PREVALENCE AND ANTIBIOGRAM OF GRAM-NEGATIVE BACTERIAL ISOLATES FROM WELL WATER IN ULA-UBIE COMMUNITY, AHOADA WEST, NIGERIA

Abstract

Antibiotic resistance has become a great burden in the health care system and has emerged as a public health challenge. The antibiotic susceptibility pattern of bacteria in well water was characterized with a view of determining the level of resistance in the environment. fifty well water samples were collected from ten different points in Ula-Ubie community, Ahoda, Rivers State for a period of five months. Standard microbiological methods were used to analyse the microbiological constituents of the water while methods recommended by the American Public Health Association (APHA) was used to determine the physicochemical parameters of the samples. The antibiotic susceptibility profile of the bacterial isolates was carried out using the disc diffusion methods. The total heterotrophic bacteria of the water samples ranged from 0.93±0.46 to 2.02±1.06 log10cfu/ml. The coliform counts ranged from 0.45±0.42 - 2.55±2.33 log10cfu/ml, respectively. Despite the variations in the counts of the different microbial population, there were no significant differences ($P > 0.05$) in the different well water samples. The physicochemical parameters except the pH were all within the permissible limits. *Klebsiella, Pseudomonas Serratia* and *Enterobacter* were identified in the well water. The antibiotic susceptibility profile showed that all the isolates were resistant to ceftazidime and augmentin, whereas Enterobacter wsa the most resistant organism to the antibiotics. Meanwhile there is an existence of multi-drug resistance. The water is not potable for drinking. Thus, proper sanitation and cleanliness of well should be encouraged.

INTRODUCTION

Water is one of the most important and most valuable natural resources. It is essential in the life of all living organisms including plants and animals [2]. Good drinking water or potable water is water that is free from microbial contaminants and other substances which could cause diseases. Due to the continued pollution of water bodies, potable water has become a public health concern in many countries, especially in developing countries [14]. The bacterial qualities of groundwater, pipe borne water and other natural water supplies in Nigeria, have been reported to be unsatisfactory, with coliform counts far exceeding the level recommendation by WHO [20]. The quality of water may vary from place to place due to the type of activities carried out in that environment. Thus, ground water sources sited close to dump sites could be more polluted than those sited far away from dumpsites. [12]. posited that the quality of groundwater is not always constant especially for different water sources since certain factors such as periodic changes, rock and soil types and areas via which the water flows from could influence the substances present in the water. Contaminants are naturally present in the rocks and sediments and as groundwater moves across the sediments, metals such as iron and manganese are dissolved and may later be found in large amounts in the water. Additionally, the pollution of most water bodies is orchestrated by certain human activities (the disposal or dumping of chemicals and microbial matter on the land surface and into soils, or via the direct injection of wastes into groundwater). This contamination caused by human activities adversely affects the health of people who consumes them without treatment [11]. More so, ground water sources like well water could be contaminated by poor hygienic practices such as the indiscriminate use of dirty fetching buckets to scoop water from deep wells as well as talking or sneezing when fetching water. Infectious diseases caused by pathogenic bacteria, viruses and parasites (e.g. protozoa and helminths) are the most common and widespread health risk associated with drinking-water [6]. Consumption of untreated water has been reported to cause different types of water borne

Comment [A1]: A suggestion for the authors Occurrence and antibiotics susceptibility profile of Gram- negative bacteria isolated from groundwater in ……

Comment [A2]: The opener is great but it's not really clear why the authors chose to study drinking water. Possibly an additional sentence will do the trick

Comment [A3]: Microbiological constituent would mean that the authors studied the entire microflora of the water samples…. I'll suggest that the authors rephrase this

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Comment [A7]: Were these bacterial species identified to species level? If yes, the authors should add the species. If no? I'll suggest that the authors add spp.

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Comment [A10]: The authors should made it clear how they arrived at this conclusion… This is an absolute statement and should be rephrased.
On what basis was this conclusion made? Physicochemical, microbiological quality or the presence of antibiotic resistant bacteria.
Also, it appears that the authors presented the results of all the 10 different points together (from the Abstract).
Do these points have the same microbiological quality?
How different are thee points in terms of physicochemical parameters?

Comment [A11]: Reference [1] is missing

Comment [A12]: Reference 1, 3-13 is missing. The authors should revise this *in-context* reference citations

Comment [A13]: Bacterial qualities or microbiological qualities? Please confirm this statement

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diseases including cholera, typhoid, hepatitis A and diphtheria [10] For instance, cholera outbreak has been reported in Zimbabwe, India and Nigeria which was caused by the presence of Vibrio cholerae in municipal taps and wells [18], 80% of sicknesses and deaths among children worldwide have been associated with the consumption of unsafe water [20].

