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Culture-Based Analysis, Phenotypic Virulence Screening and 16S rRNA -Based Bacterial Community Profiling of Human-Impacted Rural Streams in Akwa Ibom State, Nigeria

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Abstract

Human impacts on water sources are of concerns due to the associated public health risks. A study on two human-impacted rural streams was conducted, particularly investigating the bacterial community profile of the streams by both culture-based and 16S rRNA gene amplicon sequencing as well as the pathogenic potential of the bacterial isolates via phenotypic detection of virulence traits. The streams were Odiong stream in Abak LGA and Akpan Akpa Itoko stream in Oruk Anam LGA, both in Akwa Ibom State. The water samples were analyzed using culture-based methods and 16S rRNA gene sequencing. The phenotypic activity of the culture isolates was determined using enzyme activity assay. The mean heterotrophic bacterial counts of 5.91 ± 0.08 $\log_{10}$ cfu/mL from Odiong stream and 5.83 ± 0.01 $\log_{10}$ cfu/mL from Akpan Akpa Itoko stream were obtained followed by mean coliform counts of 2.37 ± 0.58 $\log_{10}$ MPN/100 mL and 2.67 ± 0.31 $\log_{10}$ MPN/100 mL, respectively from both streams. The bacterial counts of Odiong stream was significantly higher ($p<0.05$) than Akpan Akpa Itoko stream. The bacterial counts in both streams exceeded the guideline limits for heterotrophic plate counts in potable water. Different bacterial species were isolated from each stream except *Enterococcus* sp., *Pseudomonas* sp., *Micrococcus* sp., S*taphylococcus* sp. and *Bacillus* sp. that were found across both streams. *Proteus* sp, *Enterobacter* sp, *Pseudomonas* sp., *Actinomyces* sp., *Micrococcus* sp., *Vibrio* sp., *Streptococcus* sp. and *Bacillus* sp. across the streams tested positive for all three virulence-associated trait assessed. 16S rRNA–based profiling revealed substantial differences in both the number and composition of bacterial taxa between the two streams. The phylum Pseudomonadota (formerly Proteobacteria) was the most abundant in both streams. Odiong stream was dominated by Fluviibacter (12.85%) and the species *F. phosphoraccumulans* (18.39%), while Akpan Akpa Itoko stream was dominated by Comamonas (35.40%) and the species *Comamonas testosteroni* (19.73%). Most isolates from the culture method such as *Proteus* sp., *Escherichi coli*, *Actinomyces* sp., and *Vibrio* sp. were absent from the 30 most abundant species by the 16S rRNA gene amplicon sequencing. Both methods detected largely different sets of organisms. The presence of coliforms in this study suggests poor sanitary condition of the streams. The presence of virulent traits in these bacterial isolates suggests potential health risks. The study shows that relying solely on culture-based methods can lead to incomplete conclusions. Integrating phenotypic screening for virulence-associated traits provides a broader understanding of the microbial functional potential. Findings from this study therefore call for routine monitoring of these rural water sources and environmental sanitation measures to reduce contamination and safeguard public health.

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1.0 Introduction

Water is an essential substance for diverse human needs, functions and activities including consumption, industrial production, and generation of energy. It is essential for the maintenance and functioning of ecosystems and therefore must be guaranteed in quantity and quality to meet the needs of all living beings in their ecosystems (Jung *et al*., 2023). Surface water serves as an important source of water for many rural communities. These include streams and rivers which the residents rely on for domestic and recreational activities. They bathe, wash, fish, and cook from these water sources (Kalu, 2025). The sustainability and quality of the water resources remain of significant concerns because many bodies of water are neither adequately protected nor effectively managed by the rural users as they lack sufficient awareness of the need for responsible use and conservation of water (Simpson & de Loë, 2014). Limited awareness of the importance of maintaining the quality of water source is a serious environmental problem with reciprocal effects on the integrity of the ecosystem, food chain, and the health of humans and wildlife that depend on it (Rose, 2026; Putnik & Šojić Merkulov, 2026; Ezugwu and Osarumwense, 2022).

Poorly maintained natural aquatic systems act as repositories for pollutants and related impacts arising from anthropogenic pressures (Jesus & Tremblay, 2022; Uko *et al*., 2020; Häder *et al.,* 2020). Numerous studies show that environmental stressors bioaccumulate in the fauna and flora of such environments. Some of these organisms may harbour pathogenic microbes with potent antimicrobial resistance genes and virulence factors (Ekpo *et al.,* 2017; Udoekong *et al.,* 2025). Assessment of these unassessed natural bodies will serve critical functions that extend beyond contaminant detection to include public health surveillance, ecological status assessment, regulatory compliance, and environmental risk characterization (Sohail, 2026; Ekwere *et al.,* 2025).

Microbial water quality assessment uses biological indicators in aquatic communities to infer contamination sources, evaluate ecological integrity, and assess potential risks to human and environmental health. It relies primarily on indicator organisms, particularly faecal indicator bacteria (FIB), as surrogate measures for pathogens that are difficult to detect directly (Tan *et al*., 2015). A study of stream water in Afaha Nsit, Akwa Ibom State, Nigeria revealed a high level of faecal coliform well above the WHO recommended levels rendering. This indicated that the water was not good for human consumption without treatment (Akpan *et al*., 2024). Findings from similar study of surface water by Victor and coworkers also revealed a high degree of faecal pollution from bacteriological analyses (Victor *et al*., 2018).

Viable-but-nonculturable microbes remain undetected by the traditional method of microbial studies. This method can be described as 'selective' since it cannot capture all the cells within a sample. Advances in microbial studies have helped to eliminate or address this challenge of the 'Great Plate Count Anomaly.' Although culture-based and 16S rRNA gene sequencing can characterize bacterial community profiles, it remains unclear which organism present in human-impacted rural streams exhibits phenotypic virulence-associated traits, and consequently what their

virulence-associated traits may be. Therefore, this study aims to integrate culture-based isolation, phenotypic virulence screening, and 16S rRNA gene-based identification for two unprotected streams that are frequently used by local residents for domestic and recreational purposes. To our knowledge, no prior published studies have reported the bacteriological profiles and virulence-associated phenotypes of isolates from these water sources. The results will provide bacterial community profiles and the prevalence of phenotypic virulence-associated traits among isolates from these rural water sources.

2.0 Materials and Methods

2.1 Study location and sample collection

Two freshwater sources were studied. Location A was Odiong stream (located at Latitude 4°54'17"N and Longitude 7°46'8"E), Eriam Afaha Obong, Abak LGA and location B was Akpan Akpa Itoko stream (located at Latitude 4°48'31"N and Longitude 7°39'20"E), Obio Akpa, Oruk Anam LGA. Both are in Akwa Ibom state, Nigeria. One litre of water was collected from 3 points and 20 m apart in each location to make a composite sample using well labeled (one for each location) wide mouth bottles. The bottles were rinsed with the respective water sources 3 times to ensure any contaminant was removed. The bottles were opened and closed 3 m beneath the water surface to avoid surface scum and debris as described by Borja-Serrano *et al.* (2020). The collected samples were placed in an icepack and transported immediately to Microbiology laboratory, Akwa Ibom State University, for analyses. Water sampling was done in a space of one month (January 9th-morning, 16th-afternoon, and 23rd-evening, 2025). In all, 9 composites water samples were obtained from each location.

