



Bacterial Pathogen Community Profiling of Three Freshwater Bodies in Akwa North and South Local Government Areas, Anambra State, Nigeria



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Abstract	Article History
<p>The bacterial pathogen community profile of three freshwater bodies with different effluent inputs in Akwa North and South Local Government Areas, Anambra State, Nigeria was investigated. Water samples were collected and analyzed for bacteriological properties using standard procedures. The microscopic and biochemical techniques were employed in the identification of bacterial isolates. The bacteriological result revealed that water sample from Urum had the highest total heterotrophic aerobic bacteria count of 81.25 ± 22.98 logcfu/ml with percentage frequency of 22.8 % and Mgbakwu has the lowest total heterotrophic aerobic bacteria count of 75.00 ± 35.53 logcfu/ml with percentage frequency of 19.95 % respectively. For faecal Streptococci water sample from Urum has the highest value of 79.88 ± 24.92 logcfu/ml with percentage frequency of 22.46 %. Water sample from Obibia has the lowest faecal streptococci count of 66.25 ± 33.58, respectively. The results revealed that <i>Escherichia coli</i>, <i>Enterococcus faecalis</i>, <i>Salmonella enterica</i> subsp. Enterica, <i>Clostridium viride</i>, <i>Vibrio alginolyticus</i>, <i>Serratia marcencens</i>, <i>Staphylococcus aureus</i>, <i>Shigella</i> sp. And <i>Pseudomonas aeruginosa</i> were the dominant bacteria identified. Statistically, there were significant ($p < 0.05$) differences detected among the means of pathogenic groups, but non – significant ($p > 0.05$) differences detected among the means of sampling sites. Thus, the baseline data from this study showed that the pathogenic bacterial populations of the three streams were high thereby suggesting public health danger to human consumption. Strict measures should be put in place by water managers, stakeholders and government in order to avert these menaces in the three studies areas.</p> <p>Keywords: <i>Bacterial pathogens, Baseline data, Freshwater, Public health, Water quality monitoring</i></p>	<p>Received: 16 Aug 2025 Accepted: 25 Aug 2025 Published: 28 Aug 2025</p>  <p>Scan QR Code to view¹</p> <p>License: CC BY 4.0²⁴</p>  <p>Open Access article.</p>
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Introduction

Water pollution occurs when unwanted materials enter in to water, changes the quality of water (Alrumman *et al.*, 2016) and harmful to environment and human health. Water is an important natural resource used for drinking and other developmental purposes in our lives. Safe drinking water is necessary for human health all over the world. Being a universal solvent, water is a major source of infection. According to World Health Organization (WHO) 80 % diseases are water borne. Drinking water in various countries does not meet WHO standards (Bibi *et al.*, 2016). Also, about 3.1 % deaths occur due to the unhygienic and poor quality of water (Pawari and Gawande, 2015). Discharge of domestic and industrial effluent wastes, leakage from water tanks, marine dumping, radioactive waste and atmospheric deposition are major causes of water pollution (Owa, 2013). Microorganisms play a major role in water quality and the microorganisms that are concerned with water borne diseases are *Salmonella* sp., *Shigella* sp., *Escherichia coli* and *Vibrio cholerae* (Adetunde and Glover, 2010). All these cause typhoid fever, diarrhoea, dysentery, gastroenteritis and

cholera. The most dangerous form of water pollution occurs when faeces enter the water supply. Many diseases are perpetuated by the faecal-oral route of transmission in which the pathogens are shed only in human faeces. Presence of faecal coliforms of *E. coli* is used as an indicator for the presence of any of this water borne pathogens (Adetunde and Glover, 2010).

The need for good water quality has been of growing concern in Nigeria and worldwide. Urgent attention is therefore necessary to mitigate water pollution problems in Nigeria especially through microbial monitoring as well as enforcement of emission standards by industries (Ekiye and Zejiao, 2010; Okolo *et al.* 2025; Okpalaunegbu *et al.*, 2025). However, there is dearth of information on the bacteriological properties of Mgbakwu, Obibia and Urum Rivers in Awka South and North Local Government Area of Anambra State. This study was undertaken to isolate and establish the pathogenic bacterial community profile of the water samples from the three sampling sites in Akwa North and South Local Government Areas, Anambra State, Nigeria.

