

Seasonal Evaluation of the Bacteriological Quality of Agangwu Stream in Enugu State, Nigeria

Onuorah, E. R.¹, Orji, M. U.^{1,2}, Nwobu, R. A. U.^{1,3}, Oghonim, P.A.N.⁴, Abana, C. C.^{1*}

¹Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Nigeria

²Department of Microbiology, Caritas University, Enugu, Nigeria

³Deptment of Microbiology, Paul University, Awka, Nigeria

⁴Microbiology Department, Biological Sciences, University of Delta, Agbor P.M.B 2090, Agbor, Delta State, Nigeria.

*Corresponding Author

Abana, C. C.

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Abstract: Water is essential to life but many people do not have access to clean and safe drinking water while many even die of water borne bacterial infections. The sanitary quality of stream water cannot be predicted due to behavioral differences of the users and their living condition. This investigation was carried out on the seasonal evaluation of the microbiological quality of Agangwu Stream in Enugu State, Nigeria. A total of eighteen (18) stream water samples collected at wet and dry seasons comprised of six (6) water samples each from the upstream, midstream and downstream locations. They were subjected to a wide range of analytical assessments like the multiple tube fermentation method according to APHA, 2016. Then, the bacteria isolates were characterized and identified morphologically and biochemically using standard microbiological techniques. The dilution pattern showed varying coliform concentration especially for MS¹ (3-0-2) and DS¹ (3-1-2). *E. coli*, the most common fecal coliform appeared in both seasons with the highest (most probable number) MPN index of 75mpn/100ml (95% confidence limits: 14-230) in dry season and the least MPN index of 1.5mpn/100ml (95% confidence limits: 3 - 44) in wet season. Even though the values were statistically insignificant ($P > 0.05$). The molecular identification of some of the isolates from the stream water samples confirmed various pathogens, including *Salmonella enterica subsp. Enterica*, *Escherichia coli*, etc. The MPN index for both seasons reveal a clear gradient of increasing fecal contamination from upstream to downstream sections of Agangwu Stream. These findings demonstrate that Agangwu stream is definitely not safe for direct human consumption or recreational use without adequate treatment. Rather, there is need for regular monitoring, effective water treatment, and public health interventions to mitigate the risks associated with waterborne diseases.

Keywords: Stream water, fecal coliforms, *E. coli*, seasonal evaluation.

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Introduction

Water resources are indispensable for life, agriculture, environmental sustainability, human consumption, economic development and industrial activities. Globally, billions of people depend directly on groundwater and surface-water systems for drinking, domestic activities, crop production and manufacturing purposes (Khatri & Tyagi, 2015). Water is also one of the most abundant substances on Earth, with an estimated volume of approximately 1.4×10^9 km³, and plays a fundamental role in the geophysical and geochemical processes of the planet (Brown & Bhushan, 2016). At the biological level, water constitutes a substantial proportion of living cells and participates in numerous physiological processes, including molecular transport, biochemical reactions, protein folding and the interaction of metabolites and drugs with biomolecules (Brini *et al.*, 2017; Sharma & Bhattacharya, 2017).

Aquatic environments also support diverse populations of microorganisms whose occurrence and distribution are influenced by physicochemical conditions, nutrient availability and human activities. Microorganisms are ubiquitous in natural environments and may contribute positively to nutrient cycling and biodegradation, while some may constitute important environmental and public-health hazards (Awari *et al.*, 2023). Studies from Anambra State have demonstrated the diversity and adaptability of microorganisms in aquatic systems. Agu *et al.* (2017), for instance, isolated and characterised halotolerant bacteria from Ezzu River in Amansea, Awka, demonstrating the capacity of bacteria to persist under varying environmental conditions. Similarly, Agu *et al.* (2023) reported diverse microbial populations in fish ponds within Awka, further illustrating the ecological importance of aquatic microorganisms.

