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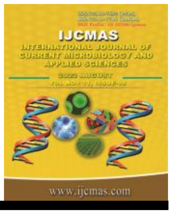

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Bacteriological Quality of Some Streams Used for Domestic Purposes in Ogoniland, Rivers State, Nigeria

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ABSTRACT

Water occupies an important place in the performance of many vital activities such as circulation, excretion, reproduction, and various other metabolic activities that take place in the body. This study aims to assess the bacteriological quality of surface water sources used for domestic purposes by rural communities in Khana Local Government Area (LGA), of Rivers State, Nigeria. The analysed streams include Bianu, Kaani-babbe, Kor, Kpong and Lubara. The water samples from the various streams were collected once a month for three months. Standard Microbiological procedures were performed to analyze for total heterotrophic bacteria, *Shigella-Salmonell*a, total and feacal coliform counts were determined using the most probable number (MPN) technique. The results of total heterotrophic bacterial counts ranged from; 1.47 ± 0.19 x 10^{7} cfu/ml to 4.60 ± 2.74 x 10^{7} CFU/ml. Total coliform counts ranged from 1.49 ± 0.50 x 10^{5}cfu/ml to 5.39 ± 2.03 x 10^{5} CFU/ml. faecal coliforms counts ranged from 0.00 ± 0.00 to 4.05 ± 2.16 x 10^{3} CFU/ml. *Salmonella-Shigella* counts ranged from 2.23 ± 1.4 ×10^{3}CFU/ml to 4.03 ± 1.77 × 10^{3} CFU/ml. The mean range of coliform bacteria for MPN ranged from 1.08 ± 9.08 to 1.60 ± 00MPN/100ml. Faecal coliform bacteria ranged from 3.9 ± 3.6 to 8.66 ± 6.35 MPN/100ml. Thirty- nine (39) bacterial species belonging to thirteen (13) genera were identified, which include: *Staphylococcus aureus* (7.7%), *Klebsiella* (2.6%), *Proteus* (7.7%), *Bacillus* (12.8%), *Salmonella* (5.1%), *Serratia* (10.3%), *Pseudomonas* (10.3%), *Escherichia coli* (23.0%), *Enterobacter* (5.1 %), *Shigella* (2.6%), *Flavobacterium* (5.1 %), *Chromobacterium* (2.6%) and *Micrococcus* (5.1%). The water from these sources is not safe for drinking and for other domestic uses because of its low-quality thresholds. Therefore, the water sources require further purification to ensure its potability for domestic uses, and there is an urgent need for a safe drinking water supply in the area, to prevent outbreaks of waterborne diseases in these communities.

Keywords

Surface water, stream, bacteria, coliform, Ogoniland

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Introduction

Water is of great importance and plays a very crucial role in all living things and some of the major functions in man's body include; transportation and dissolution of nutrients, minerals and vitamins, helping to regulate body temperature etc (Bain *et al.*, 2018). Water, forms the main component of carbohydrates, fats, and proteins in the human body (Zeyneb, 2020). In addition to all these, water occupies an important place in the performance of many vital activities such as circulation, excretion and reproduction, and 80-90% of our blood and 75% of our muscles are made up of water (Zeyneb, 2020). Water is an essential element of life and we feel uncomfortable when we are dehydrated even for a short time. Unavailability of water in terms of quality and quantity has been a major public health concern in the Niger Delta (Bain *et al.*, 2018).

The physical, chemical and microbiological characteristics of drinking water play an essential role in the health industries (Niru *et al.*, 2020). The importance of water for the existence of life is under constant threat due to increased human activities and advocacy for good water quality (Roy and Majumder, 2017).

Health risks associated with water contact activities, such as washing, swimming, include infection with harmful pathogenic bacteria that can be harmful to health (Roy and Majumder, 2017). Water is one of the compounds in the reproduction and transmission of bacteria associated with humans (Matta and Kumar, 2017). Drinking water is a basic human right and if it is contaminated with pathogenic bacteria, it can have significant negative impacts on public health (Matta and Kumar, 2017).

Thus, water pollution is the introduction of substances into the water body that generally renders water unfit for consumption (Obire *et al.*, 2017). Natural activities that can affect water quality include run-off, erosion, defoliation, and flooding, which render water unfit for consumption (Pal *et al.*, 2018). Human activities that could affect the water quality include the discharge of untreated effluents into the water bodies, the release of feacal materials directly or indirectly into the water, the release of domestic wastes and other activities carried out by man (Douglas and Longjohn, 2022). Surface water may habour human bacterial pathogens, particularly members of the coliform group (Kukrer and Mutlu, 2019).

