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HEAVY METALS INCIDENCE AND PLASMID PROFILES OF BACTERIA ISOLATED FROM BOREHOLE WATERS AROUND FEDERAL POLYTECHNIC NEKEDE COMMUNITIES

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KeyWords
Borehole waters, bacteria, culture based analysis, heavy metals, plasmid profile, public health, septic tanks, waste dumpsites

ABSTRACT

This study investigated heavy metals incidence and bacterial contamination of 21 borehole water samples collected around Federal Polytechnic Nekede community. These borehole water samples were grouped into two, BH1-16 and NK1-5, based on the media used in isolating the bacteria contaminants then the heavy metals analyzed. The result shows that Coliforms and *E. coli* were the dominant bacteria in (87.5%) of the BH1-16 borehole samples, followed by *Salmonella typhi* (18.75%), *Clostridium perfringens* (12.5%). Copper and zinc concentrations were in the safe zone while lead, cadmium and chromium were higher than WHO standards. The samples BH1-16 contaminated with lead is 100%, cadmium 100% and chromium (68.75%). The concentration of lead ranged from 0.66 – 1.93 mg/l; cadmium (0.006 – 0.298 mg/l) and cadmium concentration in the range 0.0576 – 0.567 mg/l. The 7 genera of bacteria of public health importance, isolated from samples NK1-5, include *Staphylococcus aureus* (100%), *Salmonella typhi* (40%), *Escherichia coli* (80%), *Vibrio cholerae* (100%), *Pseudomonas* spp., (40%), *Yesinia* spp., (60%), and *Shigella* spp. (40%). Lead contaminant was present in 100% of samples NK1-5, ranged from 0.08 – 0.23 mg/l, above WHO standard. Plasmid DNA was found in all seven genera from NK1-5 grown in lead supplemented nutrient agar. The total of 23 bacteria 15(65.2%) had plasmids of the size range (0.9 – 12 Kbp). The presence of plasmids confers on the bacteria the ability to survive in lead contaminated borehole waters. Lead resistant bacteria could be potential agents for bioremediation of lead polluted environment. There is need to treat these waters using both chemical methods and boiling before drinking.

Introduction

Water is an important solvent needed for the sustenance of life. There are different sources of water and borehole is one of them. Water is threatened by heavy metal and microbiological contaminants. Most of the borehole waters have acrid tastes as a result of

metal contaminants. The source of metals and the entry pathway to water sources (borehole waters) is important in public health. Cadmium is used in the steel industry and in plastics. Cadmium compounds are widely used in batteries. Cadmium is released to the environment in wastewater, and diffuse pollution is caused by contamination from fertilizers and local air pollution. Contamination in drinking-water may also be caused by impurities in the galvanized pipes and solders and some metal fittings. Food is the main source of daily exposure to cadmium. The daily oral intake is 10–35 mg. Smoking is a significant additional source of cadmium exposure. Chromium is widely distributed in the Earth's crust [1,2].

Copper is both an essential nutrient and a drinking-water contaminant. It has many commercial uses. It is used to make pipes, valves and fittings and is present in alloys and coatings. Copper sulfate pentahydrate is sometimes added to surface water for the control of algae. Copper concentrations in drinking-water vary widely, with the primary source most often being the corrosion of interior copper plumbing. Levels in running or fully flushed water tend to be low, whereas those in standing or partially flushed water samples are more variable and can be substantially higher (frequently > 1 mg/l). Copper concentrations in treated water often increase during distribution, especially in systems with an acid pH or high-carbonate waters with an alkaline pH. Food and water are the primary sources of copper exposure in developed countries. Consumption of standing or partially flushed water from a distribution system that includes copper pipes or fittings can considerably increase total daily copper exposure, especially for infants fed formula reconstituted with tap water [1,2].

Lead is used principally in the production of lead-acid batteries, solder and alloys. The organolead compounds tetraethyl and tetramethyl lead have also been used extensively as antiknock and lubricating agents in petrol, although their use for these purposes in many countries is being phased out. Owing to the decreasing use of lead containing additives in petrol and of lead-containing solder in the food processing industry, concentrations in air and food are declining, and intake from drinking-water constitutes a greater proportion of total intake. Lead is rarely present in tap water as a result of its dissolution from natural sources; rather, its presence is primarily from household plumbing systems containing lead in pipes, solder, fittings or the service connections to homes. The amount of lead dissolved from the plumbing system

depends on several factors, including pH, temperature, water hardness and standing time of the water, with soft, acidic water being the most plumbosolvent [1,2].

Zinc is an essential trace element found in virtually all food and potable water in the form of salts or organic complexes. The diet is normally the principal source of zinc. Although levels of zinc in surface water and groundwater normally do not exceed 0.01 and 0.05 mg/litre, respectively, concentrations in tap water can be much higher as a result of dissolution of zinc from pipes. However, drinking-water containing zinc at levels above 3 mg/l may not be acceptable to consumers [1,2].

Many bacterial strains contain genetic determinants of resistances to heavy metals such as Hg^{2+}, Ag^{+}, Cu^{2+}, Ni^{2+}, Cd^{2+} and undoubtedly others. These resistance determinants are often found on plasmids and transposons. The same mechanisms of resistance occur in bacteria from soil, water, industrial waste and clinical sources [3]. Plasmid determined resistance to toxic metal ions has been demonstrated for many bacterial species and is a useful selectable marker for these DNA molecules [4]. Bacteria that are resistant to and grow on metals play an important role in the biogeochemical cycling of those metal ions [5].

Plasmid profiling is one of a typing tool. Plasmid profiling of an organism involves the isolation of plasmid DNA from an organism of interest, followed by the separation of these molecules based on their size by agarose gel electrophoresis. Plasmid incidence and characterization among microorganisms are useful in genetic studies and in the development of cloning vectors [6,7]. Although plasmids can be transferred between organisms, their presence or absence can be an important epidemiological marker. In general, plasmids of 10 Kb or less occur and the function of these low molecular weight plasmids were previously not known to encode distinguishing traits and were mostly used in molecular typing as opposed to the high molecular weight ones that were known to encode distinguishing traits. It has, however, been discovered that these low molecular weight plasmids code for retron reverse transcriptase and tend to influence phage resistance [8,9].

The unavailability of treated waters for community distribution by governing agencies responsible necessitated the need for drilling of boreholes to access drinking waters for domestic uses by land owners. The fear that the boreholes may not meet the standards for portability is a major concern. The over population of a community as a result of major projects like the siting of Federal Polytechnic, in Nekede community, has led to clustered settlement, scarcity of lands, overcrowding, packed up hostels with more and more wastes containing metals generated; most of which are not properly disposed off and can be sources of heavy metals leaching into groundwater. Many septic tanks are built close to boreholes, as a result contaminating the borehole waters. These metals have become major concern to all because of their serious health effects on man. Most bacteria of public health importance acquire resistance as a result of plasmids. Therefore the need to monitor drinking water so as to prevent the spread of water borne diseases from these bacteria habouring plasmids and protect the health of the community. Therefore this project was to randomly sample borehole waters around Federal Polytechnic Nekede, Owerri community, to ascertain the level of portability and the possible health effects on the teaming human population.

Methods

Study Area
The study was conducted around Federal Polytechnic Nekede Owerri communities. The twenty onee student lodges are close to waste dumpsites situated in Nekede community, Owerri West LGA, Imo state. The Nekede community is well populated and their main source of drinking water is borehole.

Sample Collection
Water samples were collected in two sets and were given the codes BH and NK. The BH set was collected from 16 boreholes and labeled BH1-16. The set NK was collected randomly from other five boreholes and labeled NK1-5. These samples were collected with sterile bottles. The samples were thereafter transported to the laboratories in a closed ice bucket for analysis.

Media Preparation
The nutrient media were prepared according to the manufacturer's instruction and autoclaved at 121^{o}C for 30 minutes and cooled to about 48^{o}C [10].