In Nigeria, surface-water bodies are frequently subjected to diverse anthropogenic activities, including domestic washing, bathing, agricultural activities, waste disposal, religious activities and storm-water runoff. These activities can alter the microbial quality of water and promote the introduction and proliferation of potentially pathogenic microorganisms (Abana *et al.*, 2024). Microorganism-mediated water pollution has consequently become an important environmental and public-health concern. Wastewater from homes, hospitals, industries, abattoirs and livestock facilities may introduce substantial quantities of microorganisms and organic matter into surrounding aquatic environments. Ezeokoli *et al.* (2023), for example, documented bacterial contamination of abattoir wastewater in Awka, demonstrating the potential of untreated effluent to serve as a source of environmental microbial pollution.

Surface waters are particularly vulnerable because they are directly exposed to runoff and waste discharges from

surrounding communities. Where human or animal faecal material enters a stream, river or other water body, the water may become an important vehicle for transmission of enteric pathogens (Denchak, 2018). The vulnerability of water resources to anthropogenic contamination has also been demonstrated in south-eastern Nigeria. Agu *et al.* (2014) reported bacteriological contamination of groundwater associated with leachate from solid-waste dumps in Awka, while Victor-Aduloju *et al.* (2023) documented microbial contamination of water obtained from selected hostels in Ifite, Awka. More recently, Egrefa *et al.* (2024) reported bacteriological contamination of borehole drinking-water sources in Enugu metropolis. Together, these studies indicate that both surface and groundwater resources in rapidly urbanising areas may be compromised by inadequate sanitation and waste-management practices.

The microbial burden of aquatic environments is often dominated by bacteria, although fungi and other microorganisms may also be present. Abana *et al.* (2024) reported that bacteria generally occur at greater frequencies than fungi in aquatic habitats. The ability of microorganisms to survive under diverse environmental conditions is further demonstrated by studies involving organisms recovered from unusual ecological niches. Agu and Odibo (2021), for example, reported the biodegradation potential of *Aspergillus flavipes* isolated from Uburu and Okposi salt lakes, while Agu *et al.* (2017) documented halotolerant bacteria from a riverine environment. These observations demonstrate the adaptability of microorganisms and their potential persistence in aquatic ecosystems subjected to varying ecological pressures.

Coliform bacteria are widely used as indicators of the sanitary and microbiological quality of water because their detection may suggest contamination by faecal or environmental sources (Some *et al.*, 2021). Among these organisms, *Escherichia coli* is particularly important as an indicator of recent faecal contamination. The presence of faecal coliforms in a water body suggests contamination with faecal material from humans or other warm-blooded animals and may indicate the possible presence of enteric pathogens. Faecal coliforms are generally characterised as Gram-negative, non-spore-forming, rod-shaped organisms capable of lactose fermentation and growth at elevated temperatures of approximately 44.5°C (Payment *et al.*, 2015).

The detection and enumeration of indicator bacteria therefore remain fundamental to the microbiological assessment of drinking and recreational water. In addition to *E. coli*, the presence of organisms such as faecal *Streptococcus* species, *Clostridium perfringens* and *Klebsiella* species may provide additional evidence of faecal pollution. Pathogenic organisms such as *Salmonella Typhi*, *Salmonella Typhimurium*, *Salmonella Enteritidis*, *Shigella*

species and *Proteus* species may also occur in sewage-contaminated aquatic environments (Korajkic *et al.*, 2018).

Contaminated water may also harbour microorganisms with broader environmental significance. The capacity of aquatic and environmental microorganisms to interact with contaminants has been demonstrated in several studies. Ojiagu *et al.* (2018) reported the biosorption of hexavalent chromium by *Pseudomonas aeruginosa*, illustrating the ability of bacteria to interact with toxic metals. Okafor *et al.* (2016) demonstrated enhancement of crude-oil bioremediation following amendment with poultry waste, while Orji *et al.* (2022) described the utilisation of fungal species in the bioremediation of glyphosate-polluted soil. Similarly, Okeke *et al.* (2023) demonstrated the degradative potential of *Aspergillus niger* and *Aspergillus flavus* against Congo red dye, and Orji *et al.* (2014) reported cellulase production by *Penicillium* and *Aspergillus* species using unconventional substrates. Although these studies were not primarily concerned with waterborne pathogens, they highlight the broad ecological activities of microorganisms and their ability to persist and function in polluted environments.

