



Bacteriological Quality Determination of Borehole Water Supply in Hostels of Rivers State University Main Campus, Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Water serves as a vehicle for the transmission of various diseases to man and animals, and have been associated with both acute and chronic health syndromes. This study was carried out to determine the bacteriological quality of borehole water supply in the Hostels of Rivers State University, Port Harcourt, Nigeria. Borehole water samples were collected from six (6) hostels for

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each study period, and subjected to standard bacteriological Procedures. Results obtained indicated that the highest mean total heterotrophic bacterial count of $3.6 \pm 1.7 \times 10^5$ cfu/ml was recorded in the Hostel C water sample, while the least mean bacterial count of $5.5 \pm 0.35 \times 10^4$ cfu/ml was observed in FCMB Hostel. The total coliform count had the highest value ($3.3 \pm 3.82 \times 10^4$ cfu/ml) recorded in Hostel D and the lowest value of $2.0 \pm 0.14 \times 10^3$ cfu/ml was obtained in the Hostel H water sample. The faecal coliform count recorded the highest value in FCMB Hostel ($1.5 \pm 0.71 \times 10^3$ cfu/ml) and lowest in Hostel H ($8.5 \pm 4.95 \times 10^3$ cfu/ml). The staphylococcal count was highest in Hostel B ($8 \pm 9.9 \times 10^4$ cfu/ml) and lowest in Hostel C ($0.6 \pm 0.57 \times 10^4$ cfu/ml). Six bacterial species were identified, which included *Bacillus* spp (100%), *Staphylococcus* spp (100%), *Pseudomonas* spp (83.3%), *Klebsiella* spp. (66.6%), *Salmonella* spp (16.6%) and *Escherichia coli* (16.6%). Data obtained showed that all the isolates were biofilm producers, albeit at varying percentages, as 52.9%, 55.5%, 60%, 30%, 33.3%, and 100% of *Bacillus* spp, *Staphylococcus* spp., *Pseudomonas* spp., *Klebsiella* spp., *Salmonella* spp. and *Escherichia coli*, respectively were positive for biofilm formation. The hemolysis result indicated that 28.3%, 10%, and 61.7% of the isolates were alpha, beta and gamma hemolytic, respectively. The study indicated the presence of bacteria of public health importance, thereby, making the need for regular monitoring and treatment of these water sources very necessary to check bacterial proliferation and ensure continuous access to safe water in the hostels.

Keywords: Bacteriology; biofilm; borehole; hemolysis; hostels; Rivers State University; water.

1. INTRODUCTION

Water is involved in every bodily function for circulation, digestion and control of body temperature, as well as the excretion of waste. It is the only inorganic compound that appears in solid, liquid and gaseous physical state under normal conditions [1]. One cannot underestimate the significance of water to living things. Man can survive longer without food for two or more days than without water. Man requires water for different uses, including domestic activities like cooking, drinking, washing, sanitation, and for growing his crops and running his factories. Consequently, modern man like his primitive ancestors, depends heavily on water for his sustenance due to its availability. This abundance has been abused and taken for granted recently, due to unnecessary pollution impact resulting from man's daily activities. Aside from the industrial usage of water and recreational purposes, good water quality and water sanitation help to eradicate waterborne and water related diseases and ensure water use efficiencies, including the aesthetic quality of the environment [2].

The availability of useable water is threatened by several factors influencing the quality of water for human use. These factors range from physicochemical, bacteriological, heavy metals, industrial, domestic, agricultural, urbanization, constructional activities among others. Most of these factors are more of anthropogenic origin than nature and thus exercise an untold health effect on the end-users of such water bodies.

Bacteriological contaminants have however, found their way into water supplies due to deficiencies in waste management (inadequate treatment and improper disposal of the different types of wastes), agricultural practices (lives-tocks and human wastes, storage, pesticides, etc.), industrial discharges (air pollutants of various sizes and sources), and the over-use of limited water resources [3].

