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# Physico-chemical analysis and identification of antibiotics resistant Enterobacteriaceae from groundwater sources in Ayobo, Lagos, Nigeria

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## Abstract

*This study aimed at evaluating the physico-chemical parameters and detection of Enterobacteriaceae of groundwater samples in Ayobo, Lagos State. The physico-chemical and bacteriological parameters were determined using standard methods. Twenty Groundwater samples were selectively collected over a period of 6 weeks from two groundwater stations which were Anchor University and Ayobo community. Groundwater temperature values from both well and borehole ranged from 23.9-27.5°C, with an average value of 24.9°C. Conductivity and TDS had their highest values (99.1µS and 49.3ppm) recorded in well 1 of station 1 while in station 2, the result of the selected physico-chemical water quality parameter revealed that the temperature of groundwater samples ranged from 25.1°C to 26.5°C. pH range from 5.0-7.0 with the highest value (7.0) recorded in sample BOK 1 and BOK 2. Conductivity recorded the highest value of 60.9µS while TDS had the highest value of 63.8ppm. Total mean counts for total viable count, faecal coliforms and total coliforms for Station 1 were as follow: 225.00±7.07CFU/ml, 167.00±4.25CFU/ml and 131per 100ml respectively; and for Station 2: 137.00±1.41CFU/ml, 102.00±1.41CFU/ml and 250 per 100ml respectively, which are all higher than the WHO standard limits. The result of the antibiotics susceptibility profiling revealed that the isolates were resistant to more than three antibiotics. In conclusion, this study showed that borehole water and well water around Ayobo are not safe for direct consumption due to high level of the quality indicator bacteria in them.*

**Keywords:** Physico-Chemical; Groundwater; Bacterial; Enterobacteriaceae; Antibiotics;

## Introduction

Water quality has been one of the primary concerns in Nigeria today, and good drinking water is the basis for good health. Water quality is important to man since it is linked to human health[1]. The concentration of different chemical components, which are mostly obtained from the geological data of the specific region, and their impact on the quality of groundwater are interrelated[2,3]. Water quality is mostly described by its physico-chemical and biological properties[4-6]. These may include its taste, odour, colour and the amount of both organic and inorganic materials. Human activities in response to variables such as population growth, urbanization, industrial production, climate change, and others can also affect the quality of water, especially groundwater resources. Most people around the world continue to lack access to clean water, which has led to an increase in the number of waterborne illnesses. In developing nations where the majority of population reside in locations with inadequate cleanliness and waste disposal procedures, reports of both natural and man-made pollutants are common.

Having access to clean water can raise standards of life, productivity, and health[7]. The best water for human consumption originates from lakes, waterways, aquifers, rivers, and ground water. In the past, examinations of groundwater have mostly concentrated on supply with little attention paid to its microbiological condition. Meanwhile, the most frequent and pervasive health concerns linked to drinking water in underdeveloped nations are biological in nature. Some of the bacteria that contaminated surface water could also find their way into the groundwater. This has been supported by recurring occurrences of waterborne illnesses in Lagos and other Nigerian states[8]. Actually, both surface and groundwater typically contain a variety of microorganisms that are difficult to distinguish from one another in native vegetation and potentially harmful pollutants. The transmission of disease through drinking water is, therefore, one of the primary concerns for safe water supply[9], especially human bacterial gastrointestinal diseases, such as salmonellosis, shigellosis, and cholera, are spread through faecal contamination of waters[10].

Anchor University and the village of Ayobo in Lagos State both rely heavily on groundwater as a source of drinking water[11]. Alimosho is the most populated LGA in Lagos State with population of 1,319,571 people based on 2006 population census[12]. Lagos State is one of the 36 states in the country, and a costal surrounding area of the Atlantic Ocean. The state has a land mass of 3,577km$^2$ and 787km$^2$ of the inland water. Nigeria's smallest state in terms of land area is Lagos, however, the state is one of the most populated in the country.

It is therefore expedient that this study be carried out to determine the physico-chemical and bacteriological parameters of the groundwater samples in Ayobo, Alimosho Local Government Area, Lagos State. This research focuses on the physico-chemical analysis and bacterial evaluation of probable Enterobacteriaceae found in groundwater samples found in Ayobo, Lagos State. The objectives are therefore to collect ground water samples in Ayobo, Lagos, assess the total viable bacterial count from each water sample, isolate, identify and characterize the Enterobacteriaceae colonies in the ground water samples, evaluate the implication on the surrounding public health and establish the profiles of antibiotic resistance of the isolates from the groundwater samples.