Materials and Methods

Description of Study Area

The studied area include: Mgbakwu stream in Awka North Local Government Area, Obibia Stream in Awka South Local Government Area and Umuife Iyiohia Urum Stream in Awka North Local Government Area all located in Anambra Central Senatorial Zone, Anambra State Nigeria. Anthropological activities in the three sampling sites revealed that they are source of water supply for domestic and agricultural purposes in the eastern parts of the town despite being exposed to different industrial effluents (Chukwura and Udogu, 2017; Alfred *et al.*, 2023; Chukwura 2025; Dibua *et al.*, 2020; 2025a, 2025b; 2025c).

Collection of Water Sample

The samples were collected at four different points (5 m from apart) from the three sampling sites previously described above by direct random sampling method at Mgbakwu stream, Obibia stream and Umuife Iyiohia Urum, respectively at the depth of 5 - 10 cm using sterile 5 L cylindrical plastic containers, labeled, placed in a cooler and immediately transported to the laboratory for analysis (Uba, 2018a; Uba *et al.* 2019; 2020; Uba *et al.* 2021a, 2021b; Okoye *et al.*, 2024).

Bacteriological Analysis

The total heterotrophic aerobic bacterial counts, total coliform counts, total faecal coliform counts, Salmonella - Shigella counts, total Vibrio counts, *Staphylococcus aureus* counts, total clostridial counts, total faecal streptococcal counts and total *Pseudomonas aeruginosa* counts of the water samples were obtained using spread plate method (Ibo *et al.*, 2020; Umeh *et al.*, 2020; Dokubo *et al.* 2022a; 2022b; Dokubo and Uba, 2023). With the aid of a sterile pipette, 0.1 mL aliquots of the 10⁻⁴ dilution were spread plated on the surfaces of the nutrient agar (NA), eosin methylene blue agar (EMB), MacConkey agar (MA), Salmonella - Shigella agar (SSA), thiosulphate citrate bile sucrose agar (TCBS), mannitol salt agar (MSA), differential reinforced clostridial agar (DRCA), bile aesculin azide agar (DRCA) and cetrimide milk agar (CMA) plates in duplicates with the aid of a glass spreader. The spreader was sterilized after each successive spreading by dipping it in 70 % ethanol and then passing it through flame of a Bunsen burner. The inoculated plates were then incubated by inversion at 37 °C for 24 hr. The total heterotrophic aerobic bacterial counts were determined after incubation using an electric colony counter and colonies counted were expressed at CFU/mL (APHA, 2012; Nwigwe and Uba, 2022; Nwigwe *et al.*, 2023; Ibe *et al.*, 2023).

Characterization and Identification of the Isolate

The isolates were characterized and identified using their morphological description and biochemical reactions. After sub-culturing and incubation, cultural morphological properties such as appearance, edge, consistency, optical properties, elevation, and pigmentation characteristics of the selected bacterial strains were observed and noted (Willey *et al.*, 2008; Uba *et al.* 2016; Uba *et al.* 2017). Gram staining involves staining the bacterial cell to indicate if the bacteria is Gram positive or negative (Chesbrough, 2006; Uba *et al.*, 2018a; 2018b). Also, biochemical by oxidase test, catalase test, urease test, indole test, starch hydrolysis test, Nitrate reduction test, Methyl Red – Voges Proskauer (MR-VP) test, Motility test, Gelatin hydrolysis test and sugar fermentation test (Chesbrough, 2006; Uba, 2018; Uba, 2019a; 2019b; 2019c). The isolate was identified using key of identification as contained in Bergey's Manual for Determinative Bacteriology by Holt *et al.* (1994).