Faecally contaminated water represents an important vehicle for the transmission of infectious diseases. Consumption of contaminated water, or direct recreational contact with polluted streams, can expose individuals to pathogenic microorganisms through ingestion, mucosal surfaces or breaks in the skin. Such exposure has been associated with illnesses including gastroenteritis, typhoid fever, dysentery, hepatitis, poliomyelitis and other water-related infections (Rodrigues *et al.*, 2017). Consequently, microbiological assessment of community water sources is important for identifying potential public-health risks and informing appropriate environmental and disease-control measures.

Agangwu Stream, located in Enugu State, is used by inhabitants of the surrounding settlement for various domestic and other activities. Interactions with community members have suggested recurrent episodes of illnesses such as gastroenteritis, raising concerns about the microbiological safety of the stream. Given previous reports of microbial contamination of water and wastewater sources in south-eastern Nigeria (Agu *et al.*, 2014; Ezeokoli *et al.*, 2023; Victor-Aduloju *et al.*, 2023; Egrefa *et al.*, 2024), systematic evaluation of the microbiological quality of Agangwu Stream is warranted.

Therefore, this study aimed to evaluate seasonally the microbiological quality of Agangwu Stream in Enugu State, Nigeria, with a view to determining its microbial status, identifying potential indicators of contamination and providing baseline information relevant to environmental monitoring and public-health protection.

Methodology

The study area: Agangwu stream in Agangwu settlement Enugu North Local Government Area, Enugu State, Nigeria is a brownish meandering stretch of water body with topography formed on a valley and its vegetation forming canopies of basically palm trees and bamboo trees which dominate other plants.

Collection of Agangwu Stream water sample: Sterile plastic containers were used to collect a total of eighteen regular stream samples. Six each were collected from the downstream, middle stream and upstream points during the wet and the dry seasons at different sampling days. The samples were collected aseptically and transported to the laboratory immediately in ice packs for the microbiological analysis.

Analysis of the water sample for bacteria: Multiple-tube fermentation method as described by APHA (2016) was used for analyzing the stream water samples for bacteria. It involved adding specified quantities 10ml, 1ml, 0.1ml, of the sample to 3 set of tubes each containing lactose broth and incubated the tubes for 24hrs at 37°C. After which the development of gas and/or turbidity produced by the bacteria was checked upon. The presence or absence of gas was noted and used to obtain the most probable number (MPN) by comparing with a reference table (APHA, 2016).

The method of McCrady (1915) used to run the Total Indicator Bacteria Test is described as follows:

Multiple tube technique for Fecal Coliforms

Presumptive Test: A combination of lactose broth and peptone water in bijou bottle (half filled) and Durham tubes were used. The combination is necessary as the peptone water helps to determine the ability of an organism to ferment a specific carbohydrate, thus aiding in the differentiation of genera and species. A specified quantity of the stream water sample (1ml) was introduced into the medium following serial dilution of the sample. Incubation was at 35°C for 24hrs.

Confirmed/Differential Test: Medium used was Brilliant green bile broth (BGBB). Subculture was made from (a) into Brilliant Bile Broth (BGBB) in a test tool containing Durham tube. The Durham tubes were properly filled to expel any entrapped air. Incubation was at 44.5°C for 24hrs. Fecal coliforms only survived at this temperature. Gas production is confirmatory for fecal coliform e.g. *E. coli*. samples were taken from BGBB, to inoculate Eosin methylene blue agar. Incubation was at 35°C for 24hrs. Metallic sheen colonies indicated the presence of coliforms.

Multiple tube technique for *S. faecalis*

Presumptive test for fecal *Streptococci*: The medium used was Glucose Azide Broth. 5ml of the water sample was

introduced into the medium (Bijou bottle half filled) and incubated at 37°C for 72hrs. Presence of turbidity indicated growth of *S. faecalis*. The Bottle that showed no turbidity was re-incubated for two more days.

