agar were used to estimate total heterotrophic bacterial counts (THBC), while colonies on EMB agar were used to estimate total coliform counts (TCC) (Cheesbrough, 2005). Discrete colonies of distinct morphological types were sub-cultured to purity on fresh nutrient agar and stored as 10% (v/v) glycerol suspensions at –4 °C for further characterization (Amadi et al. , 2014).

Morphological, Biochemical and Molecular Characterization of Bacterial isolates

Pure isolates were characterized by Gram reaction, cell morphology, motility, and standard biochemical tests, including catalase, oxidase, citrate utilization, methyl red, Voges–Proskauer, indole and sugar (glucose, lactose, mannitol, sucrose) fermentation tests, and identified with reference to Bergey's Manual of Systematic Bacteriology. For molecular identification, genomic DNA was extracted from selected isolates by the boiling method, quantified with a Nanodrop spectrophotometer, and the 16S rRNA gene amplified using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGTTACCTTGTTACGACTT-3') (Srinivasan et al., 2015). Amplicons were resolved on 1% agarose gel electrophoresis and visualized under UV trans-illumination. Sequences obtained were compared with the NCBI non-redundant nucleotide database using

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BLASTN, aligned with MAFFT, and an evolutionary tree constructed by the Neighbor-Joining method in MEGA 6.0 with 500 bootstrap replicates (Saitou & Nei, 1987), and evolutionary distances computed by the Jukes–Cantor method (Jukes & Cantor, 1969).

Antimicrobial Susceptibility Testing

Antibiotic susceptibility was determined by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, using a bacterial suspension standardized to 0.5 McFarland turbidity. Antibiotic susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar. A bacterial suspension was standardized to 0.5 McFarland turbidity (approximately 1.5×10^8 CFU/mL) before inoculation, after which the Gram-negative antibiotic discs were applied. Ten antibiotic discs were used: streptomycin (S), cefalothrin (CFF), ofloxacin (OFX), augmentin (AU), pefloxacin (PEF), cefotaxime (CTZ), gentamicin (CN), ciprofloxacin (CPX), cefpodoxime (CFP) and trovafloxacin (TRX). Plates were incubated at 35–37 °C for 18–24 hours and zones of inhibition measured and interpreted according to Clinical and Laboratory Standards Institute breakpoints (CLSI, 2008).

The multiple antibiotic resistance (MAR) index for each isolate was calculated as the number of antibiotics to which the isolate was resistant divided by the total number of antibiotics tested.

Statistical Analysis

Data were expressed as mean ± standard deviation, and differences among mean bacterial counts were considered statistically significant at $p < 0.05$.

Results

The result of the Total heterotrophic bacterial counts (THBC) of the water samples ranged from 1.3×10^3 to 9.2×10^3 CFU/mL across the sampling period, with upstream water consistently recording the highest mean counts and downstream water the lowest (Table 1). Total coliform counts (TCC) ranged from 1.2×10^1 to 6.0×10^1 CFU/mL, with tap water from Isiala-Ngwa North and downstream water from Isiala-Ngwa South recording the highest mean counts. Both THBC and TCC exceeded recommended safe limits for potable water across all sources examined.

Table 1: Mean and standard deviation or what? Total Heterotrophic Bacterial Count and Total Coliform Count of Water Sources in Isiala-Ngwa North and South LGA, Abia State

Water source	Isiala-Ngwa North		Isiala-Ngwa South	
	THBC ($\times10^3$ CFU/mL)	TCC ($\times10^1$ CFU/mL)	THBC ($\times10^3$ CFU/mL)	TCC ($\times10^1$ CFU/mL)
Tap water	3.0 ± 1.1	3.1 ± 2.5	1.9 ± 0.9	1.4 ± 0.2
Rain water	3.4 ± 1.4	3.0 ± 0.8	3.7 ± 1.5	2.3 ± 1.4
Upstream water	5.4 ± 1.2	1.7 ± 0.4	7.6 ± 1.6	2.5 ± 0.5
Downstream water	2.2 ± 0.5	1.7 ± 0.3	1.8 ± 0.5	2.9 ± 1.7

Key: THBC= Total Heterotrophic Bacteria TCC = Total Coliform Count

Results of the morphological and biochemical characteristics of 40 bacterial isolates recovered from the domestic water samples revealed that the isolates belong to 12 genera which were; *Citrobacter, Serratia, Klebsiella, Enterobacter, Proteus, Escherichia, Pectobacterium, Erwinia, Pseudomonas, Arsenophonus, Cronobacter*, and *Yersinia*.