Statistical Analysis

The results of the data generated were expressed as mean ± standard deviation (SD) using GraphPad Prism version 8.0.2. The data means were analyzed by two-way Analysis of Variance (ANOVA) followed by Tukey's to compare differences in the diversity of the pathogen composition of the three sampling sites and different sampling points. Threshold values less than 5 % (p < 0.05) were considered statistically significant at 95 % confidence interval (Emmy – Egbe *et al.*, 2015; Anidu *et al.*, 2023; Uba and Anidu, 2023).

Results

The result of the mean bacterial distribution count of water sample collected from different sampling points at Mgbakwu sampling site is represented in Table 1. From the result, sample A had the highest total heterotrophic aerobic count of 50.00 ± 42.42 logCFU/mL with percentage frequency of 23.69 %, sample B had the highest faecal coliform count of 115.00 ± 49.49 logCFU/mL with percentage frequency of 24.84 % and highest total faecal Streptococci count of 70.00 ± 14.14 logCFU/mL with percentage frequency of 15.12 %, sample C had the highest total coliform count of 90.00 ± 42.42 logCFU/mL with percentage frequency of 27.11 % and sample D had the highest faecal coliform count of 110.00 ± 28.28 logCFU/mL with percentage frequency of 23.50 %, respectively. Statistically, there were significant (p < 0.05) differences detected among the means of pathogenic bacterial group and sampling points.

The mean bacterial count of water sample collected from different sampling points at Obibia sampling site is presented in Table 2. From the result sample A had the total heterotrophic aerobic bacteria count of 32.50 ± 13.44 with percentage frequency of 13.20 %, sample B had the highest total faecal coliform count of 105.00 ± 63.63 with percentage frequency of 22.22 %, sample C had the highest total coliform of 50.00 ± 14.14 with percentage frequency of 28.41 % and sample D had the highest total *Salmonella Shigella* count of 55.00 ± 7.07 with percentage frequency of 11.68 % and total faecal streptococci count of 100.00 ± 56.56 with percentage frequency of 21.23 % respectively. Statistically, there were significant (p < 0.05) differences detected among the means of pathogenic bacterial group and sampling points.

The result of the mean bacterial count of water sample collected from different sampling points at Urum sampling site is represented in Table 3. From the result sample A had the highest total heterotrophic bacteria count of 100.00 ± 28.28 with percentage frequency of 26.59 %, sample B had the highest total faecal coliform count of 105.00 ± 35.35 with percentage frequency of 24.5 %, sample C had the highest total coliform count of 45.00 ± 7.07 with percentage frequency of 27.1 %, and sample D had the highest total Streptococci count of 100.00 ± 28.28 with percentage frequency of 22.15 % and highest total *Salmonella Shigella* count Of 55.00 ± 7.07 with percentage frequency of 12.18 %. Statistically, there were significant (p < 0.05) differences detected among the means of pathogenic bacterial group and sampling points.

The microscopic and biochemical features of dominant bacteria strains isolated from the three-sampling site are presented in Table 4. The result of Table 4 revealed that most of the isolate were Gram negative, short rod shaped, pairs, clustered in cellular arrangement, positive to catalase, starch hydrolysis, motility, citrate, nitrate reduction, gelatin hydrolysis, glucose, fructose, maltose tests but negative to indole, oxidase and urease tests, respectively.

Table 1: Mean bacterial distribution count of water sample collected from different sampling points at Mgbakwu sampling site

