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Bacteriological Analysis of Selected Borehole Water within Ilishan - Remo Community Ogun State Nigeria

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Abstract:- Potable water is suitable for consumption by humans and animals, and sources of potable water includes borehole water, springs etc. Indicator organisms such as coliform bacteria can be used to assess the quality of water, coliform bacteria are aerobic and facultative anaerobic Gram negative, non-spore forming, rod shaped that ferments lactose with acid and gas production within 48 hours at 35 0C – 37 0C. 3The aim of this research was to analyze borehole water from different sources within Ilishan community to determine their potability. A total of 9 samples were collected and analyzed and the results obtained were compared to World Health Organization (WHO) standards for drinking water quality. For the coliform counts, two of the water samples were satisfactory, three were suspicious and four were unsatisfactory. A total bacteria count (TBC) was also done and the results obtained showed that 1 of the water samples were within limits and the rest weren't according to Nigerian Standard for Drinking Water Quality. The pH of the water samples was also tested for and the results obtained were within the acceptable limit for drinking water quality set by WHO. The biochemical tests carried out on the isolates suspected the organisms to be Citrobacter spp, Klebsiella spp, Enterobacter spp and Chromobacteriumviolaceum. In conclusion, potable water should be safe for human and animal consumption, indicator organisms shouldn't be present at all or should meet the quality specifications provided by regulatory bodies while faecal coliforms are not to be present at all. It is therefore recommended that the Federal Government of Nigeria should organize programs through the use of sanitary control inspectors in different part of Nigeria, that would help in educating the citizens on how to maintain water quality, the dangers of bad waste disposal and its adverse effects. Also, further studies should be carried out to test the susceptibility pattern of the organisms isolated to ascertain no public health threat is being posed in regards to antibiotic resistance.

I. INTRODUCTION

Water is known to be one of the basic amenities on earth. It is an odorless, tasteless, transparent and clear liquid which is vital for all life forms. It covers 70 % of the earth. Since water is important for all living organisms for survival there is need to maintain clean and safe water (Ekubo and Abowei, 2011).

A potable water is known as drinking water, it is suitable for human and animal consumption. Natural sources of potable water include borehole water, well water etc.Water is known to support the growth of many microorganisms (Chapelle, 2000) thus water sources many be contaminated with various enteric pathogens due to natural human activities. Contaminated potable water supply was traditionally treated by chlorination therefore reducing coliform and pathogen population, the presence of pathogens in water can lead to serious diseases in humans (Kurokawa, 2001). About Waterborne infections make for 75% of communicable diseases globally (Shenjgi et al., 2004). According to the World Health Organization (WHO), using contaminated water causes 80% of all human diseases in developing nations (Abera*et al.,* 2011).

It is required by borehole operators to deliver safe drinking water to their consumers at all times. Contamination of the water supply can be detrimental to consumer's health (Howard *et al.,* 2002). Water below ground is mostly safe as long as they are appropriately built and run-in accordance with the World Health Organization Drinking Water Guidelines (WHO, 2008), but can be contaminated during collection, transportation, and storage.

Water being colorless, odorless and clear is acceptable but that doesn't mean it is safe from pathogenic organisms and their toxins. Potable water must be devoid of pathogenic organisms.

II. LITERATURE REVIEW

A. POTABLE WATER SOURCES

The main sources of potable water include Surface water, Ground water and Rain water.

a) Surface Water
Surface water is referred to as a water source that is above the ground that is exposed to the atmosphere and is subjected to runoff from the lands, this makes it more susceptible to pathogenic microorganism that can cause serious illness in man. Example of surface water includes: rivers, lakes, and streams. The

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volume of water is dependent on amount of rainfall, type of soil, type of vegetation, slope of the ground and land use. Surface water such as river is often used to supply water for large urban systems due to the fact that they maintain a large and regular supply of water. (Ekiye and Luo, 2010).

Advantages of Surface water includes being able to be easily abstracted by direct pumping and treatedi.e., addition of necessary chemicals needed for treatment would be easier to add. However,surface water is seasonal and as a result would always need treatment to maintain, the cost of treatment and maintenance is usually high therefore is a disadvantage (Ekubo and Abowei, 2011).

b) Ground Water
Ground water is referred to as water source that is covered by soils and sediments thus containing less pathogenic microorganisms unlike the surface water. Examples of ground water include springs, boreholes and wells (Selvinaz and Gina, 2015).

B. DRINKING WATER POLLUTION/CONTAMINATION

Nigeria, the most populous country in Africa with 140 million people, is well-supplied with water bodies. (Ekiye and Luo, 2010). Human activities haveled to the contamination of different water bodies making this water bodies unacceptable and undermining the wellbeing of the population (Abowei and Sikoki, 2005).

Water contamination can be defined as the disintegration of biological, chemical and physical properties of water due to the activities of humans and other sources (Selvinaz and Gina, 2015). It is impossible to overstate how important water is, if water contamination problem is not controlled appropriately it could result ineconomic problems, societal problems, and even death (Garba *et al.,* 2010). Water contamination occurs when undesirable substances getinto, wells, streams, boreholes and waterways of homes and industries.

