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Bacteriological Assessment of a Near-Dumpsite River in Obohia, Omoku, River State, Nigeria

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Abstract
Water samples were collected for bacteriological assessment of a river near a dumpsite in Obohia community, Omoku, Rivers State. The study was guided by four research objectives and corresponding research questions. To achieve these objectives, four freshwater samples were collected for bacteriological analysis from two sampling stations during both the wet and dry seasons. Results revealed the presence of coagulase-negative Staphylococcus, Staphylococcus aureus, Salmonella species, Escherichia coli, Klebsiella species, Proteus species, Shigella species, and Pseudomonas aeruginosa. Bacterial counts in the water samples exceeded the standard limits set by the World Health Organization (WHO) and the Environmental Protection Agency (EPA) for surface water. Seasonal variations were observed, with bacterial isolates totaling eleven during the wet season and eight during the dry season. It was recommended that appropriate water treatment measures be implemented, and regular pollution monitoring be conducted to prevent potential outbreaks of waterborne diseases in the community.

Keywords: Bacteria, Assessment, Dumpsite, River, Obohia

Introduction

Water is one of the most well-known and widely available chemical substances found naturally on the Earth's surface (Hemant et al., 2012). It is a key component of the biosphere and plays a vital role in sustaining all living organisms (WHO, 1996). As an essential natural resource, water supports the survival of plants, animals, and humans, serving as a universal solvent that influences numerous natural processes (Ngodi, 2011). Among the primary sources of freshwater for human use are rivers, which provide essential water for domestic, industrial, and agricultural activities. However, the introduction of foreign substances into water bodies can either contribute nutrients for aquatic microorganisms or lead to pollution (Boukori et al., 1999). Rivers often face contamination due to the indiscriminate disposal of sewage, untreated industrial waste, and various human activities, which alter their physiochemical and microbiological properties.

In developing nations, particularly Nigeria, potable water is highly susceptible to contamination from pollutants. Water pollution occurs when harmful substances that pose risks to human health and ecosystems enter rivers, lakes, and streams (Fagorite et al., 2019). According to the Food and Agriculture Organization (FAO), waterborne diseases have significantly hindered human development in several African nations, especially Nigeria (APHA Washington, 2005). The mismanagement of vast amounts of waste from human activities, along with the indiscriminate disposal of refuse, fecal matter, and agricultural and industrial contaminants, continues to pose a serious threat to aquatic ecosystems. The increasing discharge of untreated waste and rapid urbanization in many developing areas have led to the progressive deterioration of water bodies in recent years (Boss, 2003). Rivers are essential freshwater systems that sustain life and serve as critical conduits worldwide, providing primary water resources for domestic, industrial, and agricultural needs. They also play a key role in maintaining soil fertility, conserving wildlife, supporting forest resources, and serving as important transportation routes (Mamun & Zainudin, 2013). However, industrialization,

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urbanization, and the continuous expansion of economic activities have contributed to the degradation and pollution of natural resources on a global scale. Bacteriological studies have revealed contamination by microorganisms, leading to overgrowth that may facilitate the spread of plant and animal pathogens in water. Microbial pollution has been linked to the transmission of infectious diseases such as dysentery, cholera, diarrhea, typhoid, shigellosis, and salmonella, along with fungal, viral, and parasitic infections (Nwachukwu & Ume, 2013).

Waterborne bacteria and the diseases they cause pose significant public health challenges worldwide due to their high mortality rates and the costs associated with prevention and treatment. Effective assessment and continuous monitoring of water quality, particularly for bacterial indicators, are crucial in mitigating these risks. According to the Washington State Department of Health (2016), the presence of specific indicator organisms like E. coli in water is a sign of recent fecal contamination, suggesting a heightened risk of pathogen presence. These contaminants can often be traced through the water supply chain from their origin to the point of consumption (Ekwere et al., 2011). The sources of bacteria pollution in river water include agricultural runoff, municipal wastewater discharge, stormwater runoff, defective septic systems, and animal waste. These sources introduce pathogenic bacteria into freshwater ecosystems, thereby degrading water quality (Johnson & Bofinger, 2020).

In recent times, evaluating the bacteriological quality of water has become essential due to its direct impact on human health. Recognizing the connection between pollution and the critical need to safeguard public health, recreational activities, and fisheries production led to the early establishment of water quality regulations and monitoring systems (Anyanwu & Okoli, 2012). Indicator bacteria are microorganisms used to assess the quality, safety, and potential contamination of various environments, including water bodies. These bacteria act as markers for the presence of other potentially harmful microorganisms or overall sanitary conditions. Common examples include coliform bacteria and Enterococci. Among them, Escherichia coli (E. coli) is widely utilized as an indicator of fecal contamination in water. The presence of coliform bacteria, particularly E. coli, suggests the possible existence of enteric pathogens such as Salmonella, Shigella, and Vibrio, which are known to cause gastrointestinal illnesses (Odonkor & Ampofo, 2013).

