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ASSESSMENT OF MICROBIOLOGICAL, PHYSICOCHEMICAL QUALITIES AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF COLIFORMS IN VARIOUS WELL WATERS IN IPETUMODU, OSUN STATE

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ABSTRACT

According to World Health Organization guideline standards for total and faecal coliform in drinking water, the indicator of faecal contamination must not be detectable in any 100 ml of samples. Such water is not potable and drinking or using such water in food preparation leads to wide spread of acute and chronic illnesses. This study was conducted to determine the microbiological, physicochemical qualities and antibiotic susceptibility pattern of Escherichia coli in various well waters in Ipetumodu, Osun state Southwestern Nigeria. A total of ten water samples labeled as A to J were collected from different locations in Ipetumodu city. The total bacterial count was determined by serial dilution and pour plate method, total and fecal coliform count and isolation were carried out using membrane filter technique. Identifications of isolates were done using cultural, Gram staining reaction and biochemical methods. Physicochemical parameters were analyzed using their various standard methods, the concentrations of some heavy metals were determined using Atomic Absorption Spectrophotometer. Total bacterial count ranged from 5.7 x 10^3 CFU/ml to 6.7 x 10^3 CFU/ml with the highest count obtained in sampling station G and J, and the least count in sampling station F. The total coliform count of the waters analyzed ranged 49 to 67 MF index of coliform/100 ml of the water samples. Fecal coliforms were detected in water sample of sampling station A, B, E, H, I and J only which range from 1.0 to 3.0 CFU/ml. From the water samples, eight genera of bacteria which include Escherichia coli, Klebsiella sp, Enterobacter sp, Salmonella sp, Citrobacter sp, Enterococcus sp, Neisseria sp, and Staphylococcus aureus were isolated. All isolates were found sensitive to Ofloxacin and resistant to Augmetin, Ceftzadime, Cefuroxime and Cefixime. The results obtained indicated that well waters are not safe for consumption and they were of poor bacteriological qualities indicative of health risk to the inhabitants of the city.

INTRODUCTION

According to [1], the availability of drinking water is an indispensable feature for preventing epidemic diseases and improving the quality of life. Groundwater which is the major source of well water has long been considered as one of the purest form of water in nature and meets the overall demand of rural and semi-urban people. Although, the significance and use of groundwater has increased during the last decades in urban and rural areas of Nigeria [2], pollution from natural and anthropogenic activities is upsetting this vital resource [3, 4]. Consequently of the inadequacy of treatment plants, unproductive management of piped water

distribution system, and direct discharge of untreated sewage into rivers and streams, the quality and available quantity of water for drinking purposes are continuously failing in Nigeria [5, 6, 7]. Pollution in water occurs from a variety of sources and contamination with pathogenic organisms remains a major cause of epidemic diseases [8]. Excessive levels of pollution are causing a lot of damage to human and animal health [9]. It is the cause of many diseases, which affect not only the old but also the young and the energetic and all animals [10]. An estimated 1.2 billion people drink unhygienic water which is the source of water related diseases that are responsible for about five to ten thousand teenage and adult killing around the world today [11].

As there is a dearth of pipe-borne water in Ipetumodu town, the inhabitants of different settlements in this town largely depend on well water as an important source of water for drinking and other purposes. Several studies has been conducted on the quality of well waters from other parts of the state [12] and the country [13, 14, 15, 16, 17], nevertheless there are little or no information on the physicochemical and microbiological quality of well waters in this town. Therefore, this study was designed to assess the physicochemical and microbiological properties of water from wells at selected sampling locations in Ipetumodu, examining the samples for the presence of coliforms (widely used and accepted indicators of water pollution).

MATERIALS AND METHOD

Study Location and Sampling

Having a geographical coordinates of latitude 7 °22′N and longitudes 4 °30′E, Ipetumodu, a town located in Osun State, South-western region of Nigeria was designated as the study location for this research. In Ipetumodu, ten sampling locations with relatively high number of open and closed wells were selected and sampled. Water samples were aseptically collected in duplicates from various residential household wells in the ten different areas within a period of two months (May to July 2017). All samples were labelled and stored in sterile media bottles. The first set of ten bottled samples was used for analysis for physicochemical properties while the other set of ten were used for microbiological analysis.