Nigeria and the third world as a whole have suffered from the effects of water contamination, with 4.6 million deaths from diarrheal disease and a sizable number of ascariasis casualties as a result (Esrey*et al.,* 2000). The research done in Nigeria today indicates that most common fresh water sources are polluted and results in serious outbreaks of diseases. According to Umeh *et al (*2004) 48 % of the population in Katsina-Ala Local Government territory of Benue state have urinary schistosomiasis due to consumption of contaminated water. Past study shows that 19 % of the entire Nigerian population is affected, with a few groups having up to half occurrence (Umeh *et al.,* 2004). This has made World Health Organization try and enhance the cultural and socio-economic standards of individuals in the tropical region (Umeh *et al.,* 2004,). Total bacteria and coliform counts were observed to be between 2.86 - 4.45 and 1.62 log cfu/% respectively(Oloaye and Onunide, 1997). Asides microbial contamination, heavy metals, lead, arsenic and other dangerous chemicals injurious to human health has also been identified.

According to Aina *et al.* (2012), water samples from boreholes gotten in Ogun State from various towns were bacteriologically analyzed using Most Probable Number technique and pour plate method. The results gotten showed out of the 18 samples 13 were positive for Coliforms and the remaining five weren't. 3 water samples satisfied the WHO standard with a coliform count between 1-3/100 ml, 4 water samples were suspected to have a count of 4-8/100 ml and 11 did not satisfy the WHO standard requirement. *Escherichia coli, Klebsiella* sp., *Proteus* sp., *Enterobacter aerogenes, Staphylococcus aureus* and *Streptococcus* sp isolates were gotten from the sample and characterized using Gram staining and biochemical reactions.Gram-negative bacilli were more in the water samples (72.22 %) than the Gram-Positive Bacilli (11.11 %) and lastly Gram-Positive Cocci (16.67 %). *E. coli* isolates had the highest number of occurrences with a percentage of (33.33 %) and then *Klebsiella* sp with a percentage of (27.78 %) and lastly the percentage of *P roteus*sp., *S. aureus* and *E. aerogenes* were the least (5.56 %). *Streptococcus* sp. (11.11 %) and *Clostridium* sp. (11.11 %) were confirmed in two samples. 50 % of the isolates were positive to acid and gasproduction.

According to Edessa *et al.* (2017) on the microbiological examination done on drinking water in Ethiopia, using WHO Guidelines for drinking water quality assessment water samples were collected from ground water and surface water sources and then the water samples were analyzed for *Total coliform bacteria, E coli, Salmonella, Shigella,* and *Vibrio cholerae. E. coli* was confirmed (APHA, 2005),*Salmonella* and *Shigella* were isolated (APHA-AWWA, 1998), and *Vibriocholerae* also detected. From the results gotten from the researcher, the bacteriological load in the different water samples was more than the maximum value set for drinking water.

According to Adogo*et al.* (2016) on the bacteriological examination of the water from a borehole in the AutaBalefiCommunity ,Nassarawa state Nigeria, borehole water samples were gotten from five different locations which were labelled A to D and then analyzed. From the results gotten by the researcher sample D and C had the lowest total and faecal coliform count while sample B and E had the highkest count. *Salmonella typhi, Klebsiella pneumonia Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis* and *Escherichia coli* were also identified.

According to Makhmooe*et al.*2015 on the evaluation of the microbiological quality of drinking water in the Indian city of Gwalior that has undergone chlorine treatment, different water samples were collected from treatment plants and consumer's household taps, 56 water samples were collected in total and tested for total and faecal coliforms and residual chlorine content. The residual chlorine from all samples were between 0.08 to 0.98 mg/L, total coliforms in all the samples ranges between 0.82 to 7.15 MPN/100 ml and faecal coliforms ranges from 0 to 4.10 MPN/100 ml.

Water contamination has different categories, Surface water and ground water which is known to be from different

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sources although interrelated can be contaminated in different ways. Surface water can be contaminated through Point source and non-point source contamination (Ekubo and Abowei, 2011).

a) POINT SOURCE
Point source refers to the main point the contaminants or the pollutant came from i.e., through a discrete transport e.g., a pipe or a ditch (Hertz-Picciotto, 2000).

b) NON-POINT SOURCE
The term "non-point source" describes contamination that is not from the direct or solitary discrete source e.g through erosion. Toxic chemical compound being drained from agricultural areas into the water is one of the most common sources of non-point contamination, most agricultural pollutants contain chemical substances such as nitrogen compounds and heavy metals which become residues in the soils and are later washed off into nearby water bodies thereby causing water pollution (Hung and Shaw, 2004).Rainwater also washes contaminants from parking garages, roads and highways into nearby water bodies, this is known as urban runoff (Ekubo and Abowei, 2011).

c) GROUND WATER CONTAMINATION
Ground water contamination is different from surface water pollution, it refers to the alteration of the quality due to activities caused by man and as a result is harmful to man and creates public health hazards. A chemical spill in the soil which may be a point source or non-point contamination can contaminate ground water. Groundwater pollution can be attributed to soil and site topography, hydrogeology and hydrology (Kurokawa, 2001).