Microbial group	A (LogCFU/mL ×10 ⁵)	Relative frequency (%)	B (LogCFU/mL ×10 ⁵)	Relative frequency (%)	C (LogCFU/mL ×10 ⁵)	Relative frequency (%)	D (LogCFU/mL ×10 ⁵)	Relative frequency (%)	WHO standard
THABC	50.00 ± 42.42	23.69	110.00 ± 14.14	23.76	70.00 ± 14.14	21.08	70.00 ± 14.14	14.96	1.0 × 10 ² /mL
TCC	25.00 ± 21.21	11.85	110.00 ± 14.14	23.76	90.00 ± 42.42	27.11	110.00 ± 28.28	23.50	Zero per 100mL
TFCC	45.00 ± 49.49	21.33	115.00 ± 49.49	24.84	100.00 ± 28.28	30.12	110.00 ± 56.56	23.50	Zero
TSSC	7.50 ± 0.71	3.55	12.00 ± 5.7	2.59	6.50 ± 4.95	1.96	55.00 ± 21.21	11.75	Zero
TVC	3.00 ± 1.41	1.42	2.00 ± 0	0.43	3.00 ± 2.83	0.90	3.00 ± 1.41	0.64	Zero
TPC	2.50 ± 0.71	1.18	3.00 ± 2.82	0.65	4.00 ± 2.83	1.20	2.50 ± 0.71	0.53	—
TFSC	55.00 ± 21.21	26.10	70.00 ± 14.14	15.12	50.00 ± 14.14	15.06	110.00 ± 56.56	23.50	—
TSC	20.00 ± 14.14	9.48	37.50 ± 10.61	8.10	6.00 ± 2.83	1.81	4.50 ± 0.71	0.96	—
TCLC	3.00 ± 1.41	1.42	3.50 ± 2.21	0.76	2.50 ± 0.71	0.75	3.00 ± 1.41	0.64	—

N.B:

THABC = Total heterotrophic aerobic bacterial count; TCC = Total coliform count; TFCC = Total faecal coliform count; TSSC = Total *Salmonella Shigella* count; TVC = Total *Vibrio* count; TPC = Total *Pseudomonas* count; TFSC = Total faecal Streptococci; TSC = Total *Staphylococcus* count; TCLC = Total *Clostridium* count; CFU = Colony forming unit per millimeter; % = Percentage, WHO = World Health Organization

Table 2: Mean bacterial distribution count of water sample collected from different sampling points at Obibia sampling site

Microbial group	A (LogCFU/mL ×10 ⁵)	Relative frequency (%)	B (LogCFU/mL ×10 ⁵)	Relative frequency (%)	C (LogCFU/mL ×10 ⁵)	Relative frequency (%)	D (LogCFU/mL ×10 ⁵)	Relative frequency (%)	WHO standard
THABC	32.50±13.44	19.94	130.00 ± 70.70	27.51	35.00 ± 7.071	19.89	100.00 ± 28.28	21.23	1.0 × 10 ² /mL
TCC	21.50 ± 2.12	13.20	100.00 ± 28.28	21.16	50.00 ± 14.14	28.41	110.00 ± 28.28	23.35	Zero per 100mL
TFCC	25.50 ± 7.780	15.64	120.00 ± 42.42	25.40	35.00 ± 7.071	19.89	95.00 ± 35.35	20.17	Zero
TSSC	10.00 ± 0	6.13	3.00 ± 2.83	0.63	10.00 ± 7.071	5.68	55.00 ± 7.07	11.68	Zero
TVC	3.00 ± 1.41	1.84	2.50 ± 0.71	0.53	2.50 ± 2.12	1.42	2.50 ± 2.12	0.53	Zero
TPC	4.50 ± 0.71	2.76	1.50 ± 0.71	0.32	4.00 ± 1.41	2.27	1.50 ± 0.71	0.32	—
TFSC	25.00 ± 7.07	15.34	105.00 ± 63.63	22.22	35.00 ± 7.071	19.89	100.00 ± 56.56	21.23	—
TSC	25.00 ± 7.07	15.34	7.50 ± 3.54	1.59	3.00 ± 1.41	1.70	4.50 ± 0.71	0.96	—
TCLC	16.00 ± 9.91	9.82	3.00 ± 1.41	0.63	1.50 ± 0.71	0.85	2.50 ± 0.71	0.53	—

N.B: THABC = Total heterotrophic aerobic bacterial count; TCC = Total coliform count; TFCC = Total faecal coliform count; TSSC = Total *Salmonella Shigella* count; TVC = Total *Vibrio* count; TPC = Total *Pseudomonas* count; TFSC = Total faecal Streptococci; TSC = Total *Staphylococcus* count; TCLC = Total *Clostridium* count; CFU = Colony forming unit per millimeter; % = Percentage, WHO = World Health Organization.

