CULTURE-BASED AND MOLECULAR DETECTION OF THE MIROBIAL QUALITY OF BOREHOLE WATER USED IN SOME HOSTELS IN IHIAGWA, IMO STATE

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Abstract: Culture-based and molecular detection of the quality of borehole water collected at random from seven hostels in Ihiagwa was undertaken. The bacterial isolates identified using culture-based analysis include *Staphylococcus* sp, *Klebsiella* sp, *Micrococcus* sp, *Salmonella* sp, *Escherichia coli,* and *Pseudomonas aeruginosa.* The presence of fungal isolates such as *Trichoderma* sp, *Aspergillus* sp *and Rhizopus* sp was also observed. Molecular identification of isolates using Polymerase Chain Reaction [PCR assay] with specific primers targeting 16sRNA of coliforms, 16sRNA of total bacteria genera, 16sRNA of *Vibrio* sp and IVsRNA of total fungi was carried out. Bands were obtained during gel electrophoresis depicting the presence of targeted organisms. The absence of *Vibrio* sp was evident. This study was carried out to ascertain the level of contamination and non potability of drinking water used within these hostels.

Key words: *Culture-based, molecular detection, borehole water, microorganisms, PCR assay, primers*

INTRODUCTION

Availability of potable water is a major social and economic concern. About a billion people around the world routinely drink unhealthy water. Pollution and poor sanitation remain the principal cause of drinking water contamination which constitute the major cause of in most developing countries. World Health Organization also estimates that safe water could prevent 1.4 million child deaths from diarrhea each year (WHO, 2008). Water quality is determined by assessing three classes of attributes such as biological, chemical and physical. It is a measure of the condition of water relative to the requirements of one or more biotic species and or to any human need or purpose (USEPA, 2001). It is most frequently used by reference to a set of standards against which compliance can be assessed. The most common standards used to assess water quality are related to drinking water, safety of human contact and for the health of eco-system. There are standards of water quality set for each of these three classes of attributes. Such guideline values have been determined for those components that are considered to have significant potential to harm human health at concentrations above the specified limits which should not be exceeded in public water supplies. More so, exceeding the guideline values may not always be a matter for immediate concern, but

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rather a trigger for follow-up action. The national standards for drinking water are developed by the Federal Government's Environmental Protection agency (FEPA) whereas the international standards are developed by World Health Organization (WHO). All municipal (public) water supplies must be measured against these standards.

Main origins of pollution of wells and boreholes are industrial, domestic, agricultural and pollution can be continuous or accidental (Ogbulie and Akujiobi, 2006; Ogbulie *et al.*, 2009). Industrial pollution may involve seepage of used water containing chemicals such as metals and radio active compounds, or contaminated water from damaged pipelines infiltrating into the borehole (Ogbulie, *et al.*, 2009). Domestic pollution may involve seepage from broken septic tanks, pit latrines, cesspools, and privics. Agricultural pollution is from irrigation water or run-off water after rains, carrying fertilizers, pesticides, herbicides and faecal matter. Environmental pollution is mainly from sea water intrusion into coastal aquifer. The WHO recommends that borehole should be located at least 30m away from latrines and 17m from septic tanks (Chukwurah, 2001, Ogbulie, *et al.*, 2009). Microorganisms of concern in contaminated water include the following bacterial agents of diarrhea and gastroenteritis namely *Salmonella* specie. *Shigella* specie, *Escherichia coli and Vibrio cholera* (Cheesbrough, 1994, Birmingham *et al.*, 1997, Okpokwasili and Akujiobi, 1996; Ogbulie, 2008; Ogbulie *et al.*, 2009).

This study was however, designed to determine the microbial quality of drinking water (borehole) consumed by students residing in hostels in Ihiagwa, with a view to create awareness to the inhabitants and general populace about the predisposed health risks of using non potable water.

