

Original Research Article

Bacteriological and physicochemical parameters of some selected borehole water sources in Abakaliki metropolis, Nigeria

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ABSTRACT

Background: Borehole water (groundwater) is the predominant source of water by the inhabitants of Abakaliki metropolis and it is generally considered a safe source of drinking water by the populace. This study was therefore, designed to assess the physicochemical and microbiological quality of borehole water samples in Abakaliki, Ebonyi State, Nigeria.

Methods: A total of twenty-five (25) water samples were collected from five (5) different locations (designated as point A, B, C, D and E) in Abakaliki and the temperature readings were taken at the site of collection. The physico-chemical parameters (turbidity, pH, total hardness, dissolved oxygen, electrical conductivity, phosphates, sulphates), microbiological and metal content were determined using standard techniques. Furthermore, the pathogens isolated were subjected to antibiotics susceptibility testing using disc diffusion method.

Results: The results of the microbiological study revealed that the bacterial pathogens isolated in this study include *E. coli* (40%), *Staphylococcus aureus* (32%), *Pseudomonas aeruginosa* (16%) and *Klebsiella spp* (12%). The borehole water was of low turbidity, colourless, odourless, and with ambient temperature. The values of the bacteria counts were 2.4x10⁴ cfu/ml for bacteria pathogen isolated from location A, 2.3x10³ cfu/ml for location B and location C, 2.1x10⁴ cfu/ml for location E and 1.0x10⁴ cfu/ml for location D. Antibiotic susceptibility studies showed that all isolated bacteria pathogens were highly resistant to most of the tested antibiotic especially nalidixic acid, ciprofloxacin, gentamicin, cefoxitin, sulphamethoxazole-trimethoprim, tobramycin, ofloxacin and erythromycin.

Conclusions: Findings from our study revealed that the borehole water analyzed within Abakaliki metropolis contained bacteria that are of public health importance including *E. coli*, *S. aureus*, *P. aeruginosa* and *Klebsiella spp*. The physical and chemical parameters of the water samples were found to be within the maximum accepted limits for drinking water with optimal physical and chemical properties. It was also discovered in this study that the isolated bacteria showed reduced susceptibility to some conventional antibiotics. It is therefore recommended that periodic assessment of the physicochemical and microbiological properties of borehole water in this area be carried out on water sources for public use in order to avoid the outbreak of some waterborne infections amongst the populace.

Keywords: Bacterial resistance, Borehole water, Coliforms, Physiochemical parameters, Waterborne diseases

INTRODUCTION

Water is vital to many life processes and they could also serve as route via which disease-causing pathogens can be transmitted. It can also contain heavy metals and other chemical substances that may adversely affect human health. Ensuring good quality of drinking water is a basic factor for guaranteeing public health.¹ Potable water is that which is odourless, colourless, practically tasteless and free from physical, chemical and biological contaminants.² Water is exploited by man for several commercial, agricultural, domestic and industrial usages; and the usage of water for any activity usually depends on the cleanness of the water.

The quality of water is determined by its physical, chemical and microbiological characteristics.³ Groundwater sources are commonly vulnerable to pollution, which may degrade their quality. Generally, groundwater quality varies from place to place, and this sometimes depends on seasonal changes.^{4,5} The quality of water also depends on the types of soils, rocks and surfaces through which it moves beneath the earth. Access to potable drinking water is a major public health issue in many parts of the world especially in most developing economies (Nigeria inclusive) where water hygiene and sanitation may still be poor.

The people living in these countries usually get their primary source of water from surface waters such as streams, ponds, rivers and the open skies when it rains. Population growth coupled with increased industrialization, livestock farming and urbanization have led to frequent contamination of rivers.⁶ And due to the inability of some governments to meet the ever increasing water demand of their populace, most people often resort to groundwater sources such as boreholes as alternative water source. Borehole water therefore, is a primary source of water in most developing nations; and the chemical, physical and biological constituents of these sources of water is a critical public health issue that needs to be ascertained on periodic basis.