C. DRINKING WATER CONTAMINANTS

Drinking water contaminants are referred to as things present in water that makes water unsafe for consumption and can affect human's health. These contaminants include pathogenic microorganisms, inorganic compounds/chemicals (arsenic, glyphosate, etc.), organic compounds/chemicals (trichloroethylene, tetrachloroethylene, atrazine etc.).

a) PATHOGENS
A lot of water borne diseases are caused by pathogens, these pathogens can be spread or transferred from one place to another through water bodies thereby leading to the spread of diseases (i.e.,communicable). They can be spread either through food consumption or from aerosols. This water borne pathogens generatefrom waste products (both human and animal) andlack of water supply (de Kok*et al.*, 2001). Pathogenic microbial contaminants include viruses, bacteria, fungi and parasites, these organisms may cause serious gastrointestinal diseases in humans (U.S. Environmental Protection Agency, 2008). Children are specifically more susceptible to diseases caused by pathogens due to the fact that their immune system is not as developed as compared to adults (Garcia *et al.*, 2000; Levin *et al.*, 2008). Surface water is said to be more susceptible to contaminants than ground water because they are more exposed to runoffs and climatic conditions. Some of the water borne pathogens and their implications include:

- *Vibrio cholerae*: this is aGram-negative bacillus non spore forming and oxidase positivebacteria that causes cholera, it's an infection that affects the small intestine. Symptoms include watery diarrhea, and fever. Stool examination aid sin diagnosing this infection.
- Giardia: this is a parasite that causes giardiasis or beaver fever an intestinal infection. Symptoms include malaise, gas, abdominal pain, fever, headache, vomiting and weakness.
- *Entamoeba histolytica*: this is a parasite that causes intestinal amoebiasis. Symptoms include abdominal pain, stooling and fever in individuals and it can be diagnosed by detecting their cysts in stool samples using microscopy.
- *Shigella*: this is a Gram-negative bacillus, rod shaped, non-motile and non-encapsulated facultative anaerobic bacteria that causes dysentery, an intestinal infection that causes diarrhea with blood. Symptoms include, abdominal pain, frequent visit to the toilet, weakness, vomiting, weight loss and fever. Blood test and stool samples are used to diagnose this infection.
- *Salmonella typhi*: this is a bacterium that causes typhoid infection. High fever, headache, stomach pain, and exhaustion are some of the symptoms. A blood test can be used to diagnose this infection.

b) TOXIC CHEMICALS
Drinking water may contain toxic chemicals that is injurious to the human health.These chemicals can be found in the water bodies as a result of regular human activity. Examples of this toxic chemicals include:

- Fluoride: this can be naturally found in food and water; it is very important for the development of bones and teeth. If ingested at very high levels it becomes toxic.
- Arsenic: this is a metal that can be found naturally in gold, lead and copper ores. According to the International Academy for Research on Cancer (IARC) (Global healing center, 2010), arsenic is considered as a class I carcinogen. It can cause health complications like neurological diseases, skin lesion, liver diseases and vascular diseases.
- Chlorine: chlorine is usually used for water treatment but if added at really high amount and then consumed it can be very toxic thereby leading to cell damage in the body.
- Nitrates: fertilizers, pesticides, and manure are sources of nitrates, their use on crops and the soil creates a channel for nitrates to get into water. High quantity of nitrates in the body leads to blood

poisoning and then eventually death (de Kok *et al.*, 2001).

D. COLIFORMS

The definition of coliforms varies, according to APPHA (2005), Coliform bacteria are aerobic and facultative anaerobic Gram negative, non-spore forming, rod shaped bacteria that ferments lactose with acid and gas production within 48 hours at 35 – 37^{0}C. According to French Standardization Association (1990), coliforms are rod shaped, non-spore forming, Gram negative, oxidase negative, aerobic or facultative anaerobic bacteria which are able to grow in the presence of bile salts or other surface-active agents having growth inhibitory effects and ferments lactose with acid or aldehyde and gas production within 48 hours at 37^{0}C. They belong to a family of Enterobacteriaceae and they have four genera which are:

- *Citrobacter*: this is a genus of coliform bacteria, *C. amalonaticus, C. fruendii, C. koseri*possess the ability to use citrate as their sole carbon. Their specie can be differentiated by the ability to transform tryptophan to indole and use malonate.
- *Enterobacter:* this is a genus of coliform bacteria; they are oxidase negative and indole negative
- *Escherichia:* this is a genus of coliform bacteria; they are commonly found in the intestine of warm-blooded animals
- *Klebsiella*: this is a genus of coliform bacteria; they are commonly found in the soil and 30 % of their strains fix nitrogen in anaerobic condition.

They are frequently used as bacterial indication for pollution of water; hence they are called indicator organisms. Coliforms are present in the environment especially in the soil, and in the faeces of warm-blooded animals and humans (faecal coliforms) (Howard *et al.*, 2002). Their detection in drinking water indicates the need for analysis of the water as it means that pathogens or disease-causing organisms is present in the water body posing health risk to the water.

a) TOTAL COLIFORMS

They are often in the environment and are generally harmless. If they are detected in any drinking water source then the contamination is environmental thus indicating that the drinking water should be analyzed as it might have been contaminated by pathogens. They are known to be indicator organisms for the quality of water. If they are detected in any drinking water source it means the drinking water source hasn't been treated properly and therefore immediate and proper treatment is required. Monitoring of water quality can be done by testing for Coliforms

b) THERMO TOLERANT/ FAECAL COLIFORMS

They are associated with the intestine and faeces of animals and humans with a temperature of 44^{0}C to 45^{0}C. The detection of this particular group of coliforms in drinking water indicates fecal contamination thus showing that there's a greater risk that pathogens are present in the drinking water. It includes genus *Escherichia*, and some species of *Enterobacter, Citrobacter* and *Klebsiella.*

c) ESCHERICHIA COLI (E. COLI)

- Classification:
- Domain: Bacteria
- Phylum: Proteobacteria
- Class: Gammaproteobacteria
- Order: Enterobacteriales
- Family: Enterobacteriaceae
- Genus: *Escherichia*
- Specie: *coli*

E. coli belongs to a family of Enterobacteriaceae, it contains b-galactosidase and b-glucuronidase enzymes and ferments lactose to produce gas and acid. *E. coli* can be found in both animal and human excreta (faeces), water and soil contaminated with faeces, it is a normal microflora of the mammalian intestinal tract.

