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Full Length Research Paper

Bacteriological analysis of borehole water from different towns in Ogun State, Nigeria

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Samples of borehole water were collected from Iperu, Ilishan, Sagamu, Ogere, Ilara, Ikenne, Irolu, Ode-remo and Babcock community, all in Ogun state. They were analyzed microbiologically using pour plate technique for total viable counts and tube fermentation technique for Most Probable Number (MPN) counts. Thirteen samples were positive for coliforms while remaining five were non-coliforms. Three samples satisfied the W.H.O. standard requirements of coliform count between 1-3/100 ml. Four samples were suspicious having coliform count ranging from 4-8/100 ml and eleven samples with too numerous counts did not satisfy W.H.O. standard requirements. Six bacteria isolates were obtained from the samples and identified as *Escherichia coli, Klebsiella* sp.*, Proteus* sp.*, Enterobacter aerogenes, Staphylococcus aureus* and *Streptococcus* sp. Characterization of isolates was carried out by Gram staining reaction and biochemical tests. Gram-negative bacilli (72.22%) were more prevalent in the water samples compared to Gram Positive Bacilli (11.11%) and Gram Positive Cocci (16.67%). *E. coli* gave the highest percentage of occurrence of isolates (33.33%) followed by *Klebsiella* sp. (27.78%) while the percentage of occurrence of *Proteus* sp.*, S. aureus* and *E. aerogenes* were the least (5.56%). Two samples were confirmed for *Streptococcus* sp. (11.11%) and *Clostridium* sp. (11.11%). 50% of the isolates tested positive to acid and gas.

Key words: Bacteriological analysis, water, bacteria.

INTRODUCTION

The health of the people depends solely on the quality of water available for consumption. The health aspects of environmental quality were among the first to receive scientific consideration through the recognition of water-borne diseases (Olawuyi, 2006). Water pollution as a result of microbial contaminants and pollutants has resulted in epidemics of water-borne diseases such as typhoid fever, cholera and dysentery (Reeves et al., 1989). Waterborne diseases are caused by pathogenic microorganisms which are directly transmitted when contaminated fresh water is consumed (Potter, 2006). Contaminated fresh water, used in the preparation of food, can be the source of food borne disease through consumption of the same microorganisms. According to the World Health Organization (2008), diarrhea disease accounts for an estimated 4.1% of the total global burden of disease and is responsible for the deaths of 1.8 million people every year. It was estimated that 88% of that burden is attributable to unsafe water supply, sanitation and hygiene, and is mostly concentrated in children in developing countries (Bagley, 1985). Waterborne disease can be caused by protozoa, viruses, or bacteria, many of which are intestinal parasites (WHO, 2000).

Coliform bacteria are commonly-used bacterial indicator of water pollution, which is present in the environment particularly in the faeces of all warm-blooded animals and humans (Howard et al., 2002). Their presence in drinking water indicates that disease-causing organisms could be in the water system and may pose an immediate health risk the water (Tebutt, 2007).

The greatest risk from microbes in water is associated

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with consumption of drinking-water that is contaminated with human and animal excreta (Ito et al., 1991). Also, infectious diseases caused by pathogenic bacteria, viruses and parasites (e.g., protozoa and helminths) are the most common and widespread health risk associated with drinking-water (De Zuane, 2009). Other microorganisms sometimes found in surface waters which have caused human health problems include; *Burkholderia pseudomallei, Cryptosporidium parvum, Giardia lamblia, Salmonella, Clostridium, Streptococcus ,* Parasitic worms (helminths), *Novovirus* and other viruses (Olawuyi, 2006; Schleifer and Kilpper-Balz, 2008).

The immunity of individuals also varies considerably, whether acquired by contact with a pathogen or influenced by such factors as age, sex, state of health and living conditions. Part of the demonstration of pathogenicity involves reproducing the disease in suitable hosts. For pathogens transmitted by the faecal–oral route, drinking-water is only one vehicle of transmission. Contamination of food, hands, utensils and clothing can also play a role, particularly when domestic sanitation and hygiene are poor. Improvements in the quality and availability of water, in excreta disposal and in general hygiene are all important in reducing faecal–oral disease transmission (Heidelberg, 2000; Amyes, 2007; Genthe and Strauss, 2007).