Borehole water and packaged sachet water serves as the easily accessed and cheap commercial sources of drinking water for a greater number of the Nigerian populace; and they also infer that the conformation of these sources of water to laid down microbiological standards is of public health interest because of their capacity to spread diseases within a large population.³ The provision of potable water to both the rural and urban population is necessary to prevent public health hazards such as the emergence and spread of waterborne pathogens.^{7,8}

Recently in Abakaliki metropolis, the outbreak of cholera infection (caused by *Vibrio cholerae*) caused several morbidity and mortality; and it was discovered that these clinical episodes of the disease were traced to contaminated water. The increase in the prevalence of

waterborne diseases across the world is alarming, and Nigeria is not left out since some outbreaks of waterborne diseases have also been reported in this part of the world.^{9,10}

Since borehole water is an important alternative source of potable water to most people in both the rural and urban areas across the world, it is vital to evaluate their physicochemical and microbiological quality since these sources of water are usually at risk of pollution from human and other environmental activities. Thus, this study determined the physical, chemical and microbiological quality of some selected borehole water sources in Abakaliki metropolis, owing to the fact that this source of water is commonly patronized in this region.

METHODS

A total of twenty five (25) water samples (250 ml each) were aseptically collected using sterile containers from five major areas [designated as point A, B, C, D, and E] located within Abakaliki metropolis, Ebonyi State, Nigeria. Prior to sample collection, the mouth of the tap was disinfected and the tap was opened for water to run for two minutes. The water samples were properly labeled and transported to the Microbiology Laboratory of Ebonyi State University, Abakaliki for bacteriological analysis. Physicochemical analysis of the water samples was carried out in the Industrial Chemistry Laboratory of Ebonyi State University, Abakaliki.

Bacteriological analysis of water samples

From each of the 25 borehole water samples that were collected, 100 ml each was filtered through 0.45 µm pore size membrane filter (Milipore, Amesterdam, Netherlands). After filtration the sterile membrane filter paper was aseptically placed onto MacConkey agar, cysteine lactose electrolyte deficiency (CLED) agar, Eosin methylene blue agar (EMB) and Mannitol salt agar using a sterile forceps and incubated aerobically at 37°C for 24 - 48 hours. All the culture media was procured from Oxoid limited (Oxoid, UK). After incubation, the culture plates were examined to access bacterial growth/colony appearance for the presence of bacteria growth. Suspect bacterial growth were subcultured onto freshly prepared CLED, MacConkey agar, EMB and MSA for the isolation of pure cultures of bacteria. The observed bacteria growths were re-subcultured on nutrient agar plates until pure isolates was obtained. The test isolates were subjected to Gram staining and further identified based on morphological characteristics and biochemical tests.¹¹

Antibiotic sensitivity testing

Antibiotic susceptibility testing was carried out by Kirby-Bauer disk diffusion technique using commercially available antibiotic disks according to clinical laboratory

standard institute (CLSI) (CLSI 2005). Briefly, a sterile cotton swab was allowed to soak in the bacterial suspension (adjusted to 0.5 McFarland turbidity standards), squeezed by the side of the tube to lose some fluid and aseptically swabbed onto Mueller Hinton agar plates (Oxoid, UK).

The inoculated plates were allowed to stand for about 30 minutes to pre-diffuse and antibiotic disks including gentamicin (30 µg), ciprofloxacin (10 µg), sulphamethoxazole-trimethoprim (25 µg), erythromycin (15 µg), clindamycin (15 µg), oxacillin (1 µg), ofloxacin (5 µg), ertapenem (10 µg), cefuroxime (30 µg), nalidixic acid (30 µg), ampicillin (10 µg), tobramycin (10 µg), cefoxitin (30 µg), mupirocin (10 µg), and vancomycin (10 µg) were aseptically placed on the inoculated plates. All the antibiotic disks were procured from Oxoid limited (Oxoid UK). The susceptibility plates were incubated for 18-24 hours at 37°C; and inhibition zone diameters were measured in millimeter.^{12,13}

Analysis of physicochemical parameters of borehole water samples

Physicochemical parameters was carried out using standard techniques, and the physical and chemical parameters detected included pH, total dissolved solids (TDS), total hardness (TH), salinity, phosphates and sulphate. The water samples were collected according to standard procedures.¹⁴ All items for collecting and storing samples and reagents were first washed with distilled deionised water (DDW), soaked overnight in 10% (v/v) nitric acid and then rinsed exhaustively with DDW. The washed items were dried and stored in zip locked polyethylene bags.¹⁵

Water samples were filtered and collected into these pre-cleaned wide mouthed containers (ca. 1 litre) with cap. The containers were completely filled with water and capped to prevent spillage. Exposure of the samples to air was minimized during the filtration to reduce the possibility of oxidation.¹⁶ Tests on each sample were carried out upon return to the laboratory, between one to six hours of collection. Whereupon not possible to meet the period of analysis, there was a re-sampling.

