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BACTERIOLOGICAL AND PHYSICOCHEMICAL ANALYSES OF SOME WELL WATER SAMPLES USED FOR DRINKING AND OTHER DOMESTIC PURPOSES IN ILE-IFE, OSUN STATE. NIGERIA.

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Abstract

The research was carried out with the aim to isolate and characterize bacteria often associated with dug wells and as well determine their susceptibility to commonly used antibiotics The physicochemical parameters of the well were also analysed to determine their influence on the well water bacteria. The multiple tube fermentation technique was used to determine the Total Coliform Count (TCC), Pour Plate Method for Total Viable Count (TBC) and the Kirby-Bauer disc diffusion method for antibiotic sensitivity determination. Based on their biochemical and morphological characteristics, fifteen genera of bacteria were isolated; Staphylococcus, Bacillus, Escherichia, Klebsiella, Pseudomonas, Enterobacter, Salmonella, Corvnebacterium, Citrobacter, Aeromonas, Vibro, Moraxella, Streptococcus, Bulkholderia and Serratia. The bacteria count was between 4000-1,21000CFU/ml of well water. The total coliform count ranged from 3-1,100MPN/100ml of water. Gentamycin, Ceftprozil and Ceftazidime were most effective antibiotics on the bacteria isolates while Ampicillin, Augmentin and Cefuroxine were least effective. The pH ranged from 5.6 to 8.42, temperature was between 24.5°C to 29.5°C. Acidity and alkalinity were between 0.7MgI-1 to 4.9MgI-1 and 0.7MgI-1 to 8.6MgI-1 respectively. Some of the parameters obtained were within the WHO (WHO, 2011) standard were some were above the standard as a result of contaminations which may be due to improper construction, shallowness, animal wastes, proximity to toilet facilities to refuse dumps and human activities around the well.

KEY WORDS: bacreriological, physicochemical, well, isolate, characterize

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Introduction

Groundwater is a widely used source of water for a lot people especially the rural and urban areas. Despite the fact that groundwater serves as an important source of drinking water, its quality is currently threatened by a combination of over-abstraction, microbiological and chemical contamination (Pedley *et al.*, 1997). There are three routes by which the water in a well may become contaminated which include: through the wellhead, lining, or water entering the intake (Appiah and

Momende, 2010). Generally, the closer the groundwater is to the surface, the more influential is the effect of heavy rain in carrying bacteria and other organisms through the soil into it. Poorly made concrete apron and water run-off can crack, and will allow leakage of waste water from the surface back into the well to contaminate it. Buckets and ropes which are used to raise the water, and often lie around the unhygienic rim of the well also pollute the water. Intensive use of natural resources and the large production of wastes in modern society often pose a threat to groundwater quality and have already resulted in many incidents of groundwater contaminations (Srivastava et al., 2012). Various activities of man have led to the contamination of groundwater. According to Khan et al(2012), sewage contamination through animal and human excreta is one of the most common and widespread cause of water contamination. The leachate produced by waste disposal sites contains a large amount of substances which are likely to contaminate groundwater. A study conducted in India to look at the impact of poor solid waste management on groundwater has revealed that the groundwater quality does not conform to the drinking water quality standards as per Bureau of Indian Standards (Vansanthi, 2008; Israel, Sunday., Mansong and Ubong, 2016; Babalola, Hinmikaiye and Ogundare, 2017). The effects of dumping activity on groundwater appeared most clearly as high concentrations of total dissolved solids, electrical conductivity, total hardness, chlorides, chemical oxygen demand, nitrates sulphates. The quality of the well water can be significantly increased by lining the well, sealing the well head, fitting a self-priming hand pump, constructing an apron, ensuring the area is kept clean and free from stagnant water and animals.

Materials and methods

Study area

The well water samples were collected from twenty-five (25) dug wells at different locations in Ile-Ife and it suburb town Modakeke. The major source of water in this area is the dug well which is basically in use for a variety of domestic and industrial activities. The wells studied are the ones sited near dirty area that is dumpsite and neat area.