Borehole operators are required to deliver safe and reliable drinking water to their consumers 24 h a day, 365 days a year. If the water supply becomes contaminated, consumers can become seriously ill (Howard et al., 2002). The underground water supplies are usually considered safe provided they are properly located, constructed and operated according to the World Health Organization Guidelines for Drinking Water (WHO, 1976). Boreholes as a low-cost technology option for domestic water supply in developing countries are generally considered as ‘safe sources’ of drinking water. However, it is the collection, transportation, storage and decanting of water that can lead to subsequent contamination. Most pathogens that can contaminate water supplies come from the feces of humans or animals (Edema and Omemu, 2001).

When coliforms and other bacteria are found there is the need to investigate and find out the sources of contamination in the water. Conformation with microbiological standards is of special interest because of the capacity of water borne disease within a large population (Edema and Omemu, 2001). Therefore, this study was aimed at characterizing bacteria isolates in borehole water of some towns in Ogun state and assessing the quality of water and potential sources of water contamination in such areas.

MATERIALS AND METHODS

Collection of samples

Eighteen borehole water samples were collected from nine different towns in Ogun state for analysis. The tap faucets were surfaced sterilized with cotton wool soaked in 75% ethanol, and then flamed. The tap was allowed to run for a 60 s before collecting in a 500 ml sterile bottle which was carefully covered with its screw cap. All samples collected from all sources were taken to the laboratory for analysis within six hours after collection. Each of the sample bottles was labeled with sample code number and they were thoroughly mixed, before testing.

Preparation of medium

Seventy-three grams of lactose broth were weighed using an analytical weighing balance and dissolved in 1000 ml of sterile distilled water inside a conical flask. 52 g of MacConkey agar powder were dissolved in 1000 ml of sterile distilled water. 36 g of Eosin Methylene Blue powder was dissolved in 1000 ml of distilled water; 28 g of nutrient agar powder was also dissolved in 1000ml of distilled water and 32 g of Mueller Hinton agar powder was dissolved in 1000 ml of distilled water. For proper dissolution and homogenization, the media were shaken vigorously and melted using a water bath at the temperature at 45°C for 40 min before sterilizing in an autoclave at 121°C for 15 min. Media were aseptically dispensed into oven-sterilized Petri-dishes and allowed to solidify under laminar air-flow.

Isolation of bacteria from water samples

Water samples were subjected to colony count (using MPN standard table procedure) and multiple tube fermentation technique (using MPN procedure).

Screening for acid and gas producing coliform bacteria

Presumptive test

1 ml of the water sample was inoculated into test tubes containing 9 ml of Lactose broth with Durham tubes which was incubated at 37°C±0.5°C for 24 to 48 h. Gas production within 24 to 48 h indicated a positive presumptive test while absence of gas production indicates negative presumptive test. The appearance of air bubbles in Durham tubes before incubation was not confused with the actual gas production. The presence of acid production within 48 h of incubation is indicated by the change in color, showing a positive presumptive test.

Confirmed test

A loopful each of the water sample in the positive presumptive test tubes was streaked on Eosin Methylene Blue and incubated at 37°C ± 0.5°C for 24 h. Growth of bacterial colonies indicated that the confirmed test is positive while absence of growth was considered as negative.

Completed test

Melted MacConkey agar was distributed into plates using pour plates technique and allowed to solidify. A loopful of broth from each of the positive tubes was streaked on each of the MacConkey agar plates. The plates were incubated at 37°C for 24 h.

Gram stained preparation from slant cultures corresponding to the positive tubes that showed acid and gas formation were thoroughly examined.

Characterization of coliform isolates

Morphological Growth and Identification of Isolates on media

The cultural characteristics of the isolates on solid MacConkey agar were examined. The growth patterns, colony size, edge, elevation on the plates were recorded after 48 h of incubation at 37°C. Gram staining technique was carried out for the identification and differentiation of each isolated bacteria. The size and arrangement of colonies into shapes (rods or round and chains) were also recorded (Ryan and Ray, 2008).

Biochemical tests for identification of bacteria isolates

Some tests were carried out namely: Catalase, Urease, Oxidase, Indole and Citrate following standard procedures with reference to Bergey's Manual of Systematic Bacteriology (Sneath, 1986).