Table 1: Description of sampling stations

Sampling Stations	Observed features
A	Location was an open well without lid in Okeola area. The water was partially clear, colorless and odourless. The water was being used for cooking, bathing and other household activities.
B	This location was a closed well with lid in Christ Army area. The environment was marshy andthere was a maize farm nearby. The water was colourless and odour free. The water was being used for the maize farm.
C	Location was in Okunola street, a deep well covered with a lid. The water was clean, colourless and odourless. The water was being used for drinking, cooking and other household activities.
D	Location was a shallow well with a rusted lid in Obada Market area. The water was colourless with foul odor. The environment was marshy and the water was being used by the traders in the market to rinse vegetables, fruits and other edible goods sold to the people of the community.
E	Location was a partially closed well with a rusted lid in Surulere area. The environment was marshy and the well was covered with algae. The water was being used for drinking, cooking and other household activities.
F	This location was a deep well in Ibugbe tutu house. The water was clear, colourless and odourless. The water was being used by the residents for drinking, cooking and other household activities.
G	Location was a shallow well in a Green hostel in Jaladugbo area. A sewage system was situated near the well. The water was clear and the water was being used for cooking, washing and other activities.
H	Location was a well with a lid in City of Harmony House in Jaladugbo Estate. The well was deep and it was being used by the residents for activities such as cooking, drinking, washing, etc.
I	Location was a shallow well in Ajisafe area. The water was clear, odourless and colourless. There was a banana plantation in the compound. The water was used for drinking, washing and other household activities the requires water.
J	Location was a closed well in Ogo Oluwa area close to a slaughter slab. This was a shallow well and the water was partially clear, colourless with a foul smell. The water was used for drinking, washing and cooking by the residents.

PHYSICOCHEMICAL ANALYSIS

Temperature:

The temperature of water samples were measured at the sites of sampling with a standard laboratory mercury thermometer.

pH:

The pH of water samples was determined using calibrated pH meter.

Total Dissolved Solid (TDS) in the water samples:

The TDS of the water samples was determined using TDS meter.

Total Suspended Solid in the water samples:

Gravimetric method was applied. Millipore membrane filter (0.45 μm) was dried to a constant weight at 105±2°C for 24 h and a known volume (100 ml) of water is filtered through the membrane filter under pressure to remove dissolved substances and re-weighed.

Determination of dissolved Oxygen (DO) content in the water samples

Glass reagent bottles (125/250 ml reagent bottles) were used to collect samples for DO. The samples were fixed with Winker's reagent A and B (Manganous sulphate and Alkaline Iodide) and the oxygen content determined by Iodometric titration using a standard sodium thiosulphate solution (0.025N $Na_2S_2O_3$) and starch as indicator which kept in a dark cupboard for 5 days for subsequent analysis as for Dissolved oxygen.

Determination of Chemical Oxygen Demand (COD) in the water samples

Wet-Oxidation digestion method: COD was determined by wet oxidation (Chromic acid), digestion of 100 ml of water sample with 10 ml of 0.1N Potassium dichromate, acidified with 20 ml concentrated sulphuric acid (chromic acid mixture) and the following day, 5 ml of concentration ortho-phosphoric acid and 100ml of distilled water was added and titrated with 0.1N $[(NH_4)_2Fe(SO_4)_2.6H_2O]$ Ferrous Ammonium Sulphate (FAS) using about 10 drops of 5% Barium Diphenylamine-Sulfonate (BDAS) solution as indicator.

Determination of Biochemical Oxygen Demand (BOD) in the water samples

Biochemical Oxygen Demand (BOD) is a measure of the change in DO concentration over 5 days in a stoppered bottle completely filled with water sample [18].

Iodemetric method: This was determined by measuring the amount of dissolved oxygen present in the water sample before and after incubation in the dark reagent bottles at 20°C for 5 days. After 5 days, the samples were fixed with Winkler's reagent A and B (Manganeous sulphate and Alkaline Iodide) and BOD determined titrimetrically using a standard sodium thiosulphate solution (0.025N $Na_2S_2O_3$) and starch as indicator.

Determination of nitrate content in the water samples

The ultraviolet spectrophotometric screening method was applied to determine nitrate concentration. The test instruction that were used in carrying out the analysis is as outlined below:

- From stock concentration of nitrate solution, series of standard nitrate solutions containing 1, 2 4, 5, 10, 20 50 ppm were prepared.
- To each 5 m of the standard solutions 0.5 ml of 1N HCl was added and mixed thoroughly, 0.5 ml of 1N HCl was added to 5ml of the sample.
- The absorbance for standards and samples after addition of 1N HCl were read at 220 nm and 275 nm using UV spectrophotometer

Determination of phosphate content in the water samples

From stock concentration of phosphate solution, series of standard nitrate solutions containing 1, 2 4, 5, 10, 20, 50, 100 ppm were prepared. The test instructions were as outlined below:

- To each 5 m of the standard solutions 0.5 ml of 1N HCl was added and mixed thoroughly, 0.5 ml of 1N HCl was added to 5 ml of the sample.
- To the 2 ml of each of standard concentrations, 2 ml of ammonium molybdate was added followed by addition of 1ml of working solution of $SnCl_2.{}_2H_2O$ the same procedure was repeated for the samples.
- The absorbance for standards and samples after addition working solution were read at 660 nm using UV spectrophotometer.