The stream is a body of water (Kachroud *et al.*, 2019), with surface waters flowing at the bottom and banks of the channel. The flow is controlled by three inputs: surface water, groundwater, and subsurface water. Surface and groundwater are highly variable between periods of precipitation. On the other hand, subsurface water has a relatively constant input and is more controlled by long-term precipitation patterns.

The stream is a major source of water for Bianu, Kpong, Kor, Lubara and Kaani-babbe with other rural communities in Khana LGA of Rivers State. Therefore, it becomes very important to regularly monitor the water quality of these rural water supplies to further establish if there are pollution problems.

Materials and Methods

Description of Study Area

The study locations were Kaani-babbe, Bianu, Kor, Kpong, and Lubara, all situated in Khana Local Government Area of Rivers State in the Niger Delta region of Nigeria. Figure 1 is the map showing the sampling locations.

Sample Collection

A total of forty-five (45) water samples were aseptically collected from the five streams in various communities in Khana Local Government Area of Rivers State. The samples were collected in sterile containers and transported immediately in ice-packed containers to the laboratory for analysis.

Samples were collected for the study from the five different communities' stream once a month for the period of three months (June-August, 2021), and the samples were collected at 3 different points along the stream. Point 1(downstream/point where women access the water), point 2 (mid-stream/ point where men access the water) and point 3 (upstream of the other points, where they fetch the water for drinking).

Bacteriological Analysis

Water samples were analyzed for Total heterotrophic bacteria (THB), total coliform bacteria (TCB), feacal coliform (FC), and *Shigella-Salmonella* (SS). The media for bacteriology were prepared according to manufacturers' standards. The following media were used; Nutrient agar, Salmonella-Shigella agar, MacConkey Agar and broth. All plates were inoculated and incubated at 37°C for THB, SS, TCB and FC at 44.5°C for 24hours. Discrete colonies which emerge were counted, recorded and sub-cultured to purify them. Pure cultures were stored in 6% glycerol for further use. Isolates were identified and characterized morphologically and biochemically. Gram staining, motility test and the following biochemical tests were carried out; catalase, oxidase, indole, urease, methyl red, Voges-Proskauer, citrate, sugar fermentation, coagulase, starch hydrolysis, haemolysis, triple sugar fermentation (Cheesbrough, 2005). Identification of the bacterial isolates was done with reference to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Results and Discussion

Results of the bacterial counts of point 1 which represent the downstream/point where women accessed the stream, point 2 which represents mid-stream/where men accessed, and point 3 which represents the upstream/ drinking point of the respective streams are presented in Table 1. Results showed that the total heterotrophic bacterial counts ranged from 2.8×10^7 to 2.1×10^8 CFU/ml, total coliform counts ranged from 1.9×10^4 to 9.8×10^4 CFU/ml, *Salmonella-Shigella* counts ranged from 0.0 to 5.7×10^3 CFU/ml, and faecal coliform counts of the stream ranged from 0.0 to 8.6×10^3 CFU/ml, respectively. Results showed that the counts at points 1, 2 and 3 of the stream varied across the streams but despite this disparity in counts, there was no significant difference ($p>0.05$) in the total heterotrophic bacterial counts. The results further showed that the total heterotrophic bacterial counts of point 1 of the Kor stream 1, is 1.4×10^8CFU/ml, which is higher than the Bianu 1, 1.0×10^8CFU/ml, Kaaani-babbe 1, is 5.3×10^7CFU/ml, Kpong 1; is 8.3×10^7CFU/ml and Lubara 1; is 2.8×10^7CFU/ml. Moreover, the total heterotrophic bacterial, total coliform, *Salmonella-Shigella* and faecal coliform counts of point 1 of all the streams were higher than those recorded from points 2 and 3 of the streams.