In Rivers State University, the main source of water for students' utilization is groundwater source (borehole water). This water passes through several networks of pipes to the various hostels. During this process, water can be interfered by contaminants or pollutants, and thus could affect the public health status of the hostel dwellers who make use of this water day in day out. Many infectious diseases could be transmitted by water through the fecal-oral route and so many other routes. However, because of the great importance of water in man's existence, it has become very domineering and thorough that the bacteriological quality of water be ascertained, since the general quality of water influences the health of any populace [4]. In a hostel or dormitory environment, different students with different approaches to water sanitation, due to their different background. All these account for the quality of water for human consumption, and has contributed to the increasing burden of waterborne and water related diseases in Nigeria. Campaigns for water, sanitation, and hygiene have therefore been intensified in rural communities, cities as well as the university community to prevent outbreak of

waterborne diseases. Various studies have however, been carried out on the quality of borehole water reticulated in hostels within Rivers University, Nigeria, and have documented the contribution of both biological and physicochemical variables in the quality of water available for students' use [5,6]. This study was therefore conducted to ascertain the current bacteriological quality of borehole water supply in hostels of Rivers State University, Port Harcourt, Nigeria. Findings from this study will provide necessary information regarding the current status and associated bacteriological risk of borehole water sources accessible to students residing in the various hostels of the university.

2. MATERIALS AND METHODS

2.1 Study Area

The study area, Rivers State University (RSU) formally called the Rivers State University of Science and Technology (RSUST) Port Harcourt, Nigeria was established in October, 1980 from the Rivers State College of Science and Technology which was itself established in 1972. It is located at Nkpolu-Oroworukwo in Port Harcourt, the capital of Rivers State, Nigeria. It is the first Technological University in Nigeria and the first state owned university in the Niger Delta region of Nigeria. The motto of the University is "Excellence and Creativity". The University runs 37 programmes at the undergraduate level and 86 at the postgraduate level. Today, the University has several outside campuses such as Emohua, Ahoada, Etche, and Sapinwa. The university lies between Latitude 4.824167, and longitude 7.033611.

2.2 Collection of Water Samples

Borehole water samples were collected from six hostels (hostel B, C, D, H, FCMB and PG hostel). A total of 12 water samples were used for the analysis. The collection of samples was spread over two months. A sterile bottle of 1.5 liters was used to aseptically collect the water samples

which were placed in an ice-packed cooler and transported to the laboratory for further analysis.

2.3 Enumeration of Bacteria in the Water Samples

A 10-fold serial dilution of the water samples was carried out aseptically and transferred into test tubes up to 10^{-3} with sterile pipette and agitated properly to allow even distribution. After serial dilution of the samples, an aliquot (0.1ml) was inoculated in duplicates on Nutrient Agar, Mannitol Salt Agar (MSA), MacConkey Agar (MCA) and Eosin Methylene Blue (EMB) using spread plate method. The inoculated plates were incubated at 37°C for 24 hours after which the colonies were counted and recorded.

2.4 Storage of Pure Cultures and Characterization of the Bacterial Isolates

Pure isolates were stored as frozen 10% (v/v) glycerol suspension at -4°C. Pure bacterial isolates were identified in accordance with the method described by Holt et al., [7] and Cheesbrough [8]. Pure cultures from respective samples were identified based on their cultural, morphological and biochemical features. This included the colonial color of the isolates, the shape of the isolates, the elevation of the isolates, the edge of the isolates, and the consistency. Gram's staining techniques was employed in characterizing the isolates to determine the morphology of the Gram negative and Gram positive bacterial species.

2.5 Biofilm Determination by the Congo Red Method

Biofilm test was done using the Congo Red Agar method. This test was used to detect the presence and growth of biofilm-forming bacteria. First, the Congo Red Indicator was prepared as a concentrated aqueous solution and autoclaved at 121°C for 15 minutes separately from the other

Table 1. GPS coordinate of sampling sites for hostels

S/N	Sample location	Borehole	Latitude	Longitude
1	FCMB Hostel	Tap water	N 4.78874	E 6.98384
2	Hostel C	Tap water	N 4.79173	E 6.98234
3	Hostel D	Tap water	N 4.79153	E 6.98224
4	Hostel H	Tap water	N 4.79599	E 6.98260
5	PG Hostel	Tap water	N 4.79588	E 6.98251
6	Hostel B	Tap water	N 4.79258	E 6.98332

Source: Author's Field Survey, 2024.

medium. Then it was added to the autoclaved brain heart infusion agar with sucrose at 55°C and aseptically poured into plates. Congo Red Agar plates were inoculated with test organisms and incubated at 37°C for 24h. After the incubation period, the morphology of colonies was differentiated as biofilm producers or not. Black colonies with a dry crystalline consistency indicated biofilm producers, whereas colonies that retained pink were non-biofilm producers.