## Materials and methods

**Study Area:** This study was carried out in Ayobo community, a settlement on the outskirts of Lagos, Nigeria. Ayobo is a highly residential community, located in Alimosho Local Government Area (LGA) of Lagos State, with co-ordinate 6°36′38″N 3°17′45″E. Within Ayobo are located Anchor University, a private University with the population of over 1000 students and teaching and non-teaching staff with population of above 500 people.

**Sample Collection:** The groundwater samples (well and borehole) were directly collected from two (2) groundwater stations in Ayobo, Lagos State namely; Anchor University, Lagos and Ayobo Community using clean stoppered and correctly labeled 50 cl plastic bottles. A total of twenty (20) groundwater samples were collected respectively from May to August, 2022. The water samples were packaged and transported to the Microbiology Laboratory of the Department of Biological Sciences, Anchor University, Lagos for analysis. The analysis was carried out according to Water and Wastewater standards method[13] for various physico-chemical parameters.

**Physico-chemical analysis of water samples:** The physico-chemical parameters analyzed include; temperature, turbidity, total dissolved solids (TDS), pH and conductivity.

**On-site Analysis:** Temperature and pH were analysed *in-situ* using the standard procedures of American Public Health Organisation (APHA) with calibrated standard instruments[13]. Water temperature was measured using the mercury in glass thermometer graduated in degree Celsius (°C). pH of the water samples was also measured using a digital pH meter. The pH meter was calibrated, with three standard solutions (pH 4.0, 7.0, and 10.0), before measurements were done.

**Laboratory Analysis:** Conductivity and TDS of water samples were measured according to the standard methods of APHA[13], using a BancTec 510 Electrical Conductivity meter.

**Microbiological Analyses:** The Microbiological analyses were carried out using standard procedures. The media used were Nutrient agar - TM media, Titan Biotech LTD, India; Endo agar, Eosin methylene blue (EMB) agar, Simmon citrate agar and Triple Sugar Ion agar - Himedia Laboratories Pvt. LTD India. The manufacturer's instructions were followed in the preparation of every media.

**Isolation, Purification, and Characterization of isolates: Isolation by Membrane Filtration:** Using a water pump (model Sartorius 16824), three (3) volumes of 100mL each were filtered through 0.45m pore size filters (cellulose nitrate membranes, advantec). These membranes were aseptically positioned on Endo plates that had already dried down and were filled with the proper selective media without any trapped air bubbles. The media are an Enterobacteriaceae-specific medium.

**Serial dilution using pour plate method:** Nutrient agar was used for total viable bacteria count, while EMB and Endo agar were used for the isolation of *Enterobacteriaceae.* All the media were prepared according to the manufacturer's instructions. Each sample was analyzed in duplicate. Serial dilution was carried out on all groundwater samples. 1ml of serially diluted samples was poured into the Petri dishes and about 20ml of the appropriate sterile agar was poured into the plates, carefully mixed and allowed to solidify. The plates were incubated at 37◦C for 24h. After incubation, the colonies were enumerated, characterized morphologically, and recorded. The isolation of pure cultures was performed by continuous sub-culturing until pure cultures were obtained, the pure cultures were then stocked in agar slants and labelled appropriately and stored in a refrigerator at 4◦C for subsequent characterization[14].

**Primary Identification and Biochemical Tests: Gram Staining Method:** The Gram staining method was done as described by Cheesbrough[15]. Using a sterile wire loop, a small colony was removed and spread on a clean glass slide before being gently heated to fix it. Crystal violet solution was then applied on the slide for a minute, rinsed under running water followed by the addition of iodine solution for a minute and also washed gently under running water. Acetone alcohol as the decolorizer was added and washed after 10 seconds, and safranin, the counter stain was added and allowed to stain for a minute. The slides were washed under running water, air dried and examined under the microscope with 100x objective.

**Triple Sugar Iron (TSI) Test:** The media were prepared based on manufacturer's instructions, and left to set. The bacteria isolates were introduced into the media, and incubated at 37◦C for 24h. presence of fermented glucose turns acid produced in the butt yellow. However, if either sucrose or lactose is fermented, both the butt and the slant turn yellow. Appearance of gas is shows either bubbles or cracking of the agar in the butt. In case of no fermentation, the slant and butt remains red. Black precipitate in the butt (a black butt) due to the bacterium indicate the formation of ferrous sulfide.