RESULTS

In this study, eighteen samples of borehole water collected from different towns in Ogun state, Nigeria were tested for the presence of bacteria. Screening for acid and gas production was carried out at 37°C (Figures 1, 2 and 3). Fifty percent (50%) of the isolates produced acid and gas with colour changes on MacConkey Lactose Broth medium and gas production was shown in Durham tubes. The isolates that produced acid only accounted for 33.33% while 16.67% isolates did not produce acid and gas (Figure 2).

From the morphological characterization of the isolates (Table 1), *E. coli* isolated were rod shaped, soft, and smooth and 2-4 mm in size, while *Klebsiella* spp. were rod shaped, mucoid, rough, heaped or doomed, opaque and pinkish colonies with sizes ranging from 2-4 mm. Isolated *Streptococcus spp*. were cocci in chains, soft, raised opaque and pink colonies with sizes ranging from 0.2-0.6 mm. *S. aureus* isolated were cocci in clusters, rough, raised, mucoid, opaque, creamy with sizes ranging from 1-1.8 mm, while *Clostridium* spp. showed rod shape, serrated flat, soft, opaque, and white colonies with sizes ranging from 4-5 mm. *E. aerogenes* showed rod shaped, rough, slightly raised, mucoid, opaque, and pink colonies with sizes from 4-5 mm, while *Proteus* also showed rod –shaped, smooth, flat, mucoid, opaque and pink colonies with sizes ranging from 2-4 mm.

In isolation and characterization of bacteria, *E. coli* gave the highest percentage of occurrence (33.3%) followed by *Klebsiella* sp. (27.78%), while percentage of occurrence of *Proteus* sp. and *E. aerogenes* were the least (5.56%). Three samples were confirmed to contain *Streptococcus* sp. (11.11%) and *Clostridium* sp. (11.11%) and 5.56% of *S. aureus* (5.56%) (Figure 2).

Table 2 showed that *E. coli* was indole and methyl red positive while, Voges prokauer, citrate, litmus milk, catalase, oxidase, coagulase, oxygen relationship, DNAse and urease tests were negative for the organism. *Streptococcus* sp. produced haemolysis on blood agar but negative to Voges prokauer, citrate, litmus milk, catalase, oxidase, coagulase, oxygen relationship tests, methyl red, urease, DNAse and spore staining tests. *Clostridium* sp. was positive for litmus milk, spore staining, oxygen relationship and haemolysis tests but negative to Voges prokauer, methyl red, citrate, oxidase and coagulase tests. *S. aureus* was positive for catalase, coagulase and DNase tests. Acid producing *Klebsiella* sp. was Voges proskauer positive but negative to other biochemical tests, while acid and gas producing *Klebsiella* sp. was positive methyl red and citrate tests but negative to other biochemical tests. *E. aerogenes* was positive to indole, Voges proskauer, and citrate but negative to other biochemical tests while Proteus was positive to Voges proskauer and urease but negative to other biochemical tests. High proportion of the isolated bacteria (72.22%) were Gram negative bacilli, 16.67% were Gram positive cocci and 11.11% were Gram positive bacilli (Figure 3). The Most Probable Number (MPN) of coliform bacteria was estimated from Monica C (2006) MPN standard Table (Table 3). Three water samples satisfied the WHO standard recommending a coliform count between 1-3/100 ml, three were suspicious, while eleven samples with too numerous to count did not satisfy the WHO standard requirements.

DISCUSSION

Water must meet internationally acceptable standards and be in line with guidelines stipulated by the World Health Organization to be fit for drinking. Water quality criteria are a function of several parameters. Appropriate parametric limits for water quality standards have been established (WHO, 1976). The bacteriological analysis of the water samples are shown in Tables 2 and 3. The total viable count (TVC) indicates that water samples from Ilishan, Ogere, Sagamu, Ikene, Irolu, and Ode, had high coliform counts far above the recommended value after incubation for 24 h. The result of the bacteriological examination of the water samples obtained also showed that three of the samples (IPR1, SG2, OGR2) are within the range of satisfactory values of 1-3/100 ml of coliform count (but the most acceptable limit is 0 coliform count which was stamped as Excellent), four samples (ILH1, ODE2, BAB1 and BAB2) were suspicious, while eleven samples (IPR2, ILH2, SG1, OGR1, IKN1, IKN2, ILA1, ILA2, IRO1, IRO2, ODE1) were unsatisfactory. This implies that they do not satisfy the World health Organization standard requirements.