The results of the bacterial population of the water samples are presented in Table 1. Results showed that the mean counts of the total heterotrophic bacteria ranged from $1.4\pm0.19\times10^7$ to $4.60\pm2.74\times10^7$CFU/ml, coliform counts ranged from $1.49\pm0.50\times10^5$ to $5.39\pm2.03\times10^5$CFU/ml, *Salmonella-Shigella* counts ranged from $2.23\pm1.4\times10^3$ to $4.03\pm1.77\times10^3$CFU/ml and faecal coliform counts ranged from $0.00\pm0.00\times10^3$ to $4.05\pm2.16\times10^3$CFU/ml. Results of the mean counts also showed that the water station with the highest total heterotrophic bacterial count was the Lubara stream $4.60\pm2.74\times10^7$CFU/ml. While the least total heterotrophic bacterial count was recorded in the Kaaani-babbe stream $1.41\pm0.19\times10^7$CFU/ml. The results of the mean counts of coliform bacteria also showed that the stream water samples from Kor had the highest counts of $5.39\pm2.03\times10^5$CFU/ml; followed by Lubara $4.87\pm2.88\times10^5$cfu/ml and Kpong stations $4.16\pm2.00\times10^5$CFU/ml while Kaani-babbe stations had the least count of $1.49\pm0.50\times10^5$CFU/ml.

In *Salmonella-Shigella* counts, the stream samples from Bianu stations had the highest counts of $4.03\pm1.77\times10^3$ CFU/ml followed by Kaani-babbe $3.67\pm1.18\times10^3$ CFU/ml and Kor $3.40\pm1.79\times10^3$ CFU/ml. While Lubara had $2.90\pm2.85\times10^3$ CFU/ml and Kpong had the least counts of $2.23\pm1.41\times10^3$ CFU/ml. Results also showed that there were no detectable faecal coliforms in stream water samples obtained from Kaaani-babbe from the 3 points while there were faecal coliforms in other samples with Bianu having the highest faecal coliform counts.

Results for the mean range of the MPN/100 index are presented in Table 3. Results showed the presence of faecal coliforms in all the streams even though the coliform index of the Lubara stream was the least ≥1075.65±908.172 and Kor and Kpong had the highest ≥1600.00±0.00. The total coliform/100ml of Kor and Kpong were ≥1600.00 ±0.00, while Kaani-babbe was ≥1233.33±635.085, and Bianu was ≥11146.67±785.19, and Lubara which is the least was ≥1075.65±908.172. For the faecal coliform/100ml Kpong had the highest ≥1366.67±404.14 followed by Bianu with ≥1110.00 ±848.71 followed by Kor with ≥1069.33±919.14 and Kaani-babbe ≥866.67±635.08 and the least was Lubara ≥39.33±36.11. These counts indicate that the water samples were highly contaminated with feacal matters, for the months of June, July and August, 2021,

After subjecting the bacterial isolates to morphological and biochemical tests, results showed that they belonged to the following species; *Micrococcus* sp, *Pseudomonas* sp, *Bacillus* sp, *Staphylococcus aureus*, *Enterobacter* sp, *Shigella* sp, *Salmonella* sp, *Escherichia coli*, *Proteus* sp, *Flavobacterium* sp *Serratia* sp, *Klebsiella* sp and *Chromobacterium* sp.

Results of the distribution of bacterial isolates across the stream are presented in Table 4. Results showed that *Escherichia coli*, *Staphylococcus* sp, *Bacillus* sp and *Pseudomonas* sp were isolated from all the streams. *Flavobacterium* sp was only isolated from the Lubara stream. *Shigella* spp was isolated from Bianu and Lubara streams. *Salmonella* sp was isolated only from Kaaani-babbe while *Proteus* sp was isolated from Lubara and Kor streams. *Klebsiella* sp was isolated from Kaaani-babbe and Kpong streams. *Serratia* sp was isolated from Bianu and Kpong streams. *Enterobacter* sp was isolated from Bianu, Kor and Kaani-babbe streams. *Micrococcus was* isolated from Bianu, Kaani-babbe and Kpong streams. While *Chromobacterium* sp was isolated from Kaani-babbe and Lubara streams. Results of the percentage occurrence are presented in Figure 1. Results showed that the percentage occurrence of *Escherichia coli* is 23.0%, *Flavobacterium* 5.1%, *Enterobacter* 5.1%, *Pseudomonas* 10.3%, *Shigella* 2.6%, *Serratia* 10.3%, *Klebsiella* 2.6%, *Chromobacterium* 2.6%, *Proteus* 7.7%, *Salmonella* 5.1%, *Micrococcus* 5.1%, *Staphylococcus* 7.7% and *Bacillus* 12.8% and 13 bacterial isolates were identified across the five (5) streams.