**Oxidase Test:** Oxidase reagent was used in carrying out this test. A pure colony isolate was positioned on a filter paper by a sterile wire loop. Test oxidase reagent (a drop) was added to it. The mixture was left for 30sec. A purple colour observed on filter paper with oxidase positive isolates is taken as presumptive *Aeromonas* and *Pseudomonas* isolates. While a colourless oxidase negative colonies were considered to be *E. coli* and other family of the *Enterobacteriaceae*.

**Citrate Test:** The capacity of a life form to make use of citrate as a source of energy was tested using citrate agar. Citrate was the only source of carbon in the medium, while inorganic ammonium salts represents supply of nitrogen. The breaking down of citrate by bacteria converts ammonium ions to ammonia, which raises alkalinity. The pH change causes a change in bromothymol blue indicator in the medium from green to blue. The 24h old isolates from nutrient agar were picked using a sterile inoculating loop and inoculated into a tube containing a solidified citrate agar slant and incubated at 37°C for 24h and the results were recorded.

**Catalase Test:** The catalase test is used to detect the presence of catalase enzyme that breakdown hydrogen peroxide to release oxygen and water. The catalase reaction is evident by the rapid formation of bubbles[16]. A sterile wire loop was used to pick a pure colony and smear on a clean slide, then 3% $H_2O_2$ was added to the slide and observed for evolution of the oxygen bubbles. Negative results yielded no reaction.

**Antimicrobial Susceptibility Test:** The CLSI[17] recommended Kirby-Bauer disk diffusion technique was utilized to conduct the antibiotic susceptibility test. The following antibiotic discs were utilized (Biomark Laboratories, Code Number: BDR009). 10mg of tetracycline, 10mg of gentamicin, 25mg of cotrimoxazole, 30mg of cefuroxime, 10mg of chloramphenicol, 30mg of ceftriaxone, 30mg of cefotaxime, 5mg of ciprofloxacin, 30mg each of amikacin and vancomycin, 30mg each of ceftazidime, and 10mg each of meropenem. Using sterile swab sticks, the standardized 18–24h old bacteria inoculum was applied to Mueller Hinton agar plates. Antibiotic discs were applied to the plates using sterile forceps. Plates were then incubated for 24 hours at 37°C. After incubation, the diameter of the antibiotic zone of inhibition was assessed. Using standard reference values from the Clinical and Laboratory Standards Institute's manual[17], isolates were then categorized as resistant, intermediate, or susceptible to a specific antibiotic. Isolates that demonstrated resistance to three or more kinds of antibiotics were given phenotypes for multiple antibiotic resistance (MAR). Escherichia coli ATCC 25922 was used as a comparison.

## Results and discussion

**Physico-chemical properties of groundwater samples from station 1:** Table-1 showed the result for the selected physico-chemical water quality parameter at station 1 (Anchor University). Groundwater temperature values from both well and borehole ranged from 23.9-27.5$^{o}$C, with an average value of 24.9$^{o}$C. The highest temperature was recorded in borehole 1 (AB1) while the lowest was recorded in well 1 (AR1). On the other hand, pH had the highest value recorded in borehole 2 (AB2) and the least in well 1 (AR1). Conductivity and TDS had their highest values (99.1µS and 49.3ppm) recorded in well 1 (AR1). The least TDS value was recorded in borehole 2 (AB2) while in Conductivity, borehole 1 (AB1) had the lowest value (39.1µS).

Table-2 showed the result of the selected physico-chemical water quality parameter at station 2. The Temperature from station 2 groundwater samples ranged from 25.1$^{o}$C to 26.5$^{o}$C. pH range from 5.0-7.0 with the highest value (7.0) recorded in sample BOK 1 and BOK 2 . Conductivity recorded the highest value (60.9µS) in sample BR2 (well) and the least value (23.1 µS) in BO2 (borehole). On the other hand, TDS had the highest value (63.8ppm) recorded in BOL2 and the least value (20.5 ppm) in BOK3.

Table-3 showed the biochemical characterization and identification of presumptive *Enterobacteriaceae* isolates from groundwater samples. 11 bacteria species were identified of which *Escherichia coli, Salmonella and Enterobacter* spp were found to be more prominent.

**Table-1:** Physico-chemical values of the Station 1 groundwater samples**.**

| Water sample | pH | Conductivity (µS) | Temperature ($^{o}$C) | TDS (ppm) |
|---|---|---|---|---|
| AB1 | 4.9 | 39.1 | 27.5 | 21.0 |
| AB2 | 5.1 | 63.7 | 24.1 | 11 |
| AR1 | 4.8 | 99.1 | 23.9 | 49.3 |
| AR2 | 5.8 | 76.4 | 24.0 | 38.5 |

Key: AB - Anchor Borehole; AR - Anchor Well.