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

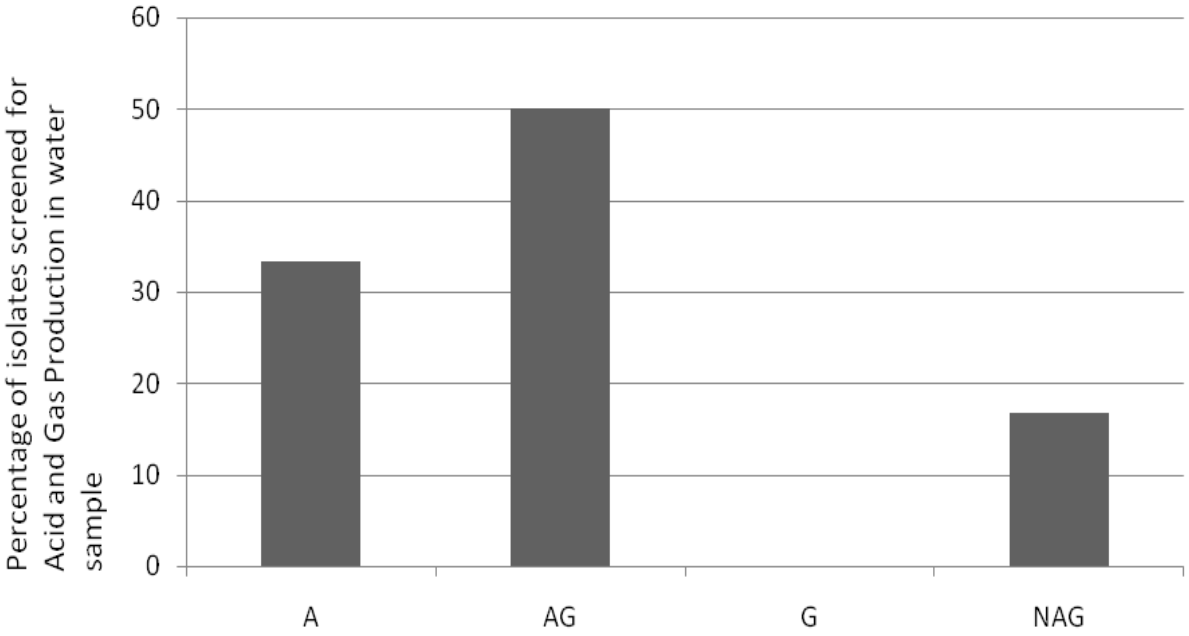

Figure 1. Percentage of isolates in water sample.