Total hardness of the water samples

Complexometric method was used.

Total Hardness = $[(Ca^{2+}\ (mg/L) \times 2.5) + (Mg^{2+}\ (mg/L) \times 4.1]$

Calcium and magnesium were calculated by complexometric titration using 0.1N EDTA as titrant and 1ml of Calcium- Magnesium Buffer and 0.2 g of Magnesium and Calcium, Erichrome Black T as indicator.

Determination of heavy metals concentration in the water samples

The concentrations of the above mentioned heavy metals manganese, iron, copper, zinc, chromium, cadmium, lead and nickel in the water samples were determined using Atomic Absorption Spectrometer method (PerkinElmer Analyst 400 Atomic Absorption Spectrometer).

MICROBIOLOGICAL ANALYSIS

Total Heterotrophic Bacteria Count

The enumeration of total aerobic heterotrophic bacteria was completed using the serial dilution and pour plate technique then incubated at 37°C for 24 h in inverted position. The number of bacterial colonies in plates containing 30 to 300 colonies were counted and this was multiplied by the dilution factor to obtain bacterial population in the water samples [19].

Enumeration of total coliform bacteria

Total coliform bacteria population which includes all aerobic and facultative anaerobic, Gram negative, non-spore forming bacteria which ferment lactose with gas formation at 37°C were determined by the membrane filter technique. Using sterile forceps, the membrane filters were aseptically placed on eosin-methylene blue agar (EMBA) and nutrient agar (NA) plates. Each sample was analyzed in duplicate. The plates were incubated at 37°C overnight afterwards each plate was observed for growth and the colonies were counted to obtain total viable count, characterized and recorded. EMBA plates containing 30-300 colonies of *Escherichia coli* were selected and about 5-10 colonies were picked for further *E. coli* purification step.

Isolation and identification of pure cultures

Bacterial colonies were sub-cultured on freshly prepared nutrient agar plates and incubated at 35°C for 24 h to obtain pure cultures. The colonial characteristics of the sub-cultured bacterial colonies were recorded. The bacterial isolates were further identified by Gram staining and biochemical characterization tests such as catalase, coagulase, indole, oxidase, methyl-red, Voges-Proskauer, and citrate utilization. The representative colonies were chosen from each plate based on the colonial morphological similarity. A Bergey's Manual of systematic Bacteriology of Conventional method was used in which eight presumptive cultures of *Escherichia coli* are randomly chosen and identified using traditional method which include cultural characteristic on selective media, gram-staining and biochemical reactions [20].

Antibiotic sensitivity testing

The Gram negative bacteria isolated were tested for antimicrobial susceptibility by disc diffusion method. Before antibiotic sensitivity testing, the isolates were revived by culturing onto EMBA plates for *E. coli* respectively. The plates were incubated at 37°C for 24 h. Three colonies were picked from each sample and each colony is transferred into 5ml of sterile normal saline to prepare bacteria suspension while obtaining 0.5 Mac Farland standards (equivalent to 1.5×10^8 CFU/100 ml). Aliquots of 100 µl from each suspension were spread-plated on Mueller Hinton agar plates. The antibiotic discs were applied on to the plates using sterile forceps and the plates were incubated at 37°C for 24 h [21] formerly (NCCLS, 1999). The antibiotic inhibition zone diameters (IZD) were measured and results obtained would be used to classify isolates as being resistant, intermediate resistant or susceptible to a particular antibiotic based on standard reference values [21]. The antibiotics used for testing were: Ceftazidime (CAZ) 30µg, Cefuroxime (CRX) 30µg, Gentamicin (GEN) 10µg, Cefixime (CXM) 5µg, Ofloxacin (OFL) 5µg, Augmetin (AUG) 30µg, Nitrofurantoin (NIT) 300µg, Ciprofloxacin (CPR) 5µg. Antibiotics disc were purchased from Abtek Biologicals Ltd, UK. These antibiotics were chosen because they are either being used in both human medicine and animal veterinary practice or because previous studies have reported microbial resistance to them.