Aim and Objectives of the Study

This study aims to evaluate the bacteriological quality of a selected river to determine the level of pollution and identify potential sources of contamination. Specifically, the research seeks to identify the bacterial species present in the river and assess whether the bacterial count falls within acceptable limits. To achieve this, the objectives of the study are to:

1. identify and characterize the bacterial species present in the river water using morphological features, Gram staining, and a series of biochemical and sugar fermentation tests.
2. determine the total viable heterotrophic and coliform bacterial counts in the river during both wet and dry seasons, and assess their variations by sampling location (mid-stream and downstream).
3. evaluate whether the observed bacterial loads in the river water are within tolerable or permissible limits for environmental and public health safety, particularly for potential domestic, recreational, or agricultural use.
4. compare seasonal variations in bacterial counts, highlighting the influence of rainfall (wet vs. dry season) on microbial contamination levels.

Materials and Methods

The research adopted an experimental design, in which controlled conditions were used to conduct the study. The experimental design consists of two times water sample collection for the dry season (February 2024) and wet season (June 2024). Water samples were collected from the central part of the river while facing the direction of the current. A total of four freshwater samples were taken from midstream and downstream locations to evaluate bacterial presence and concentration, providing insight into water quality and potential health risks associated with drinking and bathing near a dumpsite. For bacteriological analysis, samples were gathered from two stations during each season using sterilized white plastic containers with a two-liter capacity. The collection process involved wading into the river's midpoint, facing the flow, and carefully removing the container's lid before submerging it to a depth of 0.2 meters. The lid was then replaced immediately after filling. Each sample was placed in a separate, sterilized white plastic container, sealed in labeled black polystyrene bags, and promptly transported to the laboratory for analysis following standard procedures (APHA, 2000). A 0.85% sodium chloride solution (normal saline) was used to prepare bacterial

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suspensions, dilute samples, and serve as a transport medium for bacterial cultures. This solution helps maintain osmotic balance, preventing cell lysis or plasmolysis.

Results

Table 1- **Identification and characterization of bacterial isolates**

Gram Reaction			**Biochemical Test**									**Sugar Fermentation**				
Colonical feature	**Mizcroscopy**	**Cell arrangement**	**Catalases**	**Oxidase**	**Coaguloase**	**Indole**	**Citrate**	**Motility**	**Methyl red**	**Voyes-P**	**Urease**	**Glucose**	**Lactose**	**Mannitol**	**Sucrose**	**Suspected bacterial**
Wine colonies	Gram +ve	Cocci	+ve	-ve	–ve	-ve	+ve	-ve	+ve	-ve	+ve	A	NAG	NG A	NA G	*Coagulase negative Staphylococcus*
Golden yellow	Gram +ve	Cocci	+ve	-ve	+ve	–ve	+ve	–ve	+ve	–ve	+ve	AG	AG	AG	AG	*Staphylococcus aureus*
Black colonies	Gram -ve	Short rods	+ve	–ve	–ve	–ve	+ve	–ve	+ve	+ve	–ve	A	A	AG	AG	*Salmonella species*
Pink pigment	Gram -ve	Short rods	+ve	–ve	–ve	+ve	-ve	+ve	+ve	–ve	–ve	AG	AG	AG	A	*Escherichia coli*
Pale-pink Colonies	Gram -ve	Short rods	+ve	–ve	–ve	–ve	+ve	–ve	-ve	+ve	+ve	AG	AG	AG	AG	*Klebsiella species*
Swarming white colonies	Gram -ve	Short rods	+ve	–ve	–ve	–ve	+ve	+ve	+ve	–ve	+ve	A	A	NA G	AG	*Proteus species*
Brown colonies	Gram -ve	Short rods	+ve	+ve	–ve	+ve	–ve	-ve	+ve	-ve	–ve	NA G	NAG	A	A	*Shigella spp*
Creamy--grey Colonies	Gram -ve	Short rods	+ve	+ve	–ve	–ve	+ve	+ve	-ve	-ve	-ve	NA G	NAG	A	NA G	*Pseudomonas aeruginosa*

Keys: + = Positive, - = Negative, A = Acid production, AG = Acid and Gas production, NAG= No Acid and Gas production