2.6 Hemolysis Assay on Blood Agar

Blood agar was prepared using standard microbiological procedures for the preparation of blood agar and allowed to cool. Using a sterile wire loop, a colony of the test isolate was inoculated unto the freshly prepared blood agar aseptically and incubated at 37°C for 24 hours, then observed for hemolytic pattern. A greenish-grey to brownish color around the colony indicated alpha hemolysis (partial lysing of the red blood cells) while the clearance of blood on the medium indicated beta hemolysis (the complete lysing of the red blood cells). Gamma hemolysis was represented by no change on the growth medium, indicating no lysing of red blood cells.

2.7 Statistical Analysis

A one-way analysis of Variance (ANOVA) was used to check for significant differences between each of the different samples. The mean separation was analyzed using Tukey High significant difference (HSD).

3. RESULTS

3.1 Population of the Different Bacterial Groups Isolated

The Total Heterotrophic Bacterial (THB) Count indicated that PG Hostel samples had the lowest value of $1.1 \pm 1.27 \times 10^5$ cfu/ml. Hostel C sample exhibited the highest THB count ($3.6 \pm 1.7 \times 10^5$ cfu/ml) (Table 2).

Results of the Total Coliform Count (TCC) indicated that the highest was recorded at Hostel D sample which had a value of $3.3 \pm 3.82 \times 10^4$ cfu/ml while Hostel B and FCMB Hostel had the lowest value of $1.05 \pm 1.34 \times 10^4$ cfu/ml.

The lowest Faecal Coliform Counts (FCC) of $1.5 \pm 0.71 \times 10^3$ cfu/ml was obtained at Hostel H,

with FCMB Hostel having the highest FCC of $8.5 \pm 4.95 \times 10^3$ cfu/ml. Staphylococcal Counts (SC) indicated that Hostel H and PG Hostel had the lowest value of $1.5 \pm 0.71 \times 10^4$ cfu/ml, while Hostel B had the highest value of $8 \pm 9.9 \times 10^4$ cfu/ml.

However, there was no statistically significant difference ($p > 0.05$) in bacterial population between water samples.

3.2 Characterization and Presence of Bacterial General in the Water Samples

The occurrence of bacterial species in the water samples showed that *Bacillus* spp and *Staphylococcus* species were the most prevalent, with the prevalence of 100%, as it occurred in all the Six (6) samples. *Pseudomonas* species occurred in Five (5) samples with a prevalence of 83.3%. *Klebsiella* spp. had a percentage of 66.6% and it occurred in four (4) samples. *Salmonella* spp. and *Escherichia coli* were the least prevalent, showing a percentage of 16.6%, occurring in one (1) sample (Table 3). The data in Table 3 also showed that water samples from Hostel B and PG hostel had the highest (5) bacterial species each, with water samples from hostel H recording the least (3) number of bacterial species recovered.

3.3 Biofilm Formation and Hemolytic Potentials of the Isolates Recovered

Results of biofilm formation of the isolates (Table 4) showed that all the organisms were biofilm producers, albeit at varying percentages. The result indicated 52.9%, 55.5%, 60%, 30%, 33.3%, and 100% of *Bacillus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Klebsiella* spp., *Salmonella* spp., and *Escherichia coli*, respectively were positive for biofilm formation. The hemolysis result (Table 4) indicated that 2 out of the 3 *Salmonella* species isolated, representing 66.7 percent, were alpha hemolytic. While none of the species of *Salmonella*, *Klebsiella*, and *Escherichia coli* were beta hemolytic, 23.5, 5.6 and 10 percent of *Bacillus* spp., *Staphylococcus* spp., and *Pseudomonas* spp., respectively were beta hemolytic. The hemolysis report also indicated that 28.3%, 10%, and 61.7% of the isolates were alpha, beta, and gamma hemolytic, respectively.