Sterilization of Wares
The glass wares used for this experimental study were sterilized by autoclaving at 101^{o}C for 15 minutes in order to avoid cross contamination of the samples, and cooled to about 41^{o}C.

Sample preparation
The two methods used are culture based analysis. The methods (1 and 2) differed in the nutrient media that were used for culturing and isolation of the bacteria.

Microbiological analysis of the borehole water samples (Method 1)
This method 1 was applied to samples BH1-16. The microbiological analysis was done in triplicate. Pour plate count method was used for the analysis. The Petri dishes were labeled with the sample codes (BH1-16). Thereafter 1 ml of the borehole water samples were transferred to triplicate plates of different nutrient media with respect to the targeted organisms to be identified. The inocula were spread on the media surface using sterile bent glass streaking rods. The plates were incubated in an upright position at 35^{o}C – 37^{o}C. Following the incubation, the colonies that appeared were counted using a colony counter. The nutrient media used and the respective colour change for each of the micro-organisms include: Clostridia agar used for *Clostridium* with a color change to black, deoxychocolate citrate agar used for *Salmonella* with a colour change to black; eosin methylene blue agar used for *E. coli* with a colour change to pink; violet red bile lactose agar used for coliform with a colour change to red.

Microbiological analysis of borehole water samples (Method 2)
This was used for samples NK1-5. The water samples were diluted in tenfold serial dilution. Then 0.1 ml aliquots from the 10^{-4} dilution were inoculated into nutrient agar and MacConkey agar plates, using the spread plate technique. The plates were properly labeled according to the samples (NK1-5) and were incubated in inverted positions at 37^{o}C for 48 hours. Thereafter the morphology of the colonies were observed. The colonies were picked up from the plates according to their different forms and were purified by further sub culturing in different culture media using streak plates method as listed: Yesinia agar for *Yesinia*, Chromogenic agar for *E.coli* and *S.aureus,* Salmonella Shigella agar for *Salmonella* and *Shigella*, Cetrimide agar for *Pseudomonas*, Thiosulphate citrate bile salt sucrose (TCBS) agar for *Vibrio cholerae.*

Bacteria Viable Count
The total viable count was done by dividing the plates containing the bacterial isolates into four parts. Then one of the four parts was counted and then multiplied by four. The total gives the total viable count of the bacteria
*Colony forming unit/ml = Average number of colonies**/**aliquot volume × Dilution factor

Bacterial Identification
The isolated bacteria were identified using Gram staining technique and biochemical tests. The biochemical tests were carried out after the Gram staining for further identification of these bacteria based on their chemical characteristics. These include: citrate utilization test, catalase test, oxidase test, methyl red, Voges – Proskauer test, motility test, indole test and urease test [10,11]

Determination of Heavy Metals (Cd, Cr, Pb, Cu, Zn) in samples BH1-16 and NK1-5
These heavy metals were determined using Buck 205 atomic absorption spectrophotometer. The borehole ground water samples were analyzed for the presence of the following heavy metals: Zinc, copper, cadmium, chromium, lead. The control/blank was de-

termined by the analysis of a known standard sample prepared from the metals in de-mineralized distilled water and subjected to the same process as the samples. The Atomic Absorption Spectrophotometer was connected to the mains, all the power buttons was switched on and the equipment was allowed to warm up to 15 minutes, compressor and acetylene tank was put on and acetylene tank was set to between 15-20 psi. The appropriate lamp for the analysis was selected and install in upper most position of the lamp turret i.e operating position. The necessary library button was pressed and selected to put the equipment working, also selected absorbance was designated as XX-D2 wavelength. The machine was loaded and the file selected appeared on the top active screen. The lamp gave energy between 2.5 to 4.5. The flame was ignited and the equipment was ready to set for running the samples. The calibration form of element of interest was filled, for instance copper was calibrated with maximum of 5ppm concentration [12].

Test for lead tolerance of isolates from NK1-5

Nutrient Agar was prepared and different concentration of lead (II) nitrate compound was also prepared (50% and 100% mg/l). The different concentration of lead was introduced to the prepared nutrient agar with respect to the number of isolates obtained. A sterile wire loop was used to collect a loopful of each of the pure isolates and directly streaked on the surface of the heavy metal (lead) incorporated nutrient agar in accordance with their different concentration. The plates were incubated at 37^{o}C for 24 hours. The plates were observed for bacteria growth after the incubation period.

Plasmid DNA Extraction by TENS Mini Prep of isolates from samples NK1-5

The overnight culture (1.5 ml) was spinned for 2 minutes in a micro-centrifuge to pellet cells. The supernatant was gently decanted leaving about 100 µl together with cell pellet. Then 300 µl of TENS was added and mixed by inverting micro tubes 50 times until the mixture became sticky. Thereafter 150 µl of 3.0 M sodium acetate with pH 5.2 was added and vortexed to mix completely. The tubes were spinned for 10 minutes in micro centrifuge to pellet cell debris and chromosomal DNA. The supernatant was transferred into fresh labeled tubes and was mixed well with 900 µl of ice cold absolute ethanol. It was then spun again for 10 minutes to pellet plasmid DNA (white pellet is observed), after which the supernatant was discarded; the pellet was rinsed twice with 1 ml of 70% ethanol by micro centrifuging for 15 min and was dried. The pellets were re-suspended in 40 µl of Tris Boris EDTA (TBE) buffer for Gel electrophoresis of the extracted plasmid DNA [13].

(TENS composition: Tris 25 mM, Ethyl-dimethyl tetra-amine; EDTA 10 mM, Sodium hydroxide; NaOH 0.1 N and Sodium dodecyl sulphate, SDS 0.5 %).

Gel Electrophoresis of Extracted Plasmid DNA of isolates from samples NK1-5

The gel electrophoresis of extracted plasmid DNA was conducted to determine the size of the extracted plasmid DNA based on their electrical charges (positive or negative chargers). Then 0.8 g of agarose powder was weighed and 100 ml of 1X TBE buffer was added to it. The mixture was dissolved by heating for 5 minutes using microwave oven and allowed to cool to 50°C, then 10 µl of ethidium bromide was added, mixed gently by swirling. The mixture was poured into electrophoresis tray with comb in place to obtain a gel thickness of about 5 mm. It was allowed to stand for 50 mins to solidify. The comb was removed and the tray placed in the electrophoresis tank. Thereafter 1X TBE buffer was poured into the tank ensuring that the buffer covers the surface of the gel. Then 20 µl of the extracted DNA was mixed with 2 µl of loading dye which was carefully loaded into the wells. The electrodes were connected to the power pack and electrophoresis ran at 60-100 volts. Electrodes were turned off and the Gel observed on UV trans-illuminator and results were recorded [14].

Results

The mean total viable counts of the water samples in the range 74 – 102 cfu/ml, shown in Fig 1, 2, 3, were higher than the specified limit advocated by WHO, [1,2]. BH14 recorded highest total viable count, 102 cfu/ml (Fig 1). The result shown in Fig. 2 revealed the coliform count ranged from 5 – 14 cfu/ml. The total of fourteen boreholes, BH1-6 and BH9-16, (87.5%) were contaminated with coliforms. The presence of coliform vividly indicates that the boreholes are fecally contaminated. Coliforms in the infected borehole waters render the water distasteful and may cause water borne diseases such as dysentery, diarrhea, and hepatitis. Also, the microbial analysis detected the presence of *E. coli* in the range 2-7 mg/l in fourteen samples BH1-6 and BH9-16 (87.5%) (Fig. 3). This also implies that the borehole waters are fecally contaminated. *Salmonella typhi* was only detected in three samples BH14, 15 and 16 (18.8%) (Fig. 4) and recorded 2 cfu/ml, 1 cfu/ml and 1 cfu/ml respectively. *Salmonella typhi* is implicated in diarrhea diseases. *Clostridium perfringes* was isolated from two samples, BH14 and BH16 (12.5%) (Fig. 5) and recorded 1 cfu/ml and 2 cfu/ml respectively. The results of Fig. 1, 2, 3, are indications of high microbial contamination as a result of poor hygiene and sanitary conditions such as cloth and dish washing as well as defecating in and near the water bodies. The locations of the boreholes are also of atrocious concern, considering waste disposal site and sewage tanks around the water bodies which could possibly serve as route of contaminations.

