# PHYSICOCHEMICAL ANALYSIS AND ISOLATION OF COLIFORM BACTERIA IN WATER SAMPLE FROM FEMALE HOSTELS OF A TERTIARY INSTITUTION IN LAGOS, NIGERIA

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**ABSTRACT**
*Microbial quality and physico-chemical parameters of borehole water from female hostels in an unnamed tertiary institution in Lagos State were evaluated from September to October 2014. A total of seven (7) water samples were obtained and assessed for the presence of coliform bacteria using the most probable number technique (MPN). The study showed the presence of E.coli, Klebsiella spp. and Pseudomonas spp. in some of the water samples assessed. However, contrary to the World Health Organisation's standards, the physico-chemical analyses showed that some of the water samples had odours and metallic taste. The water conductivity of the samples was within standard limits ranging from 95 µm -288µm. The total hardness of the water was below acceptable limit as it ranged from 10 ppm-25 ppm which was classified as soft water. Some of the water samples were slightly acidic as the pH value of the water ranged from 5.9-7.0, while the acceptable range by WHO is 6.5-8.5. The water samples assessed in these hostels were not fit for drinking based on the microbiological and physicochemical analyses. Therefore, it is highly recommended that the water supplied to these hostels be adequately treated before distribution.*

**Keywords:** borehole water, bacteriological analysis, physicochemical parameters

## INTRODUCTION

Water plays an important role in plant, animal and human life. It is the most basic and vital resource on earth. Among the various sources of water, bore-hole is known to be more appropriate and often meet the criteria of quality water (Aydin, 2007). It is the most widely used source of water in most African countries including Nigeria. The quality of borehole water is a resultant of all the processes and reactions that act on the water from the moment it condensed in the atmosphere to the time it is discharged by a well or spring and it varies from place to place and with the depth of water table (Vijendra, 2004). Borehole water has unique features which renders them suitable for public water supply (Benjeiloun, 1997). It has excellent natural quality, usually free from pathogens and could be consumed directly without treatment (Birmingham, 1997).

Nevertheless borehole water may suffer pollution from land disposal of solid waste, sewage disposal on land, agricultural activities, urban run-off and polluted surface water (Birmingham, 1997). Water provide essential elements, but when polluted it may become undesirable and dangerous to human health (Karavoltsos *et al*., 2008). Microorganisms such as *Salmonella* spp. *Shigella* spp., *Escherichia coli* and *Vibrio cholera* play a major role in water quality (Birmingham, 1997). Many infectious diseases are transmitted by water through faecal oral route. Unsafe water is a global public health threat, placing individual at risk for a host of diarrhoea and other diseases as well as chemical intoxication (Hughes and Koplan, 2003). Report has shown that nearly 90% of diarrhoea related deaths have been attributed to unsafe or inadequate water supplies and sanitary conditions affecting a large part of the world's population (WHO, 2008).

Microorganisms of concern in contaminated water are referred to as coliform. The direct testing for harmful pathogen among faecal coliform in water supply is impractical (Birmingham, 1997), as it require lengthy, complex and expensive testing procedure, for this reason testing for indicator organism is a norm. Presence of coliform bacteria is used as an indicator for the presence of any water borne pathogen. It is recommended that no faecal coliform should be present in 100ml of a water sample (Chuwural, 2001). Continuous microbiological monitoring of drinking water is essential to ensure compliance with standards and to protect public health. This work was therefore carried out to evaluate the physico-chemical parameters as well as the bacteriological quality of bore hole water used for different important purposes in the female student hostels.

## MATERIALS AND METHODS

### Study area

This study was carried out in 7 female hostels of a tertiary institution in Lagos. Each of the hostel is inhabited by thousands of students and the borehole water is used by the students for domestic purposes such as cooking, washing, bathing and drinking. Water samples were collected from the 7 hostels which included Moremi, Honours, Kofo, Makama, Amina, Fagunwa and Madam Tinubu (MTH) hostels.

