PHYSICOCHEMICAL AND BACTERIOLOGICAL ANALYSIS OF BOREHOLE WATER FROM EIGHT SELECTED LOCATIONS WITHIN BENIN METROPOLIS, EDO STATE, NIGERIA



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ABSTRACT

In Nigeria, borehole water is a dominant source of drinking water. The quality of the water has diminished substantially due to various anthropogenic activities. Thus, this study seeks to x-ray the physicochemical and bacteriological quality of borehole water in some crowded localities in Benin City, Edo State, Nigeria. Heterotrophic plate count and total coliform count were carried out sing pour plate method. Antibiotic susceptibility test was done using the Kirby-Bauerdisc diffusion test. Except for one case each of iron (0.32 \pm 0.11), Manganese (0.16 \pm 0.06), Cadmium (0.005 \pm 0.01) and Lead (0.12 \pm 0.01) that were found to be above the recommended WHO and NAFDAC limits, the heavy metals had concentration levels that were within the stipulated limits. Isolates were phenotypically and molecularly identified as:*Klebsiella* aerogenes, strain OBANOR&OKWU 100; Streptococcus pneumoniae, OBANOR&OKWU 101: Pseudomonas aeruginosa, **OBANOR&OKWU** 102; Staphylococcus aureus, **OBANOR&OKWU** 103 and Micrococcus lylae, OBANOR&OKWU 104 using the 16S rRNA gene analysis. Isolates were deposited in the United States GenBank under accession numbers: OK017170, OK017423, OK017480, OK017486 and OK019091 respectively. Generally, the isolates showed low resistance rates (14.29 % - 19.05 %) to the antibiotics used in this study except for Cefuroxime and Ampicillin with extremely high resistance rates of 80.95 % and 85.71 % respectively. Majority of borehole water samples were found to be contaminated. This study highlights the need for continuous monitoring and quality assessment of the borehole water available for consumption.

Keywords: Borehole water, NAFDAC, Heavy metals, Microbes, Water quality

INTRODUCTION

Pindar, the Greek philosopher, described water as the "Best of all things" The expression of this view comes with little surprise since the importance and pertinence of water throughout history has remained without a doubt (Jidauna *et al.*, 2014). Portable water remains an essential ingredient for the maintenance of good health. About 67% of the human body consists of water. The human body requires up to seven litres of water per day to avoid dehydration and ensure its physiological functions. This makes water indispensable to man. Water is never entirely 100% pure; rather, it inevitably contains traces of other substances. ranging from organic compounds, particles, gasses, to minerals and ions which affect its bacteriological, chemical and even physical characteristics. The demand for water can be divided into three broad uses which include: domestic, industrial and agricultural purposes (Jidauna et al., 2014). The quality of water depends on its physical, chemical and biological characteristics, which determine its utility for different purposes (Giri and Qiu, 2016). The quality of water is continuously changing due to the reaction of water with contact media and human activities. Water that is certified fit to be consumed by humans is referred to as potable or drinking water. It is required that the water does not pose any significant health risk over lifetime consumption (Water and WHO, 2002; Yusuf et al., 2015). However, as populations increase, the problem becomes more serious and as

such, water can endanger the health and life of human beings because when polluted by faecal materials it becomes a potential carrier of pathogenic organisms (Micheal et al., 2021). Also, it has been increasingly noted that poor microbial quality water can be harmful to aquatic organisms and ecosystem function (Craun et al., 2006; Betiku et al., 2021). Moreover, there is a rising dependence on groundwater due to increasing contamination of surface water (Akhtar et al., 2021) and it is believed to be purified water moves down the bedrock. as Microorganisms (bacteria, viruses and protozoans) are ubiquitous in aquatic environments, with some being capable of producing toxigenic substances that elicit adverse effects to human health and the ecosystem. Contaminated water sources come in handy in the transmission of waterborne diseases such as: cholera, shigellosis, Campylobacteriosis and other diseases (Burgess and Pletschke, 2009). The location of the borehole very close to

open toilet systems, as well as animal husbandry in the vicinity of borehole, have been implicated as the sources of contamination borehole of water (Nkwachukwu et al., 2013; Ferrer et al., 2020). The present research is aimed at evaluating the extent of bacterial contamination of borehole water available in major commercial areas in Benin metropolis, Edo State, Nigeria.