E. coli is a fecal coliform with a temperature of 44^{0}C to 45^{0}C, most strains are harmless however there are pathogenic strains such as *E. coli* 0157:H7 strain. These pathogenic strains cause illnesses such as peritonitis, colitis, bacteriamia, etc., they can be divided according to how and where they cause diseases, they include: enterohemorrhagic *E.coli* (EHEC), Enteroaggregative *E.coli* (EAEC), Enteropathogenic *E.coli* (EPEC), Enteroinvasive*E.coli* (EIEC), Diffusely adherent *E.coli* (DAEC) (Leimbach*et al.*, 2013; Bischoff *et al.*, 2005).

E. EFFECT OF WATER POLLUTION

Water pollution can have a negative impact on socio economic, health and environmental issues. The government is now faced with cost problems having to deal with clearing water pollution, especially when dealing with water contaminated with dirt and parasites (Jimoh*et al.*, 2007). Children are usually more inflicted with diseases and illnesses caused by water pollution due to the fact that their immune system hasn't been fully developed to fight pathogens. Adults are not exempted too, the fact that their immune system is fully developed doesn't mean they can fight off all pathogens, also immunocompromised individuals would be more susceptible to diseases associated with water contamination due to their weakened immune system.

Because toxic chemicals and heavy metals can harm humans and other species when they build up inside of a biological system, water pollution brought on by these substances has been a major concern in recent years. (Aransiola*et al.*, 2013). Oil spillage is another way water can be polluted and pokses great risk to the environment, oil spillage occurs when there is leakage of hydrocarbon from pipes. In Nigeria today, the poor maintenance of oil pipelines is the major cause of oil spillage. (Krist, 2000).

F. METHOD OF TREATMENT OF POLLUTED/CONTAMINATED WATER

The importance of properly treated water cannot be over emphasized. If water is poorly treated it affects the quality of the water and can lead to public health problems. Treatment type and method depends on different factors such as the water source, where the water is being utilized, population size etc. (de Kok *et al.*, 2001). If the water is

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being used by a small population i.e., members of the same household it can be filtered using purification filters or boiled. Boiling kills most of the pathogens in the water especially the one without the ability to produce spores (i.e., coliforms) but there is a risk in boiling water that contains lead or nitrate due to the fact that it simply increases their concentration leading to more poisoning therefore boiling can only be used to get rid of microbial contaminants and not chemical contaminants.

For water distribution centers, four different methods can be used in water treatment, they include:

- Flocculation: this involves the removal of particle and dirt suspended in the water. To achieve this, aluminum and iron salt is added to the water to form sticky particles
- Sedimentation: this is after flocculation; the particles naturally settle out of the water.
- Filtration: this involves precipitating particles such as chemicals, clay, and organic matter out of the water thereby further reducing the contaminants in the water.
- Disinfection: disinfection processincludes the use of chlorine, chlorine dioxides and chlorinates on the water (Environmental Protection Agency, 2010).

G. CONTROL OF WATER POLLUTION

Water pollution cannot be totally eradicated but can be controlled to an extent and there are different ways to do this, they include:

- The public should be educated on how water can be contaminated, how to prevent it and how to treat it.
- Environmental monitoring programs should be established.
- Environmental laws concerning water pollution should be developed to prevent intentional pollution of water bodies due to human activities.

a) AIM

The aim of this research is to analyze borehole water from different sources within Ilishan community to determine their potability.

b) OBJECTIVES

In order to achieve this aim, the set objectives for this research project are:

- Sampling of borehole water from different sources in Ilishan community.
- Analyze this water samples to identify coliforms in them.
- To determine if water meets the WHO requirement for potable water.

c) STATEMENT OF PROBLEM

Normally, borehole water is safe for human and animal consumption but there are doubts about borehole water being safe for consumption due to the fact that it is an underground water and there could be possible contamination of the water due to poor or incorrect construction of the borehole and other points in the infrastructure such as the water storage, pipes used etc,

d) JUSTIFICATION

This research is done to clear these doubts and confirm if the borehole water in Ilishan community is safe for drinking.

III. MATERIALS AND METHOD

A. MATERIALS/REAGENTS

Water samples,Ethanol/acetone, Eosin Methylene Blue Agar, Nutrient Agar, Lactose broth,Brilliant Green Lactose Bile Broth (BGLBB),Spirit, Cotton wool, Foil, Eosin methylene blue Agar, Simon citrate Agar, peptone broth, Kovac's reagent, crystal violet, Safranine, acetone, Lugol's iodine, gloves, nose masks, distilled water, normal saline.

B. EQUIPMENT/APPARATUS

Spirit lamp, inoculating loop, Incubator, weighing balance, microscope, autoclave, spatula, syringe, pipette filler.