## SAMPLE COLLECTION AND HANDLING

The water samples were taken from the taps aseptically. The tap was allowed to run for five minute and sterile 75cl polyethylene tetrachloride (PET) bottles were carefully uncapped and filled with water and recapped immediately. Water

samples collected were analysed within 2hrs in Microbiology Laboratory of University of Lagos.

The assessments of the water quality were based on the physical and chemical properties as well as the microbial analyses. The physicochemical analyses included appearance, colour, taste, odour, pH, conductivity, sulphate, iron, chloride and hardness. The microbial assessment which included the faecal coliform was also determined.

## BACTERIOLOGICAL ANALYSIS

### Enumeration of Total Heterotrophic Bacteria Count

Determination of bacterial load of the water sample was carried out using a general purpose media by pour plate technique. The total heterotrophic bacteria count were determined by inoculating 0.1ml of each of the water sample on a nutrient agar aseptically, the plates were incubated at $37^oC$ for 24h. After incubation, the different culture plates were observed for microbial growth and the total colonies were counted and expressed as colony forming unit per millilitre (cfu/ml) and result were recorded (APHA, 1985).

## ESTIMATION OF TOTAL COLIFORM BACTERIAL COUNT

The coliform count was determined by the Most probable number (MPN) index method also referred to as the multiple tube method using 3-3-3 regimen. In this method series of tubes containing a suitable selective broth medium were inoculated with test portion of the water samples, initial observation was made at the end of 24h and final observation was made at the end of 48h incubation to detect the production of gas and fermentation indicated by the colour change of the medium. Three set of tubes were used per dilution. In the first set of tubes, 10mls of the water sample was inoculated in 10mls of double strength MacConkey broth, in the second set of tubes, 1ml of the sample was inoculated in 5mls of single strength MacConkey broth and the third set of tubes 0.1ml of the sample was inoculated in 5mls of single strength MacConkey broth. Positive result was indicated by acid and gas production on incubation at $37^oC$ for 48h (Cheesbrough, 2000). The MPN were determined from the MPN table for the three set of tubes.

## TOTALFAECAL COLIFORM COUNT

Faecal coliform count was determined using Eosine Methylene Blue (EMB) agar. A small portion from the positive tubes from the presumptive test was streaked on

EMB agar, for pure colonies and incubated at 37$^{o}$C for 24h. Colonies on EMB were further identified using cultural characteristics, morphology and biochemical tests. Colonies with greenish metallic sheen were Gram stained and the IMVIC (Indole test, methylred, vogesproskaeur and citrate) tests were carried out and used to identify the colonies as *E. coli.* (Speck, 1988). Other coliform colonies were also identified using these tests.

**DETERMINATION OF PHYSICOCHEMICAL PARAMETERS OF WATER**

Parameters such as turbidity, temperature, PH, conductivity, salinity, total suspended solids, total hardness, acidity and minerals (Sodium, calcium, chloride, nitrate, sulphate) content of the water samples were determined using the methods described by Dewis and Frectas (1990).

**RESULT**

The physicochemical parameters of all the water samples obtained from the female hostels are shown in Table 1. The pH of the water samples ranged from 5.9-7.0 which was between the limit approved by World Health Organization. Iron levels were more and above approved standard in the entire water samples except one.

The total bacterial count in the borehole water was sampled and it was observed that all of the borehole water samples had the presence of bacterial counts and the range of heterotrophic count varied among the water samples (Table 2).

One way ANOVA was used to statistically determine the microbial load difference and it was observed that there was significant difference between the total coliform and faecal coliform in the water samples. Both coliform bacteria and faecal coliforms were present in all the water samples but the total coliform count was higher than the faecal coliform count (*E.coli* count) however only two of the samples did not contain faecal coliform ($P > 0.038$) (Tables 3&4).

A total of three (3) coliform bacteria were isolated from the borehole water and they were differentiated using standard biochemical test into *E.coli, Klebsiella* spp. and *Pseudomonas* spp (Table 5) while the distribution of total coliform and faecal coliform in the water sample is shown in figure 1.

## DISCUSSION

The physiochemical parameters in the majority of the boreholes in this study were within the WHO water standards for domestic use. On the contrary, the pH and iron concentrations of water from the boreholes were above the required limits.