MATERIALS AND METHODS

Study Area

Benin City is the capital and the largest city of Edo State, South-South Nigeria. It is the fourth largest city in Nigeria, with a population of 1,782,000 as of 2021. It is situated approximately 40 kilometres (25 mm) north of the Benin River, with latitudes of 6.3350⁰N and 5.6037⁰E. The eight sampling locations in Benin City Metropolis were: Ekehwuan Road, Sakponba Road, Igun Street, Sapele Road (Ekae), Limit Road, Ogida Quarters, Ugbowo and New Benin environments. Locations such as Ekae, Limit and Ekehwuan are densely populated residential areas; while locations such as: Igun, Ogida, New Benin, Sakponba Road and Ugbowo were selected because of the heavy commercial activities in these localities.

Collection of water samples

Samples were collected at two weeks' intervals between February and April 2021. The outlet of each borehole was sterilized using cotton wool soaked in methylated spirit followed by the collection of water samples with sterile containers (APHA, 2005). The sample bottles were immediately placed in an icepack and transported to the laboratory.

Determination of physicochemical parameters and heavy metals

The physicochemical parameters analyzed were: pH, electrical conductivity, turbidity, total dissolved solids (TDS), total suspended solids (TSS), iron, manganese, zinc, copper, cadmium and lead. The measurements of the physicochemical parameters in the samples were carried out according to the standard methods prescribed by the American Public Health Association (APHA) (APHA, 1998).

Bacteriological analysis

Isolation and enumeration of bacteria in the samples was performed with the pour plate technique (Public Health England, 2014). Ten-fold serial dilution of samples was done up to 10⁻⁴, with the first dilution of the water samples made by mixing 25 ml of water with 225 ml of sterile physiological saline in a sterile tube. One millilitre of each of the serially diluted samples was separately poured into sterile petri dishes in duplicate and then mixed with 15 ml of tryptone soy agar (TSA) and MacConkey agar (MA) (Himedia Laboratories, India) separately. The TSA and MA Petri dishes were subsequently incubated at room temperature for 48 hours. After incubation, Petri plates were observed for the presence of colonies. Selected Petri plates

containing between 30 and 300 discrete colonies were counted and the counts were used to deduce the total viable counts (TVC) and total coliform counts (TCC), expressed as CFU/ml (colony forming units/ml).

Identification of isolates

The phenotypic techniques employed for the genus-level identification of bacterial isolates obtained from the samples were performed with water standard methods (Krieg and Holt, 1984). The phenotypic tests used in the identification of the isolates included Gram stain, citrate test, lactose test, catalase test, urease test, coagulase test and sugar fermentation test. Partial 16S rRNA gene analysis confirmed the presence of bacterial strains in the water samples. The gene analysis was done by polymerase chain reaction (PCR) and sequencing methods (Lane 1991; Schuurman et al. 2004). Ultra-pure DNA template was used to perform the polymerase chain reaction (PCR). Universal 16S rRNA bacterial

[27F-

primers

AGAGTTTGATCMTGGCTCAG;

1492R- GGTTACCTTGTTACGACTT]

(Lane, 1991) often employed for bacterial taxonomy were used to determine the presence of the 16S rRNA gene. The PCR protocol was carried out with a 50 µl reaction mixture containing 2 µL of template DNA, 10mM Tris-HCL (pH 8.3), 50mM KCl, 2 mM MgCl₂, 200 mM of each of the deoxynucleoside triphosphates (dNTPs) (Fermentas Inc., USA), 2 U of GoTaq Hot Start Polymerase (Promega, USA) and 0.5 µM of each primer. Amplification was done using a GeneAmp PCR system 9700 (Applied Biosystems) with the following cycling conditions: initial denaturation at 95°C for 2 minutes, followed by 40 cycles, with each cycle consisting of denaturation at 94°C for 45 seconds; annealing at 55°C for 60 seconds; extension at 72°C for 120 seconds; and a final extension at 72°C for 300 seconds. Ten microliters of the amplified product analyzed by were gel