**Table-2:** Physico-chemical values of the Station Two (2) Groundwater samples.

| Water sample | pH | Conductivity (µS) | Temperature ($^oC$) | TDS (ppm) |
|---|---|---|---|---|
| BOK1 | 6.1 | 51.1 | 26.5 | 25.8 |
| BOK2 | 7.0 | 45.4 | 26.3 | 22.6 |
| BOK3 | 7.0 | 38.9 | 26.3 | 20.5 |
| BO1 | 6.9 | 36.6 | 26.3 | 21.3 |
| BO2 | 6.8 | 23.1 | 26.5 | 20.8 |
| BR1 | 5.2 | 40.4 | 26.4 | 20.2 |
| BR2 | 5.3 | 60.9 | 26.4 | 37.4 |
| BA | 5.9 | 48.6 | 26.4 | 24.6 |
| BS | 6.3 | 23.3 | 26.2 | 21.0 |
| BTA | 6.8 | 45.3 | 26.4 | 32.5 |
| BKO | 5.8 | 49.5 | 26.5 | 28.1 |
| BOR | 5.8 | 49.5 | 26.5 | 28.1 |
| BOL1 | 6.3 | 60.09 | 26.4 | 37.4 |
| BOL2 | 6.5 | 53.4 | 26.3 | 63.8 |
| BOO | 5.7 | 49.8 | 25.1 | 51.9 |
| BOO2 | 5.8 | 49.73 | 25.3 | 50.7 |

**Table-3:** Biochemical characterization and Identification of presumptive *Enterobacteriaceae* isolates from groundwater samples.

| S/N | GS | Cit | Cat | Oxi | Ind | Glu | Suc | Lac | Slant | Butt | Gas | $H_2S$ | Probable bacteria |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | - | + | - | + | + | + | + | Y | Y | + | - | *Escherichia coli* |
| 2 | - | + | + | - | - | + | - | + | R | R | - | - | *Shigella* spp. |
| 3 | - | + | + | - | + | + | - | - | R | Y | - | + | *Proteus vulgaris* |
| 4 | - | - | + | - | - | + | - | + | Y | Y | - | - | *Escherichia coli* |
| 5 | - | + | + | - | + | + | + | + | Y | R | - | + | *Serratia* spp. |
| 6 | - | + | + | - | + | + | - | + | R | Y | - | + | *Salmonella typhi* |
| 7 | - | + | - | - | + | + | + | - | R | R | - | - | *Shigella* spp. |
| 8 | - | + | + | - | - | + | + | + | Y | Y | - | + | *Enterobacter* spp. |
| 9 | - | + | + | - | - | + | + | + | Y | Y | + | + | *Enterobacter* spp. |
| 10 | - | + | - | - | - | + | + | - | R | Y | - | + | *Salmonella* spp. |
| 11 | - | + | - | - | + | + | + | + | R | R | - | - | *Proteus* spp. |
| 12 | - | + | + | - | + | + | + | + | R | R | - | + | *Proteus* spp. |
| 13 | - | + | + | - | + | + | + | - | R | Y | - | - | *Salmonella* spp. |
| 14 | - | + | + | - | - | - | + | + | R | R | - | + | *Serratia* spp. |
| 15 | - | + | + | - | - | + | + | - | R | R | - | - | *Shigella* spp. |
| 16 | - | - | - | - | - | + | - | + | R | Y | - | + | *Salmonella* spp. |
| 17 | - | + | + | - | + | + | - | + | Y | Y | + | + | *Citrobacter* spp. |
| 18 | - | + | + | - | + | + | - | + | Y | Y | + | - | *Enterobacter* spp. |
| 19 | - | + | - | - | - | + | + | - | R | R | - | - | *Serratia* spp. |

Key: +: positive; -: negative; Cat: catalase; Oxi: oxidase; Glu: glucose; Suc: sucrose; Lac: lactose; Y: yellow; R: Red; GS: Gram staining.

**Total viable count (TVC) and coliform count from groundwater samples:** Table-4 shows the total viable count obtained from Borehole and well water at different points in Station 1. The highest TVC was obtained at point 3 which was too numerous to count. At a dilution of $10^{-3}$, the TV Count ($10^3$) at point 1 is 101.00±1.41, 35.50±2.12 at point 2 and 135.00± 2.12 at point 4. For dilution of $10^{-5}$, a mean Total Viable count ($10^5$) of 94.50±2.12 was obtained at point 1, 13.00±2.82 at point 2 and 36.00±1.41 at point 4.