Table 2. Bacterial Population in the water sources studied

Parameter (cfu/ml)	Period	FCMB HOSTEL	PG HOSTEL	HOSTEL C	HOSTEL H	HOSTE D	H0STEL B	p-value
THB × 10 ⁵	Month 1	0.3	0.2	4.8	5.6	0.6	3.0	0.6921
	Month 2	0.8	2.0	2.4	0.8	5.0	0.6	
	Mean	0.55±0.35	1.1±1.27	3.6±1.7	3.2±3.39	2.8±3.11	1.8±1.7	
TCC × 10 ⁴	Month 1	2.0	0.5	0.6	0.1	6.0	2.0	0.6301
	Month 2	0.1	2.1	0.5	0.3	0.6	0.1	
	Mean	1.05±1.34	1.3±1.13	0.55±0.07	0.2±0.14	3.3±3.82	1.05±1.34	
FCC × 10 ³	Month 1	12.0	1.0	2.0	1.0	2.7	10.0	0.5050
	Month 2	5.0	8.0	2.0	2.0	5.0	2.0	
	Mean	8.5±4.95	4.5±4.95	2±0	1.5±0.71	3.85±1.63	6±5.66	
SC × 10 ⁴	Month 1	0	2.0	0.2	0.3	1.0	1.0	0.6279
	Month 2	4.0	1.0	1.0	5.0	2.0	15.0	
	Mean	2.0±2.83	1.5±0.71	0.6±0.57	2.65±3.32	1.5±0.71	8±9.9	

Table 3. Occurrence of Bacterial Species in the Hostel water samples

Bacterial species	FCMB HOSTEL	HOSTEL B	HOSTEL D	HOSTEL C	HOSTEL H	PG HOSTEL	Total (%)
<i>Pseudomonas</i>	+	+	+	+	-	+	5(83.3)
<i>Bacillus</i>	+	+	+	+	+	+	6(100)
<i>Staphylococcus</i>	+	+	+	+	+	+	6(100)
<i>Escherichia coli</i>	-	+	-	-	-	-	1(16.6)
<i>Klebsiella</i>	-	+	+	+	-	+	4(66.6)
<i>Salmonella</i>	-	-	-	-	-	+	1(16.6)
Total	3	5	4	4	2	5	

Table 4. Biofilm and Haemolytic Activities of the Isolates

Probable Isolate (n)	Biofilm	Haemolysis		
	Positive No. (%)	Alpha No. (%)	Beta No. (%)	Gamma No. (%)
<i>Bacillus</i> spp (17)	9 (52.9)	5(29.4)	4 (23.5)	8 (47.1)
<i>Staphylococcus</i> spp (18)	10 (55.5)	5(27.7)	1 (5.6)	12 (66.6)
<i>Pseudomonas</i> spp (10)	6 (60)	4 (40)	1 (10)	5 (50)
<i>Klebsiella</i> spp (10)	3 (30)	1 (10)	0	9 (90)
<i>Salmonella</i> spp (3)	1 (33.3)	2 (66.7)	0	1(33.3)
<i>Escherichia coli</i> (2)	2 (100)	0	0	2(100)
Total (%)	31(51.6)	17 (28.3)	6 (10)	37 (61.7)

n = number of isolates

4. DISCUSSION

The result from the bacteriological survey indicated a high level of bacterial contamination of the water sources. The counts were observed to be higher than the acceptable counts of 1.0×10^2 cfu/ml for drinking water. This higher bacterial counts may be due to contamination from different sources like proximity to septic tanks as well as water treatment practices. Also, the variations observed in the bacterial count between the water samples obtained from the different hostels could be attributable to the location of the hostels. The water sample from Hostel C had the highest Heterotrophic bacterial count which could be owed to the proximity of the hostel to a river. Similarly, water samples from Hostel D recorded the Highest Total Coliform count which may also be attributed to the proximity of the Hostels to a river. From the study, it was therefore, noticed that the hostels situated very close to the stream had higher bacterial count compared to the other hostels, implying that the river influences the underground water supplied to the students. According to the American Public Health Association, the fecal coliform count/Heterotrophic count ratio greater than 4.0 indicates pollution mostly caused by human activity, whereas less than 1.5 suggests contamination caused by non-human sources. The human or anthropogenic activities around these hostels include solid waste disposal, which has the potential of underground water contamination through a process of leaching.