electrophoresis on a 2% agarose prepared in Tris-Borate-EDTA buffer containing 0.5 μ g/ml of ethidium bromide at 100 V for 1 hour. The DNA band in the gel was subsequently visualized and documented on the gel documentation system (Applied Biosystems). A molecular marker (100 base pair ladder) was ran concurrently. The DNA sequencing of each amplicon was performed with the dideoxy-chain termination method (Sanger et al. 1977). The amplicon was cleaned up with ExoSAP-IT (ThermoFisher Scientific). The purified amplicon was then subjected to cycle sequencing with the Big Dye Terminator version 3.1 (Applied Biosystems) using standard cycling conditions. The purified cycle sequencing product was separated by capillary electrophoresis on an ABI 3730×I DNA analyzer. The sequence was then quality checked and proofread with Sequencher version 4.10.1 (Gene Codes Corporation, USA). The sequences were classified by the basic local alignment search tool

(BLASTN 2.8.0+ program) developed by the National Center for Biotechnology Information (NCBI, USA). The classified bacterial strains were subsequently deposited in the NCBI GenBank database.

Determination Antibiotic Susceptibility

Profiles of the Isolates

The test isolates were tested for antibiotic susceptibility using the Kirby-Bauer

disc diffusion test as described by the Clinical and Laboratory Standards Institute (CLSI,

2014). An incubated saline suspension of each pure bacterial culture adjusted to 0.5 McFarland turbidity standards was inoculated on to Petri plates of Mueller– Hinton agar, and antibiotic discs were placed on the agar surface. After 16–18 hours of incubation at 35°C, inhibitory zone diameter around each of the bacterial colonies was interpreted as sensitive, intermediate, or resistant based on zone diameter interpretive standards stipulated by the Clinical and Laboratory Standards Institute. Antibiotic discs used in this study were: cefuroxime (30 μ g), ampicillin (10 μ g), amoxicillin/clavulanic acid (30 μ g), ciprofloxacin (5 μ g), pefloxacin (5 μ g), ofloxacin (10 μ g), sparfloxacin (5 μ g), gentamicin (10 μ g), erythromycin (15 μ g) and chloramphenicol (30 μ g).

Statistical analysis

Descriptive statistics of heterotrophic plate count (HPC) and total coliform count (TCC) counts was done with NCSS version 12 data analysis software. Levene test of homogeneity, Shapiro–Wilk Kruskal–Wallis test, nonparametric one-way ANOVA test for non-normally distributed datasets, as well as Fisher (F) one-way ANOVA test for normally distributed datasets with equal variances, were also performed with NCSS version 12 data analysis software. The test of the hypothesis was considered statistically significant if the achieved level of significance (p) was less than 0.05.

RESULTS *Physiochemical parameters*

Table 1 shows the results of the physicochemical analysis of the borehole water samples. Results of the analysis were compared with standards of the World Health Organization (WHO) and the National Agency for Food and Drug Administration and Control (NAFDAC). Temperature readings for all collected water samples were within recommended limits. The lowest mean reading of 26.9°C was recorded in water samples collected from Ogida environs while the highest mean reading of 28.1°C was recorded in the borehole situated in the New Benin environs. pH readings for samples were within permissible limits stipulated by WHO and NAFDAC except for water samples collected from boreholes at Limit Road, Ugbowo and New Benin with pH below standards. the Heavy metals concentrations were found to be generally within recommended limits except for iron, manganese, cadmium and lead that were above the limits at Sakponba, Igun street and Limit Road respectively.

Counts of bacterial colony-forming units

Table 2 shows the results of the heterotrophic plate and total coliform counts of the borehole water samples. Borehole water samples collected from four locations (Ekehwuan road, Sapele road, Ogida road and New Benin environ) had HPC values that exceeded the limits set by the World Health Organization. However, the TCC values recorded in all the borehole water samples were within recommended limits of WHO and NAFDAC. The mean HPC counts obtained from all the sampling locations were non-normally distributed (p < 0.05) with unequal variance (p < 0.05). Kruskal-Wallis ANOVA tests indicated а significant difference in the median HPC counts (p < 0.05). However, the mean TCC counts obtained from all the sampling locations were normally distributed (p > 0.05) with equal variance (p > 0.05), and Fisher ANOVA tests showed no significant difference in the mean TCC counts (p > 0.05).