The data presented in Table 1 outlines the bacterial species identified in the river, based on morphological characteristics, pigmentation on media, microscopy, and various biochemical tests. The isolates were analyzed using tests such as Gram staining, Citrate utilization, Motility test, Voges-Proskauer (VP) test, Methyl Red (MR) test, Indole production, Oxidase, Catalase, Urease, and Coagulase test, which were key in their identification. The results confirmed the presence of Coagulase-negative Staphylococcus, Staphylococcus aureus, Salmonella species, Escherichia coli, Klebsiella species, Proteus species, Shigella species, and Pseudomonas aeruginosa. All isolates were catalase positive and *Pseudomonas aeruginosa* and *Shigella species* were oxidase positive respectively. A motility test was applied to all isolated bacteria, *Pseudomonas aeruginosa, Escherichia coli,* and *Proteus species* are motile, while *Staphylococcus aureus* is only coagulase-positive isolates. *Escherichia coli* and *Shigella species* were indole-positive and citrate-negative. *Staphylococcus* aureus, *Coagulase negative Staphylococcus, Proteus species, Salmonella species, Escherichia coli,* and *Shigella species* were methyl red positive while *Salmonella species* and *Klebsiella pneumonia* were Voges-Proskauer positive. *Salmonella species, Escherichia* and *Pseudomonas aeruginosa, Shigella species* were urease positive, Staphylococcus *Aureus. Salmonella species, Klebsiella pneumonia, Escherichia Coli,* fermented Glucose, Lactose, Mannitol, and Sucrose with the production of acid and gas. While *Coagulase-*

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negative Staphylococcus were nonfermented Mannitol Sucrose and Lactose, *Shigella spp,* and *Pseudomonas aeruginosa* were non-fermented Lactose and glucose. Microscopy or gram staining reaction shows six-gram negative bacteria with rod shape and two-gram positives with cocci shape.

Table 2- Total Viable Heterotrophic Bacteria Counts (CFU/ML) of Water Samples

Season	Sample Source	Dilution Factor	Volume factor) /	No. of Colonies	CFU formula (No. of colonies x dilution volume	THPC (CFU/ml)	Average Count (CFU/ml)
Wet	Mid-stream	10^5	1	52	(52 * 10^5)/1	5.2 x 10^6	
		10^6	1	48	(48 * 10^6)/1	4.8 x 10^7	**26.6 x 10^6**
	Downstream	10^5	1	74	(74 * 10^5)/1	7.4 x 10^6	
		10^6	1	69	(69 * 10^6)/1	6.9 x 10^7	**38.2 x 10^6**
Dry	Mid-stream	10^5	1	37	(37 * 10^5)/1	3.7 x 10^6	
		10^6	1	31	(31 * 10^6)/1	3.1 x 10^7	**17.35 x 10^6**
	Downstream	10^5	1	65	(65 * 10^5)/1	6.5 x 10^6	
		10^6	1	50	(50 * 10^6)/1	5.0 x 10^7	**28.25 x 10^6**

Table 2 shows the total viable heterotrophic bacteria counts (CFU/ml) of water samples of the study area, results revealed that the wet season in the mid-stream has 52 and 48 number (no.) of colonies with average bacteriological counts of 26.6 x 10^6, While the dry season mid-stream has 37 and 31 number (no.) of colonies with the average bacteriological count range of 17.35 x 10^6. Therefore, the mid-stream for the wet season showed that the number (no.) of colonies and average bacteriological is higher than the mid-stream in the dry season. In the wet season, the downstream has 74 and 69 number (no.) colonies and an average bacteriological count range of 38.2 x 10^6 while the dry season water downstream has 65 and 50 no. of colonies and an average bacteriological count range of 28.25 x 10^6. Therefore, the downstream for the wet season has a higher number (no.) of colonies and average bacteriological count than the downstream for the dry season.

Table 3- Total Viable Coliform Bacteria Counts (CFU/Ml) of Water Samples

Season	Sample	Dilution Factor	Volume factor) /	No. of Colonies	CFU formula (No. of colonies x dilution volume	THPC (CFU/ml)	Average Count (CFU/ml)
Wet	Mid-stream	10^5	1	48	(48 * 10^5)/1	4.8 x 10^6	
		10^6	1	33	(33 * 10^6)/1	3.3 x 10^7	**18.9 x 10^6**
	Downstream	10^5	1	59	(29 * 10^5)/1	2.9 x 10^6	
		10^6	1	41	(41 * 10^6)/1	4.1 x 10^7	**21.95 x 10^6**
Dry	Mid-stream	10^5	1	28	(28 * 10^5)/1	2.8 x 10^6	
		10^6	1	22	(22 * 10^6)/1	2.2 x 10^7	**12.4 x 10^6**
	Downstream	10^5	1	68	(68 * 10^5)/1	6.8 x 10^6	
		10^6	1	72	(72* 10^6)/1	7.2 x 10^7	**39.4 x 10^6**