The two borehole samples BH7 and BH8 (12.5%) (Fig. 3) were free from bacterial contamination but are high in lead, cadmium and chromium (Fig 7, 8, 9) and as such not portable and good for consumption.

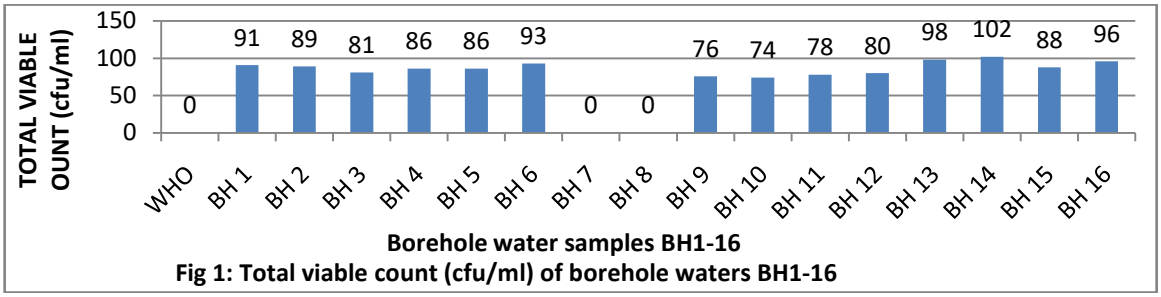

Fig 1: Total viable count (cfu/ml) of borehole waters BH1-16

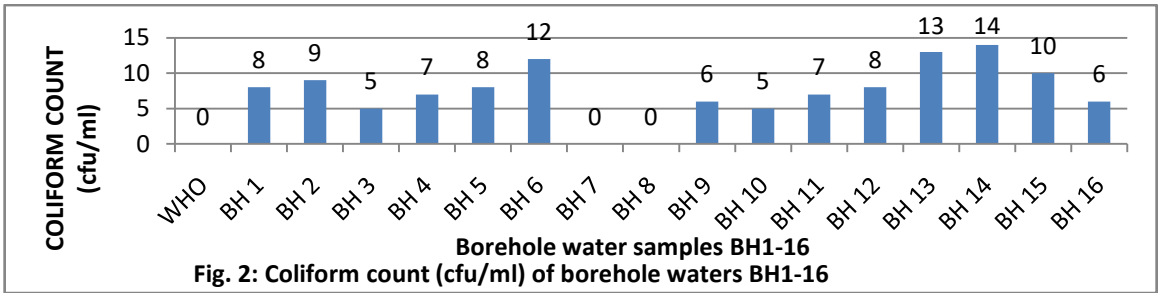

Fig. 2: Coliform count (cfu/ml) of borehole waters BH1-16

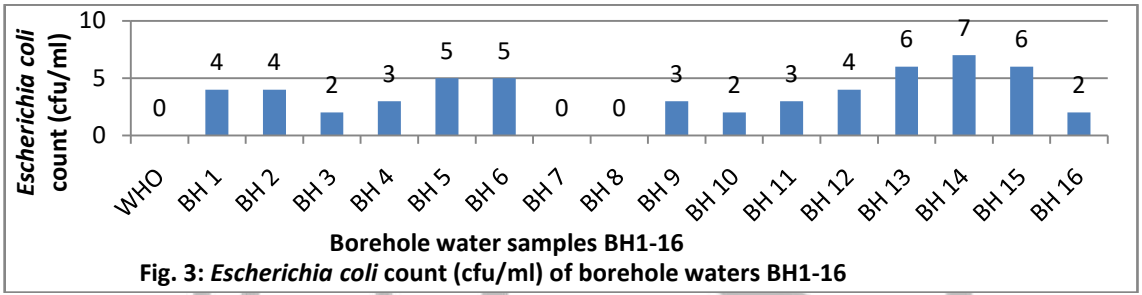

Fig. 3: *Escherichia coli* count (cfu/ml) of borehole waters BH1-16

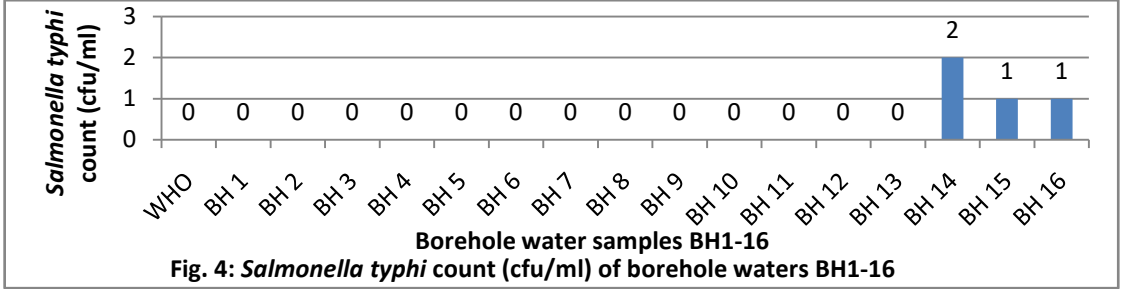

Fig. 4: *Salmonella typhi* count (cfu/ml) of borehole waters BH1-16

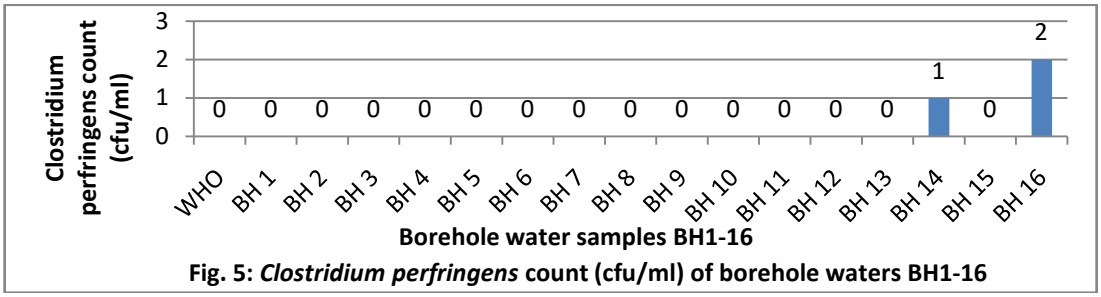

Fig. 5: *Clostridium perfringens* count (cfu/ml) of borehole waters BH1-16

The results of the heavy metals content of borehole samples BH1-16 are represented in Fig. 6-10. Fig. 6 shows that all the samples BH1-16 recorded copper concentrations (0.008 – 1.138 mg/l) less than that of WHO guideline value of 2 mg/l. The highest concentration of copper was recorded by BH7 (1.138 mg/l) and BH8 90.706 mg/l). Fig 7 shows the lead concentrations of BH1-16 samples (0.066 - 1.93 mg/l). These values recorded were higher than WHO guideline value of 0.01 mg/l. The highest lead concentration was observed in sample BH8 (1.93 mg/l).