Identified bacterial isolates

Table 3 shows the phenotypic and molecular characterizations performed on the bacterial isolates. Streptococcus pneumoniae was a common isolate obtained from the borehole water samples collected from homes along Ekehwuan road and Igun street. Pseudomonas aeruginosa and Micrococcus lylae respectively isolated. were also Staphylococcus aureus was isolated from samples collected from homes along Sapele road and Ugbowo road. Klebsiella aerogenes was isolated from samples obtained from homes along Ogida street and New Benin environment; while Bacillus species was the general contaminants that were found in all the samples but samples from Igun street.

| Parameters | W | OH | NAFDA | AFDA SAMPLING SITES | | | | | | | | | |
|----------------|--------|--------|--------|--|--|--|--|--|--|--|--|--|--|
| | HDL | MPL | С | EK | SKP | IG | SP | LM | UGB | OGD | NB | | |
| | | | | N = 15 | | |
| Temp. (°C) | 30.00 | 25.00 | - | 27.50 ± | 27.40 ± | 27.10 ± | 27.80 ± | 27.80 ± | 27.30 ± | 26.90 ± | 28.10 ± | | |
| - · · · | | | | 1.11 | 2.21 | 1.64 | 2.61 | 2.41 | 1.14 | 0.90 | 2.35 | | |
| Turbidity | 5.00 | 2.00 | - | $0.76 \pm$ | $4.60 \pm$ | $4.35 \pm$ | $0.79 \pm$ | $0.00 \pm$ | $3.00 \pm$ | 5.21 ± | $0.83 \pm$ | | |
| (NTU) | | | | 0.02 | 0.15 | 0.09 | 0.13 | 0.00 | 0.11 | 0.10 | 0.01 | | |
| рН | 8.50 | 6.50 | 6.50 - | $7.00 \pm$ | $6.20 \pm$ | $6.60 \pm$ | $6.20 \pm$ | $5.70 \pm$ | $5.30 \pm$ | $6.40 \pm$ | $5.60 \pm$ | | |
| - | | | 8.50 | 0.13 | 0.19 | 0.22 | 0.18 | 0.13 | 0.11 | 0.16 | 0.21 | | |
| EC (µs/cm) | 500.00 | 200.00 | | $19.40 \pm$ | $23.30 \pm$ | $21.20 \pm$ | $23.10 \pm$ | $16.20 \pm$ | $23.40 \pm$ | $21.80 \pm$ | $19.40 \pm$ | | |
| . | | | | 1.16 | 2.15 | 1.89 | 1.74 | 1.71 | 2.00 | 1.11 | 1.05 | | |
| TDS (mg/l) | 500.00 | - | 500.00 | $16.10 \pm$ | $13.5 \pm$ | $12.60 \pm$ | $19.30 \pm$ | $11.30 \pm$ | $16.30 \pm$ | $13.90 \pm$ | $15.80 \pm$ | | |
| | | | | 1.21 | 1.62 | 2.11 | 1.07 | 0.08 | 1.33 | 0.09 | 1.00 | | |
| Salinity | - | - | - | $7.90 \pm$ | $8.20 \pm$ | $6.60 \pm$ | $8.10 \pm$ | $7.60 \pm$ | $10.90 \pm$ | $8.40 \pm$ | $10.10 \pm$ | | |
| • | | | | 0.21 | 0.32 | 0.22 | 0.21 | 0.12 | 0.22 | 0.17 | 0.15 | | |
| TSS (mg/l) | - | - | - | $2.30 \pm$ | $3.00 \pm$ | $7.20 \pm$ | $5.90 \pm$ | $0.00 \pm$ | $10.00 \pm$ | $9.40 \pm$ | $3.80 \pm$ | | |
| | | | | 0.09 | 0.09 | 0.07 | 0.10 | 0.00 | 0.13 | 0.14 | 0.08 | | |
| Fe (mg/l) | 0.30 | 0.10 | - | $0.02 \pm$ | $0.32 \pm$ | $0.05 \pm$ | $0.04 \pm$ | $0.04 \pm$ | $0.28 \pm$ | 0.13 ± | $0.04 \pm$ | | |
| | | | | 0.01 | 0.11 | 0.02 | 0.01 | 0.01 | 0.15 | 0.08 | 0.01 | | |
| Mn (mg/l) | 0.05 | - | - | $0.02 \pm$ | $0.02 \pm$ | $0.16 \pm$ | $0.01 \pm$ | $0.02 \pm$ | $0.03 \pm$ | $0.02 \pm$ | $0.05 \pm$ | | |
| | | | | 0.01 | 0.01 | 0.06 | 0.01 | 0.00 | 0.01 | 0.00 | 0.01 | | |
| Zinc | - | - | - | $0.04 \pm$ | $0.02 \pm$ | $0.02 \pm$ | $0.02 \pm$ | $0.04 \pm$ | $0.03 \pm$ | $0.03 \pm$ | $0.02 \pm$ | | |
| | | | | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 | | |
| Cu (mg/l) | 2.00 | - | - | $0.04 \pm$ | $0.02 \pm$ | $0.52 \pm$ | $0.05 \pm$ | $0.05 \pm$ | $0.15 \pm$ | $0.05 \pm$ | $0.08 \pm$ | | |
| | | | | 0.01 | 0.01 | 0.11 | 0.02 | 0.01 | 0.04 | 0.01 | 0.02 | | |
| Cadmium | 0.003 | | - | $\begin{array}{c} 0.002 \pm \\ 0.00 \end{array}$ | $\begin{array}{c} 0.003 \pm \\ 0.00 \end{array}$ | $\begin{array}{c} 0.005 \pm \\ 0.01 \end{array}$ | $\begin{array}{c} 0.001 \pm \\ 0.00 \end{array}$ | $\begin{array}{c} 0.002 \pm \\ 0.00 \end{array}$ | $\begin{array}{c} 0.003 \pm \\ 0.01 \end{array}$ | $\begin{array}{c} 0.002 \pm \\ 0.00 \end{array}$ | $\begin{array}{c} 0.002 \pm \\ 0.00 \end{array}$ | | |