An antibiogram is a chart that displays the susceptibility test or responses of microorganisms against the antibiotics to which they were tested for [19]. With the rate at which microorganisms are becoming very resistant to antibiotics, there is a need to develop antibiogram for microbial isolates so as to ascertain the antibiotics which are more potent in treating infections caused by these microbes. Well water is the major source of drinking water in many communities in Ahoada, Rivers State, Nigeria. Thus, evaluating the bacteriological properties as well as developing an antibiogram would help us understand the extent of contamination or potability of these water sources, the prevalence of bacterial isolats especially Gram-negatives as well as the right antibiotics suitable in the treatment of diseases caused by microorganisms associated with the wells.

MATERIALS AND METHOD

Description of Study Area

The study was carried out in Ula-Ubie community. Ula-Ubie is one of the communities located in Ahoada, Ahoada West Local Government Area of Rivers state, Nigeria. Ahoada is a city in Orashi Region of Rivers State, Nigeria, found northwest of Port Harcourt. The map of the stations where samples were collected is presented in Fig. 1.

Collection of Samples

Fifty well water (underground water) samples were collected in sterile containers from ten different stations in the community. The well water was drawn out of the well using a fetching bucket (a bucket that has a rope tied to it; used in scooping water from the well). The collected samples were placed in ice pack container and sent to the microbiology laboratory of the department of Microbiology, Rivers State University for analysis.

Microbiological Analysis

The microbiological analysis of the samples involved enumeration and isolation of the bacteria present in the different samples. The microbial population in the water samples was enumerated using the tenfold serial dilution of Harrigan and McCanc as described by [19]. In this method, one milliliter of the water sample was transferred into test tube containing 9mL of prepared sterile saline. After which a step wise dilution was made by transferring 1mL from the previous dilution into another test tube containing 9mL sterile saline. This was done until a dilution of 10^{-6} was reached. was transferred into test tube containing 9mL sterile normal saline. This was done serially until the dilution of 10^{-6} was achieved.

After the serial dilutions, aliquots of 10^{-1}, 10^{-2} and 10^{-3} dilutions were seeded into prepared Nutrient agar, Brain Heart infusion agar (BHI), MacConkey agar, and Bile esculin agar plates. Swabs were inoculated directly on the respective agar plates. Plates were incubated at 37ºC for 24-48 hours. After incubation, plates were observed for microbial growth. Counts were made for the respective plates and colonies were characterized morphologically and were subcultured on freshly prepared nutrient agar plates. The counts from the different plates were used in enumerating the microbial load present in water samples.

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I will suggest that the author revisit and revise this section to provide the background essential to understand this interesting study.

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Comment [A21]: Serial dilution is a known technique in the field... I will suggest the authors w *serial dilution as described by ***

Comment [A22]: It appears that the authors just copied and pasted some sentences without proper proof-reading and editing. This observation is consistent with the Abstract and Introduction section

Comment [A23]: Repetition of 10^{-6}

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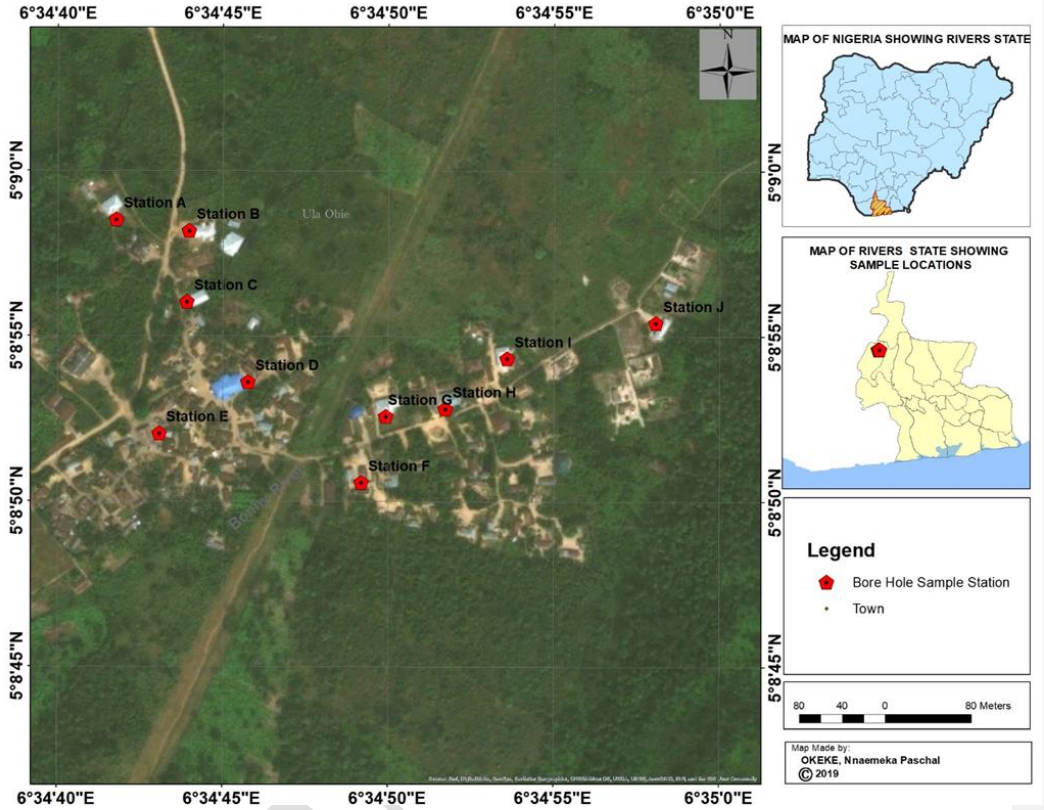