RESULTS

A total of twenty five (25) borehole water samples were analyzed in this study. The borehole water samples (250 ml each) were aseptically collected from various borehole sites in Abakaliki metropolis [the borehole sites were designated as location A, B, C, D and E] and they were bacteriologically analyzed in the Microbiology Laboratory of Ebonyi State University, Abakaliki, for the isolation of bacterial pathogens that may be source of contamination. The profile of bacteria count from the various water samples is shown in Table 1. The result of the bacteria count showed that the values of bacteria count ranged from 2.4×10^4 cfu/ml in borehole water from location A to 1.0×10^4 cfu/ml in borehole water samples from location D. The bacterial count of borehole water samples from location B, C and E is 2.3×10^4 , 2.3×10^4 , and 2.1×10^4 respectively. The highest bacteria count was observed from water samples from location A (2.4×10^4 cfu/ml), while the least bacteria count was observed in water samples from location D (1.0×10^4 cfu/ml). The physical parameters of the water sample are shown in Table 2.

Overall, the water sample is colourless and odourless. And the temperature range of the water sample was between 24°C-27°C (Table 2). The results of the bacteriological analysis of the borehole water samples revealed the presence of enteric and non-enteric microorganisms (Table 3). The enteric microorganisms isolated from the various water samples were isolates of *Escherichia coli* (40%) and isolates of *Klebsiella spp* (12%). However, isolates of *Staphylococcus aureus* (32%) and *Pseudomonas spp* (16%) were the non-enteric bacteria isolated from the borehole water samples (Table 3). The antimicrobial susceptibility profile of the isolated bacteria is shown in Figure 1 and Figure 2.

Table 1: Bacteria count.

Sampling site	Bacteria count *(cfu/ml)
A	2.4×10^4
B	2.3×10^4
C	2.3×10^4
D	1.0×10^4
E	2.1×10^4

*CFU=colony forming unit.

Table 2: Physical parameters of the water samples

Sample	Turbidity	Colour	Odour	Temperature (°C)
A	4	Colourless	Odourless	26.30
B	3	Colourless	Odourless	24.90
C	4	Colourless	Odourless	27.20
D	5	Colourless	Odourless	26.10
E	2	Colourless	Odourless	26.80

Table 3: Frequency of bacteria isolated from the water samples from different locations.

Bacteria isolates	A	C	B	E	D	Total occurrence n (%)
<i>Escherichia coli</i>	4	0	6	6	4	20 (40)
<i>Staphylococcus aureus</i>	2	6	4	2	2	16 (32)
<i>Klebsiella</i> species	2	2	0	0	2	6 (12)
<i>Pseudomonas</i> species	2	2	0	2	2	8 (16)

Key: % = percentage, n = no of occurrence of bacteria isolated.

Table 4: Physicochemical Parameters of Borehole Water Samples.