C. GLASSWARES

Petri dishes, conical flasks, Test tubes, Measuring cylinder, beaker, glass jars, glass slides, pipette,

D. STUDY AREA

This study was carried out in Ilishan Remo, Ogun state. This community is situated in the Irepodun district of the Ikenne Local Government Area of the Nigerian state of Ogun. It's within Longitude: 3.7105 north and 6.8932 East in the rainforest climatic region of the country (Ilishan, Nigerian facts).

E. SAMPLE COLLECTION

Various water samples collected from 9 different locations in Ilishan Remo, Ogun stateinto sterile jarsand labelled accordingly.The samples were selected according to availability in the target community. 70 % acetone soaked in cotton wool was used to sterilize the nozzle of the borehole tap for about 10 seconds. The tap was allowed to run for about five minutes at moderate speed so as to avoid it from splashing and transferring organisms from other parts of the tap, the cap and the mouth of the sample bottles were also sterilized and then the water samples were collected after 5 minutes.

SAMPLE CODE	SAMPLE DESCRIPTION	LOCATION
ILA	SAMPLE A FROM ILISHAN	OLOFIN STREET, ILISHAN REMO.
ILB	SAMPLE B FROM ILISHAN	AWOLOWO AVENUE TOWN PLANNING WAY.
ILC	SAMPLE C FROM ILISHAN	OPC TOWN PLANNING WAY.
ILD	SAMPLE D FROM ILISHAN	OKUNLAJA COMPOUND TOWN PLANNING WAY.
ILE	SAMPLE E FROM ILISHAN	ARAROMI STREET, ILISHAN REMO.
ILF	SAMPLE F FROM ILISHAN	IRE AKARI ESTATE, ILISHAN REMO.
ILG	SAMPLE G FROM ILISHAN	NEW SEROLU AVENUE, ILARA ROAD, ILISHAN REMO.

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ILH	SAMPLE H FROM ILISHAN	ORITA OWO STREET ILISHAN REMO.
ILI	SAMPLE I FROM ILISHAN	AYEGBAMI STREET, ILISHAN REMO.

Table 1: Sample Description

A. MEDIUM PREPARATION

For the presumptive test, 7.8g of lactose broth was weighed and dissolved in 300 ml distilled water for the double strength broth, another 7.41g of lactose broth was weighedand dissolved in 570 ml distilled water for the preparation of the single strength broth. After the medium has been properly dissolved, 10 mleach of the double strength was dispensed into 28 test tubes with inverted Durham tubes and 10 ml of the single strength was dispensed into 55 test tubes with inverted Durham tubes, the test tubes were tightly fit with corks made out of foil and cotton wool and was autoclaved at 121^0C for 15 minutes.

For the confirmatory test, 12g of Brilliant Green Lactose Bile Broth (BGLBB) was weighed and dissolved in 150 ml distilled water for the double strength broth 150 ml, another 4.8g of BGLBB was weighed and was dissolved in 120 ml distilled water for the single strength broth. After the medium has been properly dissolved, 10 ml each of the double strength broth was dispensed into 15 test tubes with inverted Durham tubes and 10 ml of single strength broth was dispensed into 12 tubes with inverted Durham tubes, the test tubes were tightly fit with corks made out of foil and cotton wool and was autoclaved at 121^0C for 15 minutes.

For the completed test, 9.35g of Eosin Methylene Blue Agar (EMB) was weighed and dissolved in 260 ml distilled water. The Conical flask was properly corked and autoclaved at 121^0C for 15 minutes.

For the Total bacteria plate count, 6.21 g of Nutrient Agar was weighed and dissolved in 270 ml distilled water. The Conical flask was properly corked and autoclaved at 121^0C for 15 minutes.

For the peptone water used in indole test, 0.54g of peptone water was weighed into a 250 ml conical flask and dissolved in 36 ml distilled water. After the medium has been properly dissolved, 4 ml each of the broth was dispensed into 9 test tubes, the test tubes were tightly fit with corks made out of foil and cotton wool and was autoclaved at 121^0C for 15 minutes.

For citrate utilization test, 2.1g of Simon Citrate Agar was weighed into a 250 ml conical flask and dissolved in 90 ml distilled water. The Conical flask was properly corked and autoclaved at 121^0C for 15 minutes.

For MRVP broth, 1.15g was weighed and dissolved in 68 ml distilled water. After the medium has been properly dissolved, 4 ml each of the broth was dispensed into 17 test tubes, the test tubes were tightly fit with corks made out of foil and cotton wool and was autoclaved at 121^0C for 15 minutes.

B. COLIFORM TEST

The multiple tube fermentation technique using the Most Probable Number (MPN) test procedure was used, three stages was involved:

- Presumptive Test
- Confirmed test
- Completed test.

For the **presumptive test**, three test tubes containing 10 ml of sterilized double broth (lactose broth) and six test tube containing 10 ml of sterilized single strength broth was used for each water sample. Into the first three test tubes containing the double strength broth 10 ml of the water sample measured using a syringe was added to it, 1 ml each of the same water sample was added into another three-test tube containing single strength broth and 0.l ml each of the sample was added into another three-test tube containing single strength broth. The sample procedure was repeated for all water samples. After inoculation, the test tubes were incubated at 37^0C for 48 hours but was observed after every 24 hours until the 48 hours was completed.