RESULTS AND DISCUSSION

Table 2 shows the temperature, levels of dissolved oxygen, biochemical oxygen demand values, chemical oxygen demand, dissolved and suspended solids, pH value, the concentration of calcium carbonate (total hardness), and the concentration of nitrate and phosphate ions present in the water samples. Eight heavy metals which included Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn), Chromium (Cr), Cadmium (Cd), Lead (Pb) and Nickel (Ni), were analyzed in the water samples during the period of study. Manganese, Iron, Copper, Chromium, Cadmium, Lead and Nickel were absent in the water samples from all sampling stations as they were all recorded as zero (Table 3). Zinc concentrations was present in the water samples from sampling station C and F only but they all recorded less than 5 mg/L which is the limit level of the Recommended Dietary Allowances (RDAs) for zinc per day can lead to stomach cramps, nausea, diarrhea and vomiting. The standard plate counts of total bacteria population recorded during the study are presented in Table 1. Total bacteria count of water sample from sampling stations A to J ranged from 5.7×10^3 CFU/100 ml to 6.7×10^3 CFU/100 ml. Bacterial count of water sample from sampling station F recorded the lowest with the value of 5.7×10^3 CFU/ml, while the highest bacterial count was recorded from water sample of sampling station G and J with the value of 6.7×10^3 CFU/ml throughout the study period.

This increase in the bacterial load might be attributed to the accumulation from microbial contamination. The total and faecal coliforms recorded during the study are shown in Table 2.

The total coliform count in the entire water samples were relatively high. Generally, the water sample of sampling station C has the lowest coliform count of 4.9 x 10 CFU/100 ml throughout the period of study, while sample G had the highest coliform count value of 6.7 x 10 CFU/100 ml. Faecal coliforms was isolated in all the water sample from sampling stations A, B, E, H, I and J. Faecal coliforms recorded during the study ranged from 1.0 CFU/100ml in water sample of sampling station E, I and J to 3.0 CFU/100 ml in water sample of sampling station H. No faecal coliform was isolated from water sample of sampling stations C, D, F and G during the study. Normally water for human consumption should be free of coliform bacteria (United States Public Health Service, 1995). A total of sixty two (62) isolates of forty-two (42) species of Gram Positive bacteria representing three (3) genera and 20 species of Gram Negative bacteria representing six (6) genera were isolated from the water samples of the ten sampling stations. The cultural, morphological and biochemical characteristics of dominant bacteria isolated from the various sampling stations during the study are shown in Table 4. The most important organisms detected were *Escherichia coli, Enterobacter* sp., *Klebsiella pneumonia* and *Enterococci* and they were identified in all the 10 samples. It is important to note that coliform bacteria are widely found in nature and do not necessarily indicate faecal pollution [22; 23]. However, the presence of *E. coli* according to [24] suggests feacal pollution, hence the quality of the wells fell strongly below the standard of safe drinking water. Enteric pathogens cannot normally multiply in water hence, water is not its mode of transmission to humans. However, the infective dose in immune-compromised individuals would be significantly low when this water serves as their source of drinking water.

The antibiotic susceptibility profile of confirmed isolates are illustrated in Table 5. About 80% of all Gram negative bacteria isolated were 100% susceptible to Ofloxacin and about 15% of the isolates were also susceptible to Gentamicin and Nitrofurantoin. Isolates were intermediate to the other antibiotics in the following order: Ciprofloxacin (70%), Gentamicin (40%), Cefixime and Ofloxacin (5%). All the isolates were 100% resistant to Augmetin, Ceftazidime and Cefuroxime while 95% of the isolates were resistant to Cefixime. Findings also indicate that the *E. coli* isolates from water sample of sampling station J recovered in this study expressed high levels of resistance to antimicrobials that are commonly used in clinical medicine such as Ofloxacin and Ciprofloxacin. This could contribute to the spread

and persistence of antimicrobial resistant bacteria and resistance determinants in humans and the environment.

Table 1: Total bacteria, Coliform and Faecal Count of the water samples

Sampling stations	Total Bacterial Count (CFU/ml)	Total Coliform Count (CFU/100 ml)	Faecal Coliform (CFU/100 ml)
A	6.2×10^3	5.7×10	2.0
B	6.5×10^3	6.1×10	2.0
C	6.2×10^3	4.9×10	0
D	6.3×10^3	6.1×10	0
E	6.4×10^3	5.5×10	1.0
F	5.7×10^3	5.1×10	0
G	6.7×10^3	6.7×10	0
H	6.4×10^3	6.6×10	3.0
I	6.5×10^3	5.0×10	1.0
J	6.7×10^3	6.5×10	1.0

LEGEND:

A = Okeola, Ipetumodu. B = Christ Army Area, Ipetumodu. C = Okunola Street, Ipetumodu.
D = Obada market Area, Ipetumodu. E = Surulere, Ipetumodu. F = Ibugbe Tutu House, Ipetumodu.
G = Green Hostel in Jaladugbo. H = City of Harmony, Jaladugbo. I = Ajisafe, Ipetumodu.
J = Ogo–Oluwa, Ipetumodu