Sample	Sample Code	pH	DO (mg/L)	EC (μS/cm)	TDS (mg/L)	Salinity (%)	TH (mg/L CaCO ₃)	Phosphate (mg/L)	Sulphate (mg/L)
B	W ₁	7.00 ± 0.004	8.55 ± 0.2	875 ± 7.4	431 ± 1.7	0.43 ± 0.0	288 ± 0.14	0.451 ± 0.002	56.785 ± 0.001
	W ₂	7.50 ± 0.0	7.97 ± 0.6	73 ± 16.2	360 ± 5.6	0.36 ± 0.002	486 ± 0.21	0.425 ± 0.001	16.938 ± 0.006
	W ₃	6.80 ± 0.001	8.24 ± 0.4	704 ± 11.6	343 ± 5.5	0.34 ± 0.0	346 ± 0.21	0.433 ± 0.001	48.402 ± 0.003
	W ₄	8.00 ± 0.003	7.80 ± 0.3	700 ± 2.9	342 ± 6.0	0.34 ± 0.007	370 ± 0.07	0.422 ± 0.002	29.742 ± 0.001
	W ₅	7.00 ± 0.0	7.91 ± 0.1	538 ± 9.3	260 ± 7.1	0.26 ± 0.003	306 ± 0.49	0.240 ± 0.002	37.895 ± 0.002
A	P ₁	8.00 ± 0.002	8.14 ± 0.1	1546 ± 11.3	774 ± 1.3	0.78 ± 0.003	70 ± 0.07	0.447 ± 0.004	19.522 ± 0.002
	P ₂	6.80 ± 0.0	7.68 ± 0.3	515 ± 3.4	250 ± 5.0	0.25 ± 0.003	306 ± 0.49	0.348 ± 0.002	19.694 ± 0.003
	P ₃	7.40 ± 0.001	7.98 ± 0.2	987 ± 1.6	485 ± 3.2	0.49 ± 0.003	294 ± 0.78	0.434 ± 0.002	39.158 ± 0.002
	P ₄	7.00 ± 0.0	8.19 ± 0.1	685 ± 3.0	334 ± 3.0	0.33 ± 0.0	472 ± 1.41	0.444 ± 0.004	19.005 ± 0.002
	P ₅	7.00 ± 0.0	6.81 ± 0.1	860 ± 2.1	421 ± 5.7	0.42 ± 0.002	342 ± 0.21	0.365 ± 0.001	33.990 ± 0.002
C	N ₁	6.00 ± 0.0	7.95 ± 0.3	543 ± 4.1	263 ± 7.1	0.26 ± 0.0	350 ± 0.49	0.356 ± 0.003	19.866 ± 0.004
	N ₂	7.00 ± 0.0	8.03 ± 0.3	188.1 ± 9.7	89.4 ± 5.5	0.09 ± 0.0	130 ± 0.21	0.989 ± 0.002	5.799 ± 0.002
	N ₃	6.50 ± 0.002	7.78 ± 0.1	801 ± 6.1	391 ± 2.1	0.39 ± 0.001	460 ± 0.14	0.429 ± 0.002	53.856 ± 0.002
	N ₄	7.00 ± 0.0	7.85 ± 0.3	767 ± 9.0	373 ± 3.9	0.37 ± 0.0	506 ± 0.49	0.469 ± 0.003	44.153 ± 0.001
	N ₅	6.00 ± 0.0	7.70 ± 0.3	837 ± 3.5	408 ± 1.7	0.41 ± 0.0	470 ± 0.07	0.428 ± 0.002	50.182 ± 0.002
E	K ₁	7.00 ± 0.001	7.55 ± 0.0	586 ± 4.5	285 ± 1.1	0.28 ± 0.0	370 ± 0.07	0.428 ± 0.002	9.416 ± 0.001
	K ₂	7.00 ± 0.0	7.71 ± 0.2	349 ± 8.1	167.9 ± 3.9	0.17 ± 0.0	202 ± 0.21	0.073 ± 0.001	15.388 ± 0.001
	K ₃	7.00 ± 0.001	8.08 ± 0.2	489 ± 2.1	237 ± 9.0	0.23 ± 0.0	398 ± 0.78	0.368 ± 0.003	4.478 ± 0.002
	K ₄	6.00 ± 0.001	8.14 ± 0.2	356 ± 4.0	170.4 ± 7.2	0.17 ± 0.0	198 ± 0.21	0.112 ± 0.002	15.445 ± 0.002
	K ₅	7.00 ± 0.001	8.17 ± 0.2	483 ± 2.2	233 ± 3.0	0.23 ± 0.0	278 ± 0.21	0.356 ± 0.002	17.340 ± 0.002
	E ₁	8.00 ± 0.002	7.79 ± 0.1	1036 ± 5.3	510 ± 3.3	0.51 ± 0.002	263 ± 0.07	0.421 ± 0.003	24.574 ± 0.001
	E ₂	7.00 ± 0.001	7.93 ± 0.1	860 ± 4.1	423 ± 8.5	0.42 ± 0.002	480 ± 0.14	0.427 ± 0.001	13.378 ± 0.001

D	E ₃	7.00 ± 0.001	7.67 ± 0.1	828 ± 3.2	405 ± 8.0	0.40 ± 0.003	388 ± 0.42	0.426 ± 0.003	7.866 ± 0.001
	E ₄	8.00 ± 0.001	7.71 ± 0.0	894 ± 0.9	399 ± 2.0	0.40 ± 0.001	320 ± 0.14	0.421 ± 0.002	6.890 ± 0.003
	E ₅	7.00 ± 0.0	7.88 ± 0.0	844 ± 1.1	413 ± 4.4	0.41 ± 0.002	396 ± 0.57	0.418 ± 0.003	13.493 ± 0.001
	WHO	6.5 – 8.5	-	1000	600	-	500	-	-
	SON	6.5 – 8.5	-	1000	-	-	150	-	100
	US-EPA	6.5 – 8.5	-	-	500	-	-	-	250

Key:DO = Dissolved oxygen; EC = Electrical conductivity; TDS = Total dissolved solids; TH = Total hardness; WHO = World Health Organisation standard (2011); SON = Standard Organisation of Nigeria (2007); US-EPA = United States Environmental Protection Agency (1994).