**Table-4:** Mean Total Viable Count in Station 1 based on Dilution Factors of $10^{-3}$ and $10^5$.

| Station 1 | Mean ± Standard Deviation | |
|---|---|---|
| | ($10^3$) | ($10^5$) |
| Point 1 | 101.00 ± 1.41 | 94.50 ± 2.12 |
| Point 2 | 35.50 ± 2.12 | 13.00 ± 2.82 |
| Point 3 | TNTC | TNTC |
| Point 4 | 135.00 ± 2.12 | 36.00 ± 1.41 |

**Faecal coliform count and total coliform:** The faecal coliform count and total coliform were also obtained from the samples. The result is present in Table-5. The dilution factor of $10^{-3}$ point 1 had the highest mean faecal count ($10^3$) of 225.00±7.07, point 2 had a mean of 87.00±4.23, point 3 (173.50±33.23) and point 4 had a mean faecal count of 90.00 ± 4.24. For dilution factor of $10^{-5}$, point 1 also had the highest faecal count ($10^5$) of 167.00± 4.25. Next is point 3 (81.50 ±2.12), point 2 (65.50±2.12) and point 1 (39.00±1.41) had the least feacel count.

The total coliform count per 100ml was also obtained, the result in Table-5 shows that a total of 131 coliform were obtained from point 1, 120 coliforms from point 2, 92 coliforms from point 3 and 31 coliforms from point 3. The result revealed that the points with the highest faecal count at both dilutions also had the highest coliform count per 100ml.

**Table-5:** Mean Faecal Coliform Count based on Dilution Factors ($10^{-3}$ and $10^{-5}$) and Total Coliform in Station 1.

| Station 1 | Mean ± Standard Deviation | | Total Coliform (Per 100ml) |
|---|---|---|---|
| | ($10^3$) | ($10^5$) | |
| Point 1 | 225.00 ± 7.07 | 167.00 ± 4.25 | 131 |
| Point 2 | 87.00 ± 4.23 | 65.50 ± 2.12 | 120 |
| Point 3 | 173.50 ± 33.23 | 81.50 ± 2.12 | 31 |
| Point 4 | 90.00 ± 4.24 | 39.00 ± 1.41 | 92 |

**Microbial load analysis:** The microbial load from water was also obtained at different points in Station 2. The mean microbial load is presented in Table-6. It showed that at points 6, 7, 17, 18, 19 and 20, the TVC obtained at dilution factor $10^{-2}$ were too numerous to count. Aside the points that had their microbial loads were too numerous to count, point 10 had largest mean TV Counts ($10^2$) of 249.00±1.41 and the least count was obtained in point 16 (55.50±0.71). For dilution factor of $10^{-4}$, points 17 and 18 had mean Total Viable counts ($10^4$) that were too numerous to count. While point 6 sampled had a mean TVC of 297.00±1.41, the least TVC ($10^4$) was obtained from Point 14 (19.50±0.71).

**Table-6:** Mean Total Viable Count in Station 2.

| Station 2 | Mean ± Standard Deviation | |
|---|---|---|
| | ($10^2$) | ($10^4$) |
| Point 5 | 148.50 ± 2.12 | 77.50 ± 0.71 |
| Point 6 | TNTC | 297.00 ± 1.41 |
| Point 7 | TNTC | 154.50 ± 2.12 |
| Point 8 | 147.00 ± 1.42 | 97.00 ± 1.41 |
| Point 9 | 228.50 ± 2.13 | 107.50 ± 0.71 |
| Point 10 | 249.00 ± 1.41 | 72.00 ± 1.43 |
| Point 11 | 207.50 ± 2.12 | 60.00 ± 2.12 |
| Point 12 | 155.00 ± 1.41 | 40.00 ± 1.46 |
| Point 13 | 90.00 ± 1.61 | 77.00 ± 1.51 |
| Point 14 | 52.00 ± 1.42 | 19.50 ± 0.71 |
| Point 15 | 110.00 ± 1.41 | 67.00 ± 1.41 |
| Point 16 | 55.50 ± 0.71 | 29.50 ± 0.71 |
| Point 17 | TNTC | TNTC |
| Point 18 | TNTC | TNTC |
| Point 19 | TNTC | 75.00 ± 0.71 |
| Point 20 | TNTC | 50.5 ± 0.71 |

**Mean Faecal Coliform Count:** Table-7 showed that the faecal coliform sampled from point 6 was too numerous to count. While the highest mean count was obtained from Point 7 (241.45±2.12), the least faecal count (39.50±0.71) was obtained from Point 20. Using $10^{-4}$, the highest faecal count was obtained from 6 (102.00±1.41) and the least count (17.50±2.12) from

Point 9. For the total coliform count, the highest count per 100ml was 278 and its obtained from Point 6. Total coliforms obtained from samples in Points 12, 13, 16 and 17 were too numerous to count. Point 19 had the least total coliform count of 20.

