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Isolation of Bacterial Pathogens from Borehole Water Sources within the University of Port Harcourt

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Authors' contributions

This work was carried out in collaboration between both authors. Author HOS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HOS and CNE managed the analyses of the study. Author CNE managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

A study about the bacteriological and physicochemical quality of borehole water at the University of Port Harcourt was carried out. Eight water samples was pooled from NUH Block B (Under graduate Hostel), Nelson Mandela Block B (Undergraduate Hostel), Intercontinental Hostel (Post Graduate Hostel), Donald Ekong Block C (Post graduate Hostel), Clinical Hostel, Staff quarters (Opposite Uniport Bottling plant), Dan Etete (Undergraduate Hostel) and Gambiama Staff quarters designated as Sample 1 to sample 8 respectively. Physical examination was conducted to as the sanitary and hygiene practices within the collection area. It was observed that the undergraduate hostels had the least sanitary practice. Total counts of heterotrophic bacteria count showed that the highest bacterial count was recorded from Sample 2 with bacterial count of $2.3x10^4$ CFU/ml while the least bacterial count was recorded from sample 8 with bacterial count of $3.0x10^2$ CFU/ml. The isolated bacterial species from the water sources were identified as *Bacillus* sp., *Micrococcus* sp., *E. coli., Serratia* sp., *Staphylococcus* sp., *Enterobacter* sp., *Citrobacter* sp. The presence of coliform bacteria in the sampled water source does not comply with the World Health Organization (WHO) standard for coliform bacteria of zero total coliform per 100 ml of water. The borehole water

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samples collected had pH values within 5.9-6.85 which does not comply with the WHO recommended range for drinking water standards which should fall between ≥7 to ≤9.2. Nitrate concentration as observed amongst the eight water samples was below the WHO standard of 50mg/l. The Total dissolved solutes was below 0.01 in all the tested waters samples. This study has revealed that borehole water from sampled sources within the University of Port Harcourt is not fit for human consumption without adequate treatment.

Keywords: Microbiological; physicochemical; borehole water; treatment.

1. INTRODUCTION

Water is the most vital element among the natural resources and the most indispensable need for existence of all living things [1]. Its decreasing availability both in quality and quantity has been a major public health concern in Africa, particularly in Nigeria [2]. No gain saying that shortage of water leads to disease outbreak and economic loss, hence water is a necessity. In a nutshell without water life is impossible. Water plays a vital role in the proper functioning of the earth's ecosystem [3]. Globally, water is known to be a scarce resource and it has been estimated that 41 of the world's population (2.3 billion people) live under water stress condition. While 1.1 billion people live without access to potable water [4]. However, access to clean water is worse in developing countries, having one third of the population without access to safe drinking water and thus, leaving near 1.87 million children to die from diarrhea annually [5]. The quality of water for drinking deteriorates due to poor treatment plants, direct discharge of untreated sewage into rivers and stream, and inefficient management of piped borne water distribution system [6]. The contaminated water therefore has critical impact on all biotic components of the ecosystem and this could affect its use for other purposes [3]. Most of drinking water sources are often contaminated with different pollutants such as faeces, animal and plant wastes, and thus making such water unfit for drinking if not treated. Groundwater provides potable water to an estimated 1.5 billion people worldwide daily and has proved to be the most reliable resource for meeting rural water demand in the sub-Saharan Africa [7]. Due to inability of governments to meet the ever-increasing water demand, most people in rural areas resort to groundwater sources such as boreholes as an alternative water resource. Thus, humans can abstract groundwater through a borehole, which is drilled into the aquifer for industrial, agricultural and domestic use. However, groundwater resources are commonly vulnerable to pollution, which may degrade their quality.

Generally, groundwater quality varies from place to place, sometimes depending on seasonal changes [8], the types of soils, rocks and surfaces through which it moves [9]. Industrial discharges, urban activities, agriculture, groundwater plumage and disposal of waste can affect groundwater quality [10]. Proximity of some boreholes to solid waste dumpsites and animal droppings being littered around them [11] could also contaminate the quality of groundwater. Therefore, groundwater quality monitoring and testing is of paramount importance both in the developed and developing world. The key to sustainable water resources is to ensure that the quality of water resources is suitable for their intended uses. The risk of outbreaks of waterborne diseases increases where standards of water, sanitation and personal hygiene are low. Contaminated drinking-water is a frequent cause of diseases such as cholera, typhoid, viral hepatitis A and dysentery. Human activity may also cause water to become contaminated with substances such as microorganisms which can cause infections [12].

The aim of this study is to assess the quality of water sources within the University of Port Harcourt.

2. MATERIALS AND METHODS

2.1 Samples Collection

Water samples were collected in sterile containers and in the process, special care was taken to obtain fair samples, by allowing borehole taps to run for about five minutes before collecting the water samples. All samples were transported to the laboratory in ice-bag and processed within 6 hours of collection.