Fig1. Map showing the various stations under study.

Preservation of Isolates

Pure cultures of the bacterial isolates were preserved in bijou bottles containing 10% prepared glycerol. Prior to storage, 5mL glycerol suspension were transferred into bijou bottles and were sterilized by autoclaving at 121ºC for 15 psi. The pure isolates were transferred into labeled bijou bottles containing the glycerol suspensions. After which, the bottles were kept frozen in the refrigerator. This was used for subsequent analysis.

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Characterization of Bacterial Isolates

The morphological and biochemical characteristics of the bacterial isolates were determined using the method of [8]. The morphological and biochemical test used include; Gram staining, motility, catalase, indole production, methyl red, citrate utilization, vogue's proskauer test, blood

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haemolysis test and sugar fermentation (raffinose, arabinose, mannitol, glucose, lactose and sucrose). The confirmed identities were gotten from the advanced bacteriological identification system (ABIS) after imputing the biochemical responses of various isolates. Prior to the use of the ABIS software, identities were first confirmed with the Bergy's manual of determinative bacteriology [7].

Antibiotic Sensitivity

This was prepared as described by [8]. Four millilitres of normal saline were dispensed into test tubes and sterilize by autoclaving at 121^{o}C for 15minutes. After sterilizing, allow to cool to room temperature. With wire loop, colonies of 24 hours old culture were aseptically picked and introduced into the sterile normal saline, turbidity of the organism in the tube was compared to the turbidity of the 0.5 McFarland Standard. Antibiotic susceptibility test was carried out on the test bacteria using Kirby Bayer disk diffusion method. Unto a sterile solid Muller Hinton agar plates, each of the test organisms from the already prepared McFarland standard was aseptically inoculated using a sterile swab stick and allowed to dry [21] for 5minutes after which antibiotic disc was aseptically placed on the solid media using sterile forceps. The inoculated plates were incubated at 37°C for 24hours. After 24 hours, the diameter of the zone of inhibition around each antibiotic was measured to the nearest millimeter and the readings recorded. The antimicrobial discs used were stored in the refrigerator according to the manufacturer's instruction. They were kept at room temperature before use to enable the viability of the antimicrobial discs. The abtek antibiotics disc was used and it contained the following antibiotics; Gentamycin (10µg), Ciprofloxacin (5 µg), Nitrofurantoin (µg), Augmentin (30 µg), Ofloxacin (5 µg), Cefixime (5 µg), Ceftazidime (30 µg), Cefuroxime (10 µg), Ceftriaxone (30 µg), Cloxacillin (5 µg) and Erythromycin (5 µg).

RESULTS

Microbial Load of Well Water

The total heterotrophic bacterial load, and coliform counts of the ten (10) well water samples is illustrated in Table 1.

The total heterotrophic bacteria of the water samples ranged from 0.93±0.46 to 2.02±1.06 log_{10}cfu/ml. The coliform counts ranged from 0.45±0.42 - 2.55±2.33 log_{10}cfu/ml.

The result for the coliform count showed that coliform was detected in all the well water samples. The counts for coliform showed that the station with the highest coliform load was well station H (2.55±2.33 log_{10}cfu/ml) followed by station I (1.90±2.75 log_{10}cfu/ml), G (1.18±0.55 log_{10}cfu/ml), E (1.10±1.14 log_{10}cfu/ml) and C (1.10±1.13 log_{10}cfu/ml). The least coliform load of 0.45±0.42 log_{10}cfu/ml was observed in station A.