The bacteriological assessment of water quality is a vital tool in determining the purity or potability of water (WHO, 2011). The results of bacteriological quality of the five streams in Khana Local Government Area showed that all the streams had very high counts of total heterotrophic bacterial which exceeded the 100cfu/ml recommended by the WHO for surface water (WHO, 2016). The high bacterial load in these streams could be due to contamination from external sources such as runoffs from surface soil and vegetation, anthropogenic activities such as swimming, and disposal of untreated sewage and wastes directly into the water (Douglas and Isor 2015; Douglas and Longjohn, 2022). The results of the total heterotrophic bacterial counts of the various streams showed disparity across the streams and these changes or fluctuations in bacterial load could be due to the anthropogenic activities going on around the streams and the deterioration of the water (Bartram *et al.*, 2003).

It has been reported in a previous study that significant fluctuations in total heterotrophic bacterial counts serve as a warning indicator that water quality may be deteriorating (Bartram *et al.*, 2003). Although, previous studies on surface waters have reported higher counts in the total heterotrophic bacteria, which agreed with this present study. The counts of $2.2x10^4$ to $2.5x10^7$ CFU/ml were reported by Douglas and Isor, (2015) in pond water in Ogoni land while total heterotrophic bacterial counts of 4.77±0.20log10 to 4.92±0.11log10 CFU/ml were reported by Obire, (2017).