Fig. 8 showed that all the BH1-16 samples recorded higher cadmium concentration than WHO guideline value of 0.003 mg/l. BH15

and BH16 recorded the highest cadmium concentrations of 0.225 mg/l and 0.298 mg/l respectively. Fig. 9 shows that five samples BH11 (0.033 mg/l), BH12 (0.042 mg/l), BH13 (0.039 mg/l), BH15 (0.009 mg/l), and BH16 (0.013 mg/l) recorded lower concentrations of chromium whereas BH7 (0.567 mg/l) recorded the highest value of chromium. These recorded concentrations are higher than WHO guideline concentration (0.05 mg/l). Fig 10 shows that all the borehole samples recorded less zinc concentration in the range of 0.042 mg/l -2.857 mg/l, below WHO standard of 3 mg/l. This implies that the water samples obtained from the boreholes are unfit for drinking due to the high lead, chromium and cadmium concentrations. This high content may suggest the presence of lead, chromium, cadmium containing materials being deposited around the borehole which may have seeped into the ground over time. High concentration of lead, cadmium and chromium in the body can cause death or permanent damage to the central nervous system, the brain and kidney.

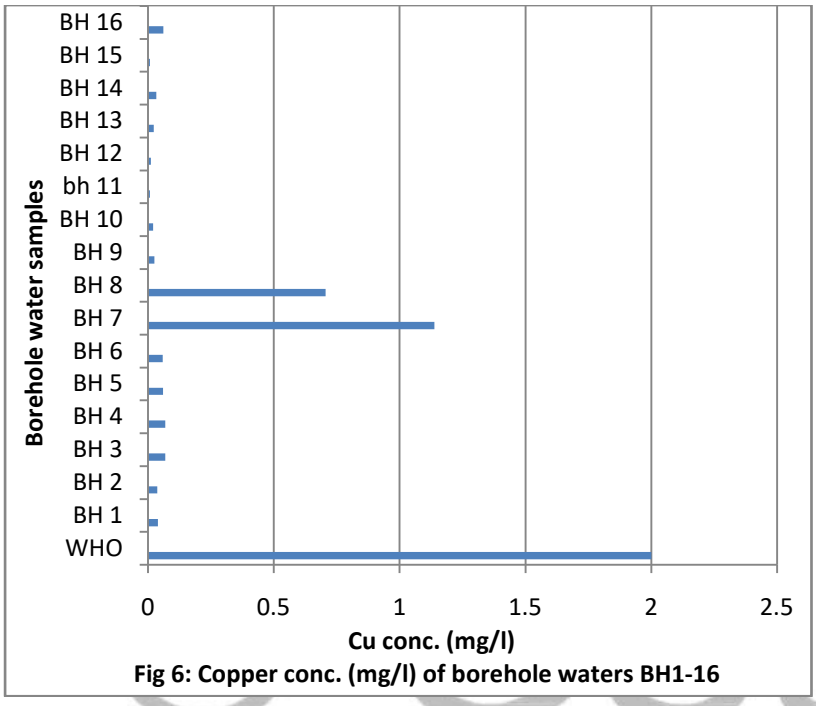

Fig 6: Copper conc. (mg/l) of borehole waters BH1-16

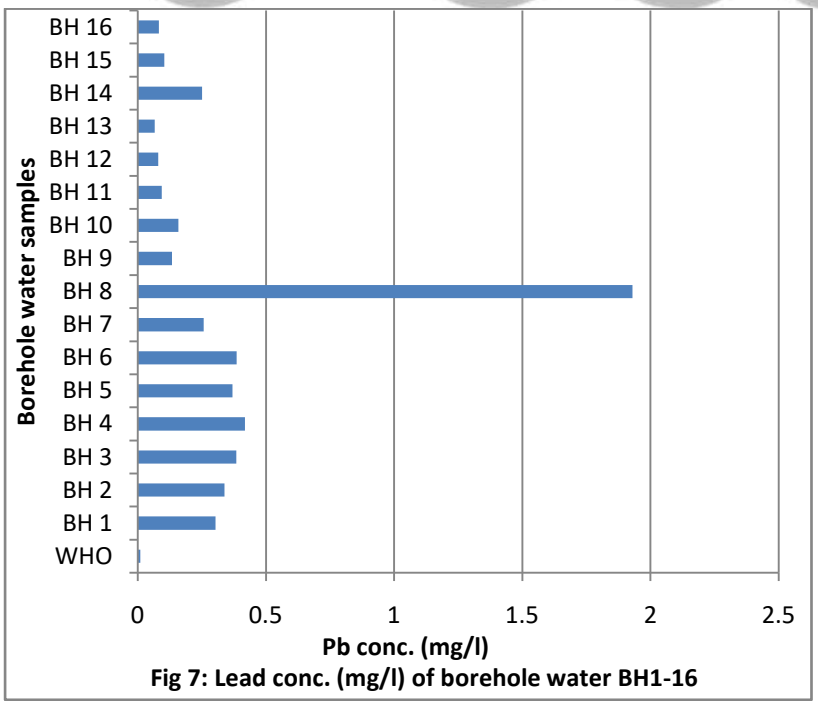

Fig 7: Lead conc. (mg/l) of borehole water BH1-16

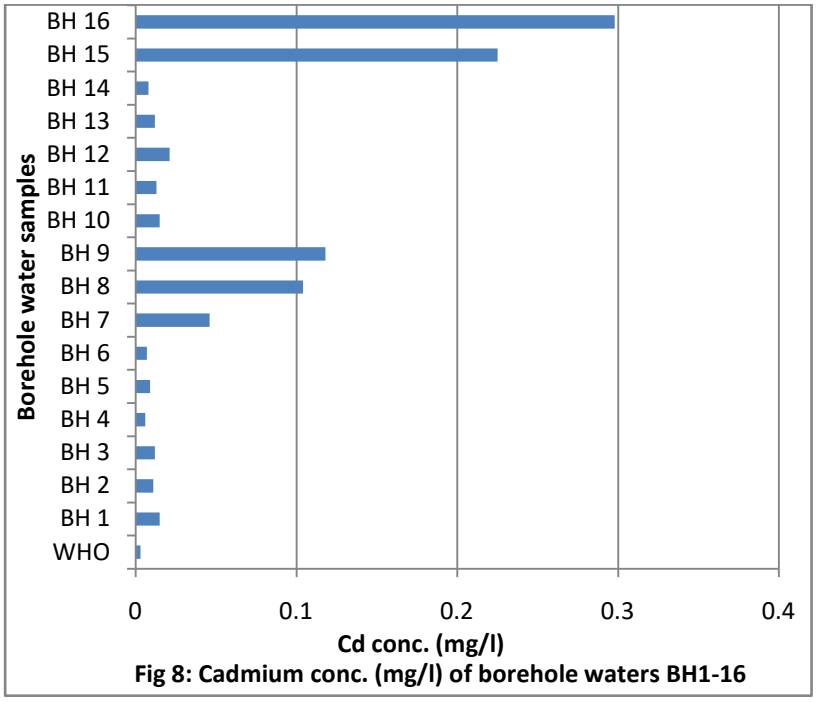

Fig 8: Cadmium conc. (mg/l) of borehole waters BH1-16

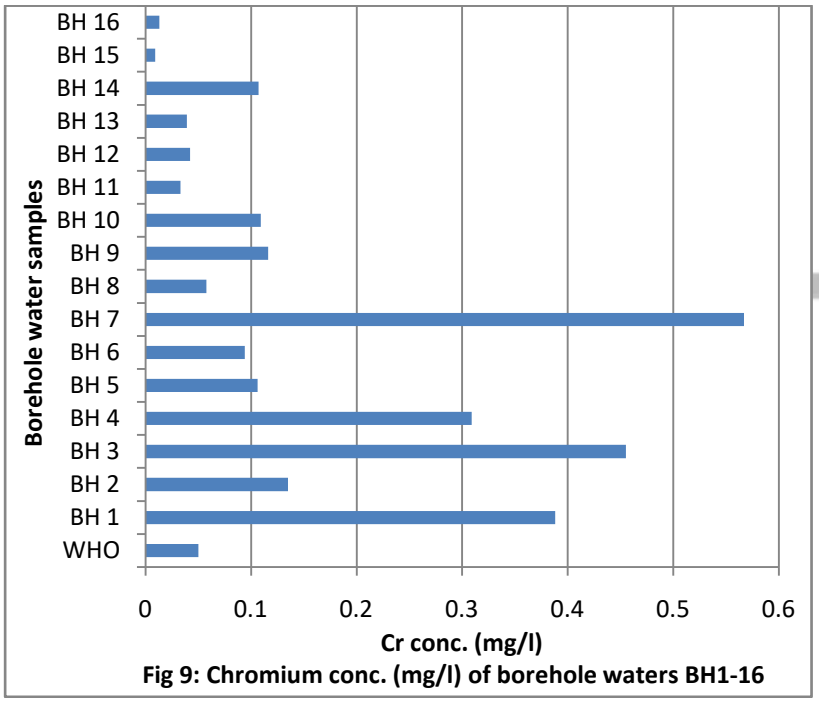

Fig 9: Chromium conc. (mg/l) of borehole waters BH1-16

Table 1, 2 and Fig 11 showed the isolated bacteria, from samples NK1-5, based on Gram staining and biochemical tests. There were a total of twenty-three (23) bacterial isolates recovered from samples NK1-5 and grouped into seven (7) genera of bacteria. *Staphylococcus aureus* (5) and *Vibrio cholerae* (5) had the highest appearance (21.7%), followed by *Escherichia coli* (4: 17.4%), *Salmonella typhi* (2), *Pseudomonas* species (2) and *Shigella* species (2) having the same number of occurrence (8.7%). *Yesinia* species (3) occurred 13% (Fig. 11). *Staphylococcus aureus* was seen to be the only Gram positive bacteria and the rest Gram negative bacteria. The results in Table 1 revealed that all the borehole samples NK1-5 were contaminated with *Vibrio cholerae.