2.2 Sample Collection Sites

The sample collection sites are presented in Table 1.

Table 1. Sample collection sites

S/N	Sample code	Sampled site
1	Sample 1	NUH Block B (Under graduate Hostel)
2	Sample 2	Nelson Mandela Block B (Undergraduate Hostel)
3	Sample 3	Intercontinental Hostel (Post Graduate Hostel)
4	Sample 4	Donald Ekong Block C (Post graduate Hostel)
5	Sample 5	Clinical Hostel
6	Sample 6	Staff quarters (Opposite Uniport Bottling plant)
7	Sample 7	Dan Etete (Undergraduate Hostel)
8	Sample 8	Gambiama Staff quarters

2.3 Bacteriological Parameters

2.3.1 Total counts of heterotrophic bacteria

Total heterotrophic bacteria counts were carried out using Nutrient agar (NA) by pour plate method. Aliquot of 1 ml (must be diluted ,where the plate can not count 3 $x10^4$)of the samples was used to inoculate the plate in triplicates; the plates were incubated at 37°C for 48 hrs. Thereafter the mean count of the bacteria colonies was taken. The bacteria isolates was further experimented in order to attain pure cultures. The pure cultures would then be characterized and identified to determine the bacteria species using the standard microbial method.

2.3.2 Total coliform counts and total fecal coliform counts

The coliform counts were determined by the multiple tubes fermentation techniques. Samples were incubated in lactose broth tubes at 37°C for 48 hrs. Measured amounts of double and single strength MacConkey broth (purple colour) were sterilized in bottles containing inverted Durham tube to indicate the gas production. The bottles were arranged in three sets 50 ml, (10 ml and 1 ml and each had 5 bottles), and incubated at 37°C. Fermentation tubes were inoculated with 50 ml, 10 ml and 1 ml of aliquot of the samples in accordance with standard methods. The tubes were incubated at 37°C for 48 hrs. Positive tubes producing acid and gas were used to obtain the presumptive result. The confirmed test for total coliform was achieved by plating a loopful of positive MacConkey broth on Eosine Methylene Blue (EMB) agar and incubated at 37°C for 24hrs, while the fecal coliform was achieved by transferring a loopful of broth from a positive tube to EC broth and incubated at 44.5°C for 24-48 hrs and the tubes were observed for gas formation. Completed test for fecal coliform was carried out by plating a loopful of broth from a positive EC tube into an Eosine methylene blue agar plate. The plates were incubated at 44.5°C for 48 hrs and observed for a dark red colour with metallic green sheen. Stock cultures of the colonies of the total and fecal coliforms were prepared on nutrient agar slants for Gram staining and biochemical test. Final fecal coliform count was calculated based on the completed test.

2.3.3 Characterization of isolates

Cultural characteristics of isolates e.g. size, shape, margin, elevation, consistency, colour, transparency were determined. Gram staining and Biochemical test such as catalase test, oxidase test**,** coagulase test, coagulases urease test**,** indole test, citrate utilization test, sugar fermentation test were carried out using standard methods, with reference to Holt et al. [13].

2.3.4 Physicochemical parameters

The physicochemical parameters of the samples [pH, biological oxygen demand (BOD), total dissolved solid (TDS) and nitrate] were determined as following the American Public Health Association (APHA) guidelines [14].

3. RESULTS

Table 2 shows the bacteriological parameters of the water samples. The highest bacterial count was recorded from Sample 2 i.e Nelson Mandela Block B (Undergraduate Hostel) with bacterial count of $2.3x10^4$ CFU/ml while the least bacterial count was recorded from sample 8 i.e Gambiama Staff quarters with bacterial count of $3.0x10^2$ CFU/ml. Sample 7 i.e. Dan Etete (Undergraduate Hostel) had the highest fecal coliform count while sample 4 Donald Ekong Block C (Post graduate Hostel).

Table 3 shows the bacterial isolates present in the water samples. The bacterial isolates include *Bacillus* sp., *Micrococcus* sp., *Enterobacter* sp.,

Table 2. Bacteriological parameters of the water samples

S/N	Sample code	CFU/ml*	MPN Index per 100 ml **
1	Sample 1	1.6 X10^4	5
2	Sample 2	2.3x10^4	5
3	Sample 3	1.1x10^3	11
4	Sample 4	1.1x10^3	<2
5	Sample 5	1.0x10^4	17
6	Sample 6	1.5x10^4	2
7	Sample 7	1.3x10^4	26
8	Sample 8	3.0x10^2	2