Collection of samples

The water samples were consistently taken weekly from selected wells with their location sites, site description and sampling code recorded. A total of twenty-five water samples were collected from October, 2015 to November, 2016. Samples were consistently taken in the morning when wells were in use by the community members. The water from the wells was collected using the receptacle used in that household. Sample collection techniques were dependent on the particular experiment to be carried out.

For the bacteriological analyses, water samples were collected in 50ml sterilized bottles and was tightly corked. For dissolved oxygen (DO) and biological oxygen demand (BOD), the samples were collected in 250ml clean white bottles to enable easy observation of chemical reaction during the test and also 250ml clean brown bottles to prevent interference with external agent respectively and lastly for other physicochemical parameters, the samples were collected in 500ml clean plastic containers. After sampling, the containers were tightly covered and were appropriately labeled and put in an ice-packed cooler and was immediately transported to the laboratory for analysis.

Physicochemical analysis of well water

A number of physicochemical parameters of well water samples were determined. They included temperature, color, total dissolved solids (TDS), total suspended solid (TSS), pH, conductivity, acidity, alkalinity, dissolved oxygen (DO), biological oxygen demand (BOD), Ca²⁺, Mg²⁺, Cl⁻.

The methods for physicochemical analysis included use of pH meter for pH determination, thermometer for temperature, colorimeter for apparent and true color, nephelometric method for turbidity and gravimetric method for TDS and TSS, iodometric titration for BOD and DO, mohr titration for Cl⁻, acid-base titration for acidity and alkalinity and complexionmetric titration for Ca²⁺ and Mg²⁺.

Bacteriological analyses

Determination of Total Viable Count (TVC)

The total viable count was determined using pour plate technique and plating was done in duplicate. This was done by pouring molten nutrient agar at 45°C aseptically which has already been sterilized in the autoclave at 121°C for 15 minutes into petridishes containing 1ml of the appropriate dilution of the water samples. They were swirled to mix and incubated at 37°C in an inverted position for 24 to 48 hours. Colonies of microorganisms that developed on the plates after incubation were counted, recorded and expressed as standard numbers of colony forming unit per milliliter (Cfu mL-1). Sub culturing was done on solidified sterile nutrient agar to obtain pure cultures. The pure cultures were maintained at 4°C as stock culture for further tests (Prescott et al., 2005).

Determination of Total Coliform Count (TCC)

A three (3) tubes dilution series using inoculum quantities of 10ml, 1ml and 0.1ml was used in the Most Probable Number Analysis. A double strength MacConkey broth was prepared for the

10ml inoculum quantities in test tubes, while a single strength MacConkey broth was prepared for 1ml and 0.1ml of the inoculum quantities. Durham tubes were placed inside the test tubes in an inverted position to indicate trapping of gas (Cheesbrough, 2005). The prepared broth were inoculated with the isolates and incubated at 37°C for 48 hours. The positive samples (that showed acid and gas production) were used for the confirmatory test For the confirmatory test, all positive presumptive cultures were streaked on sterile solidified eosin methylene blue agar (EMB) plates and incubated at 35°C for 24 hours. The plates were then observed for various cultural characterizations. For the completed test, gram staining was done and the isolate were also subcultured into MacConkey broth to test for gas formation (APHA, 2000).

Characterization and Identification of isolates

Biochemical procedures (Gram staining, catalase, coagulase, sugar fermentation, starch hydrolysis, Citrate utilization, motility, oxidase, Voges Proskauer and endospore tests) were used to characterize and identify the isolates following standard protocols as described in Bergey's manual of Systemic Bacteriology (Krieg and Holt, 1994).