METHODOLOGY

Sample collection

The samples were collected at 7am on the sampling day, prior to collection, the mouth of the tap was flamed with cotton wool dipped in ethanol. Thereafter the tap was allowed to cool off by allowing it to run off before sample collection from different points using sterile sample bottles. This was taken to the laboratory within 1hr of collection for microbial analysis.

Culture-based analysis and identification

An aliquot was inoculated into an already prepared sterile culture medium using spread plate method as described by Cheesbrough (1994). Thereafter the inoculated plates of NA and MA were incubated at 37^{0}C ± 5^{0}C for 24 - 48h whereas SDA, TCBS and SSA plates were incubated at room temperature. The colonies were counted , morphologically identified, Gram stained and biochemically characterized. Identification was as described in Bergey's Manual of Determinative Bacteriology (Holt, 1984) and Cowan and Steel's Manual for the identification of Medical Bacteria (Barrow and Feltham, 2003).

Sanitary surveillance

The area/site of collection was investigated to check the sanitary quality of the water collection point where the inhabiting students obtain their drinking water.

pH and temperature measurement

The pH and temperature were measured at the point of collection using a pocket tester model AD14 professional IP67 Waterproof.

Molecular analysis

Chromosomal DNA Extraction by Boiling Method

This was done using an 18h broth culture of the tests isolates. The eppendorf tubes were labeled and bacterial growth mixed by vortexing. One milliliter (1ml) of broth was added into pre-labeled eppendorf tubes centrifuged at 10,000 rpm for 2 minutes, supernatant was discarded. One milliliter (1ml) of sterile distilled water was added, vortexed and centrifuged at 10,000rpm for 5minutes. The supernatant was again discarded by decanting and it was blotted on a paper towel. Thereafter, 200µl of 1X Tris EDTA (TE) was added and vortexed to homogenize the pellets. The mixture was boiled at 100^{0}C for 10minutes, after which it was vortexed again and centrifuged at 10,000rpm for 5minutes. The supernatant was transferred into another pre-labeled eppendorf tubes by gentle aspiration using a micropipette and stored at -20^{0}C.

The Polymerase Chain Reaction

A mixture was created, with optimized concentrations of the DNA template, Taq polymerase enzyme, primers and dNTPs. The mixture was a combination of 4µl of master mix (comprising of HOT FIRE Pol Taq DNA polymerase, 5X blend master mix buffer, 7.5mM $MgCl_2$, 2 mM dNTPs of each of dATP , dCTP ,dGTP, dTTP, BSA, Blue and yellow dyes) , 0.2µl each of forward and reverse primers and 2µl of template DNA gave a total of 6.4µl. Hence 13.6µl of sterile nuclease free water was added to make it up to the required 20µl .The entire mixture was then vortexed and loaded into the thermal cycler (Eppendorf vapo. protect) including negative control (dH_2O). The reaction was carried out for 30 cycles at initial denaturation temperature of 95^0 C for 12min with subsequent denaturation at same temperature for 20 sec. This was followed by annealing temperature of 54 - 66^{0}C (depending on the test isolate) for 30 sec, elongation temperature of 72 ^{0}C for 30 sec and final elongation temperature of same temperature for 4 min.

At the end of the 30 cycles, the samples were analysed using gel electrophoresis

Analysis of PCR products

Agarose gel (1.5%) was prepared into which 10µl of ethidium bromide was added. It was gently mixed by swirling and casted into an electrophoresis tray with comb in place to obtain thickness. The comb was gently removed to obtain wells for loading of samples and IX TBE buffer was poured into the tank ensuring that the buffer covers the surface of the gel. Thereafter, 18µl of sample was mixed with 2ul of loading dye, and the samples were loaded into the wells created by the comb with 100bp marker which was loaded in lane 1 of the well. The electrodes of the electrophoresis tank were connected to the power pack ensuring that the negative terminal of the electrodes is at the well side where the samples were loaded. The electrophoresis was run at 75 V until the loading dye migrated up to 3/4 of the

gel field, it was turned off and the electrodes dis-connected. The gel was observed on a UV – Trans illuminator and the observed gel was documented.