Table 2: Biochemical and physical parameters of the water samples

Sampling Stations	Temperature (°C)	TDS (ppm)	pH	Nitrate (mg/L)	Phosphate (mg/L)	DO (mg/L)	BOD (mg/L)	COD (mg/L)	Total Hardness ($CaCO_3$ mg/L)	TSS (mg/L)
A	20	287.50	7.40	5.26	0.39	7.20	1.60	27.90	118.50	213.00
B	20	514.00	7.20	13.24	0.77	9.40	7.40	13.00	198.40	120.00
C	23	218.00	7.20	2.55	1.10	12.00	3.20	24.80	124.60	45.00
D	20	195.00	7.30	1.40	0.28	8.00	3.20	20.50	89.50	649.00
E	25	119.00	7.30	24.64	0.29	10.40	2.40	14.90	63.20	218.00
F	30	123.50	7.10	0.72	0.39	9.40	3.00	18.60	69.10	215.00
G	30	121.50	7.10	22.23	0.64	8.80	0.40	14.30	62.10	231.00
H	30	111.00	7.20	21.94	2.88	8.80	1.60	16.10	67.50	68.00
I	25	108.00	7.10	25.82	2.76	10.60	2.20	8.10	71.20	105.00
J	25	70.00	7.30	11.03	0.53	10.60	5.40	5.60	43.90	55.00

LEGEND: A = Okeola, Ipetumodu. B = Christ Army Area, Ipetumodu. C = Okunola Street, Ipetumodu.
D = Obada market Area, Ipetumodu. E = Surulere, Ipetumodu. F = Ibugbe Tutu House, Ipetumodu.
G = Green Hostel in Jaladugbo. H = City of Harmony, Jaladugbo. I = Ajisafe, Ipetumodu. J = Ogo–Oluwa, Ipetumodu

Table 3: Heavy metals parameters of the water samples

Sampling Stations	Mn (mg/l)	Fe (mg/l)	Cu (mg/l)	Zn (mg/l)	Cr (mg/l)	Cd (mg/l)	Pb (mg/l)	Ni (mg/l)
A	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C	0.00	0.00	0.00	**0.026**	0.000	0.000	0.000	0.000
	0.00	0.00	0.00	**0.025**	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	**0.026**	0.00	0.00	0.00	0.00
D	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	0.00	0.00	0.00	**0.019**	0.000	0.000	0.000	0.000

	0.00	0.00	0.00	**0.018**	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	**0.018**	0.00	0.00	0.00	0.00
G	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00
J	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000
	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

LEGEND:
Mn = manganese, Fe = iron, Cu = copper, Cr = chromium, Cd = cadmium, Pb = lead, Ni = nickel.

Table 4: Cultural, Morphological and Biochemical characteristics of dominant genera of bacteria isolated from the well water samples from Ipetumodu

ISOLATE CODE	CULTURAL CHARACTERISTICS ON AGAR	CELL SHAPE	GRAM REACTION	CATALASE	COAGULASE	OXIDASE	INDOLE TEST	MR	VP	CITRATE	TRIPLE SUGAR IRON TEST (TSI)				SUSPECTED ORGANISMS
	Whole colony. Edge. Elevation. Surface. Colour. Opacity										**Butt**	**Slant**	**Gas**	**H_2S**	
A01	Circular. Entire. Raised. Smooth. Cream. Opaque	C	+	_	N A	N A	N A	N A	N A	N A	NA				*Enterococcus* sp.
A02	Circular. Entire. Flat. Glistering. Yellow. Opaque	C	+	+	+	N A	N A	N A	N A	N A	NA				*S. aureus*
A03	Circular. Lobate. Raised. Smooth. Greenish Metallic sheen. Opaque	R	_	N A	N A	_	+	+	_	_	YB +	-	YS		*Escherichia coli*
A04	Irregular. Lobate. Flat. Dull. Cream. Transparent	C	+	+	+	N A	N A	N A	N A	N A	NA				*S. aureus*
A05	Puntiform. Undulate. Dull. Cream. Transparent	C	+	+	_	N A	N A	N A	N A	N A	NA				CoNS
A06	Spindle. Entire. Flat. Dull. Cream. Transparent	C	+	+	+	N A	N A	N A	N A	N A	NA				*S. aureus*
A07	Circular. Lobate. Raised. Smooth. Pink. Translucent	R	_	N A	N A	+	_	+	_	+	YB +	-	YS		*Klebsiella* sp.
A08	Spindle. Entire. Raised. Smooth. Cream. Translucent	C	+	+	+	N A	N A	N A	N A	N A	NA				*S. aureus*
A09	Rhizoid. Crenated. Low convex. Rough. Cream. Transparent	C	+	+	+	N A	N A	N A	N A	N A	NA				*S. aureus*
A10	Rhizoid. Lobate. Low convex. Rough. Yellow. Transparent	C	+	_	N A	N A	N A	N A	N A	N A	NA				CoNS
A11	Spindle. Entire. Raised. Smooth. Cream. Translucent	C	+	_	N A	N A	N A	N A	N A	N A	NA				CoNS