Fig.1 Map of Sampling Locations

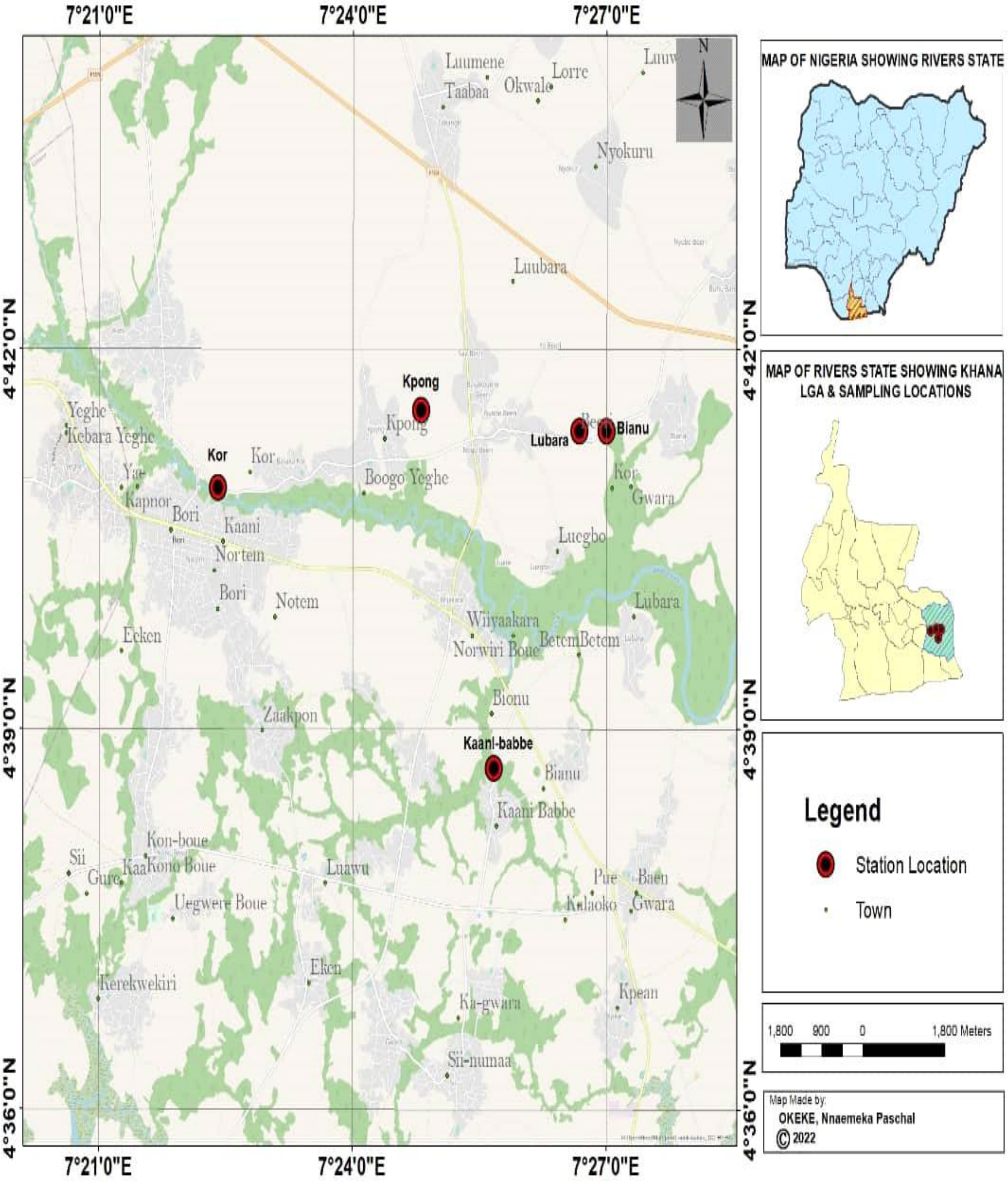


Table.1 Bacterial Counts (CFU/ml) at the different Points along the Streams

Station	THB	TCC	SSA	FC
Bianu 1	$1.0\times10^{8\ a}$	$9.2\times10^{4\ a}$	$2.8\times10^{3\ a}$	$8.6\times10^{3\ a}$
Bianu 2	1.2×10^{8}	$3.1\times10^{4\ a}$	$9.3\times10^{2\ a}$	$0.0^{\ b}$
Bianu 3	$9.13\times10^{7\ a}$	$3.9\times10^{4\ a}$	0.0	$0.0^{\ b}$
Kaaani-babbe 1	$5.3\times10^{7\ a}$	$4.7\times10^{4\ a}$	$5.3\times10^{3\ a}$	$0.0^{\ b}$
Kaaani-babbe 2	$3.7\times10^{7\ a}$	$6.7\times10^{4\ a}$	$5.7\times10^{3\ a}$	$0.0^{\ b}$
Kaaani-babbe 3	$8.2\times10^{7\ a}$	$6.2\times10^{4\ a}$	$0.0^{\ b}$	$0.0^{\ b}$
Kor 1	$1.4\times10^{8\ a}$	$6.0\times10^{4\ a}$	$7.5\times10^{3\ a}$	$1.0\times10^{4\ a}$
Kor 2	$7.7\times10^{7\ a}$	$1.9\times10^{4\ a}$	$2.7\times10^{3\ a}$	$0.0^{\ b}$
Kor 3	$1.1\times10^{8\ a}$	$3.1\times10^{4\ a}$	$0.0^{\ b}$	$0.0^{\ b}$
Kpong 1	$8.3\times10^{7\ a}$	$4.1\times10^{4\ a}$	$4.8\times10^{3\ a}$	$2.6\times10^{4\ a}$
Kpong 2	$1.7\times10^{8\ a}$	$9.8\times10^{4\ a}$	1.9×10^{3} a	$0.0^{\ b}$
Kpong 3	$5.7\times10^{7\ a}$	$5.8\times10^{4\ a}$	$0.0^{\ b}$	$0.0^{\ b}$
Lubara 1	$2.8\times10^{7\ a}$	$2.4\times10^{4\ a}$	$3.0\times10^{3\ a}$	$5.3\times10^{3\ a}$
Lubara 2	$2.1\times10^{8\ a}$	$8.2\times10^{4\ a}$	$5.7\times10^{3\ a}$	$0.0^{\ b}$
Lubara 3	$2.0\times10^{8\ a}$	$6.9\times10^{4\ a}$	$0.0^{\ b}$	$0.0^{\ b}$
P-value	0.21	0.80	0.02	0.31

Means that do not share a letter are significantly different ($P \leq 0.05$)
Keys: 1 = female, 2 = male, 3 =drinking water point.