Table. 1: The Bacterial Populations of the Water Samples across the stations

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SAMPLES	THB(X10^{4}Cfu/ml)	TCC(X10^{3}Cfu/ml)
A	1.33±0.80^a	0.45±0.42^a
B	1.25±0.93^a	0.60±0.71^a
C	1.31±1.10^a	1.10±1.13^a
D	0.93±0.46^a	0.50±0.80^a
E	1.17±0.73^a	1.10±1.14^a
F	1.68±0.64^a	0.73±0.51^a
G	1.99±1.13^a	1.18±0.55^a
H	1.90±0.94^a	2.55±2.33^a
I	2.02±1.06^a	1.90±2.75^a
J	1.33±1.01^a	0.95±0.21^a

**Means with same superscript across the column shows no significant difference at (p>0.05)*
Key:TFC(Total fungi count), THB(Total heterotrophic bacteri) and TCC(Total coliform count).
The above result is presented in Mean ± SD $Log_{10}cfu/ml$

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Antimicrobial Susceptibility Profile

The antibiotic susceptibility pattern of *Pseudomonas*, *Enterobacter, Klebsiella*, and *Serratia* sp isolated from the well water samples are presented in Tables 2, 3, 4. and 5, respectively.

The result of the antibiotics susceptibility of all bacterial isolates from well water samples showed very high resistant to the antibiotics especially on Ceftazidime and Augmentin.

Comment [A38]: First of all, it will be interesting and important to see the distribution of the probable bacterial species isolates based on the sample points. This information is missing and would be important to understand the overall occurrence of the bacterial. Perhaps there is/are wells that do not have this bacterial species

Table 2. Susceptibility Pattern of *Pseudomonas* Sp Isolated from Water Samples

Antibiotics	Resistant (%)	Intermediate (%)	Susceptible (%)
Ceftazidime	4(100.0)	0(0.00)	0(0.00)
Cefuroxime	3(75.0)	1(25.0)	0(0.00)
Gentamycin	0(0.00)	1(25.0)	3(75.0)
Ofloxacin	2(50.0)	2(50.0)	0(0.00)
Augmentin	3(75.0)	1(25.0)	0(0.00)
Cefixime	3(75.0)	1(25.0)	0(0.00)
Nitrofuriaxons	4(100.0)	0(0.00)	0(0.00)
Ceftriaxons	4(100.0)	0(0.00)	0(0.00)

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In principle, the diameter of the zone of inhibition should be compared to a reference (either CLSI, BSAC or EUCAST) this information is mission

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167 **Table 3. Susceptibility Pattern of *Enterobacter* Sp Isolated from Water Samples**

Antibiotics	Resistant (%)	Intermediate (%)	Susceptible (%)
Ceftazidime	5(100)	0(0.00)	0(0.00)
Cefuroxime	3(60.0)	2(40.0)	0(0.00)
Gentamycin	4(80.0)	1(20.0)	0(0.00)
Ofloxacin	4(80.0)	1(20.0)	0(0.00)
Augmentin	5(100)	0(0.00)	0(0.00)
Cefixime	3(60.0)	2(40.0)	0(0.00)
Nitrofurazones	3(60.0)	2(40.0)	0(0.00)
Ceftriaxone	5(100)	0(0.00)	0(0.00)

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175 **Table 4. Susceptibility Pattern of *Klebsiella* Sp Isolated from Water Samples**

Antibiotics	Resistant (%)	Intermediate (%)	Susceptible (%)
Ceftazidime	5(100.0)	0(0.00)	0(0.00)
Cefuroxime	3(60.0)	2(40.0)	0(0.00)
Gentamycin	0(0.00)	2(40.0)	3(60.0)
Ofloxacin	0(0.00)	1(20.0)	4(80.0)
Augmentin	4(80.0)	1(20.0)	0(0.00)
Cefixime	4(80.0)	1(20.0)	0(0.00)
Nitrofurantoin	0(0.00)	1(20.0)	4(80.0)

Ceftriaxone	0(0.00)	1(20.0)	4(80.0)

Table 5. Susceptibility Pattern of *Serratia* sp Isolated from Water Samples

Antibiotics	Resistant (%)	Intermediate (%)	Susceptible (%)
Ceftazidime	5(100.0)	0(0.00)	0(0.00)
Cefuroxime	3(60.0)	2(40.0)	0(0.00)
Gentamycin	4(80.0)	1(20.0)	0(0.00)
Ofloxacin	0(0.00)	1(20.0)	4(80.0)
Augmentin	5(100.0)	0(0.00)	0(0.00)
Cefixime	3(60.0)	2(40.0)	0(0.00)
Nitrofurantoin	0(0.00)	1(20.0)	4(80.0)
Ceftriaxone	5(100.0)	0(0.00)	0(0.00)