* The borehole samples NK1-2 were contaminated with *Pseudomonas* sp. The samples NK 3-4 were contaminated with *Salmonella typhi.* NK1 and NK5 were contaminated with *Shigella* sp. while *Yesinia* sp. was isolated from NK2, NK3 and NK4. The results in Table 12revealed that *Escherichia coli* was isolated from samples NK1-4 whereas *Staphylococcus aureus* was isolated from all the samples NK1-5.

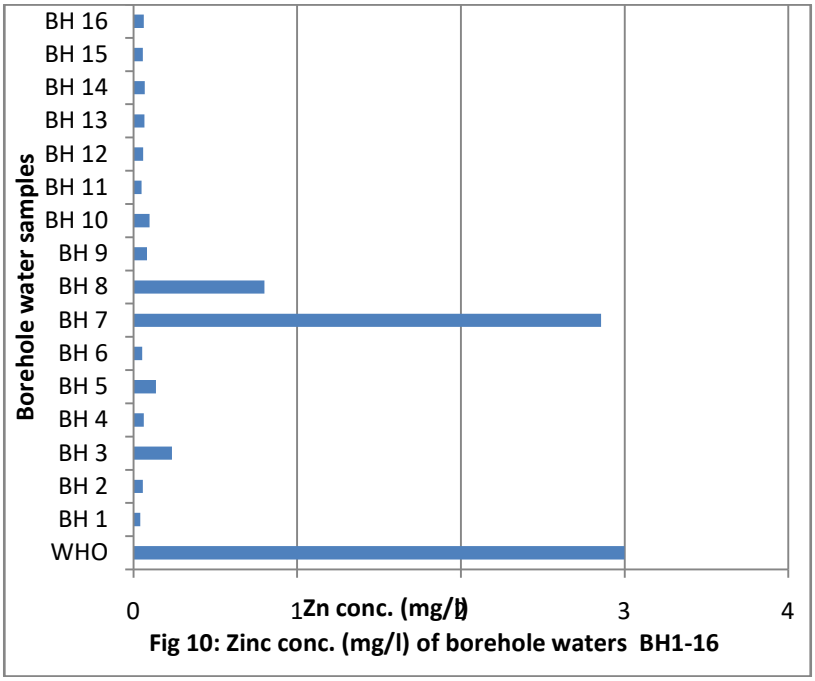

Fig 10: Zinc conc. (mg/l) of borehole waters BH1-16

Table 1: Biochemical characterization of isolates from samples NK1-5

Sample codes	Gram reaction	Citrate	Catalase	Oxidase	Methyl red	VP	Motility	Indole	Urease	Isolates
NK_1VC	-ve rods	+	+	+	+	-	+	+	-	*Vibrio cholerae*
NK_2VC	-ve rods	+	+	+	+	-	+	+	-	*Vibrio cholerae*
NK_3VC	-ve rods	-	+	+	-	+	+	+	-	*Vibrio cholerae*
NK_4VC	-ve rods	+	+	+	+	-	+	+	-	*Vibrio cholerae*
NK_5VC	-ve rods	+	+	+	+	-	+	+	-	*Vibrio cholerae*
NK_1P	-ve rods	+	+	+	+	-	+	-	-	*Pseudomonas* sp.
NK_2P	-ve rods	+	+	+	+	-	+	-	-	*Pseudomonas* sp.
NK_3Sal	-ve rods	-	+	-	+	-	+	-	-	*Salmonella typhi*
NK_4Sal	-ve rods	-	+	-	+	-	+	-	-	*Salmonella typhi*
NK_5Sh	-ve rods	-	+	-	+	-	-	+	-	*Shigella* sp.
NK_1Sh	-ve rods	-	+	-	+	-	-	+	-	*Shigella* sp.
NK_2Y	-ve rods	-	+	-	-	+	+	-	+	*Yesinia* sp.
NK_3Y	-ve rods	-	+	-	-	+	+	-	+	*Yesinia* sp.
NK_4Y	-ve rods	-	+	-	-	+	+	-	+	*Yesinia* sp.

Table 2: Biochemical characterization of isolates from samples NK1-5

Isolate code	Gram reaction	Citrate	Catalase	Oxidase	Methyl red	VP	Motility	Indole	Urease	Isolates
NK_1EC	-ve rods	-	+		+	-	+	+	-	*E.coli*
NK_2EC	-ve rods	-	+	-	+	-	-	+	-	*E.coli*
NK_3EC	-ve rods	-	+	-	+	-	+	+	-	*E.coli*
NK_4EC	-ve rods	-	+	-	+	-	+	+	-	*E.coli*
NK_1Sa	+cocci	NT	+	-	NT	NT	NT	NT	NT	*S.aureus*
NK_2Sa	+cocci	NT	+	-	NT	NT	NT	NT	NT	*S.aureus*
NK_3Sa	+cocci	NT	+	-	NT	NT	NT	NT	NT	*S.aureus*
NK_4Sa	+cocci	NT	+	-	NT	NT	NT	NT	NT	*S.aureus*
NK_5Sa	+cocci	NT	+	-	NT	NT	NT	NT	NT	*S.aureus*

Key: NT = Negative

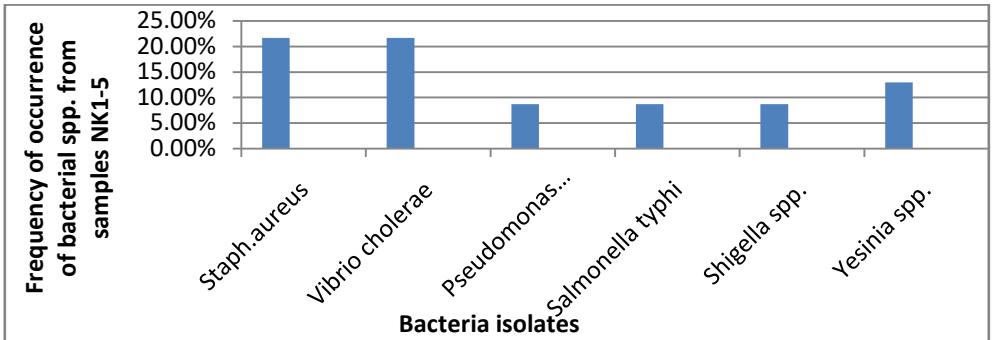

Fig 11: Frequency of occurrence of bacterial spp. from samples NK1-5

The occurrence of the bacteria in borehoe samples (NK1-5) is shown in Table 3. These genera include *Staphylococcus aureus* (5/5; 100%), *Salmonella typhi* (2/5; 40%) , *Escherichia coli* (4/5; 80%), *Vibrio cholereae* (5/5; 100%), *Pseudomonas* spp., (2/5; 40%), *Yesinia* spp., (3/5; 60%), and *Shigella* spp. (2/5; 40%).