**colony forming unit*
*** most probable number*

Table 3. Bacteria isolated from water samples

S/N	Sample code	Isolate
1	Sample 1	*Bacillus* sp., *Micrococcus* sp., *Enterobacter* sp., *Serratia* sp., *Staphylococcus* sp., *E. coli*
2	Sample 2	*Staphylococcus* sp., *Citrobacter* sp., *Micrococcus* sp., *Enterobacter* sp., *Serratia* sp., *E. coli*
3	Sample 3	*Citrobacter* sp., *Enterobacter* sp., *Staphylococcus* sp., *E. coli*
4	Sample 4	*Staphylococcus* sp., *E. coli, Micrococcus* sp., *Enterobacter* sp.,, *Serratia* sp.,
5	Sample 5	*Citrobacter* sp., *Staphylococcus* sp., *E. coli*
6	Sample 6	*Staphylococcus* sp., *Micrococcus* sp., *Enterobacter* sp.,, *Serratia* sp., *E. coli*
7	Sample 7	*Enterobacter* sp., *Staphylococcus* sp., *E. coli*
8	Sample 8	*Citrobacter* sp., *Staphylococcus* sp., *E. coli*

Table 4. Physicochemical parameters of the water samples

S/N	Sample code	Temperature (^{o}C)	pH	BOD (mg/l)	Nitrate (mg/l)	TDS (mg/l)
1	Sample 1	29.9	6.4	3.18	1.30	<0.01
2	Sample 2	28.2	6.7	3.34	3.40	<0.01
3	Sample 3	29.1	6.5	3.18	2.20	<0.01
4	Sample 4	29.4	5.9	3.50	1.20	<0.01
5	Sample 5	28.7	6.50	3.28	2.90	<0.01
6	Sample 6	28.8	6.67	2.97	3.0	<0.01
7	Sample 7	29.1	6.50	3.46	2.40	<0.01
8	Sample 8	27.9	6.85	3.19	1.97	<0.01

Serratia sp., *Staphylococcus* sp., *E. coli* and *Citrobacter* sp. Staphylococcus sp. and E. coli were detected in all the water samples.

Table 4 shows the physicochemical parameters of the water samples. The temperature values ranged from 27.9^{o}C to 29.9^{o}C; pH, ranged from 5.9 to 6.85; biological oxygen demand (BOD), 2.97 to 3.46 mg/l; nitrate, 1.2 mg/l to 3.0 mg/l and total dissolved solid (TDS), <0.01.

4. DISCUSSION

The analyzed water samples collected from water bore hole situated at University of Port Harcourt varied in quality by location, although is found free of pathogens. It was observed that the Undergraduate hostels had the least sanitary practice as reflected in the bacterial load. The bacterial count ranged from 3.0x10^2 CFU/ml (sample 8 - Gambiama Staff quarters) to 2.3x10^4 CFU/ml (Sample 2 - Nelson Mandela Block B, Undergraduate Hostel) These counts are higher than the acceptable counts of 0 CFU/ml for drinking water [15]. The risk of outbreaks of waterborne diseases increases where standards of water, sanitation and personal hygiene are low.

The isolated bacteria species from the water sources are *Bacillus* sp., *Micrococcus* sp., *E.coli., Serratia* sp., *Staphylococcus* sp., *Enterobacter*

sp., *Citrobacter* sp. The presence of coliform bacteria in the sampled water source does not comply with WHO standard for coliform bacteria in water, which is zero total coliform per 100 ml of water. The detection of *Escherichia coli*, *Bacillus* sp., *Micrococcus* sp., *Serratia* sp., *Staphylococcus* sp., *Enterobacter* sp. and *Citrobacter* sp. species in borehole water that was intended for human consumption suggests that water from these sources may pose severe health risks to consumers and is unsuitable for direct human consumption without treatment [15].

The temperature values of the water samples ranged from 27.9^{o}C to 29.9^{o}C. The water samples were collected during the hot seasons in Nigeria where the average temperature is about 29^{o}C. Temperature is one of the most important ecological and physical factor which has a profound influence on both the living and non-living components of the environment, thereby affecting organisms and the functioning of an ecosystem [16]. Although temperature generally influences the overall quality of water (physico-chemical and biological characteristics), there are no guideline values recommended for drinking water.

The borehole water samples collected had pH values within 5.9-6.85 which does not comply to the recommended ranges for WHO drinking water standards which should fall between ≥7 to ≤9.2 [15]. The pH of water is important because many biological activities can occur only within a narrow range, thus any variations beyond an acceptable limit could be fatal to a particular organism recorded by Palamuleni and Akoth [17]. The Nitrate concentration as observed amongst the eight water samples is way below the WHO standard of 50mg/l and it is an acceptable value. The Total dissolved solutes was below 0.01 in all the tested waters samples.

5. CONCLUSION

This study has revealed that borehole water from sampled sources within the University of Port Harcourt is not fit for human consumption without further and adequate treatment. The university community should routinely monitor the quality of borehole water to ensure safety of consumers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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