RESULT AND DISCUSSION

Microbiological analysis.

Water is absolutely essential to all forms of life (Fawole *et al.*, 2002). It is a basic pre-requisite for existence and if not properly treated and handled, can be a medium for breeding some organisms (Ward *et al.*, 1998). Potable water for human consumption should be free from disease causing organisms or large numbers of non pathogenic organism which most times are opportunistic. Microorganisms of concern in contaminated water include bacterial agents of diarrhea and gastroenteritis namely *Salmonella* sp, *Shigella* sp, *Escherichia coli* and *Vibrio cholerae* (Birmingham *et al.*, 1997). According to the findings of this study using the culture based method [Fig I], six bacterial species such as *E.coli, Salmonella typhimirium, Klebsiella, Pseudomonas aerugenosa, Micrococcus and Staphylococcus* were identified which indicates that the water samples were contaminated.

Total heterotrophic and bacterial count in colony forming unit is as shown in figure I. From the result, no *Vibro* colony was observed in all the water samples whereas the presence of *Salmonella sp, Shigella,* some fungi and coliforms was evident. *Salmonella-Shigella* count was highest in water sample from hostel B and E_2 with lowest count from hostel C. However, hostel E recorded the highest coliform and total heterotrophic counts of 1.60 logcfu/ml and 1.68 logcfu/ml respectively. Aside the water sample from hostel B and PG hostel, which had no fungal count, other water samples had fungal count within the range of 0.60-1.45 logcfu/ml.

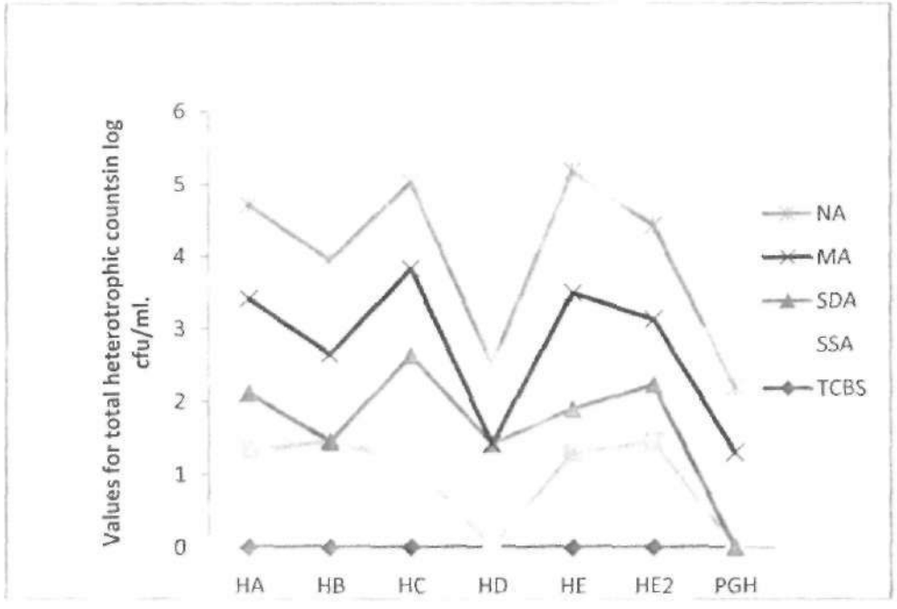

Fig I: Showing log cfu/ml on different agar medium per sampling site

Legend: HA=hostel A, HB= hostel B, HC=hostel C, HD=hostel D, HE=hostel E, PGH= post graduate hostel

The results of the microbiological analysis depicting bacterial and fungal isolates are shown in Tables 1 and 2. The isolation of these bacterial agents of diarrhea and gastroenteritis in the test water samples is in line with the findings reported elsewhere (Birmingham *et al.*, 1997; Ibe and Okpelenye., 2005; Ogbulie and Akujiobi, 2006).