2.2 Culture-Based Enumeration of Bacteria

2.2.1 Total coliform bacteria counts

Standard Method 9221B (Multiple Tube Fermentation) method was used to enumerate the Most Probable Number (MPN) of coliform bacteria as described by Dimri *et al.* (2019). Three (3) sets of 5 tubes were prepared and labeled 10 ml, 1 ml, and 0.1 ml (according to the amount of water sample to be dispensed into them). The first set contained 10 ml of double strength Lactose broth (DSLB); the second set had 1.0 ml of single-strength Lactose broth (SSLB); and the last contained 0.1 ml of the broth. A Durham tube was placed in each tube in an inverted position. The tubes were incubated for 24 – 48 hours at 35°C. After incubation, visible air bubbles trapped inside the inverted Durham tubes and a color change (from red to yellow) indicated gas and acid production, respectively and a positive result for the presumptive test. The number of tubes in each set with positive results was recorded. MPN was determined by referring to the MPN table (McBride, 2003). A confirmed test was performed by transferring a loopful of culture from the positive tubes to a fermentation tube containing brilliant green lactose bile broth (BGLB). The tubes were incubated for 48 hours at 35.5°C. Gas production following incubation indicated a positive result. The number of tubes with positive results was recorded. A completed test was done by streaking colonies from all positive BGLB tubes onto eosin methylene blue agar and incubation at 37°C for 24 hrs. The plates were observed for the growth of colonies with a greenish metallic sheen which indicated *Escherichia coli* presence and a positive test.

2.2.2 Heterotrophic bacterial counts

Total heterotrophic bacteria in the water samples were enumerated through serial dilution of the samples and pour plating on nutrient agar medium. The media were supplemented with cycloheximide (100 µg/ml and benomyl (50 µg/ml) to prevent fungal growth. Inoculated plates were incubated at 28 ± 2°C for 18-24 hrs. Visible discrete colonies ranging from 30 to 300 in the plates were multiplied by the reciprocal of the dilution factor and expressed as colony forming units per milliliter (cfu/mil) of water

samples. Discrete colonies were purified by repeatedly sub-culturing unto appropriate agar media. Pure cultures were preserved on the respective agar slants and stored in the refrigerator (4°C ± 2°C) for further tests (Udotong *et al*., 2015).

2.2.3 Culture-based characterization and identification of the bacterial isolates

Pure cultures of bacteria were identified based on cultural parameters, microscopic techniques and biochemical tests according to Nwankwo *et al.* (2020) and Bulakarima *et al.* (2016). The biochemical tests were catalase, methyl red, Voges-Proskauer, indole, oxidase, urease, and sugar fermentation tests. The characterized bacterial colonies were identified according to the methods described in *Bergey's Manual of Determinative Bacteriology* (Holt *et al*., 1994).

2.3 Determination of Virulence Characteristics of the Bacterial Isolates

The bacterial ability to produce hemolysin, protease and catalase was carried out using the method described by Vaish *et al.* (2016), Cappuccino & Welsh (2018), Ekpo *et al.,* (2017) and Selvaraj *et al. (*2018).

2.4 Molecular Identification of Bacterial Communities from the Water Samples

2.4.1 DNA extraction, PCR amplification, library preparation and sequencing

Total genomic DNA was extracted from water samples using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Irvine, CA, USA; Cat. No. D4300) according to the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop spectrophotometer prior to PCR amplification. The full-length bacterial 16S rRNA gene (V1–V9 region; approximately 1,500 bp) was amplified using the universal primer pair 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). PCR amplification was performed using a high-fidelity DNA polymerase under optimized cycling conditions. Amplicons were purified, barcoded with PacBio M13 barcode adapters by limited-cycle PCR for multiplexing, quantified, pooled in equimolar concentrations, and purified using AMPure PB magnetic beads (Pacific Biosciences, Menlo Park, CA, USA). SMRTbell libraries were prepared according to the manufacturer's instructions. Sequencing primer annealing and polymerase binding were performed using the SMRT Link workflow prior to sequencing on the PacBio Revio platform. High-fidelity (HiFi) circular consensus sequence (CCS) reads were generated for downstream bioinformatics analyses.

2.4.2 Sequence processing and quality control

Raw PacBio HiFi CCS reads were processed using QIIME 2 (version 2025.10). Demultiplexing was performed using sample-specific barcode sequences, followed by primer and adapter trimming. Sequence quality control, denoising, and chimera removal were carried out using the DADA2 plugin implemented in QIIME 2 with the recommended default parameters. A total of 25,185 raw CCS reads were obtained, of which 18,552 high-quality reads were retained after quality filtering and chimera removal for downstream analyses.

2.4.3 Taxonomic classification

High-quality sequences were taxonomically classified using the QIIME 2 feature-classifier plugin against the SILVA small subunit (SSU) rRNA reference database (release 138). Taxonomic assignments were generated from phylum to genus level using a confidence threshold of 0.70. Alpha-diversity, beta-diversity, and microbial community composition analyses were performed within the QIIME 2 environment. The resulting taxonomic profiles were further processed and visualized using the Inqaba Biotec bioinformatics pipeline.

2.4.4 Statistical Analysis

Statistical analysis was performed using IBM SPSS statistics version 20.0. Data are presented as mean ± standard deviation replicate measurements. Difference among streams were evaluated using one way analysis of variance (ANOVA) followed by Tukey's significant

difference post hoc test. Differences were considered statistically significant at $p<0.05$.

3.0 Results

3.1 Heterotrophic bacterial counts from the water samples

The mean heterotrophic bacterial counts (THBC) and total coliform counts from the three sampling occasions of Odiong and Akpan Akpa Itoko streams are shown in Figures 1 and 2.

3.2 Culture-based Identification of the Bacteria Isolates

The morphological and biochemical characteristics of the bacterial isolates from the two streams are presented on Table 1. Odiong stream had 14 isolates and Akpan Akpa Itoko had 18 isolates.

3.3 Characterization of Virulence-Associated Traits in the Bacterial Isolates

The phenotypic activities of the bacterial isolates from culture method are presented on Table 2 below. Seven (7) isolates from the Odiong stream showed complete (beta) hemolysis; 5 showed partial (alpha) hemolysis; and 5 isolates showed no (gamma) hemolysis, on blood agar. From Akpan Akpa Itoko stream, 11 bacterial isolates showed complete hemolysis; 1 isolate showed partial hemolysis; and 8 isolates showed no hemolysis. Five (5) isolates from Odiong stream and 11 isolates from Akpan Akpa Itoko stream were able to produce the enzyme, protease. Odiong stream had 8 catalase-producing isolates and Akpan Akpa Itoko had 7 same isolates.

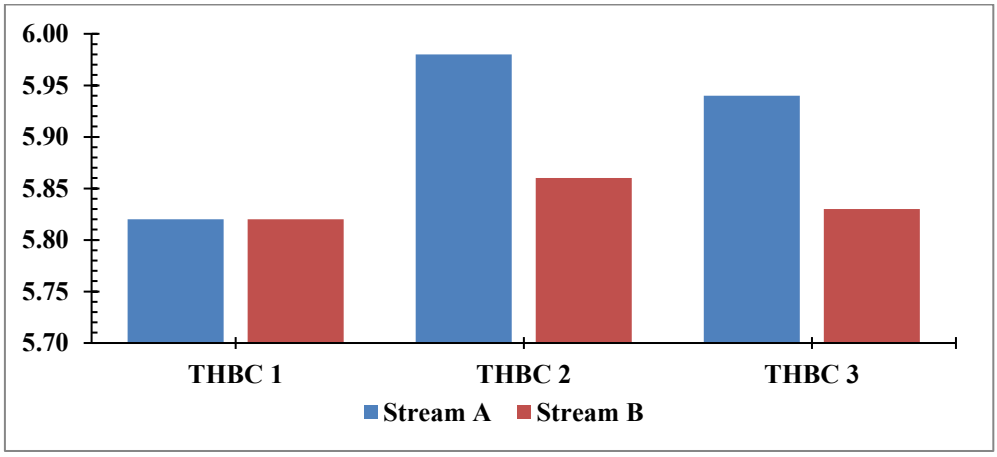

Figure 1: Total heterotrophic bacterial counts (log_{10} CFU/g) in samples A and B