**Table-7:** Mean Faecal Coliform Count in Station 2.

| Station 2 | Mean ± Standard Deviation | | Total Coliform (Per 100ml) |
|---|---|---|---|
| | **($10^2$)** | ($10^4$) | |
| Point 5 | 137.00 ± 1.41 | 77.50 ± 0.71 | 121 |
| Point 6 | TNTC | 102.00 ± 1.41 | 278 |
| Point 7 | 241.45 ± 2.12 | 49.00 ± 1.41 | 250 |
| Point 8 | 136.50 ± 2.13 | 46.50 ± 2.12 | 71 |
| Point 9 | 49.00 ± 1.41 | 17.50 ± 2.12 | 67 |
| Point 10 | 107.00 ± 1.41 | 50.50 ± 2.12 | 56 |
| Point 11 | 136.50 ± 0.71 | 48.00 ± 1.41 | 51 |
| Point 12 | 89.00 ±1.41 | 44.50 ± 2.12 | TNTC |
| Point 13 | 72.50 ± 1.61 | 90.50 ± 0.71 | TNTC |
| Point 14 | 82.00 ± 1.41 | 51.50 ± 0.71 | 30 |
| Point 15 | 66.50 ± 0.72 | 42.00 ± 1.41 | 89 |
| Point 16 | 71.50 ± 0.71 | 39.00 ± 12.73 | TNTC |
| Point 17 | 96.50 ± 0.71 | 58.50 ± 2.12 | TNTC |
| Point 18 | 53.50 ± 0.72 | 21.00 ± 1.41 | 51 |
| Point 19 | 40.50 ± 0.71 | 18.00 ± 1.41 | 20 |
| Point 20 | 39.50 ± 0.71 | 30.50 ± 0.77 | 54 |

Key: TNTC: Too numerous to count.

**ANOVA Test of Difference among the TVC and faecal coliform from Station 1:** In order to determine if there was any difference in the microbial load obtained from different points at a dilution of $10^{-5}$, an Analysis of Variance test was conducted. The result for the TVC and faecal coliform in Station 1 as well as in Station 2 (at $10^{-4}$) is presented in Table-8 and 9. Table-8 shows that there was a significant difference in the TVC obtained across the four points in Station 1 [(F (3, 4) = 13,243 $*10^5$, ρ=0.00]. Also the faecal Coliform count was significantly different across the four points in Anchor University (F (3, 4) = 845.08; ρ=0.00) while Table-9 shows that there was a significant difference in the TVC obtained across the 16 points in Station 1 [(F (15, 16) = 18145 $*10^4$, ρ = 0.00]. Also the faecal coliform count was significantly different across the 16 points sampled in Station 2 [(F (15, 16) = 84.440 * $*10^4$; ρ = 0.00).

The TVC and faecal coliform obtained from the study was compared to the expected mean count by WHO. The result is presented in Table 10. The result revealed that the values of TVC obtained in Station 1 was not significantly higher than the WHO recommended in drinking water (t (7)=2.24; p=0.06) while the faecal count obtained was significantly different (higher) (t (7)=4.32; p=0.003). From Station 2, the TVC (t (31) = 5.74; p=0.00) and faecal count (t (31)=8.85; p=0.00) were significantly higher than the WHO standard. The result shows that the well and borehole water in Station 2 is not safe for drinking.

**Antibiotics Susceptibility Profile of Enterobacteriaceae Isolates:** Table-11 shows the susceptibility of the isolated *Enterobacteriaceae* to some selected antibiotics. The highest number of resistance was recorded for CRX, CTX and CPZ.

**Profile of Antibiotic Resistant Presumptive Enterobacteriaceae Isolates:** Table-12 shows the zone of inhibition (measured in millimeters) of some selected multi-resistant Enterobacteriaceae to the selected Antibiotics.