Antibiotics sensitivity testing

Antibiotics sensitivity testing was carried out using the standardized disk agar diffusion method described by Bauer et al (2000). A 24 hours old culture was inoculated into a10ml sterile distilled water in a test tube to give a concentration of one million colony forming units per ml. It was standardized according to MacFarland standard. Gram positive and Gram negative single disk antibiotics (AB disk Abtek, Biological limited Liverpool, England) stored between 2-8°C were used for sensitivity testing. The Gram positive disk contained the following corresponding antibiotics and their concentration: Augmentin (30 µg), Gentamicin (10 μg), Ofloxacin (5 μg), Erythromycin (5 μg), Ceftriaxone (30 µg), Cefuroxime (30 µg), Ceftazidime (30 µg) and Cloxacillin, while the gram negative discs contain: Ofloxacin (5 µg), Augmentin (30 µg), Nitrofuranton (300 µg), (10µg), Ceftazidime Ampicillin (30 μg), Cefuroxime (30 µg), Gentamicin (10 µg), Ciprofloxacin (5µg), Cefprozil (30 µg). These antibiotics impregnated discs were applied to the surface of the inoculated plates using a pair of sterile forceps, which had been flamed and cooled. The discs were placed at the agar and were pressed firmly unto the agar with the sterile forceps so that it was in complete contact with the agar and incubated at 37°C for 18-24 hours. The zone of inhibition was recorded in mm and interpreted according to CLSI, 2013 standard.

Results

Table 1 below shows the highest and least values of each of the physicochemical parameters obtained for the well water samples in relation to WHO, 2011 standard

Table 1: Physicochemical parameters of the water quality with WHO standard

PARAMETER	HIGHEST VALU MEASURED	JE LEAST VALUE MEASURED	WHO LIMIT (2011)
Temperature (°C)	29.5	24.5	Ambient
Apparent Color (Pt-Co)	102	079	NS
True Color (Pt-Co)	99	88	NS
TDS(MgI-1)	485	87	<1000
TSS (MgI-1)	2.976	0.0082	500
рН	8.42	5.60	6.5-8.5
Conductivity(µs/cm)	950	130	<1000
Acidity (MgI ⁻¹)	4.9	0.7	0.3
Alkalinity (MgI ⁻¹)	8.6	0.8	500
DO (MgI ⁻¹)	5.7	1.1	
BOD (MgI ⁻¹)	3.8	0.2	30
Ca ²⁺ (MgI ⁻¹)	4.7	0.2	75
$Mg^{2+}(MgI^{-1})$	2.4	0.3	150
Cl ⁻ (MgI ⁻¹)	16.9	1.3	250
		10F C / 1)	1 1 1 1 1 1 1 1

NS= No Standard

The Total Viable Cell Counts (TVC) of the heterotrophic microorganisms calculated from the dilution factor and colony count per plate for each sample is shown in Table 2. The values for number of counts per plates were estimated by means of duplicate determination. The microbial load is highest in W 25 (1.21 x

 10^5 cfu/ml) and least load in W 24 (4 x 10^3 cfu/ml).

The most probable number (MPN) index for the 25 well water samples obtained by the three dilution series is shown in Table 3. Sample W13, W16, W23 had the most coliform count greater than 1100 coliform/100ml (MPN index/ml > 11.0), while samples W1 and W20 had the least count which was 11 coliform/ml (MPN index/ml = 0.11).

Table 4 summarizes the various genera and species isolated, the total number of each

genera or species isolated, the wells from which they were isolated and the percentage of each genera or species isolated from the twenty- six wells. Sixteen (16) different species were isolated from the 25 well water samples which are listed in Table 3.7 with their percentage occurrence. Bacillus spp had the highest percentage occurrence (40%)while Streptococcus **Pseudomonas** spp, spp, Burkhoidea spp and Enterobacter spp had the least percentage occurrence of 4%.