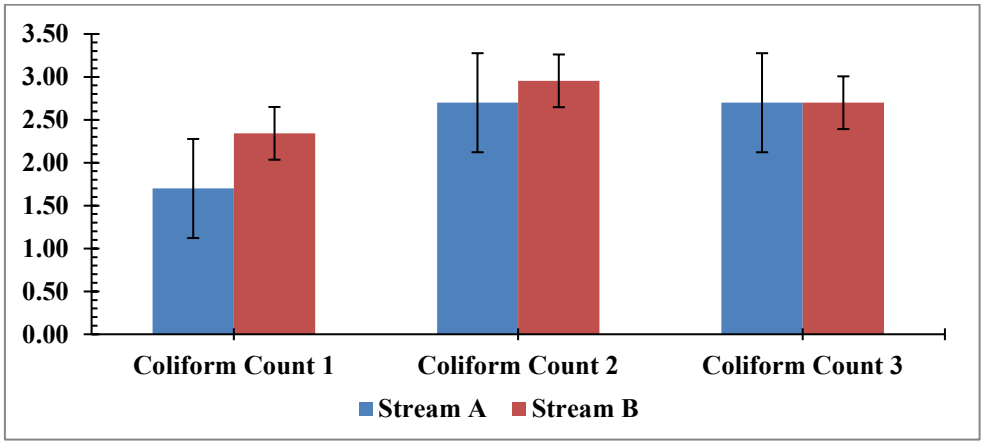

Figure 2: Total coliform counts (log_{10} CFU/g) in streams A and B

Table 1: Colony morphology and biochemical characteristics of the bacterial isolates obtained from the Odiong and Akpan Akpa Itoko streams

S/N	Isolates	Shape	Margin	Elevation	Size	Colour	Opacity	Consistency	Surface	Texture	Gram reaction	Cell type	Catalase	Methyl Red	Voges Paskauer	Indole	Oxidase	Urease	Citrate	Glocose	Lactose	Sucrose	Maltose	Probable Organisms
1	AB1	Irregular	Undulent	Raised	Medium	Cream	Opaque	Mucoid	Dull	Rough	-	Rod	+	+	-	-	+	+	+	+	-	+	-	*Proteus* sp.
2	AB2	Irregular	Rhizoid	Flat	Small	Cream	Opaque	Mucoid	Dull	Rough	+	Rod	+	+	-	-	-	-	+	+	-	+	+	*Actinomyces* sp.
3	AB3	Irregular	Undulent	Raised	Large	Cream	Opaque	Mucoid	Shiny	Rough	-	Rod	+	-	+	-	-	-	+	+	+	+	+	*Enterobacter* sp.
4	AB4	Irregular	Undulent	Raised	Large	Cream	Opaque	Mucoid	Shiny	Rough	-	Rod	+	-	+	-	-	-	+	+	+	+	+	*Enterobacter* sp.
5	AB5	Round	Entire	Raised	Small	Cream	Opaque	Mucoid	Shiny	Smooth	-	Rod	+	+	-	+	-	-	+	+	+			*Klebsiella* sp.
6	AB6	Irregular	Undulent	Raised	Large	Cream	Opaque	Mucoid	Shiny	Rough	-	Rod	+	-	+	-	-	-	+	+	+	+	+	*Enterobacter* sp.
7	AB7	Irregular	Undulent	Flat	Large	Cream	Opaque	Brittle	Dull	Rough	-	Rod	+	+	-	-	+	-	+	+	-	-	-	*Pseudomonas* sp.
8	AB8	Round	Entire	Convex	Medium	Cream	Opaque	Slimy	Shiny	Smooth	+	Cocci	+	-	-	-	+	+	+	+	-	-	-	*Micrococcus* sp.
9	AB9	Irregular	Rhizoid	Flat	Small	Cream	Opaque	Mucoid	Dull	Rough	+	Rod	+	+	-	-	-	-	+	+	-	+	+	*Actinomyces* sp.
10	AB10	Round	Entire	Raised	Medium	Cream	Opaque	Slimy	Dull	Rough	+	Cocci	+	+	-	-	-	+	+	+	-	-	-	*Staphylococcus* sp.
11	AB11	Round	Entire	Convex	Small	Cream	Opaque	Mucoid	Shiny	Smooth	-	Rod	+	+	-	+	-	+	-	+	+	-	-	*Escherichia coli*
12	AB12	Irregular	Rhizoid	Flat	Small	Cream	Opaque	Mucoid	Dull	Rough	+	Rod	+	+	-	-	-	-	+	+	-	+	+	*Actinomyces* sp.
13	AB13	Irregular	Undulent	Raised	Large	Cream	Opaque	Mucoid	Shiny	Rough	-	Rod	+	-	+	-	-	-	+	+	+	+	+	*Enterobacter* sp.
14	AB14	Round	Entire	Raised	Small	Cream	Opaque	Mucoid	Shiny	Smooth	-	Rod	+	+	-	+	-	-	+	+	+			*Klebsiella* sp.
15	BB1	Round	Entire	Convex	Medium	Cream	Opaque	Mucoid	Shiny	Smooth	+	Cocci	+	+	-	-	-	+	+	+	-	-	-	*Staphylococcus* sp.
16	BB2	Round	Entire	Convex	Large	Cream	Opaque	Brittle	Dull	Smooth	-	Rod	+	-	-	-	+	-	-	+	-	-	-	*Pseudomonas* sp.
17	BB3	Round	Entire	Raised	Medium	Cream	Opaque	Brittle	Shiny	Smooth	+	Cocci	+	+	+	-	+	-	-	+	-	-	-	*Micrococcus* sp.
18	BB4	Large	Entire	Convex	Small	Cream	Opaque	Mucoid	Dull	Smooth	-	Rod	+	+	+	-	+	-	-	+	-	+	-	*Vibrio* sp.
19	BB5	Irregular	Undulent	Raised	Medium	Cream	Opaque	Mucoid	Dull	Rough	-	Rod	+	+	-	-	+	+	+	+	-	+	-	*Proteus* sp.
20	BB6	Round	Entire	Convex	Large	Cream	Opaque	Brittle	Dull	Smooth	-	Rod	+	-	-	-	+	-	-	+	-	-	-	*Pseudomonas* sp.
21	BB7	Round	Entire	Convex	Large	Cream	Opaque	Brittle	Dull	Smooth	-	Rod	+	-	-	-	+	-	-	+	-	-	-	*Pseudomonas* sp.
22	BB8	Round	Entire	Convex	Small	Cream	Opaque	Mucoid	Shiny	Smooth	-	Rod	+	+	-	+	-	+	-	+	+	-	-	*Escherichia coli*
23	BB9	Round	Entire	Raised	Medium	Cream	Opaque	Mucoid	Shiny	Smooth	+	Cocci	-	-	-	-	-	-	+	+	+	-	-	*Streptococcus* sp.
24	BB10	Round	Entire	Raised	Medium	Cream	Opaque	Mucoid	Shiny	Smooth	-	Rod	+	+	+	-	+	+	+	+	+		+	*Enterobacter* sp.
25	BB11	Round	Entire	Raised	Small	Cream	Opaque	Mucoid	Shiny	Smooth	-	Rod	+	+	-	+	-	-	+	+	+			*Klebsiella* sp.
26	BB12	Round	Entire	Raised	Punctiform	Cream	Opaque	Brittle	Dull	Rough	+	Cocci	+	+	-	-	+	-	-	+	+	-	-	*Micrococcus* sp.
27	BB13	Irregular	Rhizoid	Flat	Small	Cream	Opaque	Mucoid	Dull	Rough	+	Rod	+	+	-	-	-	-	+	+	-	+	+	*Actinomyces* sp.
28	BB14	Round	Entire	Convex	Large	Cream	Opaque	Brittle	Dull	Smooth	-	Rod	+	-	-	-	+	-	-	+	-	-	-	*Pseudomonas* sp.
29	BB15	Round	Entire	Convex	Large	Cream	Opaque	Brittle	Dull	Smooth	-	Rod	+	-	-	-	+	-	-	+	-	-	-	*Pseudomonas* sp.
30	BB16	Round	Entire	Raised	Small	Cream	Opaque	Mucoid	Shiny	Smooth	-	Rod	+	+	-	+	-	-	+	+	+			*Klebsiella* sp.
31	BB17	Irregular	Rhizoid	Flat	Small	Cream	Opaque	Mucoid	Dull	Rough	+	Rod	+	+	-	-	-	-	+	+	-	+	+	*Actinomyces* sp.
32	BB18	Round	Entire	Raised	Small	Cream	Opaque	Mucoid	Shiny	Smooth	-	Rod	+	+	-	+	-	-	+	+	+			*Klebsiella* sp.
33	BB19	Irregular	Undulent	Raised	Medium	Cream	Opaque	Brittle	Dull	Smooth	+	Rod	+	-	-	-	-	+	-	+	+	-	-	*Bacillus* sp.
34	BB20	Irregular	Undulent	Raised	Medium	Cream	Opaque	Brittle	Dull	Smooth	+	Rod	+	-	-	-	-	+	-	+	+	-	-	*Bacillus* sp.