Table 2 presents the distribution and occurrence of the different bacteria isolated from the different domestic water sources. *Citrobacter* sp. occurred most frequently, while *Cronobacter* sp., *Pectobacterium* sp., *Pseudomonas* sp. and *Arsenophonus* sp. were each recovered only once. While the frequency of occurrence (%) of the bacteria isolated from all the domestic water sources are presented in Figure 1.

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Citrobacter sp. was the most abundant, accounting for 30.0% . *Serratia* sp. (17.5%), *Klebsiella* sp. (15.0%) and *Enterobacter* sp. (12.5%). *E. coli*, *Proteus* sp. and *Yersinia* sp. each accounted for 5.0% of isolates, while

Cronobacter sp., *Pectobacterium* sp., *Pseudomonas* sp., *Erwinia*, and *Arsenophonus* sp. each accounted for 2.5% as shown in Figure 1.

The result of the 16S rRNA gene sequencing of selected isolates for molecular identification produced amplicons of approximately 1500 bp, consistent with bacterial 16S rRNA gene size. BLASTN comparison against the NCBI database confirmed four isolates at species level, each showing 100% similarity to reference strains (Table 3).

Table 2: The Distribution of the Bacterial Isolates in the Different Domestic Water Samples

Bacteria Isolate	Domestic Water Sources								Total
	Isiala-Ngwa North				Isiala-Ngwa South				
	Tap	Rain	Up-stream	Down Stream	Tap	Rain	Up-stream	Down Stream	
Citrobacter sp.	-	1	2	1	1	1	3	2	11
Serratia sp.	1	2	1	-	-	1	1	1	7
Klebsiella sp.	-	2	-	1	1	-	2	-	6
Enterobacter sp.	-	2	1	-	1	-	-	1	5
Proteus sp.	-	-	1	-	-	-	1	-	2
Escherichia sp.	-	-	-	-	-	1	-	1	2
Yersinia sp.	-	1	1	-	-	-	-	-	2
Pectobacterium sp.	-	-	-	-	-	-	1	-	1
Erwinia sp.	-	-	1	-	-	-	-	-	1
Pseudomonas sp.	-	-	-	-	-	1	-	-	1
Arsenophonus sp.	-	-	-	-	-	-	-	1	1
Cronobacter sp.	-	-	-	-	-	1	-	-	1
Total	1	8	7	2	3	5	8	5	40

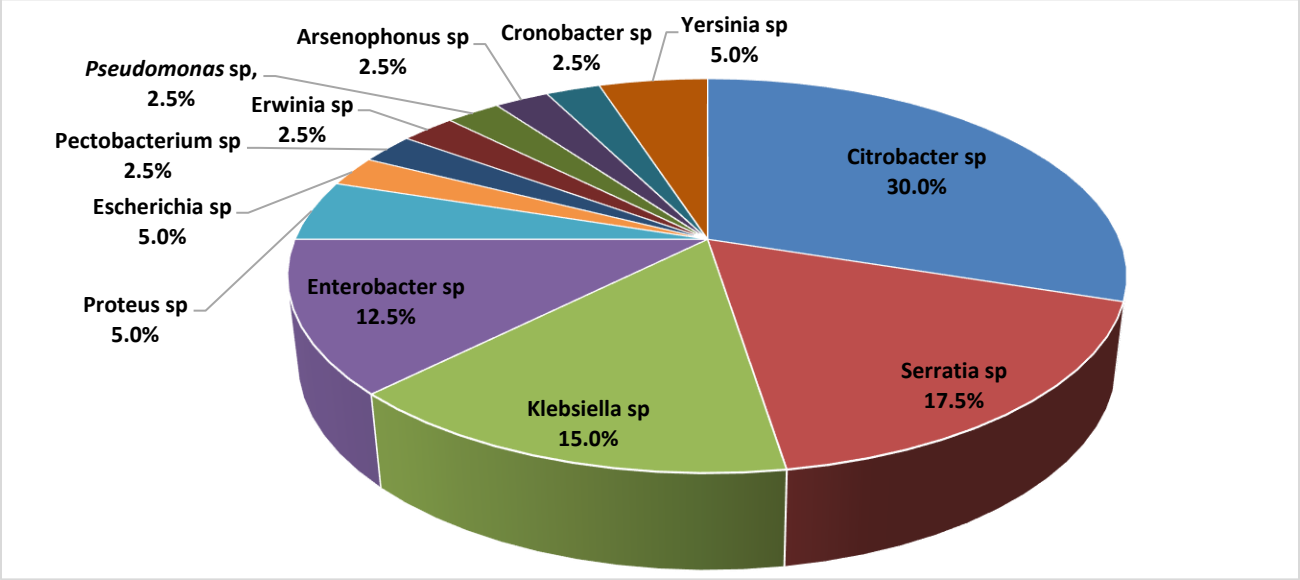

Figure 1: Percentage abundance of isolates from all the domestic water samples

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Table 3: Molecular Identification of Selected Bacterial Isolates by 16S rRNA Gene Sequencing