The pH of water is important because many biological activities can occur only within a narrow range, thus any variations beyond an acceptable limit could be fatal to a particular organism (Trivede *et al*., 2010) In the present study, all borehole water samples collected ranged from 5.9-7.0; the samples with pH value 5.9 did not conform to the WHO standard for potable water, because the pH was slightly acidic, an ideal pH for potable water is neutral to slightly alkaline and thus classified as unsuitable for drinking purposes.

The iron (Fe) values of the water sample ranged from 0.3-0.5ppm, this showed that Fe concentration in the water sample was slightly above the WHO limit of 0.3ppm. Also total hardness of the water was far below the WHO limit of specification, the water may be considered to be soft water but hard water is preferred to soft water for drinking purposes as hard water is associated with low death rate from heart diseases (ISO, 1990).

Bacteriological pure water is one which is free from faecal pollution, the result of analysis from this study showed the presence of pathogenic organisms at a level above the approved World Health Organization (WHO) standard of 0cfu/100ml of the sample, which is consequently unhealthy for human consumption (Hogan, 2016). The coliform bacteria identified in this study were *Klebsiella* spp., *Pseudomonas* sp. *and E. coli*. *Pseudomonas* sp. was present in only Madam Tinubu hostel (MTH). *Pseudomonas* sp. is not a coliform bacterium but an opportunistic organism that causes skin rash as well as some opportunistic infections therefore its occurrence in supplied water is considered as quality indicator (Penna *et al*., 2002). *Klebsiella* spp. and *E. coli* were present in most of the water samples and their presence in water indicated a risk of developing gastroenteritis (Craun, 1986). The presence of *E. coli* isolates also indicated the presence of pathogenic organism which causes water borne disease as well as diarrhoea and is directly transmitted when contaminated water is consumed. According to WHO, diarrhoea account for an estimate of 4% of total daily global burden and it is responsible for the death of 1.8 million people every year, it was estimated that 88% of the burden is attributed to unsafe water supply, sanitation and poor hygiene (WHO, 2005). The detection of coliform showed the danger of faecal pollution and consequently hazard of contracting diseases. Water suitable

for consumption should be free from disease causing organism or large number of pathogenic organisms. In table 3, result showed that water samples obtained from Moremi and Fagunwa have a lower coliform bacteria count and can be concluded to be better for domestic use than those obtained in Makama, MTH and Amina hostels. Regarding the faecal coliform, even though the water obtained from Moremi and Amina has a lower count than those obtained in Makama, MTH, and Fagunwa it can be inferred that the bore hole water from these hostels are not fit for drinking without extensive treatment before use (WHO, 1986). However coliform count obtained from water sample in Kofo and Honours hostels were considerably lower with zero faecal coliform which conforms to WHO standard for drinking water, hence may be considered fit for drinking. High coliform and faecal count obtained from the water samples in the hostels mentioned above (Makama, Moremi, Amina, MTH and Fagunwa) could be as a result of human faeces and other waste from anthropogenic activities which could be washed by rain and also presence of faecal coliform can be attributed to the proximity of the sewage located near some of the boreholes as observed in Amina and Makama hostel. Moreover the general unhygienic environment surrounding the bore hole could also be a factor. Long term usage of the borehole may lead to deterioration of the borehole water quality, because the pipe line may become corroded with random cracks and in most cases clogged with sediment which could allow, the passage of inorganic metals and bacteria (Onamano and Otun 2008). Similar observations were made in this study where most boreholes and storage tanks retaining the water in the hostels especially those found in Amina, Makama and Fagunwa have been in place for a very long time without being replaced. This could have contributed to the poor water quality in these hostels.

The total bacteria count of the borehole samples varied and only three (3) out of all the samples conformed to the specification allowed by WHO (2008). The observation in this study supports the fact that higher bacteria count in the water reflected higher coliform count.

This study showed that the water from the boreholes in the female hostels of institution did not meet the national guidelines of water for human consumption. It is therefore important that interventions such as water quality monitoring and regular testing especially for microbes be implemented in order to prevent water borne dissemination of disease as water of poor quality is a threat to the health and wellbeing of the populace.