+ = positive; - = negative; AB = Odiong stream; BB = Akpan Akpa Itoko stream

Table 2: Virulence characteristics of probable bacterial isolates from Odiong and Akpan Akpa Itoko streams

S/N	Isolates	Hemolysin	Protease	Catalase	Probable Organism
1	AB1	Beta	+	+	*Proteus* sp.
2	AB2	Beta	+	-	*Actinomyces* sp.
3	AB3	Alpha	+	+	*Enterobacter* sp.
4	AB4	Gamma	-	-	*Enterobacter* sp.
5	AB5	Beta	-	+	*Klebsiella* sp.
6	AB6	Alpha	-	-	*Enterobacter* sp.
7	AB7	Beta	+	+	*Pseudomonas* sp.
8	AB8	Alpha	-	+	*Micrococcus* sp.
9	AB9	Beta	-	-	*Actinomyces* sp.
10	AB10	Beta	-	+	*Staphylococcus* sp.
11	AB11	Gamma	-	+	*Escherichia coli*
12	AB12	Gamma	+	+	*Actinomyces* sp.
13	AB13	Gamma	-	-	*Enterobacter* sp.
14	AB14	Alpha	-	-	*Klebsiella* sp.
15	AB15	Alpha	-	-	*Enterobacter* sp.
16	AB16	Beta	-	-	*Klebsiella* sp.
17	AB17	Gamma	-	-	*Klebsiella* sp.
18	BB1	Gamma	-	+	*Staphylococcus* sp.
19	BB2	Beta	+	+	*Pseudomonas* sp.
20	BB3	Beta	+	+	*Micrococcus* sp.
21	BB4	Beta	+	+	*Vibrio* sp.
22	BB5	Beta	+	-	*Proteus* sp.
23	BB6	Beta	+	-	*Pseudomonas* sp.
24	BB7	Gamma	-	-	*Pseudomonas* sp.
25	BB8	Gamma	-	-	*Escherichia coli*
26	BB9	Gamma	+	+	*Streptococcus* sp.
27	BB10	Beta	-	+	*Enterobacter* sp.
28	BB11	Gamma	-	+	*Klebsiella* sp.
29	BB12	Beta	+	-	*Micrococcus* sp.
30	BB13	Gamma	-	-	*Actinomyces* sp.
31	BB14	Beta	+	-	*Pseudomonas* sp.
32	BB15	Alpha	-	-	*Pseudomonas* sp.
33	BB16	Gamma	-	-	*Klebsiella* sp.
34	BB17	Beta	+	-	*Actinomyces* sp.
35	BB18	Gamma	-	-	*Klebsiella* sp.
36	BB19	Beta	+	-	*Bacillus* sp.
37	BB20	Beta	+	+	*Bacillus* sp.

+ = positive; - = negative; AB = Odiong stream; BB = Akpan Akpa Itoko stream

3.4 Molecular identification of bacterial isolates from both water samples

3.4.1 16S rRNA gene - based identification of the bacterial community

The number of reads obtained from the Odiong stream sample showed similarities to 10 phyla (Figure 3). Phylum with the highest reads was Pseudomonadota (87%). Fluviibacter (12.85%) and *Fluviibacter phosporaccumulans* (12.85%) dominated among members of genera and species that were detected (Table 3 & 4). Pseudomonadota (97%) also showed dominance in Akpan Akpa Itoko stream (Figure 4) with Comamonas (35.40%) and *Comamonas testosteroni* (19.3%) leading in abundance (Tables 5 & 6).

4.0 Discussion

4.1 Bacterial Loads of the Water

Odiong and Akpan Akpa Itoko streams exhibited high heterotrophic bacterial loads. The mean total heterotrophic bacterial (THB) counts were 5.91 ± 0.08 $\log_{10}$ CFU/mL for Odiong stream and 5.83 ± 0.01 $\log_{10}$ cfu/mL for Akpan Akpa Itoko stream (Figure 1).

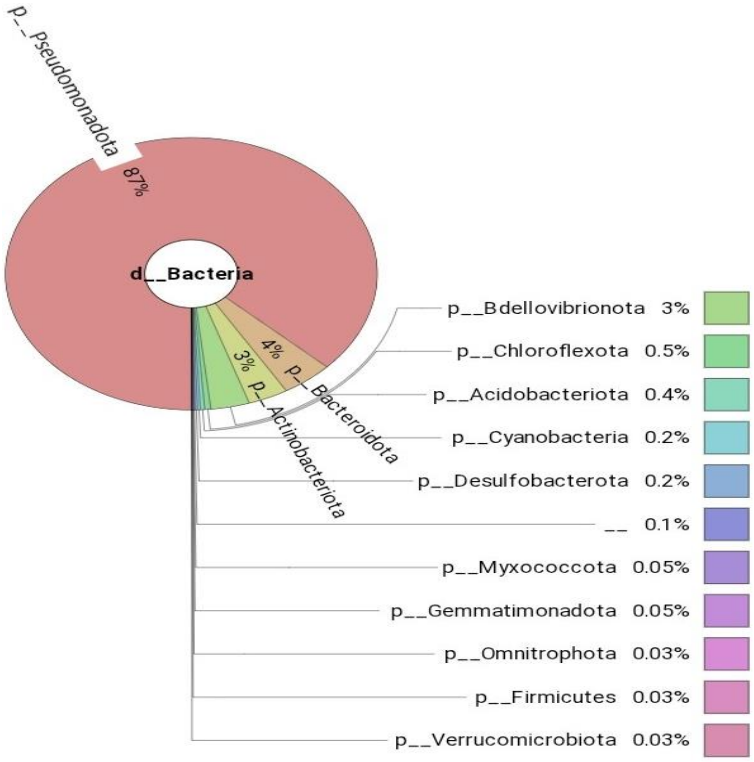

Figure 3: Relative abundance of identified bacterial phyla in Odiong stream

Table 3: Relative abundance of the top 30 identified bacterial genera from Odiong stream by 16S rRNA amplicon sequencing