The isolated bacteria from the various borehole water samples showed varying level of susceptibility and resistance to the tested antimicrobial agents. Notably, higher levels of resistance to ampicillin, ertapenem, clindamycin, sulphamethoxazole-trimethoprim, tobramycin, ofloxacin and erythromycin were observed in all the *E. coli* isolates (Figure 1). The *Klebsiella spp* isolates and *Pseudomonas spp* isolates were also resistant to the tested antibiotics especially nalidixic acid, ciprofloxacin, gentamicin, ceftioxin, sulphamethoxazole-trimethoprim, tobramycin, ofloxacin and erythromycin (Figure 1).

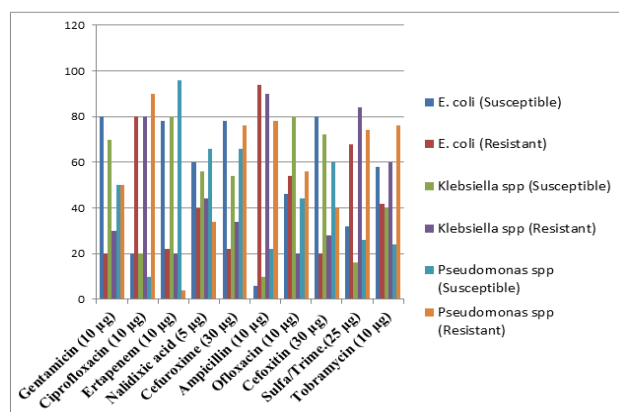


Figure 1: Percentage susceptibility patterns of *E. coli*, *Klebsiella spp* and *Pseudomonas aeruginosa* isolated from the water samples.

Figure 2 shows the susceptibility profile of the isolated *S. aureus* bacterium to the tested antimicrobial agents. The *S. aureus* isolates were more susceptible to the test antibiotics than the Gram negative bacteria (*E. coli*, *Klebsiella spp* and *Pseudomonas spp*) isolated from this study. Table 4 shows the physicochemical parameters of the borehole water sample analyzed in this study. The physicochemical parameters evaluated in this study include pH, electrical conductivity (EC), total dissolved solids (TDS), total hardness (TH), salinity, dissolved

oxygen (DO), and the presence of sulphates and phosphates.

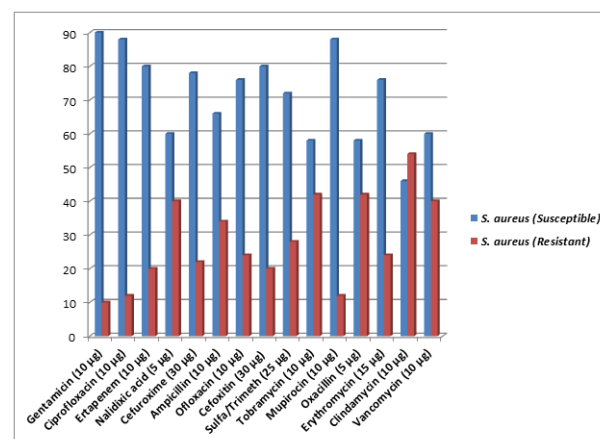


Figure 2: Percentage susceptibility patterns of *Staphylococcus aureus* isolated from the water samples.

DISCUSSION

Water, including borehole water can be described as potable if it complies with and meets certain physical, chemical and microbiological standards designed to ensure that a given water source is drinkable. Potable water is essential for good health and the socioeconomic development of man.¹⁷ Borehole water serves as the major source of drinking water in Nigeria, since only few Nigerians can actually afford treated bottled water for their daily water consumption.¹⁷ Since borehole water sources could serve as sources via which waterborne pathogens could spread and cause waterborne infections in any given community, the physicochemical and bacteriological analysis of borehole water sources are vital and necessary to prevent the spread of disease-causing agents through them. The physicochemical and bacteriological parameters of water samples from different areas [designated as point A, B, C, D and E] in

Abakaliki metropolis, Nigeria was investigated in this study.