The results of total viable coliform bacteria count (CFU/ml) of water samples, as shown in Table 3, revealed that the wet season mid-stream sample has 48 and 33 number (no.) of colonies found with an average bacteriological count range of 18.9 x 10^6 while the dry season mid-stream has 28 and 22 number (no.) of colonies with average bacteriological count range of 12.4 x $10^{6.}$ Therefore, the mid-stream for the wet season shows that the number (no.) of colonies and average bacteriological count is higher than the mid-stream in the dry season. Also, in the wet season, the downstream has 59 and 41 number (no.) colonies with an average bacteriological count range of 21.95 x 10^6, while in the dry season, the downstream has 68 and 72 number (no.) colonies with average bacteriological count range of 39.4 x $10^{6.}$ Therefore, the downstream for the dry season has a higher number (no.) of colonies and average bacteriological count than the downstream for the wet season.

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Table 4- Total Viable *Staphylococcus species* Bacteria Counts (CFU/ml) of Water Samples

Season	Sample	Dilution Factor	Volume	No. of Colonies	CFU formula (No. of colonies x dilution factor) / volume	THPC (CFU/ml)	Average Count (CFU/ml)
Wet	Mid-stream	10^5	1	23	(23 * 10^5)/1	2.3 x 10^6	
		10^6	1	31	(31 * 10^6)/1	3.1 x 10^7	**16.65 x 10^6**
	Downstream	10^5	1	47	(47 * 10^5)/1	4.7 x 10^6	
		10^6	1	53	(53 * 10^6)/1	5.3 x 10^7	**28.85 x 10^6**
Dry	Mid-stream	10^5	1	19	(19 * 10^5)/1	1.9 x 10^6	
		10^6	1	26	(28 * 10^6)/1	2.8 x 10^7	**14.95 x 10^6**
	Downstream	10^5	1	54	(54 * 10^5)/1	5.4 x 10^6	
		10^6	1	61	(61 * 10^6)/1	6.1 x 10^7	**33.2 x 10^6**

Table 4 results on total viable staphylococcus species bacteria counts (CFU/ml) of water samples revealed that in the wet season, the mid-stream has 23 and 31 number (no.) colonies found with an average bacteriological count of 16.65 x $10^{6,}$ while the dry season the mid-stream has 19 and 26 number (no.) of colonies found with an average bacteriological count of 14.95 x 10^6. Therefore, the mid-stream for the wet season is higher than the midstream for the dry season. In the wet season, the downstream has 47 and 53 number (no.) colonies with an average bacteriological count range of 28.85 x 10^6, while in the dry season, the downstream has 54 and 61 number (no.) colonies with an average bacteriological count range of 33.2 x 10^6. Therefore, the downstream for the dry season is higher than the downstream for the wet season.

Table 5- Total Viable *Salmonella Shigella* Species Bacteria Count (CFU/ml) of Water Samples

Season	Sample	Dilution Factor	Volume	No. of Colonies	CFU formula (No. of colonies x dilution factor) / volume	THPC (CFU/ml)	Average Count (CFU/ml)
Wet	Mid-stream	10^5	1	0	(0 * 10^5)/1	No Growth	
		10^6	1	0	(0 * 10^6)/1	No Growth	No Growth
	Downstream	10^5	1	17	(17 * 10^5)/1	1.7 x 10^6	
		10^6	1	12	(12 * 10^6)/1	1.2 x 10^7	**6.85 x 10^6**
Dry	Mid-stream	10^5	1	0	(0 * 10^5)/1	No Growth	
		10^6	1	0	(0 * 10^6/1	No Growth	No Growth
	Downstream	10^5	1	9	(9 * 10^5)/1	9.0 x 10^6	
		10^6	1	5	(5 * 10^6)/1	5.0 x 10^7	**2.95 x 10^6**

Table 5 shows the total *Salmonella shigella* species bacteria counts (CFU/ml). Wet season and dry season in the mid-stream showed zero (0) colonies, with average bacteriological counts of zero (0) indicating no growth. During the wet season, downstream has 17 and 12 number (no.) colonies with an average bacteriological count range of 6.85 x 10^6 while in the dry season, the downstream has 9 and 5 number (no.) colonies with an average bacteriological count range of 2.95 x 10^6. Therefore, the downstream for the wet season is higher than the downstream for the dry season. In general, downstream water samples showed maximum bacteriological colony counts compared to midstream water samples.