Confirmatory test for *S. faecalis*: The medium used here was Ethyl violet azide broth. Subcultures were made from glucose azide broth into Ethyl violet azide broth. Incubation was at 45°C for 48hrs. Purple button formation at the bottom of the Bijou bottle indicated positive result. Occasionally, denser turbidity was observed.

Enumeration of *Clostridium perfringes* using a multiple tube technique.

Presumptive test for *Clostridium perfringes*: The media used include: Differential reinforced clostridium medium (DRCM) and Litmus milk. Five milliliters (5mls) of the stream water sample was inoculated into Bijou bottle, half filled with differential reinforced clostridium medium (DRCM). It was subjected to heat for 10mins at 85°C to kill all non-spore formers. Later, it was incubated at 37°C for 48hrs. Blackening of the medium due to the reduction of the sulphate and precipitation of ferrous sulphate indicated a positive result.

Confirmatory Test for *Clostridium perfringes*: The medium used is Litmus Milk. Subculture from differential reinforced clostridium medium (DRCM) was made into litmus milk in bijou bottles (half filled). Incubation was at 37°C for 5 days. Formation of stormy clot indicated the presence of *Clostridium perfringes* due to the production of gas bubbles that broke up the clot of the milk.

Characterization and Biochemical identification: The pathogenic bacteria isolates were characterized through their morphological characteristics. The distinct colonies of the

isolates were Gram stained using the standard Gram staining procedures as described in Cheesbrough, 2002. Then, the enlisted biochemical tests were carried out. They include: catalase test, coagulase test, oxidase test, indole test, motility test, methyl red test, citrate test, Nitrate test, Voges-Proskauer (V.P) Test and sugar fermentation test. Then, for the fungal studies, serial dilution of the stream water sample was made using sterile water up to (10^{-5}) in sterile test tubes to reduce the microbial load of the sample before they were cultured in Potatoes Dextrose Agar (PDA) prepared according to the manufacturer's instruction. Incubation was done at 37°C for 4 days. The different fungal growths were observed, and the morphological characteristics, macro and microscopic findings noted. The bacterial and fungal isolates were further identified molecularly at the Bioinformatics Laboratories, Ibadan, Nigeria.

Antimicrobial susceptibility test:

Kirby-Bauer method of Disk diffusion was used to determine the antimicrobial susceptibility pattern of the microbial isolates. The levels of zones of inhibition present after incubation were noted as slightly sensitive (+), moderately sensitive (++), and very sensitive (+++) as the case may be.

Results

The water samples collected from Agangwu stream in Enugu State, Nigeria revealed the presence of both coliforms and non-coliforms organisms. The cultural and biochemical characterization of the bacteria isolates both wet and dry season are represented in tables 1 and 2 respectively while the cultural and microscopic characterization of the isolated fungi is displayed in table 3.

Table 1: Cultural Characteristics of the bacterial isolates (Wet Season)

S/N	Sample ID	Media	Colonial morphology	growth	Gram staining
1.	Up stream	Eosin methylene blue agar	Metallic green sheen colonies	+++	-ve rods
2.	Up stream	EMB agar	Blue-green colonies with black center	++	-ve rods
3.	Mid Stream	MacConkey Agar	Dark red colonies	++	-ve rods
4.	Mid stream	Salmonella-Shigella (SS) Agar	Smooth opaque	++	-ve rods
5.	Down stream	MacConkey agar	Colourless colonies	++	-ve rods
6.	Down stream	EMB agar	Pink & mucoid colonies	++	-ve

Table 2: Biochemical Characteristics of bacterial isolates (Wet Season)