B01	Puntiform. Undulate. Low convex. Rough. Pink. Translucent	R	_	NA	NA	_	_	_	+	+	YB +	-	YS	*Enterobacter* sp.
B02	Circular. Entire. Raised. Smooth. Cream. Opaque	C	+	+	_	NA	NA	NA	NA	NA	NA			CoNS
B03	Irregular. Entire. Low convex. Smooth. Purple. Opaque	C		_	NA	NA	NA	NA	NA	NA	NA			*Enterococcus sp.*
B04	Irregular. Undulate. Convex papillate. Rough. Greenish Metallic sheen. Translucent	R	_	NA	NA	_	+	+	_	_	YB +	-	YS	*Escherichia coli*
B05	Irregular. Entire. Low convex. Smooth. Purple. Opaque	C	+	_	NA	NA	NA	NA	NA	NA	NA			*Enterococcus sp.*
B06	Spindle. Entire. Low convex. Smooth. Cream. Translucent	C	+	+	_	NA	NA	NA	NA	NA	NA			CoNS
B07	Irregular. Lobate. Flat. Rough. Cream. Opaque	C	+	+	_	NA	NA	NA	NA	NA	NA			CoNS
C01	Circular. Entire. Dome shaped. Rough. Pinkish purple. Translucent	R	_	NA	NA	+	_	+	_	+	YB +	+	RS	*Salmonella typhii*
C02	Circular. Entire. Convex. Rough. Pink. Translucent	R	_	NA	NA	_	_	_	+	+	YB +	-	YS	*Enterobacter* sp.
C03	Circular. Entire. Flat. Smooth. Cream. Opaque	C	+	+	+	NA	NA	NA	NA	NA	NA			*S. aureus*
C04	Circular. Entire. Flat. Smooth. Cream. Transparent	C	+	+	+	NA	NA	NA	NA	NA	NA			*S. aureus*
C05	Circular. Entire. Flat. Glistering. Cream. Translucent	C	+	+	_	NA	NA	NA	NA	NA	NA			CoNS
C06	Spindle. Entire. Flat. Smooth. Cream.	C	+	+	+	NA	NA	NA	NA	NA	NA			*S. aureus*
C07	Opaque Circular. Crenated. Raised. Smooth, Red. Translucent	C	+	+	+	A NA	A NA	A NA	A NA	A NA	NA			*S. aureus*
C08	Circular. Entire. Flat. Smooth. Cream. Transparent	C	+	_	+	NA	NA	NA	NA	NA	NA			*Enterococcus* sp.
D01	Circular. Entire. Low convex. Glistering. Cream. Opaque	C	+	+	_	NA	NA	NA	NA	NA	NA			CoNS

D02	Circular. Entire. Flat. Smooth. Cream. Transparent	C	+	+	+	N A	N A	N A	N A	N A	NA				*S. aureus*
D03	Circular. Entire. Flat. Smooth. Cream. Transparent	C	+	+	_	N A	N A	N A	N A	N A	NA				CoNS
D04	Circular. Entire. Convex. Smooth. Cream. Transparent	C	+	+	_	N A	N A	N A	N A	N A	NA				CoNS
D05	Circular. Entire. Low convex. Smooth. Purple. Opaque	R	_	N A	N A	+	_	_	+	+	YB +	-	YS		*Enterobacter* sp.
D06	Circular. Entire. Dome shaped. Smooth. Pink. Translucent	R	_	N A	N A	_	_	+	_	+	YB +	+	YS		*Citrobacter* sp.
E01	Irregular. Lobate. Raised. Rough. Cream. Opaque	C	+	+	_	N A	N A	N A	N A	N A	NA				CoNS
E02	Circular. Entire. Raised. Dull. Cream. Transparent	C	+	+	+	N A	N A	N A	N A	N A	NA				*S. aureus*
E03	Irregular. Fimbriate. Flat. Glistering. Cream. Transparent	C	+	+	+	N A	N A	N A	N A	N A	NA				*S. aureus*
E04	Circular. Entire. Raised. Glistering. Light Purple. Translucent	C	+	+	+	N A	N A	N A	N A	N A	NA				*S. aureus*
E05	Circular. Entire. Raised. Smooth. Cream. Transparent	C	+	+	+	N A	N A	N A	N A	N A	NA				*S. aureus*
E06	Circular. Entire. Convex. Smooth. Cream. Transparent	C	+	+	_	N A	N A	N A	N A	N A	NA				CoNS
E07	Circular. Entire. Flat. Dull. Cream. Transparent	C	+	_	N A	N A	N A	N A	N A	N A	NA				*Enterococcus* sp.
E08	Circular. Entire. Convex. Smooth. Cream. Translucent	C	+	+	_	N A	N A	N A	N A	N A	NA				CoNS
E09	Circular. Entire. Unbonate. Smooth. Cream. Transparent.	C	+	_	N A	N A	N A	N A	N A	N A	NA				*Enterococcus* sp.
E10	Circular. Entire. Convex. Smooth. Greenish Metallic sheen. Translucent	R	_	N A	N A	_	+	+	_	_	YB +	-	YS		*Escherichia coli*
E11	Circular. Entire. Low convex. Smooth. Greenish Metallic sheen. Opaque	R	_	N A	N A	_	+	+	_	_	YB +	-	YS		*Escherichia coli*
F01	Irregular. Tentate. Flat or Effuse. Dull. Cream. Translucent	C	_	N A	N A	+	_	_	+	_	YB +	-	RS		*Neisseria* sp.