Table 1: Physicochemical parameters of collected water samples compared with WHO and NAFDAC standards

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|--|-------------------|
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| Lead | 0.05 | 0.02 | - | $0.03 \pm$ | $0.03 \pm$ | $0.05 \pm$ | $0.03 \pm$ | $0.12 \pm$ | $0.04 \pm$ | $0.05 \pm$ | $0.03 \pm$ |
|------|------|------|---|------------|------------|------------|------------|------------|------------|------------|------------|
| | | | | 0.01 | 0.01 | 0.02 | 0.01 | 0.01 | 0.00 | 0.01 | 0.00 |

Temp.: Temperature; EK: Ekehwuan road; SKP: Sakponba; IG: Igun Street; SP: Sapele road; LM: Limit Road; UGB: Ugbowo; OGD: Ogida; NB: New Benin; N: total number of samples collected from each of the sampling locations; WHO: World Health Organization; NAFDAC: National Agency for Food and Drug Administration; HDL: Highest Desired Level; MPL: Minimum Permissible Level; EC: Electrical conductivity; TDS: Total Dissolved Solids; TSS: Total Suspended Solids; Fe: Iron; Mn: Manganese; Zn: Zinc; Cu: Copper; Cd: Cadmium; Pb: Lead.

| T | | |
|----------------------|-------------------|-------------------|
| Locations/Limits | Mean HPC \pm SE | Mean TCC \pm SE |
| | (CFU/ml) | (CFU/ml) |
| | (N = 15) | (N = 15) |
| | | |
| Ekehwuan road | 104.67 ± 2.91 | 8.30 ± 0.40 |
| Sakponba road | 73.00 ± 2.19 | 0.00 ± 0.00 |
| Igun Street | 17.70 ± 1.74 | 2.00 ± 0.26 |
| Sapele road | 125.00 ± 4.51 | 0.00 ± 0.00 |
| Limit road | 35.00 ± 1.48 | 2.00 ± 0.15 |
| Ugbowo environ | 33.67 ± 1.55 | 1.00 ± 0.15 |
| Ogida environ | 181.00 ± 4.10 | 4.50 ± 0.47 |
| New Benin environ | 225.00 ± 5.88 | 5.00 ± 0.63 |
| WHO HPC limits | ≤ 100 | |
| NAFDAC HPC limits | None | |
| WHO TCC limits | | ≤ 10 |
| NAFDAC TCC limits | | ≤ 10 |

Table 2: Bacterial counts

HPC: heterotrophic plate count; TCC: total coliform count; N: total number of samples collected from each of the sampling locations; SE: standard error of mean; CFU/ml: bacterial colony forming units per millimeter; WHO: World Health Organization; NAFDAC: National Agency for Food and Drug Administration and Control.