Sample ID	Catalase test	Coagulase test	Oxidase test	Indole	Motility	Methyl red	Citrate test	Nitrate test	V.P test	Lactose	Glucose	Sucrose	Fructose	Suspected organisms
Up stream	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	+	+	+	-	<i>E. coli</i>
Up stream	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-	+	+	-	<i>Proteus mirabilis</i>
Mid Stream	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve		+				<i>Serratia marcescens</i>
Mid stream	+ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-	-	+	-	<i>Shigella flexneri</i>
Down stream	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-	+	-	-	<i>Salmonella enterica subsp.</i>
Down stream	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+	+	+	-	<i>Klebsiella pneumoniae</i>

p-value $\approx$ 0.143

Since the p-value is greater than 0.05, we fail to reject the null hypothesis. There is no significant association between the colony morphology and the agar type.

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Table 3: Cultural Characteristics of Bacterial Colonies (Dry Season)

S/N	Sample ID	Media	Colonial morphology	growth	Gram staining	Suspected organisms
1	Up stream	Eosin methylene blue agar	Metallic green sheen colonies	+	-ve rods	<i>E. coli</i>
2	Up stream	EMB agar	Blue-green colonies with black center	+	-ve rods	<i>Proteus mirabilis</i>
3	Mid Stream	MacConkey Agar	Dark red colonies	+	-ve rods	<i>Serratia marcescens</i>
4	Mid stream	Salmonella-Shigella (SS) Agar	Smooth opaque	+	-ve rods	<i>Shigella flexneri</i>
5	Down stream	MacConkey agar	Colourless colonies	+	-ve rods	<i>Salmonella enterica subsp.</i>
6	Down stream	EMB agar	Pink & mucoid colonies	+	-ve	<i>Klebsiella pneumoniae</i>

Table 4: Biochemical Characteristics of the Bacterial isolates (Dry Season)

Sample ID	Catalase test	Coagulase test	Oxidase test	Indole	motility	Methyl red	Citrate test	Nitrate test	V.P test	Lactose	Glucose	Sucrose	Fructose	Suspected organisms
Up stream	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	+	+	+	-	<i>E. coli</i>
Up stream	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-	+	+	-	<i>Proteus mirabilis</i>
Mid Stream	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+	+	+	+	<i>Serratia marcescens</i>
Mid stream	+ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-	-	+	-	<i>Shigella flexneri</i>
Down stream	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-	+	-	-	<i>Salmonella enterica</i> subsp
Down stream	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+	+	+	-	<i>Klebsiella pneumoniae</i>

p-value ≈ 0.91

Since the p-value is greater than 0.05, we fail to reject the null hypothesis. There is no significant association between the suspected organisms and colonial morphology.

Table 5: Cultural characteristics of Isolated Fungi

ID	Colour (R/F)	Texture/Colonial morphology	Microscopy	Phenotypic Identity	Molecular identity
F1	Black/light Brown	Black, powdery and velvety surface with white mycelia margins. Reverse side of the agar plate: light brown	Conidia and sporangium present with septate hyphae	<i>Aspergillus</i> species	<i>Aspergillus tubingensis</i>
F2	Black/dark brown	Black, powdery colonies. Edges are pale yellow. Reverse side: pale yellow/creamy white	Black spherical conidia on the smooth walled conidiophores, septate hyphae	<i>Aspergillus</i> species	<i>Aspergillus niger</i>
F3	Dark green	Velvety surface: Reverse yellowish grey coloured and smooth	Branched conidiophores with septate Pseudohyphae. Smooth conidia	<i>Penicillium</i> species	<i>Penicillium georgiense</i>
F4	Jet black	Black, Velvety/powdery surface. Reverse: pale yellow	Black spherical conidia (non-branching) with rough walls. Has septate hyphae	<i>Aspergillus</i> species	<i>Aspergillus awamori</i>

Using a chi-square distribution, we find p-value ≈ 0.083

Since the p-value is less than 0.1, we reject the null hypothesis. There is a significant association between the phenotypic identity and molecular identity.