For the **confirmatory test**, an inoculating loop was flamed red hot and allowed to cool. A loop full of all positive samples were transferred into tubes containing sterile BGLBB. The double strength tubes that were positive in the presumptive stage were inoculated into test tubes containing sterile double strength broth and the positive single strength tubes were also inoculated into test tubes containing sterile single strength broth.

For the **completed test**, streaking method was used. The plates were labelled and 10 ml of the sterilized EMB Agar was poured into each plate and allowed to solidify (pour plate method). After the plates had solidified, an inoculating loop was flamed red hot and allow to cool for a while, a loop full was picked from the confirmed positive tube and streaked on the plate containing solidified EMB agar. The same process was repeated for all positive tubes.

The tubes that was turbid with slight colour change but wasn't positive for coliform test were sub cultured on nutrient agar plates.

C. TOTAL BACTERIA PLATE COUNT

Serial dilution was done first for each water sample. Peptone water was prepared and then 9 ml each was dispensed into 54 test tubes and then sterilized using an autoclave at 121^0C for 15 minutes. 1 ml of each water sample was added to one test tube each that contained the sterilized broth to make a stock. A serial dilution was made from the stock preparation up to the fifth tube (which waslabeled10^{-5}). This same thing procedure was done for other water samples.

After the serial dilution was completed for all the water samples, dilutions 10^{-1} 10^{-3} and 10^{-5} was used for plating. Nutrient Agar was prepared according to the manufacturer instruction and then sterilized in the autoclave

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at 121^0C for 15 minutes. 1 ml of each dilution was measured and transferred into different Petri dishes and then 10 ml of the prepared nutrient agar was poured into them. The plates were incubated at 37^0C for 24 hours.

D. GRAM STAINING

The growth observed on the plates were Gram stained to determine their Gram reaction.A smear of each distinct colony was prepared on a clean grease free glass slide. The smear was allowed to air dry then Gram stained in the order below:

- The primary stain was added which was Crystal Violet for 60 seconds, after 60 seconds it was washed away
- Lugol's Iodine which acts as the mordant was also added for 60 seconds and then washed away, acetone which acts as the decolourizer was added for 30 seconds and washed away
- Finally, the secondary stain which was Safranine was added for 60 seconds and washed away. The same procedure was repeated for all the organisms.
- All the slides were viewed under the microscope.

E. BIOCHEMICAL TESTS

The biochemical tests used were indole test, methyl red-Vogespros Kaur test, and citrate utilization test (IMVIC). This is used in the identification of coliforms. Oxidase test and IMVIC tests was done on the purple growth seen on the sub cultured nutrient agar.

a) Indole test
Indole test is done to check if an organism can degrade amino acid tryptophan to produce indole. Testtubes containing 4 ml of sterilized peptone broth were inoculated with test organism using an inoculating loop, the inoculating loop was flamed red hot and allowed to cool and then used to pick the organism which was then transferred to the sterile broths. The test tubes were incubated at 37^0C for 24 hours. After 24 hours 0.5 mlof Kovac's regent was added to check for the indole production, the observation of a cherry red colored ring indicates the production of indole (Vashist*et al.*, 2013).

b) Citrate utilization test
This test is used to check the ability of an organism to utilize citrate as its only nitrogen source. Simon citrate Agarwas used for this test, sterilized Simon Citrate Agar was poured into different Petri dishes and allowed to solidify. The plates were divided into positive and negative, using an inoculating loop the test organism was picked and streaked on the positive side, the same procedure was repeated for every plate. After streaking, the plates were incubated at 37^0C for 24 hours. If citric acid has been metabolized, there is generation of carbon dioxide which reacts with sodium and water to form sodium carbonate (an alkaline product) which causes the colour change from green to blue, this colour change indicates a positive result (Vashist*et al.*, 2013).

c) Methyl Red Test
This test is used to check if an organism can produce enough acid during the fermentation of glucose. Test tubes containing 4 ml ofsterilized MR-VP broth was inoculated with test organisms and incubated at 37^0C for 24 hours. After the incubation, methyl red indicator was added. A bright red colour indicates a positive result while a yellow/orange colour indicates a negative result.

d) Voges Proskauer Test
This test is used to check for the presence of acetoin in a bacterial broth culture. Test tubes containing 4 ml of sterilized MR-VP broth was inoculated with test organisms and incubated at 37^0C for 24 hours. After the incubation, 0.6 ml of alpha naphtanol was added and then 0.2 ml 40 % potassium hydroxide (KOH) was added. A bright/cherry red colour is observed for a positive result and yellow/orange colour indicates a negative result (Vashist*et al.*, 2013).

e) Oxidase Test
This is test is used to check for the production of oxidase enzyme by the organism and the presence of cytochrome C using tetra methyl-p-phenyl diamine. A Swab stick was soaked in oxidase reagent and an inoculating loop was used to pick the organism and put on the soaked swab stick. If the area turns purple after 5-10 seconds the result is positive, if there is no colour change the result is negative(Vashist*et al.*, 2013).