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| Sampling locations | Representative | | | Biochemical characteristics of bacterial colonies | | | | | | | nies | Molecular : | malysis | Identified bacteria |
|--------------------|----------------|-------------------------------|----------------------------|---|----|-------|----|----|----|----|------|-------------|-----------|--------------------------|
| | bacterial | | | CA | CO | CO OX | IN | MR | VP | UR | LA | 16S | 16S | |
| | colonies | Growth on TSA Petri plates | Gram staining | | | | | | | | | homology | identity | |
| Ekehwan Road | 1 | Mucoid colony | Positive cocci in chains | - | - | - | - | + | - | - | + | 99 - 100% | 95 - 99% | Streptococcus pneumoniae |
| | 2 | Mucoid colony | Positive bacilli | - | - | + | - | + | - | - | + | NP | NP | Bacillus sp. |
| | 3 | Mucoid colony | Negative bacilli | + | - | - | - | + | - | - | - | 98 - 100% | 95 - 99% | Pseudomonas aeruginosa |
| Sakponba Road | 1 | Mucoid Colony | Positive bacilli | - | - | + | - | + | - | | + | NP | NP | Bacillus sp. |
| | 2 | Mucoid Colony | Positive bacilli | - | - | + | - | + | - | - | - | NP | NP | Bacillus sp. |
| | 3 | Dry Colony | Positive bacilli in chains | - | - | + | - | + | - | - | - | NP | NP | Bacillus sp. |
| | 4 | Dry Colony | Positive bacilli | + | - | + | - | + | - | - | - | NP | NP | Bacillus sp. |
| | 5 | Dry Colony | Positive bacilli | + | - | + | - | + | - | - | + | NP | NP | Bacillus sp. |
| Igun Street | 1 | Mucoid Colony | Positive bacilli in pairs | - | | | - | + | - | | + | 96 - 100% | 95 - 100% | Streptococcus pneumoniae |
| | 2 | Yellow colony | Positive cocci | + | - | + | - | + | - | - | + | 97 - 100% | 99 - 100% | Micrococcus hylae |
| Sapele Road | 1 | Mucoid Colony | Positive cocci in bunches | + | + | | - | + | | | | 98 - 100% | 97 - 99% | Staphylococcus aureus |
| | 2 | Mucoid Colony | Positive bacilli | + | - | + | - | + | - | - | - | NP | NP | Bacillus sp. |
| Limit Road | 1 | Dry Colony | Positive bacilli | + | - | + | - | + | - | + | + | NP | NP | Bacillus sp. |
| | 2 | Dry Colony | Positive bacilli | + | - | + | - | + | - | - | + | NP | NP | Bacillus sp. |
| Ugbowo Road | 1 | Dry Colony | Positive bacilli | + | - | + | - | + | | + | + | NP | NP | Bacillus sp. |
| | 2 | Yellow Colony | Positive cocci in bunches | + | + | + | - | + | - | + | - | 99 - 100% | 100% | Staphylococcus aureus |
| Ogida Street | 1 | Dry Colony | Positive bacilli | + | - | - | - | + | + | | + | NP | NP | Bacillus sp. |
| _ | 2 | Mucoid Colony | Negative bacilli | - | - | + | - | | + | | + | 97 - 100% | 97 - 100% | Klebsiella aerogenes |
| | 3 | Mucoid Colony | Positive bacilli in chains | | - | | | | | | | NP | NP | Bacillus sp. |
| New Benin environ | 1 | Mucoid Colony | Negative bacilli | | | | - | | + | | + | 97 - 100% | 97 - 100% | Klebsiella aerogenes |
| | 2 | Dry Colony | Positive bacilli | + | - | - | | + | 2 | + | _ | NP | NP | Bacillus sp. |

Table 3: Characterization of bacterial isolates obtained from the borehole water

CO: Coagulase test; CA: Catalase test; UR: Urease test; IN: Indole test; MR: Methyl red test; VP: Voges Proskauer test; -: negative reaction; +: positive reaction;

TSA: Tryptone Soya Agar; LA: Lactose test.; NP: not performed

Representative bacterial isolates of public health importance such as: K. aerogenes strain OBANOR&OKWU 100; S. pneumoniae, OBANOR&OKWU 101; P. aeruginosa, OBANOR&OKWU 102; S aureus, OBANOR&OKWU 103 and M. lylae, OBANOR&OKWU 104, as revealed by 16S rRNA gene analysis, have been deposited in the United States GenBank under accession numbers OK017170, OK017423, OK017480, OK017486 and OK019091 respectively.