S/N	Bacterial Genus	Number of Reads	Percentage Abundance (%)
1	Fluviibacter	1081	12.85
2	Curvibacter	910	10.82
3	Polynucleobacter	767	9.12
4	Cupriavidus	475	5.65
5	Novosphingobium	327	3.89
6	Flavobacterium	241	2.86
7	Parvibaculum	202	2.40
8	Bacteriovorax	198	2.35
9	Aquabacterium	188	2.23
10	Enterobacter	152	1.81
11	Rhodoferax	149	1.77
12	Comamonas	110	1.31
13	Nevskia	84	1.00
14	Limnohabitans	81	0.96
15	Methylocystis	70	0.83
16	Ideonella	47	0.56
17	Limnohabitans_A	41	0.49
18	Herbaspirillum	26	0.31
19	Geothrix	22	0.26
20	Stenotrophobium	22	0.26
21	Achromobacter	19	0.23
22	Hydrogenophaga	18	0.21
23	Hylemonella	17	0.20
24	Rhodopseudomonas	16	0.19
25	Reyranella	16	0.19

26	Burkholderia	16	0.19
27	Microbacterium	15	0.18
28	Aquitalea	14	0.17
29	Acinetobacter	13	0.15
30	Ralstonia	12	0.14
	Total	**5349**	

Table 4: Relative abundance of the top 30 identified bacterial species from Odiong stream by 16S rRNA amplicon sequencing

S/N	Bacterial Species	Number of Reads	Percentage Abundance (%)
1	*Fluviibacter phosphoraccumulans*	1081	12.85
2	*Curvibacter gracilis*	910	10.82
3	*Polynucleobacter* sp 018687515	518	6.16
4	*Cupriavidus* sp 018729255	350	4.16
5	*Novosphingobium* sp 001421325	306	3.64
6	*Flavobacterium* sp 002256385	241	2.86
7	*Bacteriovorax stolpii*	198	2.35
8	*Parvibaculum sediment*	196	2.33
9	*Aquabacterium* sp000768065	170	2.02
10	*Cupriavidus metallidurans*	118	1.40
11	*Polynucleobacter* sp 018687095	113	1.34
12	*Enterobacter roggenkampii*	93	1.11
13	*Polynucleobacter acidiphilus*	87	1.03
14	*Nevskia* sp 013823855	84	1.00
15	*Comamonas testosteroni_F*	74	0.88
16	*Methylocystis parvus*	63	0.75
17	*Limnohabitans curvus*	49	0.58
18	*Rhodoferax sp003415675*	43	0.51
19	*Polynucleobacter cosmopolitanus*	43	0.51
20	*Rhodoferax* sp002163715	42	0.50
21	*Rhodoferax* sp002943465	40	0.48
22	*Limnohabitans* sp003063545	32	0.38
23	*Ideonella* sp000333615	30	0.36
24	*Limnohabitans*_A sp003063335	28	0.33
25	*Enterobacter kobei*	26	0.31
26	*Enterobacter chengduensis*	23	0.27
27	*Stenotrophobium rhamnosiphilum*	22	0.26
28	*Achromobacter aegrificiens*	19	0.23
29	*Comamonas testosterone*	19	0.23
30	*Hydrogenophaga* sp014773545	18	0.21
	Total	**5036**	

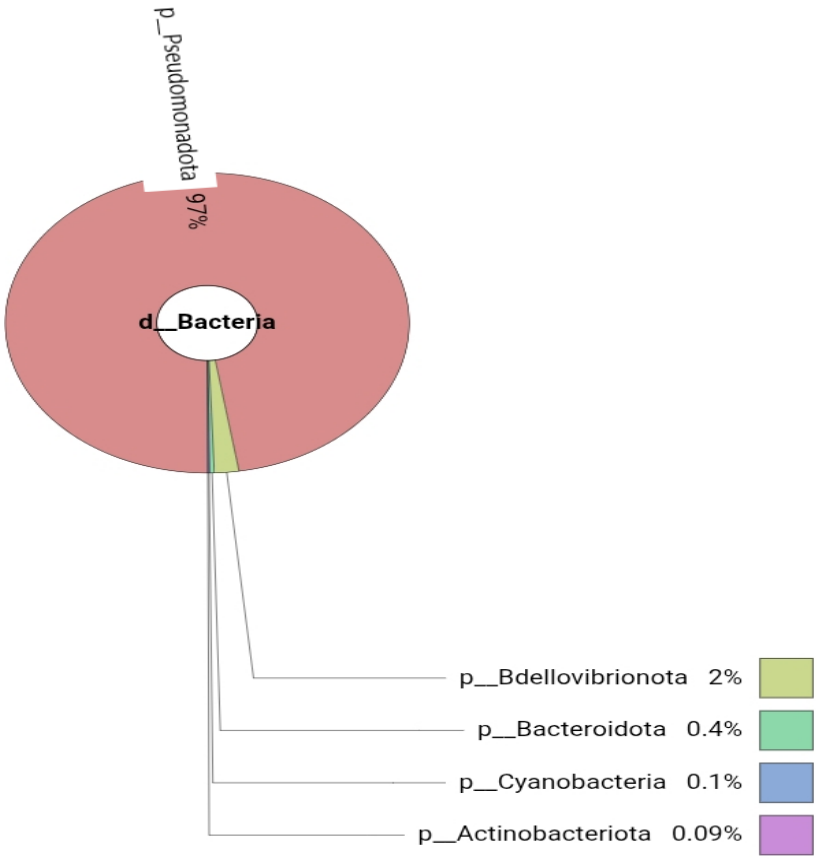

Figure 4: Relative abundance of identified bacterial phyla in Akpan Akpa Itoko stream

Table 5: Relative abundance of top 30 identified bacterial genera in Akpan Akpa Itoko stream by 16S rRNA amplicon sequencing

S/N	Bacterial Genus	Number of Reads	Percentage Abundance (%)
1	Comamonas	3589	35.40
2	Enterobacter	1628	16.06
3	Aquabacterium	1302	12.84
4	Limnohabitans	617	6.09
5	Curvibacter	531	5.24
6	Cupriavidus	321	3.17
7	Duganella	311	3.07
8	Hylemonella	279	2.75
9	Acinetobacter	265	2.61
10	Bdellovibrio	197	1.94
11	Pseudomonas_E	122	1.20
12	Pseudoduganella	95	0.94
13	Rhodoferax	89	0.88
14	Aquitalea	88	0.87
15	Burkholderia	54	0.53
16	Novosphingobium	49	0.48
17	Polynucleobacter	44	0.43
18	Ideonella	26	0.26
19	Aquirhabdus	25	0.25
20	Pelomonas	19	0.19
21	Herbaspirillum	18	0.18
22	Stenotrophomonas	17	0.17
23	Flavobacterium	17	0.17

24	Ralstonia	8	0.08
25	Sediminibacterium	8	0.08
26	Nitrospirillum	7	0.07
27	Hydrogenophaga	7	0.07
28	Sphingomonas	6	0.06
29	Microcystis	6	0.06
30	Chromobacterium	6	0.06
	Total	**9751**	