Table 5 shows the susceptibility profile of the isolates. Gram positive isolates were tested with Augmentin (AUG), Gentamicin (GEN), Ofloxacin (OFL), Erythromycin (ERY), Ceftriaxone (CTR), Cefuroxime (CRX), Ceftazidime (CAZ) and Cloxacillin (CXC). Gram negative isolates were tested with Ofloxacin (OFL), Augmentin (AUG), (NIT). Ampicillin Nitrofuranton (AMP). Gentamicin (GEN), Ceftazidime (CAZ), Cefuroxime (CRX) and Cefprozil (CPR).

Discussion and conclusion

The values of total viable and total coliform count range from 4.0 x 10³ cfu/ml to 1.41×10^3 cfu/ml and 11 coliform/ml to 1100 coliform/ ml respectively. The total coliform counts far exceed WHO standard of zero count of total coliform in 100ml of drinking water and also the total viable bacterial count of the wells studied exceeded the recommended limit of 1 x 10²cfu/ml (WHO, 2010). High total viable bacteria count and total coliform in water sample from well have also been reported in recent studies within Nigeria (Osho and Fagade, 2000; Akinyemi et al., 2006; Idowu et al., 2011; Odeyemi et al., 2011) and also in other developing countries (Akoachere et al., 2013; Gwimbi, 2011).

The potential reasons for the high presence of microbiological contaminants in the

wells especially the one sited near dirty surrounding was due to poor sanitation conditions and practice such as washing laundry near water sources, using dirty containers to collect water from the well and citing wells close to sanitary facilities have been reported to contribute significantly to high pollution of wells resulting in deterioration of its water quality (Ayantobo et al., 2012). The high level of faecal coliform in these wells indicated significant and increasing risk of contamination of water-borne diseases. Using water from these wells for domestic purposes such as drinking, cooking and washing of fruits and vegetables could predispose users to water borne diseases such as diarrhea, cholera and dysentery. The microbial contaminant was reduced in wells sited in neat surroundings compared to those sited in dirty surroundings in this study which supports that good hygienic practices is essential for good water quality.

The pathogens present were enteric organisms such as Escherichia coli which were a major public health concern as it is not only an indication of recent contamination with faecal matter but the possible presence of other dangerous intestinal pathogens. Others include Klebsiella spp, Enterobacter spp, Citrobacter spp, Bacillusspp, Staphylococcus spp, Salmonella spp, Corynebacterium spp and Aeromonas spp, Vibrio Streptococcus spp, Moraxella spp, Pseudomonas spp, Burkhoidea spp, Serratia spp and Enterobacter spp. Their isolation further confirms the contaminantion of water with faecal material.

Table 2: Total viable count for heterotrophic microorganisms in well water samples

Sample code	W	W	W	W	W	W	W	W	W	W	W	W	W
	1	2	3	4	5	6	7	8	9	10	11	12	13
Dilution coefficient	1 x10 ⁻²	1x10-	1 x 10 ⁻²	1 x10 ⁻	1 x 10 ⁻³	1x10 ⁻³	1x 10 ⁻³	1 x 0 ⁻²	1 x10 ⁻	1x10 ⁻²	1 x 10 ⁻²	1x10 ⁻²	1x 10-
Dilution factor	100	100	100	100	1000	1000	1000	100	100	100	100	100	100
Counts per plate	198	280	106	169	83	31	32	59	90	100	70	160	125
Colony forming unit(10000) in CFU/ml.	1.98	2.8	1.06	1.69	8.3	3.1	3.2	5.9	0.9	1.0	0.7	1.6	1.25

sample code	w 14	w 15	w 16	w 17	w 18	w 19	w 20	w 21	w 22	w 23	w 24	w 25
dilution coefficient	1x10 ⁻²	1x10 ⁻	1x10 ⁻²	1x10 ⁻³								
dilution factor	100	100	100	100	100	100	100	100	100	100	100	1000
counts per plate	162	244	80	250	90	143	95	86	99	141	40	121
colony forming unit(10000) in cfu/ml.	1.62	2.44	0.8	2.5	0.9	1.43	0.95	0.86	0.99	1.41	0.4	12.1

Table 3: MPN index and 95% confidence limit (3 tube dilution series; 10ml:1ml:0.1ml) for each sample.