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**Table 1: physicochemical parameters of the water samples**

| Physico-chemical parameters | Specification WHO(2009) | Moremi | Honours | Makama | Fagunwa | MTH | Amina | Kofo |
|---|---|---|---|---|---|---|---|---|
| Appearance | Colourless clear liquid | The liquid is cloudy at first and appear clear after some minute | Colourless clear liquid | A clear colourless liquid | A clear colourless liquid | A clear colourless liquid | Slightly coloured liquid | A clear colourless liquid |
| Odour | None | Metallic odour | Odourless | Odourless | Odourless | Odourless | Metallic odour | Odourless |
| Taste | None | Slightly taste | Slightly taste | Slightly taste | Slightly taste | Very tasty | Slightly tasty | Tastless |
| pH | 6.5- 8.5 | 7.0 | 6.0 | 5.9 | 6.5 | 5.9 | 6.03 | 6.5 |
| Conductivity | ≤1000µs/ml | 89.2 | 135.2 | 288.0 | 120.0 | 87.70 | 101.40 | 95.00 |
| Total dissolve solid | ≤ 500mg/l | 44.6 | 67.60 | 144.0 | 60.0 | 43.85 | 50.70 | 47.50 |
| Chloride | Not more than 100mg/l | 15ppm | 15ppm | 20ppm | 14ppm | 12ppm | 12ppm | 15ppm |
| Iron | Not more than 0.3mg/l | 0.5ppm | 0.4ppm | 0.4ppm | 0.4ppm | 0.4 | 0.5 | 0.3 |
| Sulphate | Not more than 100g/L | 20ppm | 15ppm | 15ppm | 18ppm | 20ppm | 20ppm | 12ppm |
| Total hardness | Not more than 500mg/L | 10ppm | 10ppm | 25ppm | 20ppm | 20ppm | 12ppm | 20ppm |

**Table 2: Total heterotrophic bacteria count obtained from the water samples**

| Sample source (hostels) | Colony forming unit/ml cfu/ml | Microbiological limit by WHO standard |
|---|---|---|
| Moremi | 282 colonies | 0-100cfu/ml |
| Aminat | 300 | |
| Kofo | 45 | |
| Honours | 62 | |
| Fagunwa | 108 | |
| MTH | 230 | |
| Makama | 85 | |

The table shows total bacterial count obtained from each hostel and their microbiological acceptable limit by WHO standard. The samples from Moremi, Aminat, MTH and Faguwa were not within the acceptable limit of WHO standard of 0-100cfu/ml.

**Table 3: Total coliform and faecal coliform count obtained from the water samples**

| Sample source | Number of tubes with positive count | | | MPN/100ml | Faecal coliform count |
|---|---|---|---|---|---|
| Permit for no risk | | | | 0-10cful/100 ml | 0.cfu /100ml |
| Moremi | 2 | 3 | 1 | 36 | 24 |
| Amina | 3 | 1 | 1 | 43 | 20 |
| Kofo | 2 | 0 | 0 | 9 | 0 |
| Honours | 2 | 0 | 0 | 9 | 0 |
| Makama | 3 | 2 | 1 | 150 | 36 |
| Fagunwa | 2 | 2 | 2 | 35 | 27 |
| MTH | 3 | 2 | 1 | 150 | 43 |

The table shows the number of tubes positive for coliform count, the total coliform count per 100ml of the sample obtained from an MPN table and also the total faecal coliform count obtained from the positive tube with the acceptable limit for which no risk of infection can occur when consumed.

**Table 4: statistical analysis of coliform present in the water samples**

**Descriptives**

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| MPN/100ml | 7 | 74.5714 | 58.20326 | 21.99876 | 20.7424 | 128.4005 | 9.00 | 150.00 |
| Faecal coliform count | 7 | 21.4286 | 16.51118 | 6.24064 | 6.1583 | 36.6989 | .00 | 43.00 |
| Total | 14 | 48.0000 | 49.49437 | 13.22793 | 19.4228 | 76.5772 | .00 | 150.00 |

Mean total coliform count and faecal coliform count is 75±58 and 21±16 per 100ml.