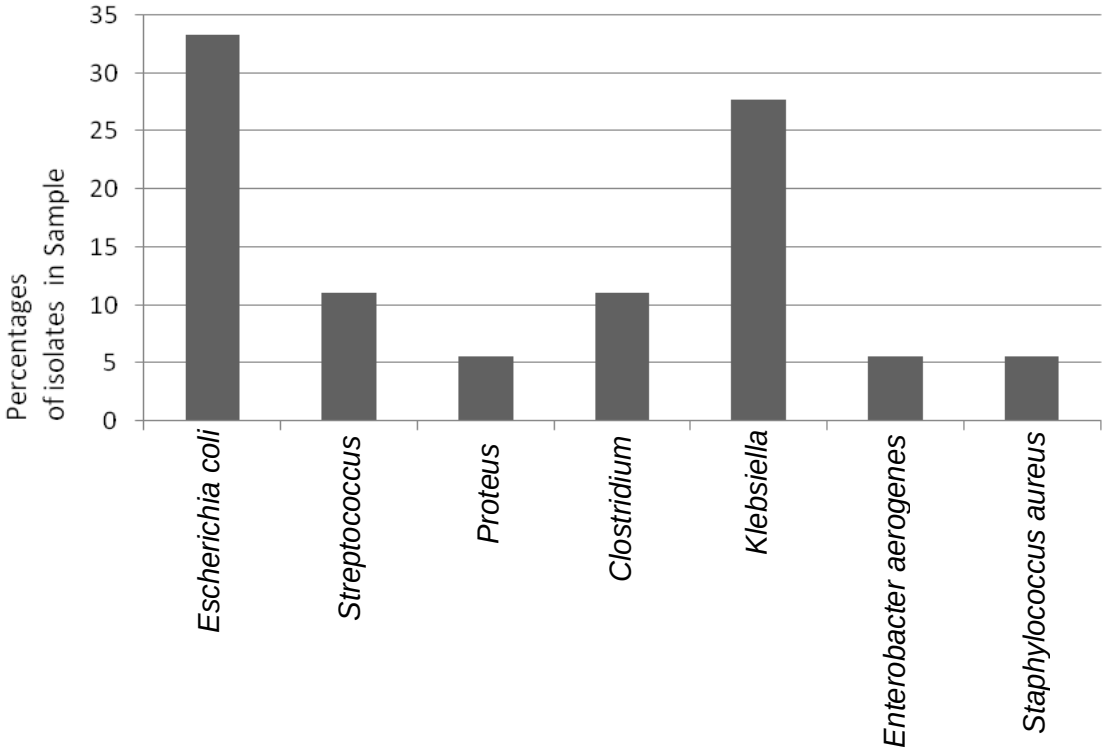

Figure 2. Percentage of isolates screened for Acid and Gas production in water samples. A: Acid Only; AG: Acid and Gas; G: Gas Only; NAG: No Acid, No Gas.

management, faecal deposit and waste dumps also affect bacterial concentration in run-off water. Faecal contamination caused by *E. coli* was dominant. The pollution caused by human activities includes the indiscriminate habit of the people in the use of latrines and siting wells close to the toilets. Bagley and Seidler

Table 1. Morphological Characteristics of isolated bacteria.

Sample code	Colour	Size (mm)	Surface	Shape	Elevation	Consistency	Odour	Opacity
IPR1	Pink	3-4	Rough	Rod	Heaped	Mucoid	No odour	Opaque
IPR2	Pink	3-4	Rough	Rod	Heaped	Mucoid	No odour	Opaque
ILH1	Pink	2-4	Smooth	Rod	Slightly Raised	Soft	Flowery	Opaque
ILH2	Pink	2-4	Rough	Rod	Heaped	Mucoid	No odour	Opaque
SG1	Pink	2-4	Smooth	Rod	Slightly raised	Soft	Flowery	Opaque
SG2	Pink	2-4	Smooth	Rod	Slightly raised	Soft	Flowery	Opaque
OGR1	Pink	2-4	Smooth	Rod	slightly raised	Soft	Flowery	Opaque
OGR2	White	0.2-0.6	Smooth	Cocci	Raised	Soft	Flowery	Opaque
IKN1	Pink	3-4	Rough	Rod	Heaped	Mucoid	Flowery	Opaque
IKN2	Pink	2-4	Smooth	Rod	Slightly raised	Soft	Flowery	Opaque
ILA1	White	4-5	Serrated	Rod	Flat	Soft	Flowery	Opaque
ILA2	White	4-5	Serrated	Rod	Flat	Soft	No odour	Opaque
IRO1	Pink	2-4	Smooth	Rod	Slightly raised	Soft	Flowery	Opaque
IRO2	Pink	4-5	Rough	Rod	Slightly raised	Mucoid	Flowery	Opaque
ODE1	PINK	3-4	Rough	Rod	Heaped	Mucoid	No odour	Opaque
ODE2	Creamy	1-1.8	Rough	Cocci	Raised	Mucoid	Flowery	Opaque
BAB1	Pink	2-4	Smooth	Rod	Flat	Mucoid	Flowery	Opaque
BAB2	Creamy	0.2-0.6	Smooth	Cocci	Raised	Soft	Flowery	Opaque

Table 2. Gram reaction and Biochemical tests of Bacteria isolates.