Sample code	W	W	W	W	W	W	W	W	W	W	W	W	W
	1	2	3	4	5	6	7	8	9	10	11	12	13
Combination of positive tube	1-2-0	3-2-2	3-3-2	3-2-2	2-2-1	2-2-1	2-2-1	3-3-2	3-3-2	3-3-2	3-3-2	2-2-1	3-3-3
MPN index/ml of sample Lower Upper	0.11	2.1	11.0	2.1	0.35	0.35	0.35	11.0	11.0	11.0	11.0	0.35	>11.0
	0.036	0.40	1.8	0.4	0.087	0.087	0.087	1.8	1.8	1.8	1.8	0.087	0.37
95% Confidence limit	0.42	4.3	41.0	4.3	0.94	0.94	0.94	41.0	41.0	41.0	41.0	0.94	4.2

Sample code	W	W	W	W	W	W	W	W	W	W	W	W	W
	14	15	16	17	18	19	20	21	22	23	24	25	13
Combination of	2-1-1	3-2-0	3-3-3	3-3-2	2-2-1	2-2-1	1-2-0	2-1-1	2-2-1	3-3-3	2-1-1	2-1-1	3-3-3
positive tube													
MPN index/ml	0.20	0.093	>11.0	2.4	0.28	0.35	0.11	0.20	0.28	>11.0	0.20	0.15	>11.0
of sample	0.045	0.18	4.2	0.42	0.087	0.087	0.036	0.045	0.087	4.2	0.045	0.037	0.37
Lower Upper													
95% Confidence	0.42	4.2	-	10.0	0.94	0.94	0.42	0.42	0.94	-	0.42	0.42	4.2
limit													

Table 4: Probable organisms isolated and their percentage occurence

PROBABLE	NI	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	%
ORGANISM		1	2	3	4	5	6	7	8	9	1 0	1 1	1 2	1 3	14	5	6	7	1 8	9	2 0	2 1	2 2	3	24	25	
Staphylococcus aureus	2				+															+							8
Staphylococcus spp	5	+								+							+				+			+			20
Salmonellaspp	6	+	+			+		+	+																	+	24
Vibriospp	4						+	+							+							+					16
Klebsiellaspp	2			+							+																8
Aeromonasspp	7				+	+			+				+							+	+				+		28
Citrobacterspp	2			+								+															8
Corynebacteriumspp	4			+								+											+			+	16
Bacillusspp	10						+	+	+						+	+			+			+	+		+		40
Moraxella spp	2									+			+														8
Burkholderiaspp	1													+													4
Pseudomonasspp	1															+											4
Enterobacterspp	1																	+									4
Escherichia coli	3													+			+							+			12
Seratiaspp	2										+							+	-								8
Streptococcusspp	1		+																-								4

Table 5: zone of inhibition and antibiotic susceptibility pattern of isolates from each of the samples

Antibiotics tested/	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Isolates	1	2	3	4	5	6	7	8	9	1 0	1 1	1 2	1 3	14	1 5	1 6	1 7	1 8	9	0	2 1	2 2	3	24	25
Erythromycin	R	I	R	R	R	I	I	I	R	I	I	I	R	R	R	R	R	R	I	R	I	R	I	R	R
Cloxacillin	R	I	R	R	R	I	I	I	R	I	I	I	R	R	R	R	R	R	I	S	I	R	I	R	R
Augmentin	R	R	R	R	R	R	R	R	I	I	R	R	S	R	R	R	R	R	R	S	R	R	R	R	R
Ceftazidime	R	S	R	R	R	R	S	R	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
Gentamycin	R	R	R	S	R	S	S	R	S	S	S	R	S	S	S	S	S	S	S	S	S	R	S	S	S
Ofloxacin	1	S	I	I	R	I	S	R	S	S	S	R	S	S	I	I	I	I	S	S	S	I	I	S	S
Cefuroxime	R	S	R	R	R	R	R	R	S	S	R	R	S	R	R	R	R	R	R	S	R	R	R	R	R
Ceftriaxone	R	1	R	R	I	I	I	I	I	I	I	I	I	I	I	R	S	R	I	R	I	S	I	R	I
Nitrofuratoin	R	S	S	S	I	I	I	S	S	S	S	I	S	I	I	I	R	I	I	I	I	I	I	I	R
Ampicillin	S	R	R	R	R	R	R	R	R	S	R	R	S	R	R	I	R	I	R	I	R	I	R	I	R
Cefprozil	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	I	S	I	S	I	S	S	S