**ANOVA**

| | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 9884.571 | 1 | 9884.571 | 5.401 | **.038** |
| Within Groups | 21961.429 | 12 | 1830.119 | | |
| Total | 31846.000 | 13 | | | |

Significant difference (P-value =0.038) was deduced between total coliform count and faecal coliform count.

**Table 5: Biochemical characteristics of isolate obtained from the water samples**

| Sample source | Morphology | Gram stain | Indole test | Citrate test | Methyl red | Vogesproskaeur | Isolate obtained |
|---|---|---|---|---|---|---|---|
| Moremi (A) | Rod shaped | -ve | +ve | -ve | +ve | -ve | *E. coli* |
| Moremi (B) | Short rods | -ve | -ve | +ve | -ve | +ve | *Klebsiella*sp. |
| Amina(A) | Rod shaped | -ve | +ve | -ve | +ve | -ve | *E. coli* |
| Amina(B) | Rod shaped | -ve | -ve | +ve | -ve | +ve | *Klebsiella* sp. |
| Honours (A) | Rod shaped | -ve | -ve | +ve | -ve | +ve | *Klebsiella*sp. |
| Kofo | Rod shaped | -ve | -ve | +ve | -ve | +ve | *Klebsiella*sp. |
| Fagunwa | Rod shaped | -ve | +ve | -ve | +ve | -ve | *E. coli* |
| MTH | Rod shaped | -ve | -ve | +ve | -ve | -ve | *Pseudomonas* |
| MTH(B) | Rod shaped | -ve | -ve | +ve | -ve | +ve | *Klebsiella*sp. |
| Makama(A) | Rod shaped | -ve | +ve | -ve | +ve | -ve | *E. coli* |
| Makama (B) | Rod shaped | -ve | -ve | +ve | -ve | +ve | *Klebsiella*sp. |

***Key:***
-ve = negative
+ve = positive

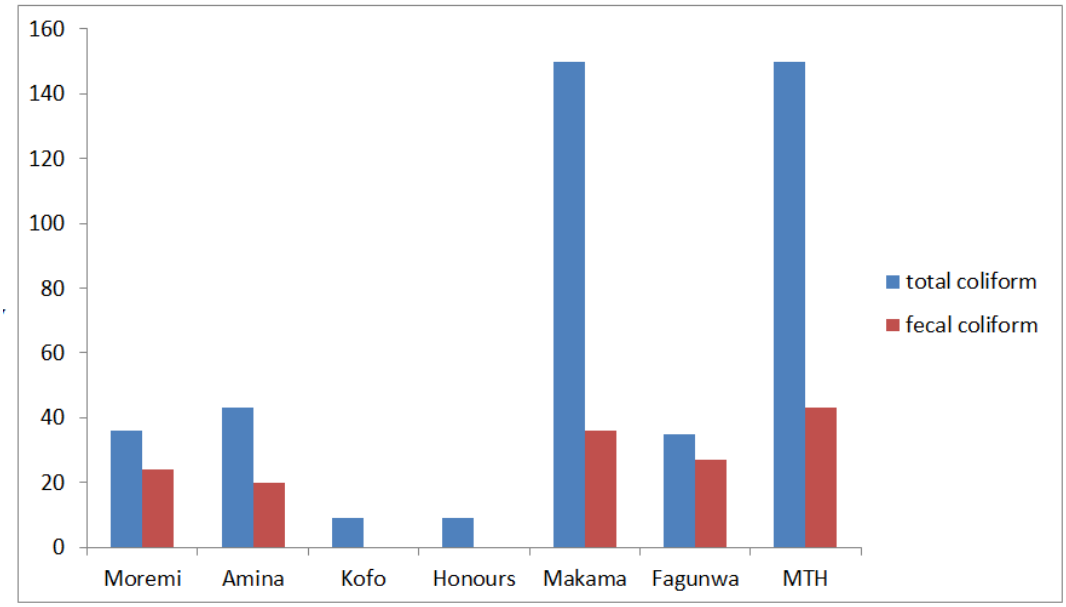


**Figure 1: Distribution of total coliformand faecal coliform in the water sample**