Sample code	Gram reaction	Indole	Methyl red	Voges proskauer	Citrate	Catalase	Oxidase	Coagulase	Haemolysis	Urease	Dnase test	Acid and gas production	Lactose	Organism
IPR 1	GNB	-	-	+	-	-	-	-	-	-	-	A	-	*Klebsiella* sp.
IPR 2	GNB	-	+	-	+	-	-	-	-	-	-	AG	+	*Klebsiella* sp.
ILH 1	GNB	+	+	-	-	-	-	-	-	-	-	AG	+	*E. coli*
ILH 2	GNB	-	+	-	+	-	-	-	-	-	-	AG	+	*Klebsiella* sp.
SG 1	GNB	+	+	-	-	-	-	-	-	-	-	AG	+	*E. coli*
SG2	GNB	+	+	-	-	-	-	-	-		-	AG	+	*E. coli*
OGR 1	GNB	+	+	-	-	-	-	-	-	-	-	AG	+	*E. coli*
OGR 2	GPC	-	-	-	-	-	-	-	-	-	-	A	-	*Streptococcus* sp.
IKN 1	GNB	-	+	-	+	-	-	-	-	-	-	AG	+	*Klebsiella*
IKN 2	GNB	+	+	+	+	-	-	-	-	-	-	AG	+	*E. coli*
ILA1	GPB	-	-	-	-	-	-	-	+	-	+	NAG	-	*Clostridium*
ILA2	GPB	-	-	-	-	-	-	-	+	-	+	NAG	-	*Clostridium*
IRO1	GNB	+	+	-	-	-	-	-	-	-	-	AG	+	*E. coli*
IRO2	GNB	-	-	+	+	-	-	-	-	-	-	A	+	*Enterobacter aerogenes*
ODE1	GNB	-	-	+	-	-	-	-	-	-	-	A	-	*Klebsiella* sp.
ODE2	GPC	-	-	+	-	+	-	-	-	+	-	NAG	-	*Staphylococcus aureus*
BAB1	GNB	-	-	+	+	-	-	-	-	-	+	A	-	*Proteus* sp.
BAB2	GPC	-	-	-	-	-	-	-	+	-	-	A	-	*Streptococcus* sp.

+: Positive; GNB: Gram Negative bacilli; -: Negative; A: Acid only; GPC: Gram Positive cocci; AG: Acid and Gas; GPB: Gram Positive bacilli; NAG: No Acid and Gas.

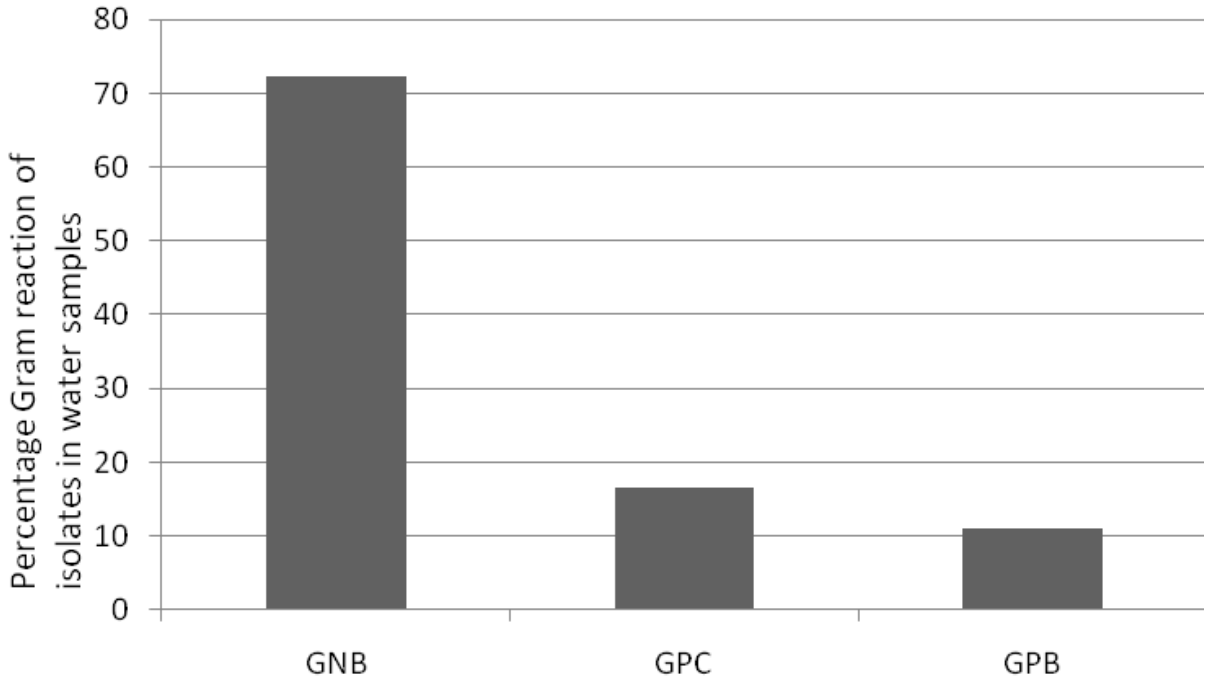

Figure 3. Percentage Gram reaction of isolates in water samples. GNB: Gram Negative Bacilli; GPC: Gram Positive Cocci; GPB: Gram Positive Bacilli.