Table 1: Culture-based identification and biochemical analysis of bacterial isolates.

S/N	Gram Rxn	Ca	Ci	Ur	In	MR	VP	Ox	Mo	La	Ma	Man	Su	Xy
Identified isolates														
A_1	-ve Rods	+	-	-	+	+	-	-	+	+	+	+	+	+
Escherichia coli														
B_1	+ve cocci	+	NT	NT	NT	-	+	-	NT	+	-	+	+	-
Staphylococcus sp in singles & clusters														
C_1	-veRods	+	+	+	+	+	-	-	-	+	+	+	+	+
Klebsiella sp														
D_1	+ve cocci	+	NT	NT	NT	+	-	+	-	-	-	-	+	-
Micrococcus sp														
E_1	-veRods	+	-	-	-	+	-	-	+	-	+	+	-	+
Salmonella sp														
F_1	-ve Rods	+	+	+	-	+	-	+	+	-	-	+	-	+
Pseudomonas aeruginosa														

Legend: A1= Smooth pink colony on MacConkey agar, B1= Small round yellowish to milkfish colony on nutrient agar, C1= Large mucoid colony on MacConkey agar, D1= Bright yellow colony on nutrient agar, E1= Black colony on salmonella-shigella agar, NT=not tested, - =no reaction; Ca = Catalase; Ci= Citrate; Ur= Urease; In= Indole; MR= Methyl Red; VP= Voges Proskauers; Ox= oxidase; Mo= Motility; La= lactose; Ma= Maltose; Man= Mannitol; Su= sucrose and Xy= xylose .

Table 2: Culture-based identification of fungi

Colonial morphology	Probable isolate
Army green mold with milkfish background, with no pigmentation on the reverse of the culture plate	*Trichoderma* specie
Black fluffy colony	*Aspergilllus* sp
Whitish round colony	*Rhizopus* sp
Green colony with line strikes on it	*Penicillium* sp

Sanitary surveillance

The sanitary surveillance revealed that some of the point were littered with dirt with one sank close to a soak away pit at 8m away which is in contrast with WHO recommended

distance in sinking boreholes close to septic tanks [Table 3]. Sanitary surveillance reveals that other sampling points were littered with waste (dump site). Five out of the borehole samples were manually operated and was constructed long before the last two (Hostel E_2 and PGH). Generally, the high heterotrophic and total coliform count observed in this study could possibly be linked to the biofilm/ bacterial regrowth in the connecting/distribution pipe as well as storage overhead tanks as reported by OECD WHO (2003) and Ogbulie and Akujiobi, (2006)

Table 3 Sanitary surveillance of water sampling site.

Borehole water sample	Solid waste dump	Soak away pit
HA	-	+
HB	+	-
HC	+	-
HD	+	-
HE	+	-
HE_2	+	-
PGH	+	-

Legend: HA=hostel A, HB= hostel B, HC=hostel C, HD=hostel D, HE=hostel E, PGH= post graduate hostel.

pH and temperature measurement

The pH and temperature were measured indicated that the pH values were within the range of 6.4-7.6 whereas the temperature was within 24-28°C [Fig II a&b], which can support the growth of some microbes of public health concern. The high heterotrophic count as observed in this study however could be due in part to this range of pH and temperature which was within the range that encouraged microbial proliferation as reported by Ogbulie, *et al.*, (2007) and USFDA, (2015).