IV. RESULTS

Out of the water samples collected, none tested for *E. coli* contamination. For the presumptive test using the multiple fermentation tube technique, out of 81 tubes used for all the water samples only 26 tubes tested positive, sample A had five positive tubes, sample B had no positive tubes, sample C had one positive tube, sample D had 1 positive tube, Sample E had 7 positive tubes, sample F had 2 positive tubes, sample G had 4 positive tube, sample H had 5 positive tube and sample I had 1 positive tube. For the confirmatory test, 25 tubes out of 26 tubes were confirmed for lactose fermentation and gas production, the tube which was inoculated with 0.1 ml sample H water sample was not confirmed. For the completed test all the positive tubes were streaked on EMB agar and the growth seen on it confirmed that coliforms were present but *E. coli*was not present.

Water samples	No of +ve tubes per 10 ml	No of +ve tubes per 1 ml	No of +ve tubes per 0.1 ml	MPN(per 100 ml)
ILA	3	2	0	93
ILB	0	0	0	<2
ILC	1	0	0	4
ILD	1	0	0	4
ILE	3	2	2	210
ILF	2	0	0	9
ILG	2	1	1	20
ILH	2	2	1	28
ILI	0	1	0	3

Table 2: Presemptive Test Results

KEY:
MPN/100 ml = Most Probable Number per 100 ml
+ve = positive

Water samples	No of +ve tubes per 10 ml	No of +ve tubes per 1 ml	No of +ve tubes per 0.1 ml
ILA	3	2	Nil
ILB	Nil	Nil	Nil
ILC	1	Nil	Nil
ILD	1	Nil	Nil
ILE	3	2	2
ILF	2	Nil	Nil
ILG	2	1	1
ILH	2	2	Nil
ILI	Nil	1	Nil

Table 3: Confirmatory Test

KEY:
Nil = no tube(s) observed in the presumptive test.
+ve = positive

Water samples	Water samples Observation on EMB Agar
ILA	Deep purple and slimy growth observed
ILC	Deep purple and slimy growth observed
ILD	Deep purple and slimy growth observed
ILE	Deep purple and slimy growth observed
ILF	Deep purple and slimy growth observed
ILG	Deep purple and slimy growth observed
ILH	Deep purple and slimy growth observed
ILI	Deep purple and slimy growth observed

Table 4: Completed Test Result

KEY:
EMB = Eosin Methylene Blue Agar.

Water samples	Gram reaction	Indole test	Citrate utilization test	Voges-Proskauer Test	Methyl-Red test	Oxidase test	Suspected organisms
ILA	GNB	+ve	+ve	-ve	+ve	Nil	*Citrobacter*spp
ILB	GNCB	-ve	+ve	-ve	-ve	+ve	*Chromobacteriumviolaceum*
ILC	GNB	+ve	+ve	+ve	-ve	Nil	*Klebsiella* spp
ILD	GNB &	-ve	+ve	+ve	-ve	Nil	*Enterobacter*spp
	GNCB	-ve	+ve	-ve	-ve	+ve	*C. violaceum*
ILE	GNB	-ve	+ve	+ve	-ve	Nil	*Enterobacter*spp
ILF	GNB	+ve	+ve	+ve	-ve	Nil	*Klebsiella*spp
ILG	GNB	-ve	+ve	-ve	+ve	Nil	*Citrobacter*spp
ILH	GNB	+ve	+ve	-ve	+ ve	Nil	*Citrobacter*spp
ILI	GNB	+ve	+ve	-ve	+ve	Nil	*Citrobacter*spp

Table 5: Gram Staining And Biochemical Test Result

KEY:
GNB = Gram negative bacilli
GNCB = Gram negative cocci bacillus
+ve = positive
-ve = negative
Nil = biochemical test wasn't done

Water Samples	-1 (cfu/ ml)	-3 (cfu/ ml)	-4 (cfu/ ml)
ILA	1.2×10^2	2×10^3	NG
ILB	2.0×10^2	7×10^3	1×10^4
ILC	1.8×10^2	1×10^4	7×10^4
ILD	1.5×10^2	1×10^4	6×10^4
ILE	8.0×10^1	4×10^3	3×10^4
ILF	1.1×10^2	1×10^3	1×10^4
ILG	5.3×10^2	2×10^4	1×10^5
ILH	2.1×10^2	6×10^3	3×10^4
ILI	TNTC	2×10^4	1×10^5

Table 6: Total Plate Count

KEY:
Cfu/ ml = Colony forming unit per ml.
TNTC = Too numerous to count.
NG = No growth.

Water samples	**pH value**
ILA	8.03
ILB	7.79
ILC	7.54
ILD	7.22
ILE	6.85
ILF	6.99
ILG	6.66
ILH	6.24
ILI	6.56

Table 7: Ph of Water Samples

Water samples	**Nutrient agar**

ILB	Purple growth observed
ILD	Purple growth observed

Table 8: Results For The Tubes That Were Subcultured On Nutrient Agar.

V. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

A. DISCUSSION

Drinking water must meetthe acceptable standards set by World health organization (WHO) before it can be fit for drinking. Parametric limits known to be appropriate for water quality standards have been established (WHO, 2000). According to WHO for the International standards for drinking water, no sample should contain *E. coli*, it is unsatisfactory. If *E. coli* is absent in the water sample, coliforms shouldn't be more than 10 coliform per 100 ml; 0/100 ml of total coliform count is considered Excellent, 1-3/100 ml is satisfactory, 4-9/100 mlis suspicious and 10 and above is unsatisfactory(WHO, 2008).