Coliform bacteria were present in all six (6) sample locations, and it indicates the presence of other potentially harmful bacteria and threat to water quality. Sule et al., [9] also reported coliform bacteria in stored drinking water

reservoir tanks, which is consistent with the findings of this investigation. The presence of coliform in these water samples could serve as an indication of contamination of faecal origin and could as well report the possible presence of disease causing bacterial populations in the water sources [10].

Staphylococcus species have been widely reported to be associated with food poisoning. Their presence in high numbers could cause water related illness in humans. The staphylococcal counts obtained in this study may however be attributed to inadequate sanitation practices as well as treatment methods and frequencies. The findings from this work agrees with that of previous researchers [11]. In their study on the bacteriological assessment of drinking water sources in Opuraja Community of Delta State, Nigeria observed that all the water sources fell below standard approved by WHO and NAFDAC. They reported that the total heterotrophic bacterial count ranged from 1.45×10^3 cfu/ml to 1.5×10^6 cfu/ml for all the water sources.

From the study, six bacterial species, including *Bacillus* spp, *Staphylococcus* spp, *Pseudomonas* spp, *Escherichia coli*, *Klebsiella* spp and *Salmonella* spp were isolated from the different water samples analysed. These organisms are of public health concern. *Bacillus* is known for its predominance in soil [12, 13], hence its high frequency in this study could also indicative of environmental sources of contamination.

Coliforms like *Klebsiella* spp. and *Escherichia coli* have been reported to cause gastroenteritis. There detection in these water samples calls for measures to prevent waterborne illnesses among

those exposed to this water in the various hostels.

Klebsiella spp. and *Salmonella* spp. may be found in the intestinal tract of man and this strongly confirms the fact that the Hostel water sources may be mainly polluted by faecal matter. Their presence in water could be due to some natural phenomenon and other anthropogenic activities which include but are not limited to the following; inappropriate siting of boreholes close to dumping sites, extraction of groundwater from very shallow aquifers, discharge from septic tanks close to the source of the taps, storage system and piping units [14]. The presence of *Salmonella* spp in waterways suggests the dissemination of the agent in the environment, emphasising the role of faecal contamination in the spread of salmonellosis [15].

Most of the bacterial species isolated in this study were identified to belong to the members of coliform bacteria, which is a gram-negative facultative anaerobes, non-spore forming that ferment lactose within 48 hours [16].

The investigation into biofilm formation and haemolytic potentials of bacterial isolates yielded critical insights into their pathogenic capabilities. Biofilms are structured communities of bacteria encased in a self-produced matrix that adheres to surfaces. The ability of bacteria to form biofilm has profound implications for public health. It has been revealed that bacteria that form biofilm have catalytic ability in chemical and microbial activities which could lead to the corrosion of pipelines and water reservoirs [17]. Biofilm formation also confers some virulence potentials including antibiotic resistance of the organisms [18]. The presence of biofilm-forming bacteria in the hostel water samples suggests a heightened risk of chronic and recurrent infections among the residents, particularly if the water quality management and sanitation practices are inadequate.

According to Encyclopedia [19], haemolysis is the breakdown of red blood cells by a bacterial protein called haemolysin. These substances cause membrane damage, cell lysis, and destruction of neighbouring cells and tissue to deliver nutrients, primarily iron, to toxin – producing bacteria [20]. The result of this study showed that some of the isolates were alpha haemolytic, which was in agreement

with previous studies [21] that reported the presence alpha haemolytic bacteria in water samples.

5. CONCLUSION

This study on the bacteriology of borehole water in hostels of Rivers State University revealed that the bacteria load in the water samples exceeded regulatory standards/limits, thereby accentuating the importance of bacteriological examination of water. The presence of some harmful bacterial species is a major public health concern. The study also showed that *Bacillus* spp, and *Staphylococcus* spp were the most abundant, as they were prevalent in all the samples analysed. The study reported *Escherichia coli* and *Salmonella* spp as the least occurring bacteria species.

The biofilm and haemolysis assessment showed that all the bacterial isolates are biofilm producers and some of the isolates were beta haemolytic. The prevalence of biofilm producers and beta haemolytic activity among the isolates suggests a heightened risk of chronic and difficult –to- treat infections.

Regular treatment of hostel water sources is therefore required to prevent microbial growth in water channels. Also, regular inspection of pipes for slime development and decontamination of the hostel water at regular intervals using chloride dioxide (ClO₂) is required to eliminate biofilm forming bacteria.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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