Table.2 Mean Bacterial Counts (CFU/ml) of the Streams

Stations	**THB ($\times10^{7}$/CFU/ml)**	**TCC($\times10^{5}$/CFU/ml)**	**SSA ($\times10^{3}$/CFU/ml)**	**FC ($\times10^{3}$/CFU/ml)**
Bianu	2.85 ± 1.19^{a}	2.81 ± 1.70^{a}	4.03 ± 1.77^{a}	4.05 ± 2.16^{a}
Kaaani-babbe	1.41 ± 0.19^{a}	1.49 ± 0.50^{a}	3.67 ± 1.18^{a}	0.00 ± 0.00^{a}
Kor	3.15 ± 1.06^{a}	5.39 ± 2.03^{a}	3.40 ± 1.79^{a}	1.70 ± 0.50^{a}
Kpong	3.11 ± 1.79^{a}	4.16 ± 2.00^{a}	2.23 ± 1.41^{a}	1.86 ± 0.50^{a}
Lubara	4.60 ± 2.74^{a}	4.87 ± 2.88^{a}	2.90 ± 2.85^{a}	1.76 ± 0.05^{a}
P-value	0.244	0.517	0.972	0.160

*Means with the same alphabets shows no significant difference ($P\geq0.05$)
Keys: THB = total heterotrophic bacteria, TCC = total coliform counts, SSA = Salmonella-Shigella counts, FC =faecal coliform

Table.3 Results of Total coliform using MPN (MPN Index/100ml)

Sampled Locations	**TC (37°C) MPN Index/100ml**	**FC (44.5°C) MPN Index/100ml**
Bianu	$\geq1146.67\pm785.19$	$\geq1110.00\pm848.71$
Kaani-babbe	$\geq1233.33\pm635.085$	$\geq866.67\pm635.08$
Kor	$\geq1600.00\pm0.00$	$\geq1069.33\pm919.14$
Kpong	$\geq1600.00\pm.000$	$\geq1366.67\pm404.14$
Lubara	$\geq1075.67\pm908.172$	$\geq39.33\pm36.11$

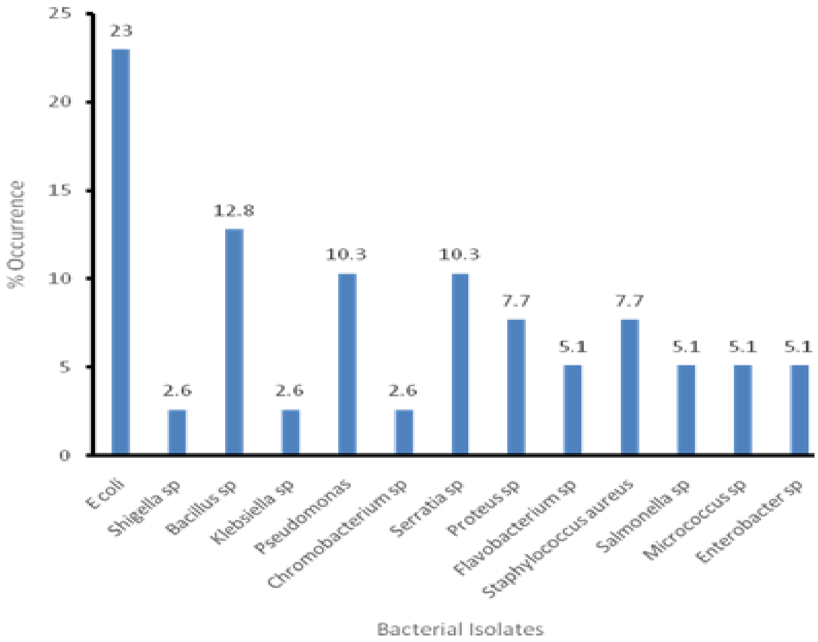
Keys: TC (Total Coliform), FC (Feacal Coliform), MPN (Most Probable Number).
T-test shows no significant difference ($p\geq0.05$) in the MPN Values

Table.4 Distribution of Bacterial Isolates from the Five Stream Samples

Isolates	**Bianu**	**Kaaani-babbe**	**Kpong**	**Kor**	**Lubara**
Escherichia coli	+	+	+	+	+
Flavobacterium sp	-	-	-	-	+
Enterobacter sp	+	+	-	+	-
Pseudomonas sp	+	+	+	+	+
Shigella sp	+	-	-	-	+
Serratia sp	+	-	+	-	-
Klebsiella sp	-	+	+	-	-
Chromobacterium sp	-	+	-	-	+
Proteus sp	-	-	-	+	+
Salmonella sp	-	+	-	-	-
Micrococcus sp	+	+	+	-	-
Staphylococcus aureus	+	+	+	+	+
Bacillus sp	+	+	+	+	+
Total bacteria isolated from each stream	8	9	7	6	8

Keys: + = isolated; - = not isolated

Fig.2 Percentage Occurrence of Bacterial Isolates identified from the samples



Despite the high counts of total heterotrophic bacterial counts in the present study, the total heterotrophic bacterial counts of 1.24×10^9 CFU/ml to 5.53×10^9cfu/ml were reported by Obire and Abigail (2015) from a hand dug well, were higher than those reported in the present study. Despite the variations in the total heterotrophic bacterial counts of the various streams, there was no significant difference ($P\geq0.05$).