Prevalence of antibiotic resistance isolates

Table 4 shows the percentage of antibiotic resistance demonstrated by a total of 21 test isolates. For ampicillin and cefuroxime, the isolates exhibited extremely high resistance rates of 85.71% and 80. 95% respectively while low rates (< 20 %) to the fluoroquinolones (ciprofloxacin, ofloxacin, pefloxacin, sparfloxacin), gentamycin, erythromycin, chloramphenicol and amoxicillin/clavulanic acid.

| Table 4: Prevalence of antibiotic | resistance exhibited by | y some bacterial isolates | obtained from |
|-----------------------------------|-------------------------|---------------------------|---------------|
| the sampling locations | | | |

| Antibiotics | Bacterial | isolates |
|-----------------------------|-----------|----------|
| | F | (%) |
| Ampicillin | 18/21 | 85.71 |
| Amoxicillin/clavulanic acid | 4/21 | 19.05 |
| Ciprofloxacin | 3/21 | 14.29 |
| Cefuroxime | 17/21 | 80.95 |
| Chloramphenicol | 4/21 | 19.05 |
| Erythromycin | 4/21 | 19.05 |
| Gentamycin | 4/21 | 19.05 |
| Ofloxacin | 3/21 | 14.29 |
| Pefloxacin | 3/21 | 14.29 |
| Sparfloxacin | 3/21 | 14.29 |

F: fractional prevalence; % percentage prevalence; prevalence interpretive criteria (Szewzyk *et al.*, 2000): > 80%: Extremely high; > 40%: High; > 20%: Moderate; $\ge 10\% \le 20\%$: Low: < 10%

DISCUSSION

Eighty-eight per cent (88%) of global diarrheal diseases burden have been attributed to unsafe water supply (Water and WHO, 2002). Temperature readings for all collected water samples were within recommended standards (Table 1).

The pH values obtained from the collected samples tended to be slightly acidic for most of the samples which are below the WHO and NAFDAC permissible limits of 6.5 - 8.5 (Table 1). The acidity of the borehole water may be due to the acidic rainwater that percolates through the soil to underground water. The pH characterization is essential because biological activities can only thrive and survive within narrow pH ranges (WHO, 1993). In the present study. pHconcentrations were largely below acceptable limits. This is a variation from the findings of Foka et al., (2018) who carried out a study on borehole water in Benin City.

Heavy metal concentrations such as those that were obtained for iron, manganese, zinc, copper, cadmium and lead were mostly within recommended limits of WHO and NADAC.

Findings from this research indicated that TCC values for all the sampling locations were within stipulated WHO and NAFDAC limits, but 4 out of the 8 sampling locations had HPC values that exceeded the WHO limits (Table 2). There was a high prevalence of contaminants such as the Bacillus spp. in all but Igun street water samples collected for this study (Table 3). Similar of borehole cases water contaminated with *Bacillus* spp. have been reported in different localities in Nigeria (Woke and Bolaji, 2015). The boreholes in sampling locations such as those in Ogida street, Igun street, Ekehwuan road and NewBenin environ were dug close to sewage and waste disposal systems. This may have contributed to the isolation of some indicators of fecal pollution such as *Klebsiella* and *Streptococcus* species from the borehole water collected from these localities; thus, indicating the poor sanitary conditions of these environments.

The bacterial isolates obtained from the borehole water samples were extremely resistant to ampicillin and cefuroxime (Table 4). The detection of antibioticresistant bacteria from borehole water has been reported in several localities in the world (Ateba and Mbewe, 2011).

CONCLUSION

The present study indicated that most of the borehole water samples were found to be contaminated with bacterial organisms, though at levels that were within the limits stipulated by WHO and NAFDAC. Thus, this study highlights the need for continuous monitoring and quality assessment of borehole water available for consumption.

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