Isolate	Closest match (BLASTN)	Reference strain	% Similarity
Iso6	*Citrobacter freundii*	Strain B38	100%
Iso7	*Klebsiella pneumoniae*	ATCC 13883	100%
Iso10	*Proteus mirabilis*	NCTC 11938	100%
Iso13	*Enterobacter cloacae*	ATCC 13047	100%

Overall, 47.5% of the isolates produced β-hemolysis, 40.0% produced α-hemolysis and 12.5% produced γ-hemolysis (non-hemolytic) on blood agar (Table 5).

β-Hemolysis predominated among *Enterobacter* sp. (80.0%), *Proteus* sp. (100%), *Pectobacterium* sp. (100%), *Cronobacter* sp. (100%) and *Yersinia* sp. (100%). The antimicrobial susceptibility and multiple antibiotic resistance (MAR) index revealed that, all isolates (100%) were resistant to streptomycin and augmentin, while resistance to cefpodoxime, cefalothrin, pefloxacin, gentamicin and cefotaxime ranged from 82.5% to 97.5% (Table 6). Ciprofloxacin retained the highest activity, with only 2.5% resistance (97.5% susceptibility), followed by ofloxacin (47.5% resistance). No isolate showed an intermediate response to any of the ten antibiotics tested.

Table 5: Hemolytic Pattern of the Bacterial Isolates from the Water Sample

Bacterial genus	N	α-hemolysis n (%)	β-hemolysis n (%)	γ-hemolysis n (%)
Citrobacter sp.	11	4 (36.4)	6 (54.5)	1 (9.1)
Serratia sp.	7	4 (57.1)	2 (28.6)	1 (14.3)
Klebsiella sp.	6	3 (50.0)	1 (16.7)	2 (33.3)
Enterobacter sp.	5	1 (20.0)	4 (80.0)	0 (0)
Proteus sp.	2	0 (0)	2 (100)	0 (0)
Escherichia sp.	2	1 (50.0)	0 (0)	1 (50.0)
Pectobacterium sp.	1	0 (0)	1 (100)	0 (0)
Erwinia sp.	1	1 (100)	0 (0)	0 (0)
Pseudomonas sp.	1	1 (100)	0 (0)	0 (0)
Arsenophonus sp.	1	1 (100)	0 (0)	0 (0)
Cronobacter sp.	1	0 (0)	1 (100)	0 (0)
Yersinia sp.	2	0 (0)	2 (100)	0 (0)
Total (n = 40)	40	16 (40.0)	19 (47.5)	5 (12.5)

Table 6: Antibiotic Susceptibility Pattern of the Bacterial Isolates (n = 40) from Water Sources in Isiala-Ngwa North and South LGA, Abia State

Antibiotic	Resistant n (%)	Susceptible n (%)
Streptomycin (S)	40 (100)	0 (0)
Augmentin (AU)	40 (100)	0 (0)
Cefpodoxime (CFP)	39 (97.5)	1 (2.5)
Cefalothrin (CFF)	38 (95.0)	2 (5.0)
Pefloxacin (PEF)	36 (90.0)	4 (10.0)
Gentamicin (CN)	34 (85.0)	6 (15.0)
Cefotaxime (CTZ)	33 (82.5)	7 (17.5)
Trovafloxacin (TRX)	25 (62.5)	15 (37.5)
Ofloxacin (OFX)	19 (47.5)	21 (52.5)
Ciprofloxacin (CPX)	1 (2.5)	39 (97.5)

Note: All 40 isolates (100%) were resistant to more than two of the ten antimicrobials tested and were therefore classified as multidrug-resistant (MDR), with MAR index values ranging from 0.25 to 1.0.

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Discussion

The total heterotrophic and coliform bacteria counts recorded across all the water sources examined in this study consistently exceeded recommended safe limits, with upstream stream water recording the highest total heterotrophic counts, likely reflecting inputs from soil runoff, decaying plant matter, animal waste and biofilm growth (Bartram et al., 2003). Using the CDC benchmark of <500 CFU/mL for potable water, the counts recorded across tap, stream and rain water sources in this study indicate poor microbiological quality. This finding aligns with a closely related study in Eziama/Mgbaja Ossah, Abia State, which similarly reported total viable bacterial counts exceeding recommended limits across borehole, tap and river water sources, with the lowest counts in public tap water (Ndukauba et al., 2025). That tap water recorded the highest total coliform counts in the present study, despite being nominally treated, is consistent with the possibility of pipe network breaches, biofilm detachment within ageing infrastructure, and inadequate residual disinfection, which can favor the persistence of resistant coliform strains.