Table 6: Relative abundance of top 30 identified bacterial species in Akpan Akpa Itoko stream by 16S rRNA amplicon sequencing

S/N	Bacterial Species	Number of Reads	Percentage Abundance (%)
1	*Comamonas testosterone*	2000	19.73
2	*Aquabacterium* sp 005502875	1161	11.45
3	*Comamonas testosteroni*_F	1147	11.31
4	*Enterobacter roggenkampii*	1040	10.26
5	*Limnohabitans curvus*	615	6.07
6	*Curvibacter gracilis*	531	5.24
7	*Comamonas aquatica*	326	3.22
8	*Cupriavidus* sp 018729255	321	3.17
9	*Duganella danionis*	298	2.94
10	*Enterobacter chengduensis*	271	2.67
11	*Hylemonella delicate*	271	2.67
12	*Bdellovibrio* sp013752535	197	1.94
13	*Acinetobacter seifertii*	177	1.75
14	*Undibacterium* sp018139685	163	1.61
15	*Pseudomonas*_E *hunanensis*	103	1.01
16	*Aquabacterium* sp000768065	102	1.01
17	*Enterobacter kobei*	94	0.93
18	*Comamonas* sp019186805	87	0.86
19	*Enterobacter asburiae*_B	87	0.86
20	*Enterobacter* sp000568095	59	0.58
21	*Aquitalea magnusonii*	58	0.57
22	*Pseudoduganella* sp 900113115	54	0.53
23	*Rhodoferax bucti*	54	0.53
24	*Enterobacter* sp. N18-03635	47	0.46
25	*Pseudoduganella* sp 009674485	41	0.41
26	*Acinetobacter brisouii*	32	0.32
27	*Acinetobacter bereziniae*	30	0.30
28	*Aquitalea pelogenes*	30	0.30
29	*Aquabacterium fontiphilum*	30	0.30
30	*Enterobacter cloacae*_M	30	0.30
	Total	**9456**	

The counts in Odiong stream were significantly higher ($p<0.05$) than that of Akpan Akpa Itoko. The heterotrophic plate counts for both samples were substantially higher than the recommended guideline value for potable water (generally <500 cfu/mL), suggesting that neither stream is suitable for direct human consumption without treatment (WHO, 2022). Similar findings have been reported by Edokpayi *et al*. (2018), who observed elevated heterotrophic bacterial counts in rural water sources

due to anthropogenic activities, emphasizing that such waters require treatment before domestic use. According to Molale-Tom *et al.* (2024), elevated heterotrophic bacterial counts generally indicate high organic matter availability, favourable environmental conditions for bacterial growth, and possible contamination from human, animal, or agricultural activities. A high heterotrophic bacterial count is indicative of deteriorating water quality, and high probability of the presence and persistence of opportunistic pathogens. The higher THB counts observed in Odiong stream than Akpan Akpa Itoko stream may indicate higher organic matter composition and nutrient enrichments. This observation agrees with studies conducted on rural freshwater systems in Nigeria and other developing countries, where increased bacterial populations were associated with runoff, domestic waste discharge, washing activities, and livestock access to streams (Edokpayi *et al*., 2018; Okon *et al*., 2022). However, the mean THB counts obtained in this study are lower than those reported by some Nigerian studies on heavily polluted urban rivers, where bacterial counts frequently exceeded 10^6–10^7 cfu/mL due to intense industrial and municipal wastewater discharge (Idu *et al*., 2026). Total coliform counts for Odiong stream (2.37 ± 0.58 $\log_{10}$ MPN/100 mL) and Akpan Akpa Itoko stream (2.67 ± 0.31 $\log_{10}$ MPN/100 mL) varied across sampling occasions (Figure 2). This detection in these streams agrees with reports by Odonkor & Ampofo (2013) that certain surface water samples contain detectable coliform bacteria and is a reflection of poor sanitary quality. Similarly, Edokpayi *et al.* (2017) reported total coliform contamination in rural South African water sources used by surrounding communities. Total coliforms are widely accepted as indicators of contamination and the possible presence of pathogenic microorganisms capable of causing waterborne diseases such as diarrhoea, typhoid fever, and gastroenteritis. According to the World Health Organization, drinking water should contain no detectable coliforms or *Escherichia coli* in 100 mL of water intended for human consumption. Therefore, the coliform levels recorded in this study indicate that both streams do not meet international microbiological standards for drinking water (Tambi *et al.,* 2023; Oon *et al.,* 2023).

4.2 Culture-based Characterization of the Bacterial Isolates

Phenotypic characterization of the bacterial isolates using colony morphology on agar, Gram staining, and biochemical tests (such as urease, citrate, Voges–Proskauer, methyl red, oxidase, and sugar fermentation) revealed substantial phenotypic heterogeneity among isolates (Table 1).

The Odiong stream was dominated by Enterobacter sp., while the Akpan Akpa Itoko stream was dominated by Pseudomonas sp. species of Enterobacter, Pseudomonas, Micrococcus, Staphylococcus, Actinomyces, and Bacillus were detected across both streams. In the Odiong stream, additional species from Proteus, Klebsiella, and Escherichia were also detected, whereas in the Akpan Akpa Itoko stream, Vibrio and Streptococcus were also detected. These differences may reflect variations in surrounding land use, nutrient availability, hydrological conditions, and sources of contamination (Sadeghi *et al*., 2026; Zeglin, 2015). The presence of these bacteria reflects a complex microbial ecology of the freshwater ecosystem and suggests the exposure of these streams to multiple contamination sources according to Staley *et al*. (2013). The detection of indicator organisms in this study implies fecal contamination, potential public-health risk and unsuitability of the water for direct consumption without adequate treatment (Dudle *et al*., 2014).

Species of Enterobacter and Klebsiella showed higher occurrence in Odiong stream. Similar observation has been reported in surface waters across Nigeria and other developing countries, where these organisms have been associated with domestic wastewater discharge, agricultural runoff, and poor sanitation (Ashbolt, 2015; Edokpayi *et al*., 2018).

Table 7: Bacterial diversity as revealed by the culture-based characterization and 16S rRNA amplicon sequencing analysis of water samples from the Odiong and Akpan Akpa Itoko streams