Table 3. Estimation of number of coliforms using MPN standard (Monica Cheesbrough, 2006).

Sample code	(Volume of Sample in each bottle) Number of bottle used		Coliform count using MPN coliform/100 ml	Remark
	1 (50 ml)	5 (10 ml)		
IPR 1	1	1	3	Satisfactory
IPR2	1	5	TNTC	Unsatisfactory
ILH1	0	3	4	Suspicious
ILH2	1	5	TNTC	Unsatisfactory
SG1	1	5	TNTC	Unsatisfactory
SG2	1	1	3	Satisfactory
OGR1	1	5	TNTC	Unsatisfactory
OGR2	1	0	3	Satisfactory
IKN1	1	5	TNTC	Unsatisfactory
IKN2	1	5	TNTC	Unsatisfactory
ILA1	1	5	11	Unsatisfactory
ILA2	1	3	TNTC	Unsatisfactory
IRO1	1	5	103	Unsatisfactory
IRO2	1	5	TNTC	Unsatisfactory
ODE1	1	5	TNTC	Unsatisfactory
ODE2	1	0	4	Suspicious
BAB1	1	2	6	Suspicious
BAB2	1	3	8	Suspicious

(1976) and Olawuyi (2006), observed that irrespective of other organisms present in water, only the isolation of faecal indicator bacteria coupled with appreciable levels of coliform MPN signifies faecal pollution as an observation. In the tropics, a fairly high proportion of coliform organisms in water are often found to be of faecal origin. Since careful sanitary surveys have been shown that such water may be free from exposure to excretal contamination, it is clear that reliance on the presumptive coliform count will result in unnecessary

condemnation of a number of unpolluted water.

Differentiation will generally be necessary and attention should be mainly to the numbers of faecal coliform by carrying out confirmed and completed tests. The absence of evidence indicating faecal contamination does not necessarily indicate that contamination has not taken place. There might be no detectable evidence of contamination as at the time the sample was examined. Due to this reason, thorough and frequent bacteriological examination of water samples is desirable with the inclusion of tests for faecal Streptococcal and some *Clostridium* sp. since growth on selective media such as Blood agar and various biochemical tests carried out had revealed the presence of *Streptococcus* sp. and *Clostridium* sp. (Table 2).

CONCLUSION AND RECOMMENDATIONS

The need for controls over the quality of water meant for drinking purposes has been recognized by public health and environmental officials for many years. The results of this study demonstrate clearly the presence of microorganisms in borehole water. Contamination from faecal origin is the major and prominent source of contaminant in water samples due to poor method of faecal waste disposal (Olawuyi, 2006).

The presence of pathogenic organisms and indicator organisms in some of the water samples renders it unfit for drinking due to contamination. Water should meet different quality specifications depending on the particular use. Potable and domestic water should be harmless to man. Water quality should be controlled in order to minimize acute problems of water related diseases, which are endemic to the health of man. Faecal waste disposal along the major surface water should be discouraged. However, the Federal Government of Nigeria should educate people through the sanitary control inspectors on the health hazards posed by indiscriminate faecal waste disposal and they should be educated on proper waste disposal management. Also, more borehole systems should be constructed to replace the shallow hand-dug wells. The result of their industrial and domestic use after treatment and purification makes borehole water free from pathogenic organisms. Coliform bacteria, and in particular *Enterobacter*, *Citrobacter*, *Klebsiella* and *Serratia* species, can bring on, as agents with facultative pathogenicity, a large number of infections in medical areas with predisposed or immunodeficient patients. The present state of knowledge concerning facultative pathogenic significance is subject to a considerable process of change, with microbiological research, in particular, resulting in new insights and assignments of individual species. The spectrum of nosocomial infections that they bring on covers, among others: wound infections, catheter-related infections, pneumonia and septicemia.

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