The results for the coliform counts showed detectable numbers which could imply that the streams were heavily polluted with feacal matters. The coliform counts were higher than the acceptable limits of coliform in drinking. Women's point which is point 1/downstream of the streams showed higher counts than the men's point 2 and drinking points of the streams which could be attributed to its usage, frequency of visitation and closeness with the vegetation. It could also be attributed to it being the first point of contact, where women wash and take their bath. This could be the reason why water fetchers go beyond this point before they could fetch the water based on the assumption that point 1 is usually more contaminated. The WHO recommended limit for the total coliform counts for water should not exceed 10 CFU/ml and that the faecal coliforms should be 0 in drinking water (WHO, 2010). Also, the results of the most probable number (MPN) techniques of the streams were very high and serve as an indicator of possible contamination of the streams with faecal matter from either human or animal sources. Coliforms are gram-negative non-spore-forming bacteria and lactose fermenters whose presence in water bodies represents contamination of water bodies with faecal particles of animal origin (Prescott *et al.,* 2011).

Moreover, the detection of coliforms in these streams could serve as an alarm for the presence of pathogenic microorganisms which implied that the streams are not safe for drinking. This study showed detectable numbers of *Escherichia coli*, *Salmonella* and *Shigella* counts. Thus, ingestion of water from these streams without treatment could lead to ingestion of pathogens which could be associated with the onset of diseases. The streams are readily used for drinking and other domestic and agricultural activities. According to Obire and Abigail (2015), majority of Nigeria's rural population lacks access to potable water, relying instead on well, stream, and river water for residential purposes.

The bacterial genera identified from this study include: *Micrococcus, Pseudomonas, Bacillus, Staphylococcus aureus, Enterobacter, Shigella, Salmonella, Escherichia coli, Proteus, Flavobacterium, Serratia, Klebsiella,* and *Chromobacterium.* The isolates from the various streams have been reported in streams and other water bodies in previous studies. Douglas and Longjohn, (2022) reported the presence of *Bacillus, E. coli, Salmonella,* and *Klebsiella* in Bonny river, Rivers State which are similar to those identified in the present study. Although, *Staphylococcus, Enterococcus, Shigella, Proteus, Flavobacterium,* and *Serratia* identified in this study were not identified by Obire and Abigail, (2015). Furthermore, Obire *et al.,* (2021) also identified *Vibrio, Salmonella, Shigella, Serratia, Proteus, Providencia, Streptococcus, Enterobacter, Staphylococcus, Bacillus, Pseudomonas, Escherichia coli* and *Klebsiella* spp. from Azumini Odumaya stream, in Port Harcourt. Douglas and Isor, (2015) also reported the presence of *Staphylococcus, Enterobacter, Shigella, Salmonella, Klebsiella, Escherichia coli, Proteus, Bacillus, Micrococcus, Pseudomonas* and *Streptococcus* in pond water in Ogoniland which are also identified in this study except *for Streptococcus.* Research has shown that these microbes could pose serious public health problems, especially when consumed without proper processing (Douglas and Isor, 2015).

Also, the bacterial genera identified in this study have been reported in previous studies in underground and surface waters (Shittu *et al.,* 2008; Kolawole and Afolayan, 2017; Douglas and Longjohn, 2022; Iyerite *et al.,* 2021; Obire *et al.,* 2021). Diseases caused by bacterial isolates from these water samples may include: traveller's diarrhea, pneumonia, urinary tract infection, bacteremia, salmonellosis, typhoid fever, respiratory tract infections, gastroenteritis, septicamia, shigellosis, meningitis, and endocarditis (Douglas and Longjohn, 2022; Iyerite *et al.,* 2021; Obire *et al.,* 2021). The presence of these microorganisms in these streams is of public health concern as these bacterial genera could be pathogenic to those who drink the water, without any form of treatment. It is recommended to treat the water at least by boiling and periodic monitoring of the water body is important for early detection of pollution problems.

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