Table 3: Bacteria isolated in samples NK1-5

Borehole Sample	*Staph. Aureus*	*Vibrio cholerae*	*Pseudomonas* spp.	*Salmonella typhi*	*Shigella* spp.	*Yesinia* spp.	*E.coli*
NK1	+	+	+	No	+	No	+
NK2	+	+	+	No	No	+	+
NK3	+	+	No	+	No	+	+
NK4	+	+	No	+	No	+	+
NK5	+	+	No	No	+	No	No

Key: + = present, No = absent

Fig. 12 shows the concentrations of lead present in the borehole water samples NK1-5. NK1 recorded 0.9 mg/l, NK2 recorded 0.11 mg/l, NK3 recorded 0.08 mg/l, NK4 recorded 0.23 mg/l and NK5 recorded 0.20 mg/l of lead concentration. These borehole samples NK1-5 recorded lead concentration far above WHO guideline concentration (0.01 mg/l). The highest lead value was recorded in sample NK4 (0.23 mg/l).

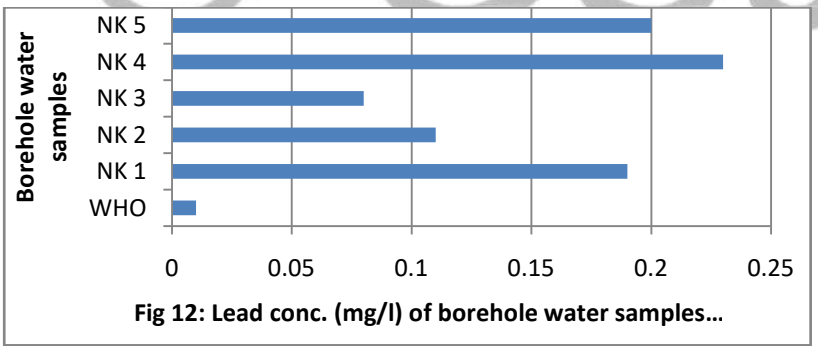

Fig 12: Lead conc. (mg/l) of borehole water samples…

Table 4 shows the total heterotrophic counts of microorganisms isolated from the water samples. NK1 recorded 4.4×10^7, NK2 had 3.2×10^7, NK3 had 2.2×10^7, NK4 had 1.0×10^7 and NK5 recorded 4.7×10^7cfu/ml. NK5 recorded the highest total heterotrophic count of 4.7×10^7cfu/ml while NK4 had 1.0×10^7cfu/ml. This indicates that all the isolates recorded TVC above WHO (2006) specification.

Table 4: Total heterotrophic count of of isolates from samples NK1-5

Sample code	THC cfu/ml
NK_1	4.4×10^7
NK_2	3.2×10^7
NK_3	2.2×10^7
NK_4	1.0×10^7
NK_5	4.7×10^7

Plasmid profile of isolates from sample NK1-5

Lead tolerant bacteria, from NK1-5, were isolated on lead supplemented nutrient medium. The presence of plasmids was determined using TENS method. The size estimates of the isolated plasmids were obtained by comparing their relative mobilities on agarose gel with standard molecular weight DNA marker. The total of twenty three lead tolerant bacteria belonging to seven genera: *Staphylococcus aureus* (5) and *Vibrio cholerae* (5) *Escherichia coli* (4)*, Salmonella typhi* (2)*, Pseudomonas* species (2) and *Shigella* species (2), were identified. The plasmid profiles of the bacteria are shown in Fig 13, 14, and 15. The samples NK1-5 had considerable levels of lead contamination and this could have necessitated the bacteria to develop adaptive feature against the lead contaminant. The Fig. 13, 14, and 15 show typical electrophoretic separation of the plasmids in some of the isolates.

The plasmid profile Fig 13 showed *Staphylococcus aureus* (B, C and E) isolated from samples NK2, NK3, NK5, have single plasmid band size 12,000 bp. *Salmonella typhi* (F and G) isolated from NK2 and NK4 showed single plasmid band size of 12,000 bp.

Fig. 14 showed *Escherichia coli* (No. 1, No. 3, No. 4) isolated from NK2, NK4 and NK1 showed no plasmid bands but *E. coli* (No. 2) from NK 3 has a faint single band of 12,000 bp. *Vibrio cholerae* (No. 5, No. 6, No. 8, No. 9) isolated from NK1, NK2, NK4 and NK5 showed single plasmid band sizes of 12,000 bp., but *Vibrio cholerae* (No. 7) from NK3 had no plasmid. *Pseudomonas* species (No. 10) isolated from NK1 showed single band size of 900 bp while the *Pseudomonas* species (No. 11) isolated from NK5 showed no plasmid band.

Fig 15 showed *Yesinia* sp. (a, c) isolated from NK4 and NK2 showed single plasmid band of 11,000 bp while *Yesinia* sp. (b) from NK3 had no plasmid. *Shigella* sp. (d and e) isolated from samples NK1 and NK5 showed single plasmid band size of 11,000 bp. There were a total of 23 bacterial isolates, 15(65.2%) of them showed presence of plasmids (Table 5). These plasmids are the reasons of their survival in lead contaminated borehole waters. The other bacterial isolates 8 (one *Pseudomonas* species, three out of the four *E.coli* spp., two out of five *Staph. aureus* spp., one out of the three *Yesinia* spp., and one out of five *Vibrio cholerae*) that showed no plasmids could be that the genes to adapt in the lead contaminated borehole waters were chromosomal DNA mediated.

Table 5: Plasmid DNA of bacterial isolates from NK1-5

Bacteria spp.	Number of isolated species	Number with plasmids	Plasmid DNA sizes
Staphylococcus aureus	5	3	12 Kbp
Salmonella typhi	2	2	12 Kbp
E.coli	4	1	12 Kbp
Vibrio cholerae	5	4	12 Kbp
Pseudomonas spp.	2	1	0.9 Kbp
Yesinia sp.	3	2	0.9 Kbp
Shigella sp.	2	2	11 Kbp
TOTAL	23	15	

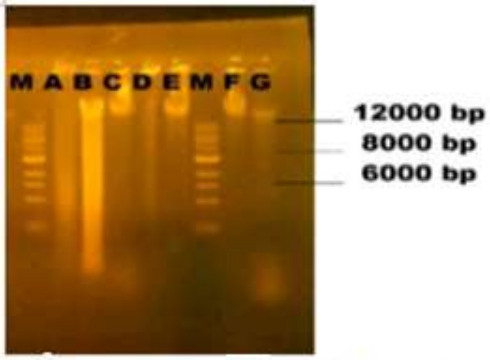

Fig. 13: Plasmid profile of bacteria from borehole water samples collected from Nekede. M = DNA maker/ladder. A-E = *Staph. aureus* from NK1-NK5 samples; F-G = *Salmonella typhi* from NK2 and NK4