F02	Circular. Entire. Convex papillate. Rough. Purple. Opaque	R	_	NA	NA	+	_	_	_	+	YB +	-	YS	*Klebsiella* sp.
F03	Spindle. Lobate. Flat. Smooth. Cream. Translucent	C	+	_	NA	NA	NA	NA	NA	NA	NA			*Enterococcus* sp.
F04	Circular. Entire. Convex. Smooth. Cream. Translucent	C	+	_	NA	NA	NA	NA	NA	NA	NA			CoNS
G01	Puntiform. Entire. Low convex. Smooth. Purple. Opaque	R	_	NA	NA	+	_	_	+	+	YB +	-	YS	*Enterobacter* sp.
G02	Puntiform. Entire. Low convex. Smooth. Purple. Opaque	R	_	NA	NA	_	_	_	_	+	YB +	-	YS	*Klebsiella* sp.
G03	Circular. Entire. Convex. Smooth. Cream. Translucent	C	+	+	+	NA	NA	NA	NA	NA	NA			*S. aureus*
G04	Circular. Entire. Convex. Smooth. Cream. Translucent	C	+	+	_	NA	NA	NA	NA	NA	NA			CoNS
H01	Circular. Entire. Unbonate. Smooth.	C	+	_	NA	NA	NA	NA	NA	NA	NA			*Enterococcus* sp.
H02	Cream. Transparent Circular. Entire. Low convex. Smooth. Cream. Translucent	C	+	_	NA	NA	NA	NA	NA	NA	NA			*Enterococcus sp.*
H03	Puntiform. Entire. Convex. Rough. Purple. Transparent	R	_	NA	NA	_	+	_	+	+	YB +	+	YS	*Citrobacter* sp.
H04	Puntiform. Lobate. Low convex. Rough. Greenish Metallic sheen. Transparent	R	_	NA	NA	_	+	+	_	_	YB +	-	YS	*Escherichia Coli*
I01	Circular. Entire. Raised. Rough. Cream. Translucent	C	+	+	NA	NA	NA	NA	NA	NA	NA			*S. aureus*
I02	Circular. Entire. Low convex. Smooth. Cream. Transparent	C	+	+	NA	NA	NA	NA	NA	NA	NA			*S. aureus*
I03	Irregular. Fimbrate. Low convex. Rough. Greenish Metallic sheen. Opaque	R	_	NA	NA	_	+	+	_	_	YB +	-	YS	*Escherichia Coli*
I04	Circular. Entire. Flat. Dull. Cream. Translucent	R	_	NA	NA	_	_	_	_	+	YB +	-	YS	*Klebsiella* sp.
J01	Puntiform. Entire. Raised. Rough. Greenish Metallic sheen. Opaque	R	_	NA	NA	_	+	+	_	_	YB +	-	YS	*Escherichia coli*
J02	Circular. Lobate. Low convex. Smooth. Greenish Metallic sheen. Translucent	R	_	NA	NA	_	+	+	_	_	YB +	-	YS	*Escherichia coli*

J03	Circular. Entire. Raised. Smooth. Cream. Translucent	C	+	+	+	NA	NA	NA	NA	NA	NA	*S. aureus*
J04	Circular. Entire. Low convex. Smooth. Cream. Opaque	C	+	_	NA	NA	NA	NA	NA	NA	NA	*Enterococcus* sp.