Table 6: MPN Determination (1) From Multiple Tube Test (Wet Season)

Number of tubes giving positive reactions out of				MPN	95 Percent Confidenc Limits	
Sample ID	3 of 10ml each	3 of 1ml each	3 of 0.1ml each	Index per 100ml	lower	Upper
US ¹	2	1	0	15	3	44
US ²	2	2	1	28	10	150
MS ¹	3	0	2	64	15	180
MS ²	3	1	1	75	14	230
DS ¹	3	1	2	120	30	380
DS ²	3	2	1	150	30	140

Using a chi-square distribution, we find p-value ≈ 0.27

Since the p-value is greater than 0.05, we fail to reject the null hypothesis. There is no significant association between the Sample ID and the number of tubes giving positive reactions.

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KEY:

US ¹	=	Upstream 1
US ²	=	Upstream 2
MS ¹	=	Midstream 1
MS ²	=	Midstream 2
DS ¹	=	Downstream 1
DS ²	=	Downstream 2

Table 7: MPN Test (Dry Season)

Number of tubes giving positive reaction out of				MPN	95 Percent Confidenc Limits	
Sample ID	3 of 10ml each	3 of 1ml each	3 of 0.1ml each	Index per 100ml	Lower	Upper
US ¹	1	1	0	7	1	21
US ²	1	1	1	11	3	23
MS ¹	2	1	0	15	3	44
MS ²	2	1	1	20	7	89
DS ¹	3	1	0	43	7	210
DS ²	3	1	1	75	14	230

Using a chi-square distribution, we find p-value ≈ 0.06

Since the p-value is less than 0.1, we reject the null hypothesis. There is a significant association between the Sample ID and the number of tubes giving positive reactions.

SPSS v23

KEY:

US ¹	=	Upstream 1
US ²	=	Upstream 2
MS ¹	=	Midstream 1
MS ²	=	Midstream 2

DS¹ = Downstream 1
 DS² = Downstream 2

Table 8: MPN Value Per 100ml Of The Stream Sample For The Wet Season.

S/N	Sample ID	Organisms	MPN Index per 100ml	MPN value per 100ml	95 Percent Confidenc Limits	
					Lower	Upper
1	US ¹	E. Coli	15	1.5	3	44
2	US ²	Proteus	28	2.8	10	150
3	MS ¹	Serratia	64	6.4	15	180
4	MS ²	Shigella	75	7.5	14	230
5	DS ¹	<i>Salmonella</i>	120	12	30	380
6	DS ²	<i>Klebsiella</i>	150	15	30	140

Using a chi-square distribution, we find p-value ≈ 0.07

Since the p-value is less than 0.1, we reject the null hypothesis. There is a significant association between the Sample ID and the type of organism.

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N/B: MPN Value = MPN Index per 100ml x lowest dilution factor (0.1ml)

Table 9: MPN Value Per 100ml Of The Stream Sample For The Dry Season.

S/N	Sample ID	Organisms	MPN Index per 100ml	MPN value per 100ml	95 Percent Confidenc Limits	
					Lower	Upper
1	US ¹	<i>Salmonella</i>	7	0.7	1	21
2	US ²	<i>Salmonella</i>	11	1.1	3	23
3	MS ¹	Proteus	15	1.5	3	44
4	MS ²	Shigella	20	2	7	89
5	DS ¹	<i>Klebsiella</i>	43	4.3	7	210
6	DS ²	E. Coli	75	7.5	14	230

Using a chi-square distribution, we find p-value ≈ 0.13

Since the p-value is greater than 0.1, we fail to reject the null hypothesis. There is no significant association between the Sample ID and the type of organism.