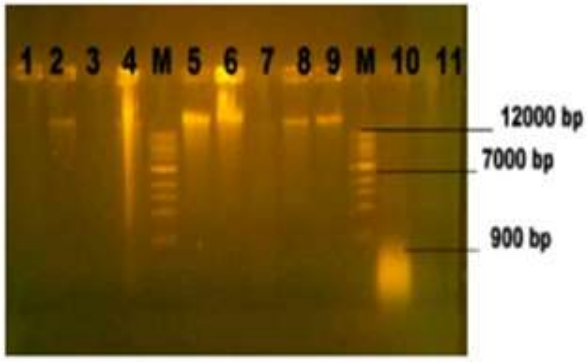

Fig 14: Plasmid profile of bacteria from borehole water samples collected from Nekede. M = DNA maker/ladder. 1-4 = *E.coli* from NK2, NK3, NK4 and NK1 samples respectively. 5-9 = *Vibrio cholerae* from NK1 and NK5. 10-11 = *Pseudomonas* spp., from NK1 and NK2

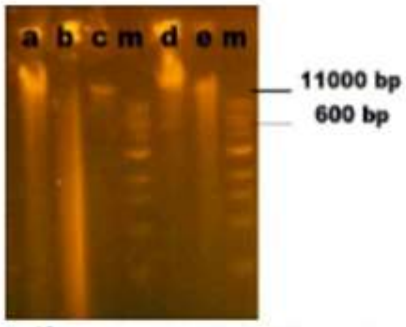

Fig. 15: Plasmid profile of bacteria isolated from borehole water samples in Nekede. M = DNA maker/ladder. a - c = *Yesinia* spp., from NK4, NK3 and NK2 samples respectively. d - e = *Shigella* spp., from NK1 and NK5

Discussion

The proximity of the water sources to dumpsite and latrines consequently deteriorate the water through seeping and leaching [15,16,17,18,19,20,21,22,23,24,25]. Faecal contamination appears to be the most serious form of water contamination obtained from various sources. Poor methods of faecal waste management like refuse disposal, shallow depth of well and uncontrollable use of inorganic fertilizers are possible source of contamination. However, illegal dumping of domestic wastes, livestock management, faecal deposit and waste dumps also affect bacterial concentration in run-off water. The pollution caused by human activities includes the indiscriminate habit of people in the use of latrines and siting wells close to the toilets [17,21,22,26,27,28]. The absence of faecal coliforms in borehole water means that it was accurately dug to get clean water. This borehole perhaps might not be located close to a toilet or suck away system; had consistent washing and disinfection of the water tank, and might also had a water purifier which increased water quality [18].

According to the WHO Fact sheet on septic tanks (see Fact sheets 3.9, http://www.who.int/water_sanitation_health/sanitation-waste/fs3_9.pdf), the minimum acceptable distance between borehole and septic tanks should be 30 m.

Most of the bacterial species isolated in ours study were identified to belong to the members of coliform bacteria, which are Gram negative facultative anaerobes, non-spore formers that ferment lactose within 48 hours [10], agrees with the findings of Odeyemi *et al.* [16] who isolated *Escherichia coli, Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp., *Bacillus* spp., *Staphylococcus* spp., and *Enterococcus* spp . A higher number of *Vibrio cholerae* and coliform bacteria are an indication that the water samples are not portable and thus, unfit for domestic uses [1,2, 15,16,17,18,19,20,21,22,23,24,25,26,27,28]. The isolation of *E. coli* from the water sample in this study is in correlation with the past studies that have presented *E. coli* as a common encounter in different water sources be it rivers, streams, rain water, well water, underground water and even borehole water [1,2, 15,16,17,18,19,20,21,22,23,24,25,26]. The report of other researchers [15,16,17,18,19,20,21,22,23,24,25,27,28] that most of the bacteria isolated during this research work as potential pathogens, is significant enough to admit that the quality of these water sources has been adversely deteriorated thereby subjecting the individual in the communities to greater health risk.

The result showed that *Staphylococcus aureus, Vibrio cholerae, Escherichia coli,* and *Yesinia* species were most frequently isolated bacteria species from the water samples of which Bashir *et al*. [18] confirmed. Bashir *et al*. [18] isolated *E.coli* and coliforms from borehole waters. The isolation of *E.coli* which is pathogenic, can cause serious diarrhea infection in human. *Vibrio cholera*e is a water-borne pathogen responsible for causing a toxin-mediated profuse diarrhea in human, leading to severe dehydration and death in unattended patients which is supported by Ibo *et al.,* [19] who identified *Vibrio cholerae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shigella flexineri, Proteus vulgaris* and *Klebsiella pneumoniae* in their study on borehole water samples.

Ekhosuehi *et al.,* [20], *isolated Escherichia coli* (34%), *Klebsiella* sp. (23%), *Pseudomonas* spp. (19%), *Proteus* sp. (5%), *Enterobacter* sp. (3%) and Feacal *Streptococci* (12%) from both boreholes and storage tanks.

Clostridium perfringens was detected in two samples (12.5%) and this is in contrast with the works of [16,18,23,24,25] who detected no *C*lostridium *perfringens i*n any of the samples analyzed.

The isolated bacteria are implicated in the following health problems: Enterohaemorrhagic *E. coli* causes diarrhoea that ranges from mild and non-bloody to highly bloody, which is indistinguishable from haemorrhagic colitis. WHO, [1,2] noted that between 2% and 7% of cases can develop the potentially fatal haemolytic uraemic syndrome (HUS), which is characterized by acute renal failure and haemolytic anaemia. Children under 5 years of age are at most risk of developing HUS [1,2].

Pseudomonas aeruginosa can cause a range of infections but rarely causes serious illness in healthy individuals without some predisposing factor. It predominantly colonizes damaged sites such as burn and surgical wounds, the respiratory tract of people with underlying disease and physically damaged eyes. From these sites, it may invade the body, causing destructive lesions or septicaemia and meningitis. Cystic fibrosis and immunocompromised patients are prone to colonization with *P. aeruginosa,* which may lead to serious progressive pulmonary infections [1,2].

Salmonella infections typically cause four clinical manifestations: Gastroenteritis (ranging from mild to fulminant diarrhoea, nausea and vomiting), bacteraemia or septicaemia (high spiking fever with positive blood cultures), typhoid fever/enteric fever (sustained fever with or without diarrhoea) and a carrier state in persons with previous infections [1,2].

Shigella spp. can cause serious intestinal diseases, including bacillary dysentery. Most cases of *Shigella* infection occur in children under 10 years of age. The incubation period for shigellosis is usually 24–72 h. The ingestion of as few as 10–100 organisms may lead to infection, which is substantially less than the infective dose of most other enteric bacteria. These signs, abdominal cramps, fever and watery diarrhoea occur early in the disease [1,2].

The multiplication of *Staphylococcus aureus* in tissues can result in manifestations such as boils, skin sepsis, post-operative wound infections, enteric infections, septicaemia, endocarditis, osteomyelitis and pneumonia. The onset of clinical symptoms for these infections is relatively long, usually several days. Gastrointestinal disease (enterocolitis or food poisoning) is caused by a heat-stable staphylococcal enterotoxin and characterized by projectile vomiting, diarrhoea, fever, abdominal cramps, electrolyte imbalance and loss of fluids [1,2].

V. cholerae symptoms are caused by heat-labile cholera enterotoxin carried by toxigenic strains of *V.cholera*e. The initial symptoms of cholera are an increase in peristalsis followed by loose, watery and mucus-flecked “rice-water” stools that may cause a patient to lose as much as 10–15 litres of liquid per day. Non-toxigenic strains of *V. cholerae* can cause self-limiting gastroenteritis, wound infections and bacteraemia [1,2].

Yersinia spp., penetrates cells of the intestinal mucosa, causing ulcerations of the terminal ilium. Yersiniosis generally presents as an acute gastroenteritis with diarrhoea, fever and abdominal pain. Other clinical manifestations include greatly enlarged painful lymph nodes referred to as “buboes.” The disease seems to be more acute in children than in adults [1,2].