LEGEND: C=Cocci, R=Rod, CoNS= Coagulase Negative *Staphylococcus*, YB= Yellow butt, YS= Yellow slant, RS= Red slant, *S. aureus*= *Staphylococcus aureus*, sp= specie, NA= Not Applicable

Table 5: Antibiotic Susceptibility Pattern of Gram negative isolates of the water samples

Suspected organisms/Isolate code	CAZ (30µg)	CRX (30µg)	GEN (10µg)	CXM (5µg)	OFL (5µg)	AUG (30µg)	NIT (300µg)	CPR (5µg)	Sensitivity Percentage (%)
	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition(mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	
A01(*Escherichia coli*)	R (6)	R (6)	R (13)	R (6)	S (18)	R (6)	R (6)	R (20)	S 12.5, R 87.5
A02 (*Klebsiella* sp)	R (17)	R (6)	I (14)	R (6)	S (24)	R (6)	R (13)	I (27)	S 12.5, I 25, R 62.5
B01 (*Enterobacter* sp)	R (6)	R (6)	R (11)	R (6)	S (16)	R (6)	R (11)	I (24)	S 12.5, I 12.5, R 75
B02(*Escherichia coli*)	R (12)	R (6)	I (13)	R (22)	S (21)	R (6)	R (14)	R (16)	S 25, I 12.5, R 62.5
C01(*Salmonella typhii*)	R (6)	R (6)	I (13)	R (6)	S (20)	R (6)	R (6)	I (21)	S 12.5, I 25, R 62.5
C02 (*Enterobacter* sp)	R (6)	R (6)	R (11)	R (6)	S (16)	R (6)	R (6)	I (24)	S 12.5, I 12.5, R 75
D01 (*Enterobacter* sp)	R (13)	R (6)	I (14)	R (6)	S (26)	R (6)	R (13)	I (23)	S 12.5, I 25, R 62.5

Do2 (*Citrobacter* sp)	R (6)	R (6)	I (13)	R (6)	S (25)	R (6)	R (6)	I (26)	S 12.5, I 25, R 62.5
Eo1 (*Escherichia coli*)	R (16)	R (6)	S (15)	R (15)	S (21)	R (6)	R (12)	I (25)	S 25, I 12.5, R 62.5
Eo2 (*Escherichia coli*)	I (20)	R (6)	R (6)	R (6)	S (6)	R (6)	R (6)	I (23)	I 25, R 75
Fo1 (*Neisseria* sp)	R (18)	R (8)	R (12)	R (21)	I (27)	R (6)	R (16)	I (30)	I 25, R 75
Fo2 (*Klebsiella* sp)	R (6)	R (6)	S (11)	R (6)	S (16)	R (6)	R (6)	I (22)	S 25, I 12.5, R 62.5
Go1 (*Enterobacter* sp)	R (6)	R (6)	R (7)	R (6)	S (16)	R (6)	R (13)	I (21)	S 12.5, I 12.5, R 75
Go2 (*Klebsiella* sp)	R (6)	R (6)	R (11)	R (7)	S (16)	R (6)	R (6)	I (24)	S 12.5, I 12.5, R 75
Ho1 (*Citrobacter* sp)	R (13)	R (6)	I (15)	R (21)	S (14)	R (6)	R (6)	R (26)	S 12.5, I 12.5, R 75
Ho2 (*Escherichia coli*)	R (13)	R (6)	S (15)	R (21)	S (18)	R (6)	S (20)	I (26)	S 37.5, 12.5, R 50
Io1 (*Escherichia coli*)	R (6)	R (6)	I (13)	R (6)	S (19)	R (6)	R (11)	I (21)	S 12.5, I 12.5, R 75
Io2 (*Klebsiella* sp)	R (6)	R (6)	I (13)	R (6)	S (17)	R (6)	R (11)	R (14)	S 12.5, I 12.5, R 75

LEGEND: CAZ= Ceftazidime (30 µg), CRX=Cefuroxime (30 µg), GEN=Gentamicin (10 µg), CXM=Cefixime (5 µg), OFL=Ofloxacin (5 µg), AUG=Augmentin (30 µg), NIT=Nitrofurantoin (300 µg) and CPR=Ciprofloxacin (5 µg).

CONCLUSION AND RECOMMENDATION

This investigation revealed that nearly all sampled groundwater tested positive to bacterial contamination indicating that most of the well waters were of poor bacteriological qualities indicative of health risk to the inhabitants of the geographical location, warranting treatment of the groundwater before it could be suitable for consumption. Surface phenomena, majorly indiscriminate waste dumps and improper disposal of faeces were responsible for the pollution of groundwater in the study area. The sites of wells are very important to be clean, and hygienic environment promote safety of water. Well water drilling contractors have to be educated on the importance of ensuring that dump sites are not used for well water site. In addition, the populace needs to be educated on the importance of maintaining clean and hygienic environment around well waters to ensure the safety of water from such sources. There is need to carry out a comprehensive epidemiological study to determine the number of people suffering from diseases or illnesses related to the microbial water quality problems identified in the area of study, as this will provide information on the actual health problems on ground caused by the use of untreated groundwater.

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