The bacteria load for all the water samples analyzed in this study were generally high, and they exceeded the acceptable limit; which is the standard limit of coliform bacteria including heterotrophic bacteria in water bodies including borehole water.^{18,19} Conformation with microbiological standard is of special interest because of the capacity of water to spread waterborne diseases within a large human population. The highest bacteria count was observed from water samples from point A, while the least bacteria count was observed in water samples from point D. This result agrees with the findings of Nwachukwu *et al.* that there are high counts of bacteria pathogens in most borehole water samples in some parts of Nigeria.¹⁷

In a related development, bacteria counts that were unacceptably high from several boreholes in Nigeria and elsewhere have been previously reported; and these studies showed similar results of high bacteria counts as obtained in this study.^{8,9,10} The major reasons that could be responsible for the high bacteria counts obtained from the various borehole water samples analyzed in this study could be as a result of human or animal fecal matter contamination resulting from surface water runoffs, and seepages from broken septic tanks. Effluents from abattoirs and seepage from sewage or septic tanks which are in close proximity to boreholes could also contribute to such contamination.

The result of the bacteriological analysis of the borehole water samples revealed the presence of both enteric and non-enteric microorganisms. *Escherichia coli* was the predominant enteric bacteria isolated from this study; and this was followed by *Klebsiella* spp. *Staphylococcus aureus* was the predominant non-enteric bacteria isolated from the borehole water samples followed by *Pseudomonas aeruginosa* isolates. These organisms have been previously identified as important human pathogens responsible for causing a handful of microbial-related diseases including but not limited to urinary tract infections (UTIs), gastroenteritis and dysentery.^{1,2,7,17,20}

The bacterial pathogens isolated from the various water samples in this study are of public health importance because these microbes are implicated in a plethora of human infections. The presence of these microbes in water (especially above the acceptable limits) also signifies that these water sources are not potable and thus could not be used for drinking purposes. The presence of *E. coli* in a water sample (as obtainable in this study) is an indication of fecal contamination of the water source.^{3,21}

It was observed in this study that water samples from point E had the highest number of *E. coli* isolates compared to the other water sources analyzed. However, *E. coli* was not isolated from water samples collected

from point C. *Klebsiella* spp was not isolated in the water samples from point B and point E but *Pseudomonas* species was not isolated in the borehole water sample from point B. This result conforms to an earlier study conducted in Enugu State, Nigeria where the presence of similar organisms in borehole water samples was reported.²

All the isolated bacteria from the various borehole water samples showed varying levels of susceptibility to the tested antimicrobial agents. Notably, higher levels of resistance to ampicillin, ertapenem, sulphamethoxazole-trimethoprim, tobramycin, and ofloxacin were observed in all the *E. coli* isolates, *Klebsiella* spp. isolates and *Pseudomonas aeruginosa* isolates. This is similar to a previous work carried out in Abakaliki where high levels of resistance amongst coliform bacteria isolated from sachet water samples sold in Abakaliki metropolis, Ebonyi State, Nigeria was reported.²²

The result of the physicochemical analysis showed that the pH of the water samples was alkaline, and was within the limits of the prescribed water quality standards; and the temperature range of the water samples were at ambient temperatures. There were slight increase in the electrical conductivities of water samples from point D and A; and these were found to be higher than the acceptable limit.

The results obtained for phosphate and sulphate were within the water quality standards prescribed limits. The study of the levels of phosphate and sulphate concentrations in the samples was important for various reasons. Phosphate enrichment of water bodies contributes to ecological impacts and their presence in water bodies contributes to eutrophication of natural waters.^{23,24}

This study revealed the presence of enteric and non-enteric bacterial pathogens in the water samples analyzed; and this signifies that the borehole water samples are not too fit for consumption. Most of the physicochemical parameters of the water samples were within the acceptable limits set by World Health Organization (WHO), Standard Organization of Nigeria (SON) and the United States Environmental Protection Agency (USEPA).^{18,19}

CONCLUSION

Conclusively, this study show that borehole water samples in the study locations require proper treatment before domestic use especially to eliminate bacterial pathogens which are usually implicated in waterborne disease outbreaks.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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