Clostridium perfringens causes gastroenteritis and food poisoning. The bacterium releases toxins which result in diarrhea while in small intestine [29].

Odeyemi *et al.*, [16] found zinc in the range 0.02 - 0.23 mg/l, lead (0.01 - 0.02 mg/l), and cadmium (0.001 - 0.01 mg/). There were no traces of copper detected in any of the borehole water samples. Their study showed that most of the water samples in Ekiti-North senatorial district are unfit for drinking and domestic use hence should be treated prior to usage. In our study lead (0.06 - 1.93 mg/l)

and cadmium (0.006 - 0.298 mg/l) were above WHO standard and higher than their concentration while, zinc (0.042 - 2.857 mg/l) was within the range recorded in their study. We detected copper but less than WHO standard (0.008 - 1.138 mg/l). Many researchers [21,22,23,24,25,27,30] have reported the presence of heavy metals in borehole waters.

Lead is a highly poisonous metal (whether inhaled or swallowed), affects the nervous system. Acute exposure to lead causes Encephalopathy (brain dysfunction), nausa, vomiting. The long term exposure in adults leads to decreased performance, the strong oxidant PbO_2 can cause nephrophy, and colic-like abdominal pains. It may also cause weakness in fingers, wrists or ankles and increase blood pressure particularly in middle-aged and older people and can cause anaemia. Lead exposure in children causes damage to the brain and nervous system, behavior and learning problems, low IQ, weight loss, anaemia, slowed growth, hearing problems [30,31]. The acute exposure (usually a day or less) of cadmium causes pneumonitis (lung inflammation) while chronic exposure (often months or years) causes lung cancer, osteomalacial (softening of bone) proteinura (excess proteinin urine, possible kidney damage) [30,31]. The acute exposure of chromium causes Gastrointestinal hemorrhage (bleeding), Hemolysis (red blood cell destruction), acute renal failure whereas the chronic exposure causes Pulmonary fibrosis (lung seaming) [30,31].

It is well known that plasmid are most important mediators facilitating the fast spreading of heavy metal resistances among bacteria [3,4,6,7,8,9,13,16,28,32,33,34,]. Bacteria carry out chemical transformations of heavy metals. These transformations are sometimes by-products of normal metabolism and confer known advantage upon the organism responsible. Sometimes, however, the transformations constitute a mechanism of resistance. Microorganisms may play a large role in the biogeochemical cycling of toxic heavy metals also in cleaning up or remediating metal contaminated environments [4]. Plasmid analysis of all the isolates provided a general picture that revealed that almost all the isolates contained plasmids DNA. The molecular sizes of the plasmid ranged from 600 bp - 12,000 bp and these plasmids code for lead resistance.

Odumosu *et al*., [34], determined the plasmid profile using alkaline lysis method, of water-borne Enterobacteriaceae recovered from some borehole water samples in 6 towns in Ogun State (Nigeria), which include: *Enterobacter* spp, *Escherichia coli, Klebsiella* spp, *Salmonella* spp, *Citrobacter freundii, Serratia.* Odumosu *et al***.,** [34] noted that out of 40 Enterobacteriaceae investigated for the presence of plasmids, 18 isolates were positive for the presence of plasmids, *E. coli* (4), *Enterobacter aerogenes* (7), *K. oxytoca* (3), *K. pneumoniae* (4) with sizes range of 33.5 – >33.5 Kbp. The presence of bacteria with resistance plasmids in drinkable water is a cause for concern due to the possible health risks to humans and animals. The incidence of plasmids among bacteria isolated from drinking water is troubling because plasmids often allow development and spread of resistance in niches irrespective of previous exposure to antibiotics and they also have high capacity for transference among bacteria of unrelated genus and communities [6,7,16,28,34].

Odeyemi *et al.*, [16] found in their plasmid profile study, a little above half (54%) of the total isolates posses plasmids with very high molecular weight varying between 4.361 Kbp and 23.13 Kbp, with the application of TENS protocol. The result of plasmid study showed that *Escherichia coli* has plasmid band of 23.13 Kbp and 4.361 Kpb, *Pseudomonas aeruginosa* strains has 23.13 Kpb plasmid, *Staphylococcus aureus* has 21.13 Kbp. In this current study the *E.coli* had plasmid band sizes of 12,000 bp (12 Kbp) smaller than those from previous borehole studies and 65.2% of the total bacteria isolates had plasmids.

Conclusion

The majority of borehole waters are contaminated with heavy metals and bacteria of public health importance, as a result of nearness to wastes dumpsites, septic tanks and many anthropogenic activities. The heavy metals detected in this current study are lead, cadmium, chromium, zinc and copper but lead, cadmium and chromium were very above WHO standard.

The acute exposure to lead causes Encephalopathy (brain dysfunction), nausa, vomiting. Chronic exposure of cadmium (often months or years) causes lung cancer osteomalacial (softening of bone). The chronic exposure to chromium causes pulmonary fibrosis (lung seaming). There were a total of 21 borehole waters analyzed and bacteria contaminants detected in the borehole waters are *coliforms, E.coli, Clostridium perferingens, Vibrio cholerae, Salmonella typhi, Shigella* spp., *Staphylococcus aureus, Pseudomonas* spp., and *Yesinia* spp. These bacteria are implicated in public health risks. *E. coli* causes diarrhoea that ranges from mild and non-bloody to highly bloody. The immunocompromised patients are prone to colonization with *P. aeruginosa,* which may lead to serious progressive pulmonary infections. *Salmonella* infections cause gastroenteritis, bacteraemia or septicaemia. *Shigella* spp. can cause serious bacillary dysentery. *Staphylococcus aureus* causes boils, skin sepsis, post-operative wound infections, enteric infections, septicaemia, endocarditis, osteomyelitis and pneumonia. *V. cholerae* cause self-limiting gastroenteritis, wound infections and bacteraemia. *Yersinia* spp., causes ulcerations of the terminal ilium, acute gastroenteritis with diarrhoea, fever and abdominal pain. *Clostridium perfringens* causes gastroenteritis and food poisoning. Plasmids, of the size 6 Kbp - 12 Kbp, were found in all bacteria species isolated. Plasmids incidence in bacteria are useful in genetic studies and in the development of cloning vectors, their presence or absence can be an important epidemiological marker.

There is an urgent need for awareness to be created about the present situation of these boreholes, to enlighten the people on the necessity for further treatment of borehole waters before they can be used for drinking and domestic purposes.

There is the need for portable water to be treated periodically before consumption. Therefore to reduce contamination in drinking water, a comprehensive laboratory test should be carried out to ensure the safety of various borehole waters. This can be enforced and supported by the government and on the other hand indirectly reduce inordinate drilling of boreholes. Also borehole water samples that do not meet the FEPA/WHO standard should be treated before consumption. Seminars and talk forum can be held on

the necessity of consumption of portable and treated water. This would help to insight inhabitants on the importance of portable water and its adequate quality.

Constant and Frequent monitoring of water in these communities is therefore recommended so as to reduce the long term effect of these toxic metals in humans. Boreholes should also, be dug deeper and far away from refuse dump sites to reduce the risk of fecal and heavy metal contamination. The inhabitants who use the water for both consumption and domestic purposes should also be enlightened on the life – threatening effects of these heavy metals and the health effects. The enlightenment on the concentration of heavy metals in borehole waters is vital because it will help in its management.

The regular analysis to ensure uniformity to world health organization standards to assure the public of the portability of their water is necessary. In fact, simple test carried out regularly at short intervals are of most valued than detailed test made occasionally.

It is advisable that the most effective means to reduce the health risk from unsafe water remains the households based water treatment and safe storage. Borehole should also be dug deeper and far away from refuse dump sites to reduce the risk of fecal and heavy metal contamination. House owners should adhere to the FEPA/WHO standard of siting boreholes 32 m away from septic tanks and waste dumpsites.

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