KEY: R=Resistant, I=Intermediate, S=Sensitive,

R1=Staphylococcus, R2=Salmonella, R3=Corynebacterium, R4=Staph.spp, R5=Aeromonas, R6=Bacillus, R7=Salmonella, R8=Bacillus, R9=Moraxella, R10=Serratia, R11=Citrobacter, R12=Moraxella, R13=Burkholderia, R14=Vibrio, R15=Pseudomonas, R16=Staphylococcus, R17=Serratia, R18=Bacillus, R19=Bacillus, R20=Aeromonas, R21=Vibrio, R22=Corynebacterium, R23=Escherichia, R24=Aeromonas, R25=Salmonella.

The most effective of the antibiotics tested was Gentamicin. Other antibiotics with high activity against the isolates are Cefprozil, Ceftazidime. High susceptibility of the isolates Gentamicin in this study compares with findings from another study where it has also been reported to be strongly active against bacterial pathogens isolated from well water samples (Akoachere et al., 2013; Odeyemi et al., 2011). The resistance of the bacterial isolates to majority of the antibiotics tested could be because of the diversity of resistant mutant among bacterial pathogens and indiscriminate use of antibiotics.

The results of the physicochemical analysis of the water samples from the 25 wells show that the pH of the water samples ranged from 5.60 to 8.42 and this indicated mild to moderate acidity of the well waters for the majority of cases. The total dissolved solids (TDS) as shown in Table.1, the ranged from 87 to 485mg/l. Therefore, the ranges measured for the well water samples were not above WHO guideline value of 1000mg/l.

Magnesium is usually abundant in waters than calcium, since magnesium is found in the earth's crust in much lower amounts as compared with calcium but ground water, the mass concentration of Ca2+ is usually several times higher compared to that of Mg. The Ca²⁺ to Mg²⁺ ratio measured for this study is approximately 3.3 for all the well samples. Kozisek (2006) as well as Monarca and Donato (2005) provided comprehensive reviews of the health significance of Mg²⁺ and Ca²⁺ ion in drinking water

The temperature of any water body affects the rate of proliferation of

microorganisms (Pelczar, 2005) and also a factor of great important in aquatic ecosystem, as it affect the organisms as well as the chemical and physical characteristics of water. The temperature range for this study is 24.5 to 29.5°C with a mean value of 26.7 which correlates with ambient temperature of 28°C at time of collection.

Biochemical oxygen demand (BOD) and Dissolved oxygen (DO) values obtained for points varied between 0.2 to 3.8 mg/l and 1.1 to 5.7mg/l respectively. BOD and DO are directly linked with decomposition of dead organic matter present in the wastewater and hence anaerobic conditions that cause bad odours (Waziri, 2010).

Having assessed the quality of the well water in the study area, the following recommendations are proposed to enhance the quality:

- Wells should be sited far away from toilet facilities and dump sites. The environs of the wells should be kept clean.
- Communities within the study area should be enlightened on the health hazards of consuming impure water hence the need for water purification
- The government should create agencies which can be effective in monitoring wells from a health stand point, compel adherence to regulations on the part of the owners.

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