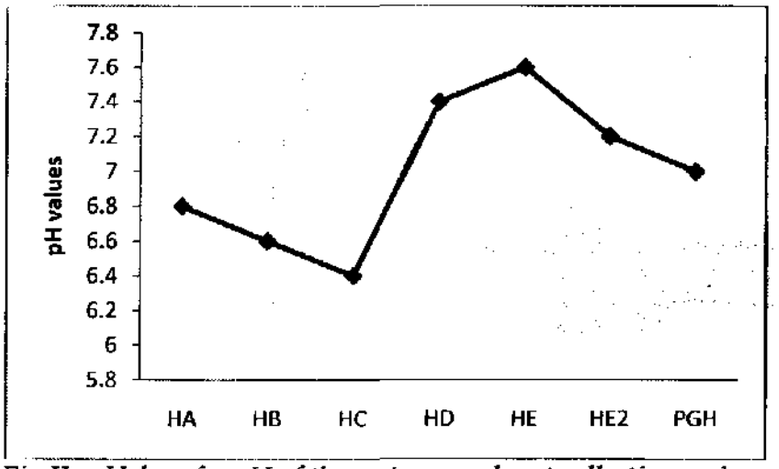

Fig IIa : Values for pH of the water samples at collection and sampling time.

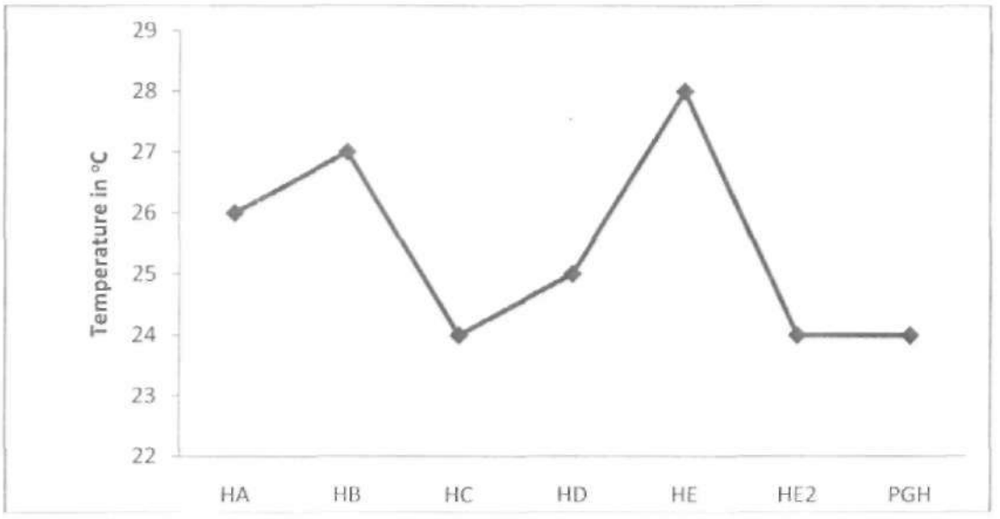

Fig IIB : Values for Temperature of the water samples at collection and sampling time.

Legend: HA=hostel A, HB= hostel B, HC=hostel C,HD=hostel D, HE=hostel E,PGH= post graduate hostel

Molecular identification

Molecular identification of some probable microorganisms was carried out to confirm whether or not they were present in the water samples that were analyzed. Figure III*b*; also depict the presence of bacterial isolates in all the water samples as evident in culture-based method carried out. The presence of *Salmonella typhimirium* was also depicted by bands using FimA forward and reverse primers as shown in fig IIIc. However, the absence of *Vibrio sp* as observed in culture-based method was also evident in molecular analysis using rxtA(F&R) primers specific for *Vibrio* sp which showed no visible band [fig IIId].