Table 3: Mean bacterial distribution count of water sample collected from different sampling points at Urum sampling site

Microbial group	A (LogCFU/mL ×10 ⁵)	Relative frequency (%)	B (LogCFU/mL ×10 ⁵)	Relative frequency (%)	C (LogCFU/mL ×10 ⁵)	Relative frequency (%)	D (LogCFU/mL ×10 ⁵)	Relative frequency (%)	WHO standard
THABC	100.00 ± 28.28	26.59	105.00 ± 21.21	24.50	25.00 ± 7.071	24.50	95.00 ± 35.35	21.04	1.0 × 10 ² /mL
TCC	45.00 ± 35.35	11.97	95.00 ± 21.21	22.20	45.00 ± 7.07	27.10	100.00 ± 14.14	22.15	Zero per 100mL
TFCC	94.00 ± 8.49	25.00	105.00 ± 35.35	24.50	45.00 ± 7.07	27.10	95.00 ± 35.35	21.04	Zero
TSSC	15.00 ± 7.071	3.99	1.50 ± 0.71	0.35	6.50 ± 4.95	3.92	55.00 ± 7.07	12.18	Zero
TVC	7.00 ± 4.24	1.86	7.00 ± 4.24	1.64	1.50 ± 0.71	0.90	1.50 ± 0.71	0.33	Zero
TPC	3.00 ± 1.41	0.79	1.00 ± 0	0.23	3.50 ± 0.71	2.11	1.50 ± 0.71	0.33	—
TFSC	79.50 ± 28.99	21.14	105.00 ± 35.35	24.50	35.00 ± 7.07	21.10	100.00 ± 28.28	22.15	—
TSC	26.00 ± 22.63	6.91	5.50 ± 0.71	1.29	2.50 ± 0.71	1.51	2.50 ± 0.71	0.56	—
TCLC	6.50 ± 4.95	1.73%	2.50 ± 2.12	0.58	2.00 ± 1.41	1.20	2.50 ± 0.71	0.55	—

N.B: THABC = Total heterotrophic aerobic bacterial count; TCC = Total coliform count; TFCC = Total faecal coliform count; TSSC = Total *Salmonella Shigella* count; TVC = Total *Vibrio* count; TPC = Total *Pseudomonas* count; TFSC = Total faecal Streptococci; TSC = Total *Staphylococcus* count; TCLC = Total *Clostridium* count; CFU = Colony forming unit per millimeter; % = Percentage, WHO = World Health Organization.

Table 4: Microscopic and biochemical feature of the dominant bacterial strains isolated from the three-sampling sites

Isolate code	Gram reaction	Cellular shape	Cell arr.	C A T	M O T	IN	S. H	O XI	U R.	CI T	N R T	M R	V P	G H	G L	L A	F R	M A	X Y	S U C	M A L T	Tentative identity
MAES	Gram -	Short rod	Pairs	+	+	+	+	-	-	-	+	+	-	-	+	+	+	+	+	-	+	<i>Escherichia coli</i>
UDSF	Gram +	Cocci	Chains	-	-	-	+	-	-	-	-	+	+	+	+	+	-	-	+	+		<i>Streptococcus faecalis</i>
OBPS	Gram -	Short rod	Clusters	+	+	-	+	-	-	+	+	+	-	+	+	-	-	-	+	-	-	<i>Pseudomonas aeruginosa</i>
UBCL	Gram +	Short rod	Pairs	-	-	-	-	-	+	+	-	+	-	-	+	+	+	-	-	+	+	<i>Clostridium sp.</i>
MCVI	Gram -	Curved rod	Clusters	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	<i>Vibrio cholerae</i>
UASA	Gram -	Short rod	Clusters	+	+	-	+	-	+	+	+	-	+	+	-	-	+	+	+	-	+	<i>Salmonella sp.</i>
OAST	Gram +	Cocci	Clusters	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	<i>Staphylococcus aureus</i>
MCSH	Gram -	Short rod	Pairs	+	-	-	+	-	-	-	+	+	-	-	+	+	-	-	-	+	+	<i>Shigella sp.</i>
OCBA	Gram -	Short rod	Chains	+	+	-	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	<i>Bacillus sp.</i>