Twelve bacterial genera were recovered and biochemically characterized in this study, with several including *Klebsiella, Enterobacter, Citrobacter, Proteus, Serratia, Yersinia, Pseudomonas* and *Cronobacter* recognised as opportunistic pathogens of public health concern, while *Pectobacterium and Arsenophonus* are not typically considered human pathogens (Croxen et al., 2013).

Molecular identification by 16S rRNA sequencing confirmed *Citrobacter freundii, Proteus mirabilis, Klebsiella pneumoniae* and *Enterobacter cloacae* among the isolates, moving beyond presumptive biochemical identification to provide molecularly verified evidence of the specific enteric organisms present. *Citrobacter freundii,* the most abundant organism recovered in this study, is commonly found in soil, water, sewage and the intestinal tracts of humans and animals, and its presence in environmental water often correlates with fecal contamination and poor water hygiene (Anderson et al., 2018); it is also increasingly implicated in urinary tract, respiratory, wound and bloodstream infections, particularly in neonates and immunocompromised patients.

The predominance of β-hemolysis (47.5%) over α-hemolysis (40.0%) and γ-hemolysis (12.5%) among the isolates suggests that a substantial proportion of the bacterial population may possess enhanced capacity for tissue invasion and iron acquisition, both recognized virulence-associated traits, although hemolysis is generally a less discriminatory diagnostic feature in Gram-negative bacteria than in Gram-positive cocci (Forbes et al., 2016).

Antibiotic susceptibility testing revealed very high resistance among the isolates, with 100% resistance to streptomycin and augmentin, and resistance exceeding 80% for five of the remaining eight antibiotics tested; only ciprofloxacin retained appreciable activity (97.5% susceptibility). This pattern is broadly consistent with the recognized effectiveness of fluoroquinolones such as ciprofloxacin against Gram-negative organisms including *Escherichia coli, Klebsiella* and *Enterobacter* (Owens & Ambrose, 2005). The finding that all 40 isolates (100%) were multidrug-resistant, with MAR index values of 0.25–1.0, is a striking indicator of antibiotic selection pressure in the sampled environment; MAR indices above 0.2 are conventionally interpreted as indicating isolates from environments of high antibiotic usage (Afunwa et al., 2020). This 100% MDR rate exceeds several comparable reports for example, an 84.7% MDR rate among *E. coli* from household wastewater and a 96.07% rate among Enterobacter isolates from hospital-acquired infections showing MAR indices above 0.2 (Singh et al., 2025); a difference that may reflect the smaller, more localized sample source, the size of the antimicrobial panel used, or the comparatively lenient MDR definition applied (resistance to more than one antimicrobial) relative to the stricter, category-based definition of Magiorakos et al. (2012). Taken together, these findings show that treated and traditionally trusted sources such as tap water in this Study area are not exempt from harboring multidrug-resistant, and hemolytic enteric bacteria, underscoring the need for routine bacteriological and antibiotic-resistance surveillance of domestic water sources rather than reliance on treatment status alone.

Conclusion

This study revealed that, despite largely acceptable physicochemical quality reported for the same water sources, the bacterial load of tap, rain and stream water

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in Isiala-Ngwa North and South Local Government Areas, Abia State, exceeded recommended safe limits, underscoring the importance of routine bacteriological examination of water. *Citrobacter* sp. (subsequently confirmed as *Citrobacter freundii* by 16S rRNA sequencing) was the most prevalent organism across all sampled sources, alongside *Proteus mirabilis, Klebsiella pneumoniae* and *Enterobacter cloacae*. Isolates showed β-hemolytic activity, and all 40 isolates tested were multidrug-resistant, with MAR index values of 0.25–1.0, and near-total resistance to streptomycin and augmentin. Only ciprofloxacin retained substantial activity against the isolates. These findings demonstrate that physicochemical compliance alone is an insufficient indicator of water safety, and it is recommended that the water from the sources examined be properly treated (e.g., by chlorination, whose efficacy should be evaluated under the prevailing physicochemical conditions) before domestic use, and that regular bacteriological and antibiotic-resistance monitoring of both surface and tap water sources be instituted in the study area.

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