Method	**16S rRNA amplicon sequencing**				**Culture Analysis**	
Stream	**Odiong**		**Akpan Akpa Itoko**		**Odiong**	**Akpan Akpa Itoko**
Taxon	**Genera**	**Species**	**Genera**	**Species**	**Genera**	**Genera**
1	Fluviibacter	*Fluviibacter phosphoraccumulans*	Comamonas	*Comamonas testosterone*	*Proteus*	*Staphylococcus*
2	Curvibacter	*Curvibacter gracilis*	Enterobacter	*Aquabacterium* sp 005502875	*Enterobacter*	*Enterobacter*
3	Polynucleobacter	*Polynucleobacter* sp 018687515	Aquabacterium	*Comamonas testosteroni*_F	*Klebsiella*	*Pseudomonas*
4	Cupriavidus	*Cupriavidus* sp 018729255	Limnohabitans	*Enterobacter roggenkampii*	*Pseudomonas*	*Vibrio*
5	Novosphingobium	*Novosphingobium* sp 001421325	Curvibacter	*Limnohabitans curvus*	*Micrococcus*	*Streptococcus*
6	Flavobacterium	*Flavobacterium* sp 002256385	Cupriavidus	*Curvibacter gracilis*	*Staphylococcus.*	*Micrococcus*
7	Parvibaculum	*Bacteriovorax stolpii*	Duganella	*Comamonas aquatica*	*Escherichia*	*Bacillus*
8	Bacteriovorax	*Parvibaculum sediment*	Hylemonella	*Cupriavidus* sp 018729255	*Bacillus*	*Actinomyces*
9	Aquabacterium	*Aquabacterium* sp000768065	Acinetobacter	*Duganella danionis*	*Actinomyces*	*Streptococcus*
10	Enterobacter	*Cupriavidus metallidurans*	Bdellovibrio	*Enterobacter chengduensis*		
11	Rhodoferax	*Polynucleobacter* sp 018687095	Pseudomonas_E	*Hylemonella delicate*	Species	Species
12	Comamonas	*Enterobacter roggenkampii*	Pseudoduganella	*Bdellovibrio* sp013752535	*Proteus* sp.	*Staphylococcus* sp.
13	Nevskia	*Polynucleobacter acidiphilus*	Rhodoferax	*Acinetobacter seifertii*	*Enterobacter* sp.	*Pseudomonas* sp.
14	Limnohabitans	*Nevskia* sp 013823855	Aquitalea	*Undibacterium* sp018139685	*Klebsiella* sp.	*Vibrio* sp.
15	Methylocystis	*Comamonas testosteroni_F*	Burkholderia	*Pseudomonas*_E *hunanensis*	*Pseudomonas* sp.	*Streptococcus* sp.
16	Ideonella	*Methylocystis parvus*	Novosphingobium	*Aquabacterium* sp000768065	*Micrococcus* sp.	*Enterobacter* sp.
17	Limnohabitans_A	*Limnohabitans curvus*	Polynucleobacter	*Enterobacter kobei*	*Staphylococcus* sp.	*Micrococcus* sp.
18	Herbaspirillum	*Rhodoferax sp003415675*	Ideonella	*Comamonas* sp019186805	*Escherichia coli*	*Bacillus* sp.
19	Geothrix	*Polynucleobacter cosmopolitanus*	Aquirhabdus	*Enterobacter asburiae*_B	*Bacillus* sp.	*Actinomyces* sp
20	Stenotrophobium	*Rhodoferax* sp002163715	Pelomonas	*Enterobacter* sp000568095	*Actinomyces* sp	*Streptococcus* sp
21	Achromobacter	*Rhodoferax* sp002943465	Herbaspirillum	*Aquitalea magnusonii*		
22	Hydrogenophaga	*Limnohabitans* sp003063545	Stenotrophomonas	*Pseudoduganella* sp 900113115		
23	Hylemonella	*Ideonella* sp000333615	Flavobacterium	*Rhodoferax bucti*		
24	Rhodopseudomonas	*Limnohabitans*_A sp003063335	Ralstonia	*Enterobacter* sp. N18-03635		
25	Reyranella	*Enterobacter kobei*	Sediminibacterium	*Pseudoduganella* sp 009674485		
26	Burkholderia	*Enterobacter chengduensis*	Nitrospirillum	*Acinetobacter brisouii*		
27	Microbacterium	*Stenotrophobium rhamnosiphilum*	Hydrogenophaga	*Acinetobacter bereziniae*		
28	Aquitalea	*Achromobacter aegrificiens*	Sphingomonas	*Aquitalea pelogenes*		
29	Acinetobacter	*Comamonas testosterone*	Microcystis	*Aquabacterium fontiphilum*		
30	Ralstonia	*Hydrogenophaga* sp014773545	Chromobacterium	*Enterobacter cloacae*_M		

These bacteria are common members of the family Enterobacteriaceae and are widely distributed in water, soil, vegetation, and the intestinal tracts of humans and animals. Their abundance suggests contamination from runoffs in the environment, particularly faecal inputs. Their presence is of public health concern because several species of these genera are opportunistic pathogens capable of causing urinary tract infections, septicemia and respiratory infections, particularly in immunocompromised individuals (Okere *et al.,* 2021).

Escherichia coli are considered key microbiological indicators of recent faecal contamination from human or animal waste (Zarić *et al*., 2023). *E. coli* is a Gram-negative, rod-shaped, facultative anaerobe, coliform bacteria from the genus Escherichia. Generally, they live as commensals in the lower intestine of warm-blooded animals (Biswas *et al*., 2025) and are involved in the synthesis of many important products within the human system such as Vitamin-K2, which helps in blood clotting (Abby *et al*., 2020). However, impact of *E. coli* strains on human health and the society by their involvement in disease burden and antibiotic resistance has been reported. They are the predominant causative agent of Urinary tract infections (UTIs) among others (Biswas *et al*., 2025). Virulent strains of *E. coli* are reportedly responsible for most diarrheal infections, meningitis, and septicemia (Makvana and Krilov, 2015). The presence *E. coli* in this study indicates public health risks and reinforces concerns regarding the microbiological safety of both streams according to Tambi *et al*. (2023) and Oon *et al*. (2023).

Several species of Pseudomonas were isolated from Akpan Akpa Itoko stream. *Pseudomonas* spp. are common freshwater bacteria with remarkable metabolic versatility. They thrive in nutrient-rich aquatic environments. Similar findings have been reported by Vaz-Moreira *et al*. (2017). These authors observed widespread distribution of *Pseudomonas* species in untreated surface waters. Their presence is often associated with organic pollution and ability to form biofilm under stressful environmental conditions as adaptation strategy. Some species, particularly *Pseudomonas aeruginosa*, are recognized as opportunistic pathogens capable of causing infections of the skin, urinary tract, and respiratory tract (WHO, 2022).

The recovery of *Bacillus* sp. from both streams agrees with numerous studies adjudging Bacillus as common inhabitants of freshwater ecosystems. Their endospore-forming ability enables them to survive adverse environmental conditions, making them frequently isolated from water, sediments and soils. Although many *Bacillus* species are harmless environmental organisms, some of them like *Bacillus cereus*, have been implicated in food borne illness and opportunistic infections (Logan & De Vos, 2015).

The occurrence of *Proteus* sp., *Staphylococcus* sp. and *Streptococcus* sp. further indicates environmental and possible anthropogenic contamination. *Proteus* species are commonly associated with sewage contamination and decomposing organic matter, while the presence of *Staphylococcus* and *Streptococcus* species may indicate contamination arising from human or animal activities around the streams. Similar findings were reported by Douglas *et al*. (2022), who isolated *Proteus, Staphylococcus, Pseudomonas, Escherichia coli*, and other potentially pathogenic bacteria from streams used for domestic purposes in southern Nigeria, suggesting significant environmental contamination. Also, Onyemaechi & Ejikeme (2019) detected species of Proteus, Staphylococcus, and Streptococcus in freshwater streams and attributed their occurrence to anthropogenic activities and inadequate sanitation around the water sources.

4.3 Virulent Characters by the Bacterial Isolates

The virulence-associated phenotypes of the culture isolates were assessed by screening for the red-blood cell lysing toxin, hemolysin as well as for extracellular enzymes (protease and catalase) activities (Table 2). Findings indicated that the isolates possess the assessed phenotypes. While some of the isolates

tested positive to one (e.g. *Actinomyces* sp.) or two (e.g. *Streptococcus* sp.) of the phenotypes, *Proteus* sp., *Enterobacter* sp., *Pseudomonas* sp., *Micrococcus* sp., *Vibrio* sp., and *Bacillus* sp. across the streams tested positive for the three (hemolysin production, protease activity, and catalase activity) phenotypes indicating the possession of virulence associated traits that may contribute to host damage under conducive conditions (Umana *et al*., 2017) if the water is consumed. A high hemolytic activity was observed among the isolates. This finding is highly consistent with Uguomore *et al.* (2025), who reported hemolytic activity among bacterial isolates recovered from domestic water sources in Choba, Nigeria. Ramessar & Olaniran (2025) also demonstrated that freshwater isolates harbor hemolysin genes as survival mechanisms that also serve as opportunistic virulence factors. Protease producers like species of Pseudomonas and Bacillus can degrade host tissues and evade immune responses (Galdino *et al.,* 2017). Sundar & Natarajan (2023) highlighted that proteases enable environmental pathogens to colonize human skin and mucosal surfaces upon contact. The detection of these exoenzymes in in this study aligns with reports by Tahoun & Hamza (2022).