**Table-8:** ANOVA Test of Difference among the TVC and faecal coliform from Station 1.

| ANOVA Test | | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| TVC | Between Groups | 144019.375 | 3 | 48006.458 | 13243.161 | .000 |
| | Within Groups | 14.500 | 4 | 3.625 | | |
| | Total | 144033.875 | 7 | | | |
| Feacal Coliform | Between Groups | 18380.500 | 3 | 6126.833 | 845.080 | .000 |
| | Within Groups | 29.000 | 4 | 7.250 | | |
| | Total | 18409.500 | 7 | | | |

**Table-9:** ANOVA Test of Difference among the TVC and faecal coliform from Station 2.

| ANOVA Test | | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| TVC | Between Groups | 365744.969 | 15 | 24382.998 | 18145.487 | .000 |
| | Within Groups | 21.500 | 16 | 1.344 | | |
| | Total | 365766.469 | 31 | | | |
| Feacal Coliform | Between Groups | 16307.500 | 15 | 1087.167 | 84.440 | .000 |
| | Within Groups | 206.000 | 16 | 12.875 | | |
| | Total | 16513.500 | 31 | | | |

**Table-10:** One sampled T-test of the TVC and faecal coliform count.

| Location | T-test | Mean ± SD ($10^{-5}$) | t | df | p |
|---|---|---|---|---|---|
| Station 1 | TVC | 123.38 ± 143.44 | 2.236 | 7 | 0.06 |
| | Faecal Count | 88.25 ± 51.28 | 4.316 | 7 | 0.003 |
| Station 2 | TVC | 120.28 ± 108.62 | 5.743 | 31 | 0.00 |
| | Faecal Count | 46.13 ± 23.08 | 8.85 | 31 | 0.00 |

**Table-11:** Antibiotics Susceptibility Profile of Presumed Enterobacteriaceae Isolates (n=48).

| Antibiotics used | Number of Resistant isolates (%) | Number of Intermediate Isolates (%) | Number of Susceptible Isolates (%) |
|---|---|---|---|
| TET | 24/48 (50) | 1/48 (2) | 23/48 (47) |
| COT | 11/48 (22) | 12/48 (25) | 25/48 (52) |
| GEN | 10/48 (20) | 11/48 (22) | 27/48 (56) |
| CRX | 47/48 (98) | 0/48 (0) | 1/48 (2) |
| CHL | 16/48 (32) | 9/48 (19) | 23/48 (47) |
| CTR | 29/48 (60) | 8/48 (16) | 12/48 (24) |
| CTX | 40/48 (83) | 7/48 (14) | 1/48 (2) |
| CIP | 2/48 (4) | 3/48 (6) | 43/48 (89) |
| AMK | 4/48 (6) | 7/48 (14) | 37/48 (77) |
| VAN | 30/48 (62) | 5/48 (12) | 13/48 (27) |
| CPZ | 40/48 (83) | 4/48 (8) | 4/48 (8) |
| MEM | 25/48 (52) | 2/48 (4) | 21/48 (43) |

Key: TET: tetracycline, COT: Cotrimoxazole, GEN: Gentamicin, CRX: Cefuroxime, CHL: Chloramphenicol, CTR: Ceftriaxone, CTX: Cefotaxime, CIP: Ciprofloxacin, AMK: Amikacin, VAN: Vancomycin, CPZ: Ceftazidime, MEM: Meropenem.

**Table-12:** Zone of Inhibition (mm) Profile of some multi-resistant *Enterobacteriaceae* Isolates.

| S/N | TET | COT | GEN | CRX | CHL | CTR | CTX | CIP | AMK | VAN | CPZ | MEM | Probable organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | R | S | S | R | S | S | I | S | S | R | S | S | *Escherichia coli* |
| 2 | S | S | S | S | I | I | R | S | S | R | R | R | *Shigella spp* |
| 3 | R | R | I | R | R | R | R | S | S | R | R | R | *Serratia spp* |
| 4 | S | I | R | R | S | R | R | S | S | R | R | S | *Shigella spp* |
| RF | R | R | R | R | I | R | S | S | R | R | R | R | *Escherichia coli* ATCC® 25922 |

Key: TET: tetracycline, COT: Cotrimoxazole, GEN: Gentamicin, CRX: Cefuroxime, CHL: Chloramphenicol, CTR: Ceftriaxone, CTX: Cefotaxime, CIP: Ciprofloxacin, AMK: Amikacin, VAN: Vancomycin, CPZ: Ceftazidime, MEM: Meropenem, S: Susceptible, I: Intermediate, R: Resistant, RF: Reference Organism.