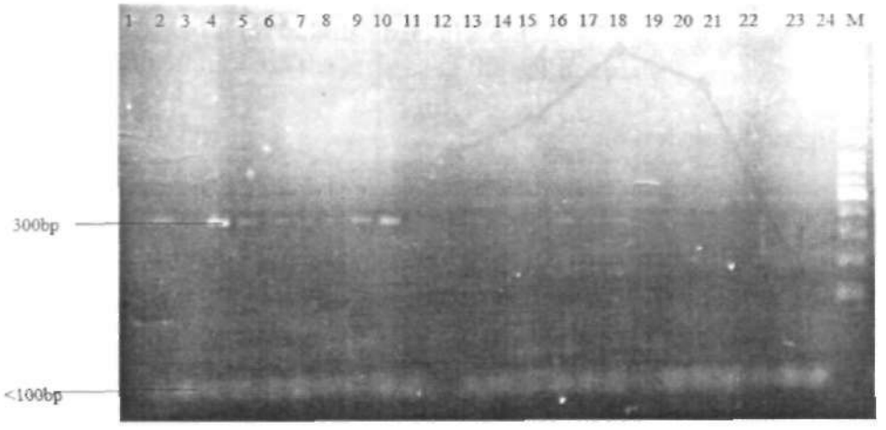

Fig IIIa: Gel electrophoresis of PCR product using NS7F&NS8R(forward and reverse primer.targeting 17sRNA of fungi species

Lane 1= negative ocnt, 2-24 are isolates from SDA plates and M is the marker.

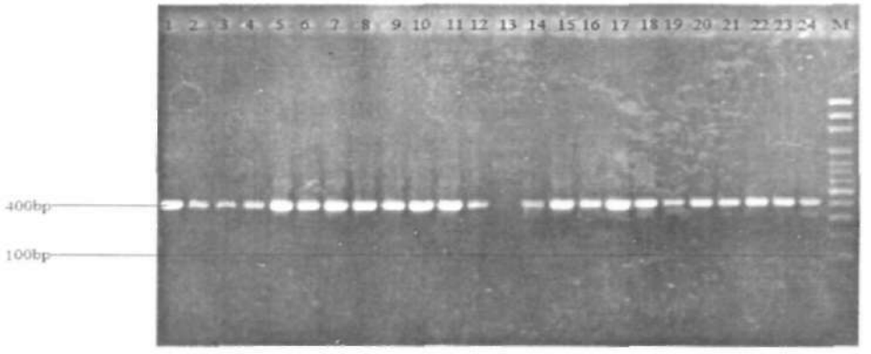

Fig IIIb Gel electrophoresis of PCR product using DNA COM 1 & DNA COM 2 forward and reverse primer targeting 16sRNA gene generally for bacteria. Bands were obtained in all samples at 400bp except in lane 13.

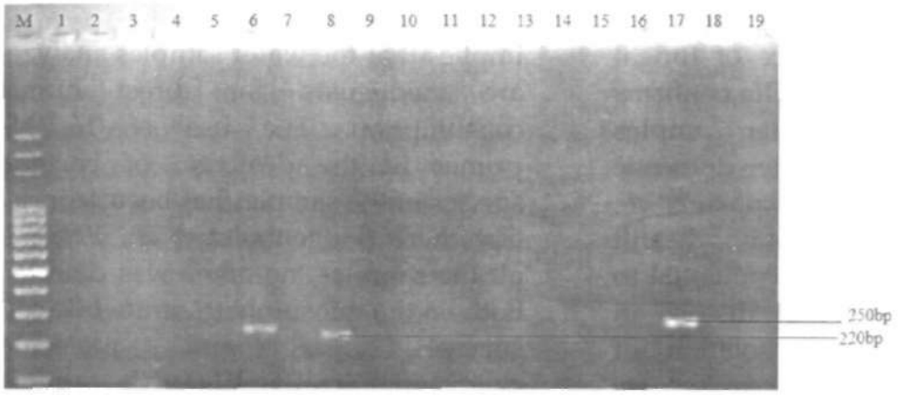

Fig IIIc: Gel electrophoresis of PCR product using FimAF & FimAR (forward and reverse) primer targeting only *S. typhi*, shows bands on lane 3, 6, 8 and 17..

