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

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 Hostels of Rivers State University, Port Harcourt, Nigeria. Borehole water samples were collected from six (6) hostels for each study period, and subjected to standard bacteriological Procedures. Results obtained indicted that the highest mean Total heterotrophic bacterial count of 3.6±1.7 X 10^5cfu/ml was recorded in Hostel C water sample while the least mean bacterial count of 5.5±0.35 × 10^4cfu/ml was observed in FCMB Hostel. Total coliform count had the highest value (3.3±3.82 ×10^4cfu/ml) recorded in Hostel D and lowest value of 2.0±0.14 ×10^3cfu/ml was obtained in Hostel H water sample. The Faecal coliform count, recorded highest value in FCMB Hostel (1.5±0.71× 10^3cfu/ml) and lowest in Hostel H (8.5±4.95× 10^3 cfu/ml). The Staphylococcal count was highest in Hostel B and lowest in Hostel C (8±0.9 × 10^4cfu/ml and 6.0±0.57×10^3cfu/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.

1.0 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 condition [1]

Several factors influence the quality of water for human consumption. Such factors ranged 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 exercises an untold health effect on the end-users of such water body.

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 his domestic activities like cooking, drinking, washing, sanitation, and for growing his crops and running his factories. Consequently, modern man like his primitive ancestors, depend 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 the industrial usage of water and recreational purposes, good water quality and water sanitation helps to eradicate water borne and water related diseases, and ensures water use efficiencies, including the aesthetic quality of the environment [2].

Bacteriological contaminants have however, found their ways 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 the Rivers State University, the main source of water for students' utilization is groundwater source (borehole water). This water passed through several network 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 existence, it has become very domineering and thorough that the bacteriological quality of water be ascertained, since the general quality of water influence the health of any populace [4].

In a hostel or dormitory environment, different students with different approach to water sanitation, due to their different background. Some may exhibit good behaviour while others may not. All these account for the quality of water for human consumption. 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].

Due to the increasing burden of waterborne and water related diseases in Nigeria, campaigns for water, sanitation and hygiene has been intensified in rural communities, cities as well as the university community. 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.

2.0 Materials and Methods

2.1Study 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 State 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 samples were used for the analysis. Collection of samples was spread over two months. 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.

Table 1 GPS Coordinate of Sampling Sites for Hostels

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

Source: Author's Field Survey, 2024.

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 24hours after which the colonies were counted and recorded.

2.4 Storage of Pure CulturesandCharacterization of the Bacterial Isolates

Pure isolates was 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 was identified based on their cultural 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.

2.5 Biofilm Determination by the Congo Red Method

Biofilm test was done using Congo Red Agar method. This test was used to detect the presence and growth of biofilm–forming bacteria. First, Congo Red Indicator was prepared as a concentrated aqueous solution and autoclaved at 121°C for 15 minutes separately from the other 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 incubation period, 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 are 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 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 is 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 difference between each of the different samples. The mean separation was analyzed using Tukey High significant difference (HSD).

3.0 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 least 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 percentage of 16.6%, occurring in one (1) sample (Table 3).

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
	Month 1	0.3	0.2	4.8	5.6	0.6	3.0	0.6921
THB $\times 10^5$	Month 2	0.8	2.0	2.4	0.8	5.0	0.6	
	Mean ±std	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 $\times 10^4$	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 ±std	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 $\times 10^3$	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 ±std	8.5±4.95	4.5±4.95	2±0	1.5±0.71	3.85±1.63	6±5.66	
SC $\times 10^4$	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 ±std	2±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 (%)

Pseudomonas	+	+	+	+	-	+	5(83.3)
Bacillus	+	+	+	+	+	+	6(100)
Staphylococcus	+	+	+	+	+	+	6(100)
Escherichia coli	-	+	-	-	-	-	1(16.6)
Klebsiella	-	+	+	+	-	+	4(66.6)
Salmonella	-	-	-	-	-	+	1(16.6)
Total	3	5	4	4	2	5	

3.3 Biofilm formation and hemolytic potentials of the isolates recovered

Result 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 isolates, 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 4 Biofilm and Haemolytic Activities of the Isolates

	Biofilm	**Haemolysis**		
Probable Isolate (n)	**Positive**	**Alpha**	**Beta**	**Gamma**

	No. (%)	No. (%)	No. (%)	No. (%)
Bacillus spp (17)	9 (52.9)	5(29.4)	4 (23.5)	8 (47.1)
*Staphylococcus*spp(18)	10 (55.5)	5(27.7)	1 (5.6)	12 (66.6)
Pseudomonas spp(10)	6 (60)	4 (40)	1 (10)	5 (50)
Klebsiella spp (10)	3 (30)	1 (10)	0	9 (90)
Salmonella spp (3)	1 (33.3)	2 (66.7)	0	1(33.3)
Escherichia coli (2)	2 (100)	0	0	2(100)
Total (%)	31(51.6)	17 (28.3)	6 (10)	37 (61.7)

n = number of isolates

4.0 DISCUSSION

The result from the bacteriological survey indicated a high level of microbial contamination of the water sources. The counts were observed to be higher than the acceptable counts of 1.0 × 10^2cfu/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. Water sample from Hostel C had the highest Heterotrophic bacterial count and 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 American Public Health Association, Faecal count/ Heterotrophic count ratio greater than 4.0 indicates pollution mostly caused by human activity, whereas less than 1.5 suggest contamination caused by non-human sources.

Coliform bacteria were present in all the six (6) samples, 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 population in the water sources [10].

Staphylococcus species have mean widely reported to be associated with food poising. Their presence in high number 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 bacteriology 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× 10^6cfu/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 analsed. 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 as well be 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 these 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 includes but not limited to the following; inappropriate sittings of boreholes close to dump site, extraction of ground water from very shallow aquifers, discharge from septic tanks close to the source of the taps, storage system and piping units [14]. 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 antibiotics 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 in order 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.

5.0 Conclusion

This study on the bacteriology of borehole water in hostels of Rivers state university had 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 it was prevalent in all the samples analysed. The study reported *Escherichia coli and Salmonella* sppas 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 (ClO_2)is required to eliminate biofilm forming bacteria.

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