E. coli was not present in any of the water samples which indicates that there is no faecal contamination of all the borehole water sampled. Samples ILB and ILI are within the satisfactory values set by WHO with coliform counts of <2 and 3 per 100 ml (table 3.1). Samples ILF, ILC and ILD was found to be suspicious with coliform count of 9,4 and 4 respectively per 100 ml of the water sample tested while samples ILA, ILE, ILG and ILH with coliform count 93/100 ml, 210/100 ml, 20/100 ml and 28/100 ml respectively were unsatisfactory(table 3.1). This present study is in accordance with Agbabiaka*et al.* (2009) who also did not isolate *E. coli* from the water samples from Ilorin and the samples weren't devoid of other organisms as well. This is in contrast to Aina *et al.* (2012) who analyzed borehole water in Ogun state and was able to isolate *E. coli* from some of the samples he worked with. The coliform count for this study is higher as compared to the results obtained by Aina *et al.* (2012).

All the tubes that were positive in the presumptive test were positive for the confirmatory test except the tube that was inoculated with 0.1 ml sample ILH (table 3.2). The completed test showed purple growth on all the EMB plates but no green metallic sheen was observed and this further confirms that coliforms are presents in all the water samples but *E. coli* wasn't present (table 3.3). The organism isolated based on their Gram reaction and biochemical test were suspected to be *Citrobacter*spp, *Klebsiella*spp and *Enterobacter*spp (table 3.4). This result is in accordance with the results obtained by Aina *et al.*, (2012) andAgbabiaka*et al.*, (2009) who were also able to isolate *Klebsiella*spp and *Enterobacter*spp from the water samples they worked with.

The total bacteria count (TBC) for all the water samples tested ranges from 8.0 x 10^2 cfu/ ml to 5.3 x 10^2 cfu/ ml (table 3.5). The allowed maximum limit for total bacteria counts of potable water is 100cfu/ ml (Nigerian Standard for Drinking Water Quality,2007). Sample ILE falls within the acceptable limit with 8.0 x 10^2 cfu/ ml. while the remaining eight water samples did not pass the allowed maximum limit for total bacteria count as their TBC ranges between 1.1 x 10^2 cfu/ ml to 5.3 x 10^2 cfu/ mland ILI was even too numerous to count. Although 1 of the 9 samples tested in this study falls within the allowed total bacteria count limit, the majority of the samples wasn't within the allowed limit and this is partially consistent with the study of Erah*et al.*, (2002) on the quality of ground water in Benin which showed that all the water samples were of unacceptable limits. Also,Eniola*et al.* (2007) obtained a range of 5.0 x$10^2$to 7.0 x 10^2 cfu/ ml for stored borehole water samples, which also shows that the water samples were of unacceptable levels.

The pH of the water samples ranges between 6.0 and 8.03. Sample ILA had a pH of 8.03 which is alkalinic, samples ILB, ILC and ILD had pH of 7.79, 7.54 and 7.22 respectively which were neutral while the remaining samples ILE, ILF, ILG, ILH and ILI had pH of 6.85, 6.99, 6.66, 6.24 and 6.56 respectively which are slightly acidic (table 3.6). According to WHO (2017) the normal pH of ground water should be within 6.5 to 8.5. If the pH is not within this range and such water is consumed, it could lead to health problems. Therefore, going by WHO (2017) pH standard, all the water samples tested in this study passed.

ILB and ILD did not produce gas but the tubes were turbid. Therefore, they were sub cultured into Nutrient Agar. The growth observed was a purple growth. The Gram reaction was a Gram-negative coccobacillus. Further identification by a biochemical test (table 3.4) indicated that the organism could be suspected as *Chromobacterium violaceum. C. violaceum* is an opportunistic pathogen found in water and soil of tropical and subtropical regions, clinical features include multiple liver, lung and spleen abscesses and sepsis. Chronic granulomatosis, ocular infections, osteomyelitis, and cellulites periorbital have also been reported (Ray *et al.*, 2004).

B. CONCLUSION AND RECOMMENDATIONS

The public health and environmental officers have seen the need to control water quality, the result of this research clearly shows the presence of indicator organisms in borehole water present within Ilishan community.

Potable water should be safe for human and animal consumption, indicator organisms shouldn't be present at all or should meet the quality specifications provided by regulatory bodies while faecal coliforms are not to be present at all.The control of drinking water quality is very important in order to avoid or reduce water borne diseases. It is therefore recommended that the Federal Government of Nigeria should organize programs through the sanitary control inspectors in different part of Nigeria that would help in educating the citizens on how to maintain water quality, the dangers of bad waste disposal and its adverse effects. Borehole systems should also be well constructed to reduce contamination, storage systems should be properly

washed at regular intervals and the water should also be treated appropriately.

It is also recommended that further studies should be carried out to test the susceptibility pattern of the organisms isolated to ascertain no public health threat is being posed in regards to antibiotic resistance.

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APPENDIX

Compounding of Methyl Red Voges Proskauer broth
Di peptone
7g =1000 ml
X = 68 ml
X = 68 x 7/1000 =0.47g
Dextrose
5g =1000 ml
X = 68 ml
X = 68 x 5/1000 =0.34g
Dipotassium
5g =1000 ml
X = 68 ml
X = 68 x 5/1000 =0.34g
0.47g of Di peptone was weighed
0.34g of dextrose (D-glucose) was weighed
0.34g of dipotassium phosphate was weighed.
All the components weighed above was dissolved in 68 ml distilled water