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Table 10: Molecular identification of selected bacteria and fungi isolates

Sample ID	Molecular (BLAST) identity	% Identity	GENE bank Accession Number
D2	<i>Serratia nematodiphila</i>	87.22	KC122708.1
D3	<i>Salmonella enterica</i> subsp. <i>Enterica</i>	99.73	MG663472.1
M3	<i>Proteus mirabilis</i>	99.19	OR432090.1
U1	<i>Escherichia coli</i>	97.91	MN314211.1
U3	<i>Escherichia coli</i>	99.48	KX602648.1
U4	<i>Proteus mirabilis</i>	98.38	MH760802.1
M5	<i>Salmonella enteric</i>	98.38	MG663457.1

Discussion

Analysis of water samples from Agangwu Stream was carried out in two seasons (dry and rainy seasons) to determine the bacteriological agents of the stream. A total of eighteen (18) stream water samples were collected and analyzed, six (6) from the upstream, six (6) from midstream and six (6) from the downstream. Bacteria detected in both seasons include *Escherichia coli*, *Proteus mirabilis*, *Serratia marcescens*, *Shigella flexneri*, *Salmonella enteric subsp.* and *Klebsiella pneumonia*, with *Escherichia coli* having the highest MPN value (7.5 per 100ml) during the dry season. The presence of *E. coli* in the sample which suggests that there is fecal contamination upstream, may indicate a potential health risk, as it can cause a range of illnesses, from mild to severe (Balogun *et al.*, 2016). The upstream contamination suggests that the source of the contamination may be from a wastewater or sewage source, agricultural runoff, or other environmental sources (Patel *et al.*, 2018). The reduced growth intensity compared to the wet season suggests that the contamination may be less severe than during periods of heavy rainfall or flooding.

The presence of *Proteus* species in the sample aligns with the research on characterization of bacterial communities in waste water conducted by Obayiuwana *et al.*, 2018 who equally reported *Proteus* species as being most prevalent in their research. This truly may indicate a potential health risk, especially if the contamination is widespread or if the bacteria can infect the body through open wounds or other means. It can also cause a range of infections, including: Urinary tract infections (UTIs), Wound infections, Respiratory infections and Gastrointestinal infections, especially in individuals with compromised immune systems or underlying medical conditions. *Proteus* species are commonly found in the environment, particularly in water and soil, and can also be part of the normal flora in the human gut. The less intense growth compared to the wet season suggests that the contamination may be less severe than during periods of heavy rainfall or flooding. It is important to note that *Proteus* species are commonly found in the environment and can be introduced into water systems through various means, including sewage overflows, agricultural runoff, and wildlife waste. The presence of *Proteus* in the water supply may indicate a need for improved water treatment and sanitation measures to prevent the spread of infection

The presence of *Serratia* in the midstream samples during the dry season indicates ongoing contamination, suggesting that the water supply may be compromised. *Serratia* species are commonly found in the environment and can be introduced into water systems through various means, including sewage overflows, agricultural runoff, and wildlife waste. The presence of *Serratia* in the water supply may indicate a need for improved water treatment and

sanitation measures to prevent the spread of infection. It is important to take appropriate measures to address the contamination, prevent further spread of potential pathogens, and ensure proper infection control practices. Further testing and analysis may be necessary to determine the extent of the contamination and to identify any additional pathogens that may be present.

The presence of coliform bacteria, which include *E. coli*, *Klebsiella*, and *Enterobacter*, suggests potential fecal contamination. The frequency of occurrence and microbial load of coliforms are greatly influenced by seasonal variations (Mishra *et al.*, 2018; Oghonim, 2023). This is a concern because fecal contamination can indicate the presence of pathogens that can cause waterborne illnesses. However, the moderate level of contamination and the variability in bacterial counts suggest that the water may not be severely contaminated, but rather may have intermittent or patchy contamination. Overall, the results suggest that further testing and monitoring are necessary to determine the source and extent of contamination and to ensure the safety of the water supply. Additional measures such as water treatment and sanitation may be necessary to reduce the risk of waterborne illnesses. Even, Mishra *et al* (2018) advised that for public health and economic reasons, the fecal pollution of water bodies especially those used for recreational and drinking purposes should be regularly monitored. The fecal coliforms and *E. coli* isolated in this study corresponds with the discovery of Salamande *et al* (2020) in their research on High fecal contaminants in water consumed in their city, where fecal coliforms and *E. coli* were equally isolated in high numbers exceeding the approved WHO limit.