**Discussion:** The result from these study shows that pH ranged from 5.03-7.01 and falls below the standard limits of 6.5–8.5, hence, the water was slightly acidic water. According to WHO[18], acidic pH could lead to gastrointestinal disorder such as ulcer, hyperacidity and burning sensation. However an average pH of 6.21 is recorded for the entire study area which is similar to result obtained by Enyoh *et al*[19] in borehole water in Orji, Owerri Imo State, Nigeria. The temperature values recorded during the sampling period is within the acceptable range for drinking water (between 20˚C and 35˚C) and also in line with the works of Mbugua *et al.*[20] and Oyekunle *et al.*[21] on Lokichar Basin, Turkana County, Kenya and groundwater in Ifetedo and Garage Olode, Osun State, Nigeria respectively. The study also showed that the mean ECs for all samples ranged from 39.1 to 60.01µS/cm, which is lower in comparison to standard drinking water guideline of 250µS/cm[18]. Hence, they were all within the limit of no risk and this is in line with what was reported by Titilawo[22]. The TDS of all samples were also below the maximum allowable limits. Based on the TDS content, the water samples are classified as good drinking water in line with the work of Egbueri[23]. However, results from microbial analysis showed that some of the water samples are unfit for consumption.

The mean values of microbiological parameters (total and faecal coliforms and TVC counts) obtained for the ground water samples which are 123.38±143.44, 88.25±51.28 for Station 1 and 120.28±108.62, 46.13±23.08 for Station 2 were significantly higher than WHO recommended standard. However, there was significant difference in the means of all microbiological parameters. This result is almost in agreement with Emmanuel-Akerele and Francis's work[11], having a mean value of 72.00±26.87, 36.00±44.37 and 27.17±31.75 in Ayobo Community and Anchor University for ground water and sachet and Bottle water sources respectively but very low compare to the values recorded by Taoufq *et al.*[24] in the Angads Aquifer (Northeast Morocco) where significant faecal contamination was reported. This study also describes the isolation, identification, characterization and prevalence of antibiotic resistant *Enterobacteriaceae* present in Groundwater samples in Ayobo, Lagos. It is obvious that *Enterobacteriaceae* have the ability to thrive in groundwater and this observation agrees with the report of Emmanuel-Akerele and Francis[11] that *E. coli*, *Proteus vulgaris*, *Shigella* sp and S*almonella* sp as principal members of the *Enterobacteriaceae* in Groundwater present in Ayobo, Lagos state, Nigeria. This is also in relation with Okonko *et al*[25] on Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella sp, Escherichia coli, Bacillus sp, Proteus sp, Klebsiella sp, Flavobacterium sp, Enterobacter aerogenes, and Acinetobacter sp. found in well water samples from Abeokuta and Ojota, Lagos State, Nigeria., *E. coli* and other *Enterobacteriaceae* showed multidrug resistance to Cefuroxime, Ceftriaxone, Cefotaxime with 98%, 63% and 83% respectively. Resistance of *Enterobacteriaceae* in groundwater sources in this study were in line with the report of Lourenco *et al.*[26] in Brazil, Mckeon *et al.* in United States and Lin *et al.*[28] in South Africa where *E. coli* recorded the highest occurrence, followed by *Citrobacter*, *Enterobacte*r, *Shigella* and *Klebsiella*, major health threats to human, animal and environment globally. Because environmental bacteria can start antibiotic resistance processes, and because human and animal infections can infect the surrounding area, several studies have demonstrated the significance of water on the antibiotic resistance cycling in nature[29].

Antibiotics find their way into the ground waters as a result of the release of hospital discharges, city sewage, and wastewater from animal farms or other agricultural uses. The discovery of bacteria with acquired resistance in surface water, ground water, and drinking water in several nations supports this reality[27]. In this study, a total of 48 isolates from borehole waters were examined for their resistance against the traditionally employed antibiotics and was compared with the reference organism *Escherichia coli* ATCC® 25922 which complied with the standard of Clinical and Laboratory Standards Institute (CLSI) 28th Edition.

## Conclusion

This study examined the physico-chemical and bacteriological parameters of groundwater sources (well and borehole). It was observed that groundwater had higher microbial load, which is due to lack of proper hygiene, in terms of handling, cleaning and disinfecting of the storage tanks. The effect of on the environment may be irreversible even t a very low concentration. In addition, few bacteria species in water are non-cultivable but viable and this is why only a little part of the aquatic environment can be studied using antibiotic resistivity. Evidences have also shown that resistant bacteria in waterways hold the potential of transferring the gene from non-harmful species to pathogenic ones and even to humans through interaction with aquatic environment.

**Recommendation:** It is recommended that the direct borehole water be treated and the wells cleaned at intervals to ensure water quality and safety. Also, simple treatment method such as boiling and general public education on proper hygiene are recommended in the study area.

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