N.B. Cell ARR. = Cell arrangement, CAT = catalase, MOT = motility, IN = indole, S.H = starch hydrolysis, OXI = Oxidase, UR = urease, CIT = citrate, NRT = nitrate reduction test, M.R = methyl red, V.P = Voges Proskauer, G.H = gelatin hydrolysis, GLU = glucose, LACT = lactose, FRUC = fructose, MAN = mannitol, XYL = xylose, SUC = sucrose, MALT = maltose, + = positive, = negative, Gram + = Gram positive, Gram = Gram negative

Discussion

In this research study and with respect to the Tables 1 - 3 results obtained from three streams' Mgbakwu, Obibia and Urum Awka, Anambra State, the mean total heterotrophic aerobic bacteria count was 75.00 ± 19.95 logCFU/mL for Mgbakwu, 74.38 ± 29.88 logCFU/mL for Obibia and 81.28 ± 25.98 LogCFU/mL for Urum, respectively indicating high level of pollution of stream water due to human and animal activities. These counts were higher than the acceptable counts of 0 CFU/mL for drinking water (NIS, 2007). These sources of bacterial contamination include surface runoff, animal waste deposition and pasture. Other human activities like swimming, waste disposal, domestic activities and faecal discharge (Egberongb *et al.*, 2012) are also possible ways of introducing foreign microorganisms in the water thereby making more nutrients available for the microorganisms in the water thus enhancing their growth at all the various water sources.

Coliform counts give an indication of extent of water quality degradation. In the present study, the results of the total coliform counts (TCC) exceeded that of the WHO standard for coliform bacteria in water, which is zero total coliform per 100 mL of water. The presence of coliform counts obtained from the samples is an indication of faecal contamination. None of the stream samples complied with the WHO standard for coliform in water, and this is in agreement with previous work by Onajite *et al.* (2018) who had earlier reported high microbial counts on water containing higher organic matter. Faecal streptococci count indicated more contamination with human excrement than animal excrement. The fecal counts for all samples were extremely higher than the WHO standard for coliform bacteria in water which is zero total coliform per 100 mL of water. Isolate code MAES has the highest frequency of 27.51 % and isolate code OBPS has the lowest frequency of 0.32 %, respectively. Statistically, there were significant ($p < 0.05$) among the means of pathogenic groups and sampling points but non - significant ($p > 0.05$) among the means of sampling sites.

The results in Tables 4 - 5 revealed that the identified isolates from three sampling sites include: *Escherichia coli*, *Enterococcus faecalis*, *Salmonella enterica* subsp. *enterica*, *Clostridium viride*, *Vibrio alginolyticus*, *Serratia marcescens*, *Staphylococcus aureus*, *Shigella* sp. and *Pseudomonas aeruginosa* and in line with published work of Ike *et al.* (2021) who in their study on bacteriological examination of Obibia Stream during wet and dry seasons in Awka, Anambra State, Nigeria identified the presence *Shigella*, *Salmonella*, *Escherichia coli*, *Vibrio*, *Enterobacter*, *Klebsiella*, *Enterococcus*, *Staphylococcus*, *Pseudomonas* and *Serratia* with their respective frequencies of occurrence as 4.55 %, 18.18 %, 13.64 %, 27.27 %, 4.5 %, 9.09 %, 4.55 %, 4.55 %, 9.09 % and 4.55 %.

Conclusion

The results of the present study indicated that fresh water bodies of Mgbakwu, Obibia and Urum Streams in Awka North and South LGA Anambra State, respectively were contaminated with various pathogenic bacteria and unfit for human consumption and agricultural purposes. As a result, public enlightenment on the consequences of pollution of these

sites should as a matter of necessity be made clear and severe lawful actions should be exercised against those individuals or organizations which pollute these fresh water bodies.

Conflict of Interest

The authors declare no conflicts of interest.

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