4.4 Molecular Identification of the Bacterial Diversity in the Water

The 16S rRNA amplicon sequencing revealed that Pseudomonadota accounted for 87% of the bacterial community in Odiong stream (Fig. 3) and 97% in Akpan Akpa Itoko stream (Fig. 4). This dominance agrees with numerous metagenomic studies of freshwater with consistent report that Pseudomonadota is one of the most abundant bacterial phyla in this ecosystem because of its remarkable metabolic versatility and ability to colonize diverse aquatic environments (Wang *et al.,* 2021). Although both streams were dominated by Pseudomonadota, differences were observed in the other taxa compositions. For the Odiong stream, the top 30 genera and species accounted for 5,349 and 5,036 sequence reads, respectively (Table 3 & 4). In the Akpan Akpa Itoko stream, the 30 genera and species had 5,349 and 5,036, reads, respectively (Table 5 & 6). As observed, Akpan Akpa Itoko showed higher relative abundance. This abundance reflects a greater environmental heterogeneity, increased nutrient inputs, or wider ecological niches within the stream. Environmental factors such as vegetation cover, organic matter availability, physicochemical characteristics, and surrounding human activities can influence stream bacterial richness/diversity according to Jones *et al.* (2020 and Hamdan *et al*. (2024).

Similar organisms were detected across the two streams within the top 30 taxa presented (Table 3 & 5) namely Enterobacter, Curvibacter, Cupriavidus, Limnohabitans, Polynucleobacter, and Flavobacterium. This was also observed among top species e.g. *Enterobacter roggenkampii*, *Comamonas testosteroni* (F), *Limnohabitans curvus*, *Cupriavidus* sp. 018729255, *Aquabacterium* sp. 000768065, and *Enterobacter kobei*. The genera Curvibacter, Polynucleobacter, Aquabacterium, Limnohabitans, Cupriavidus, and Novosphingobium are commonly reported in freshwater metagenomic studies. Many members of these genera contribute to carbon mineralization, nitrogen transformation, and the degradation of dissolved organic matter, indicating that the bacterial communities identified are involved in the maintenance of ecosystem productivity (Reinl *et al.,* 2022; Wang *et al.,* 2022). However, the detection of *Enterobacter roggenkampii* and *Enterobacter chengduensis* in Akpan Akpa Itoko stream also suggests the presence of bacteria with opportunistic pathogenic potential, supporting the findings obtained through culture-based isolation (Cirkovic & Brkic, 2025). *Fluviibacter phosphor-accumulans* was the most abundant species in Odiong stream, while *Comamonas testosteroni* dominated in Akpan Akpa Itoko stream. *Fluviibacter phosphoraccumulans* is commonly associated with freshwater habitats and is recognized for its ability to accumulate phosphorus, suggesting active phosphorus cycling within Odiong

Stream. Likewise, *Comamonas testosteroni* possesses broad metabolic capabilities, including the degradation of diverse organic compounds, and its dominance may indicate adaptation to organic inputs from human activities in the environments (Wu *et al.,* 2015).

As observed in other studies of the freshwater, bacterial presence is recorded in high counts and diverse community compositon (Ulrichet al., 2016; LeChevallier & McFeters, 1985) including the sediments as recorded by Uko *et al*. (2020). Similarly, the bacterial populations in this study were observably high in both streams.

4.5 Culture-Based Vs. 16S rRNA-Based Bacterial Characterization of Both Streams

The standard microbiological procedures and 16S rRNA amplicon sequencing showed diverse bacterial community profiles (Table 7) of the two streams, Odiong and Akpan Akpa Itoko. A substantial discordance occurred in the composition of organisms detected by the two methods across the streams. The disparity is largely attributable to the inherent differences in each method. Bacterial profile of the streams by the 16S rRNA amplicon sequencing revealed a taxonomic composition of phylum, genera and species. The identity of the organisms was obtained from their sequence homology to the already identified sequences in the gene database. The genera Enterobacter Curvibacter, Cupriavidus, Limnohabitans, Polynucleobacter, and Flavobacterium were recovered by both methods. Different Species of Enterobacter (e.g. *Enterobacter chengduensis*, *Enterobacter chengduensis*, and *Enterobacter asburiae*_B) were observed among the 16S rRNA amplicon sequencing results against just *Enterobacter* sp. in the culture method. In the culture-based analysis, a reference text, *Bergey's Manual of Determinative Bacteriology* (Holt *et al*., 1994) was use to confirm the identity of the isolates (at species level) after a step–by-step cultivation process that ranged from sample collection and inoculation to isolation, microscopy and biochemical characterization/enzymatic assays. Culture-base method therefore presents isolate-based composition of bacteria that are described as probable (source from the phenotypic identification) organisms. By this description the isolates' identification is presumptive and may require confirmatory testing. Culture-base bacterial composition from both streams as shown on Table 7 revealed species of Proteus, Enterobacter, Klebsiella, Pseudomonas, Escherichia, Micrococcus, Vibrio, Bacillus, streptococcus, etc. as the bacterial members of the streams. From these, only Enterobacter and Pseudomonas are found among the top 30 genera from the 16S rRNA amplicon sequencing, an indication that only a subset of microbial composition is detected by culture-base method.

Most microbial cells in samples are not recovered as isolates by culturing. This gives rise to the "great plate count anomaly- GPCA (Junkins and Stevenson, 2021). Culture method captures only a few viable cells (Sansupa *et al.,* 2021) that can grow under the given laboratory conditions, thereby missing other important members of the streams. 16S rRNA gene amplicon sequencing is DNA-based and therefore detects 16S rRNA gene fragments from both viable and non-viable bacterial cells in a sample (Zheng *et al*. (2024), the reason for the high breadth of taxa observed by the method in this study. Among the 30 most abundant species from the 16S rRNA gene amplicon sequencing, *Proteus* sp, *Micrococcus* sp., *Staphylococcus* sp. *Escherichi coli*, *Actinomyces* sp., *Vibrio* sp., *Streptococcus* sp. and *Bacillus* sp. which were recovered by the culture method were not detected. This discrepancy may be attributed to limitations of amplicon sequencing, including DNA extraction/template-related biases (Biesbroek *et al*., 2012) and insufficient amplification of low-abundance taxa near detection thresholds (Dopheide *et al*.., 2019).

16S rRNA sequencing does not recover whole living cells but generates sequence data from DNA extracted from both live and dead cells. 16S rRNA amplicon sequencing determines the taxonomic identity of the samples' bacterial

composition or contents by comparing the cells 16S rRNA sequences to curated reference databases using sequence homology (Agnihotry *et al.,* 2020; Dueholm *et al.,* 2020). Taxonomic identities were reported using the closest match identifiers (database IDs/accession numbers). The relative abundance of each assigned taxon in each stream sample was quantified based on the proportion of sequence reads (in percentage). In contrast, the cultural method incubates the samples under defined conditions to recover, nurture, and purify viable microbial cells into isolates. Culture method made viable bacterial isolates available for microscopy and phenotypic characterization in this study. Each method demonstrates notable differences in the detected taxa across the two streams.

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