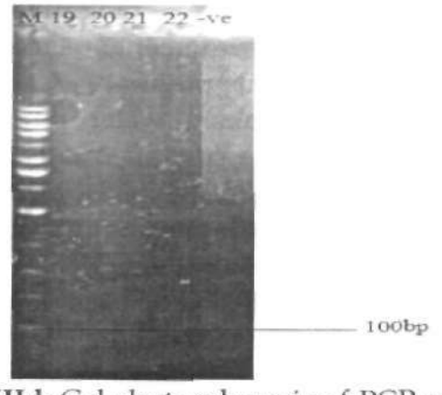

Fig IIId: Gel electrophoresis of PCR products for *vibrio* showing no bands for only raw sample 19-22

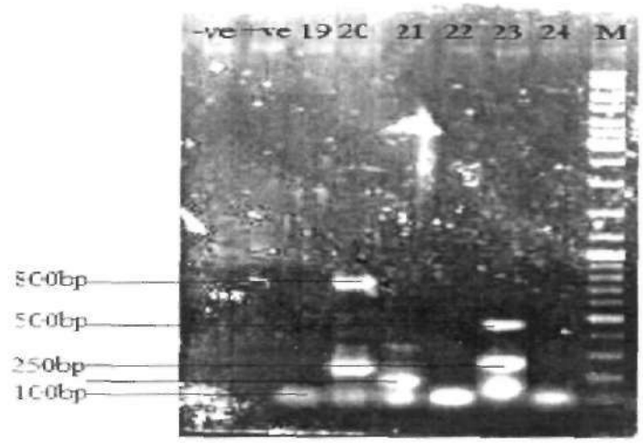

Fig IIIe: Gel electrophoresis of PCR Product using LZ forward and reverse primer targeting all coliforms

The possible presence of fungal isolates as shown in Figure IIIa confirms that fungi can be in water samples meant for drinking and other domestic purposes as reported by Okpako *et al.*, (2009). Its presence is of public health concern since they have the potential to cause allergic reactions or disease in humans. Therefore, the isolation of fungi (*Trichoderma sp, Aspergillus sp, Rhizopus sp and Penicillum sp*) from all the samples except one (PGH) of the samples is in accordance with the findings of Okpako *et al.*, (2009). The use of NS7F and NS8R primer, a universal single sub unit primer confirmed the presence of fungal isolates generally by targeting the 17sRNA of fungal community present in water.

Molecular characterization using specific forward and reverse primers targeting 16sRNA of coliform (LZ primer) and com 1 and com 2 targeting 16sRNA for all bacterial species showed amplified bands. This indicates their presence in the water samples which implies that the water samples analyzed are inadequate for direct human consumption. The use of 16sRNA primer in the detection of bacterial species in the samples has been reported elsewhere (Hugenholtz *et al.*, 1998). In all the samples, no *vibrio* was detected, both on the thiosulphate citrate-bile-salt-sucrose agar and molecular characterization [Fig IIId].

Generally, the frequent occurrence of coliforms as observed in this study however, poses sanitary questions and is indicative of contaminated environment or sinking of borehole at inappropriate level of aquifer for drinking water purposes. Furthermore, the identified organisms are of public health significance which is in line with the findings of Agbabiaka, and Sule (2010) and Ibe and Okpelenye (2005) who researched on bacteriological assessment of selected borehole water samples. *Escherichia coli* isolated in this study may not be of human faecal origin since it grew at 37°C incubation, although mutant specie may grow at

such temperature. The isolation and high coliform observed however does not conform to drinking water standard of WHO and United States Environmental Protection Agency Standard of zero coliform of faecal origin per 100ml of potable water (USEPA, 2001, WHO 2008). This finding however, is in contrary to NSDWQ drinking water standard of 100cfu/ml for Nigerian potable water (NSDWQ, 2007).

The results presented in the present paper shows that improved monitoring of water and frequent application of chlorine and other water treatment agent should be adopted. However, it is important to be aware that several of the bacterial and fungal species which are of clinical concern are also present in water. Since most fungi species survive disinfection and water treatment, it is thus suggested that treatment techniques that would eliminate not only fungi but all forms of microorganisms that could cause water related diseases should be used to treat borehole water before distributing to the consuming population. Also, proper sanitation practices should be implemented within the vicinity of borehole water and reservoirs and sitting of latrines/toilets close to borehole systems should be avoided.

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