Discussion

The findings of this study identified a total of eight bacterial species, comprising both Gram-positive and Gram-negative bacteria. According to Ike (2021), the most frequently occurring bacterial genera included Vibrio species, Salmonella species, and Escherichia coli. However, in this study, Escherichia coli and Staphylococcus aureus were the most prevalent species detected in the sampled river. These results align with previous research, which has also

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reported the presence of these bacterial species in river water (Smith, 2019). In contrast, Johnson (2020) identified Bacillus subtilis as one of the dominant species in river water in Europe.

Similarly, Asionye (2020) documented the presence of Bacillus subtilis, Vibrio species, Escherichia coli, Salmonella species, Proteus species, Klebsiella species, and Enterobacter species in river water, some of which were also detected in this study. The findings also correspond with research conducted by Jimoh and Olatunji (2021), which confirmed the presence of similar bacterial species in another river.

The identified Gram-positive bacteria, including Coagulase-negative Staphylococcus and Staphylococcus aureus, were present in significant numbers, aligning with a study by Davis et al. (2018), who also reported Staphylococcus aureus in river water. Additionally, previous studies by Kim (2020) and Patel (2019) highlighted the presence of Gram-negative bacteria in river bodies, which supports this study's findings of Salmonella species, Escherichia coli, Klebsiella species, Proteus species, Shigella species, and Pseudomonas aeruginosa. The Environmental Protection Agency (EPA) set heterotrophic plate count (HPC) as a primary standard due to its relevance to public health. HPC is used to measure a variety of bacteria naturally present in the environment (EPA, 2012). The total bacterial counts observed in all water samples were significantly high, surpassing the acceptable limit of 1.0×10^2 cfu/ml, which is the standard threshold for drinking water (EPA, 2012). The elevated heterotrophic count suggests a high presence of organic matter and dissolved salts in the water. Jimoh and Olatunji (2021), who conducted a similar study on heterotrophic plate counts, reported values ranging from 1.2×10^4 to 7.8×10^4 cfu/ml, while total coliform counts varied between 4.0×10^2 and 1.0×10^4 cfu/100ml, some of which exceeded the limits set by the World Health Organization (WHO) for river water. Likewise, research by Olatunji and Anani (2020) found bacterial counts exceeding WHO's recommended safe limits. These studies are in agreement with the findings of this research.

The EPA maximum contamination level (MCL) for coliform bacteria in drinking water is zero total coliform per 100ml of water while for recreational water the EPA recommends that *E. coli* levels should not exceed 126 Cfu/100ml as a geometric mean over 30 days and a single sample maximum should not exceed 235 Cfu/100ml for *E. coli* (EPA, 2012). The high coliform count obtained in the samples may be an indication that the water sources are faecally contaminated (Osunde & Eneuzie, 2014). According to Adieze et al. (2016), high counts of bacterial load reflect the level of water pollution as it indicates the amount of organic matter present. Likewise, Ekhaise and Omoigberale (2011) reported higher bacterial counts than the acceptable limit of the WHO standards, these studies along with present results support the notion that the bacterial load in Obohia River exceeded the WHO (2017) standard limits. According to EPA standards, any water sample containing coliform bacteria must be tested for Escherichia coli to determine contamination from human or animal waste (EPA, 2012). The presence of Escherichia coli, Staphylococcus aureus, and Shigella species in the analyzed water samples does not align with EPA standards for recreational water use, such as swimming or bathing. These pathogens pose a significant public health risk when present in substantial amounts. Salmonella species are of particular concern, as they can cause gastrointestinal infections, including typhoid fever, diarrhea, and dysentery, even at low exposure levels.

Other bacterial species isolated from the samples, including Klebsiella species, Proteus species, Pseudomonas aeruginosa, Salmonella species, and coagulase-negative Staphylococcus, also have public health implications (EPA, 2012). Findings from Olatunji et al. (2011), Esharegoma et al. (2018) reported higher bacterial counts in river samples during the wet season compared to the dry season. This pattern, which aligns with the present study, is likely due to increased runoff carrying nutrients and debris from surrounding areas into the water during rainfall.

Conclusion

The findings of this study on bacteriological assessment indicate that the river may not be suitable for human consumption, bathing, or other uses. The presence of bacteria species of public health concern was detected in both the wet and dry seasons, with bacterial counts exceeding the EPA and WHO standard limits for river water.

Recommendations

Based on these findings, the researcher recommended that:

1. appropriate water treatment processes, such as filtration, disinfection, or other effective technologies, be implemented to remove or inactivate harmful bacteria.

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2. regular bacteriological assessments should be conducted to monitor water quality and track any changes or trends in bacterial contamination levels.

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