The presence of *Salmonella* in the sample highlights the need for immediate action to address the contamination and prevent further spread of the bacteria. This may involve improved wastewater treatment and management, enhanced sanitation and hygiene practices, proper disposal of human and animal waste, education on proper hand hygiene and personal hygiene practices, and water treatment and testing to ensure safe water quality for downstream users.

The presence of *Klebsiella* downstream suggests contamination with bacteria capable of causing pneumonia and urinary tract infections, highlighting potential health risks for individuals using the water downstream, like the wet season but with reduced growth. This suggests that the water supply may be compromised, and there may be ongoing sources of contamination that need to be addressed. *Klebsiella* species are commonly found in the environment and can be introduced into water systems through various means, including sewage overflows, agricultural runoff, and wildlife waste. *Klebsiella pneumoniae*, *Serratia marcescens*, *Escherichia coli*, isolated in this study aligns with the research output of Ehrhardt *et al.* (2017), Abana *et al.*, 2024

and Oghonim *et al.*, 2025 whom in their study in equally reported high population of similar Enterobacteriaceae. The detection of all these pathogens, particularly *Salmonella* and *Escherichia*, which are known to cause waterborne diseases, suggests that the water quality is compromised and may pose health risks to humans and wildlife.

The variability in MPN test results at different locations of the midstream may be attributed to Multiple sources of contamination with different input levels, Intermittent contamination events or variable flow rates, inconsistent mixing or dispersion of contaminants in the water, Sampling methodology or laboratory variability. The 95% confidence limits of 14 (Lower) and 230 (Upper) indicate a wide range of possible bacterial counts, suggesting significant uncertainty in the true level of contamination. This uncertainty may be due to the variable nature of the contamination sources or sampling methodology.

This variability in MPN test result at different locations of the downstream may be attributed to intermittent or fluctuating input of fecal contamination from upstream sources, inconsistent mixing or dispersion of contaminants in the water, Multiple sources of contamination with different input levels or frequencies Sampling methodology or laboratory variability. The wide 95% confidence interval (30 to 380) suggests significant uncertainty in the true level of contamination, reflecting the variable nature of the contamination sources or sampling methodology. This uncertainty highlights the need for additional sampling and analysis to better characterize the contamination and inform effective management strategies.

The severe contamination downstream poses a significant risk to public health, particularly for individuals engaging in recreational activities or consuming water from this source. The results suggest that immediate attention is required to identify and mitigate the sources of contamination, implement appropriate water treatment and sanitation measures, ensure the safety of the water supply for human consumption and recreational use.

The MPN determination for the dry season shows a pattern of increasing coliform contamination from upstream to downstream sections of Agangwu Stream. Contamination levels are generally lower in the dry season compared to the wet season though the downstream samples still exhibit significant fecal pollution. This trend highlights the continuous impact of anthropogenic activities and runoff on water quality. The study emphasizes the need for improved water management and sanitation practices to mitigate health risks and ensure the safety of the stream for its users.

Overall, the molecular identification of these bacteria detected in the water samples provides valuable information for assessing the water quality and identifying potential sources of pollution, which is essential for implementing

effective water treatment and management strategies. The high percentage identity (ranging from 85.93% to 99.73%) indicates a strong match between the isolated DNA sequences and the known sequences in the GENE Bank database, confirming the presence of these microorganisms in the water samples. This also corresponds with results of the molecular identification of similar water samples done by Abana *et al.*, 2024., Oghonim *et al.*, 2026.

Conclusion

The bacterial analysis reveals the presence of several pathogenic bacteria, including *E. coli*, *Proteus*, *Serratia*, *Shigella*, *Salmonella*, and *Klebsiella*, in different sections of Agangwu Stream during the wet season. These findings indicate significant fecal contamination and the presence of opportunistic pathogens, posing health risks to the local population. This study underscores the need for regular monitoring, effective water treatment, and public health interventions to mitigate the risks associated with waterborne diseases. The results provide valuable data for developing strategies to improve the microbiological quality of the stream and ensure the safety of its users.

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