183 **Table 6. Chemical Parameters of the Well Water Stations**

Parameters	Well Water Stations									
	SASA	**SBSA**	**SCSA**	**SDSA**	**SESA**	**SFSA**	**SGSH**	**SHSA**	**SISA**	**SJSA**
pH	5.50±0.00	5.34±0.00	5.14±0.00	4.66±0.00	5.48±0.00	5.91±0.00	5.32±0.00	5.40±0.00	6.17±0.00	5.80±0.00
Temperature (ºC)	24.5±0.00	24.0±0.00	24.1±0.00	24.1±0.00	24.4±0.00	24.2±0.00	24.7±0.00	23.9±0.00	24.4±0.00	24.3±0.00
Electrical Conductivity (µS/cm)	219±0.00	59.6±0.00	98.0±0.00	289±0.00	115±0.00	22.9±0.00	73.7±0.00	55.4±0.00	58.2±0.00	91.4±0.00
Salinity (ppt)	0.10±0.00	0.03±0.00	0.05±0.00	0.13±0.00	0.05±0.00	0.01±0.00	0.03±0.00	0.02±0.00	0.03±0.00	0.04±0.00
Dissolved Oxygen (mg/ml)	4.80±0.00	4.50±0.00	4.90±0.00	4.60±0.00	4.80±0.00	4.70±0.00	4.80±0.00	4.70±0.00	4.80±0.00	4.70±0.00
Total Hardness (mgCaCO3/l)	22.0±0.00	7.00±0.00	5.00±0.00	8.00±0.00	14.0±0.00	7.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	7.00±0.00
Alkalinity (mg/ml)	3.00±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00
Total Suspended Solids (mg/ml)	<0.01±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00	<0.01±0.00
Biochemical Oxygen Demand (mg/ml)	62.0±0.00	84.5±0.00	55.8±0.00	68.5±0.00	49.6±0.00	66.5±0.00	57.4±0.00	65.0±0.00	50.5±0.00	52.0±0.00
Nitrate (mg/ml)	5.05±0.00	4.26±0.00	3.39±0.00	17.4±0.00	6.76±0.00	1.00±0.00	3.58±0.00	1.93±0.00	2.73±0.00	2.85±0.00
Chloride (mg/ml)	24.5±0.00	5.00±0.00	14.5±0.00	25.0±0.00	12.5±0.00	3.00±0.00	8.50±0.00	9.00±0.00	9.00±0.00	12.5±0.00
Calcium (mg/ml)	12.4±0.00	6.94±0.00	6.61±0.00	12.9±0.00	7.33±0.00	5.57±0.00	6.20±0.00	5.64±0.00	4.25±0.00	7.96±0.00
Magnesium (mg/ml)	1.49	0.753	0.754	1.55	1.03	0.819	0.997	0.830	0.722	0.834

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Key: SASA: Station A Water Sample, SASB :Station B Water Sample, SASC: Station C Water Sample, SASD: Station D Water Sample, SASE: Station E Water Sample, SASF: Station F Water Sample, SASG: Station G Water Sample, SASH: Station H Water Sample, SASI: Station I Water Sample, SASJ: Station J Water Sample

Table 7. MAR indices of Bacterial isolates from the water samples

MAR Index	Number (%)			
	Pseudomonas	Enterobacter	Klebsiella	Serratia
0.3	0(0.00)	2(40)	2(40)	0(0.00)
0.4	0(0.00)	0(0.00)	0(0.00)	2(40)
0.5	2(50)	3(60)	3(60)	0(0.00)
0.6	1(25)	0(0.00)	0(0.00)	0(0.00)
0.7	1(25)	0(0.00)	0(0.00)	0(0.00)

DISCUSSION

Microbial Load

The aerobic bacteria (total heterotrophic bacteria) of all the well water samples in this study were very high and exceeds the limits of 1.0×10^2 CFU/mL, which is the limit of aerobic bacteria accepted in water [24]. The level of heterotrophic bacteria in the ten sampled stations varied across the wells. Some of the wells which are major drinking source are covered with metal lids to prevent run off from the ground. Also, the fluctuation and high microbial load could be attributed to the fluctuation in rainfall. Also, the water might be contaminated from the scoop (felting bucket) which is usually used in fetching water. It could also be that these microorganisms got into the well water via activities like talking or coughing especially when fetching water from the well. The heterotrophic bacteria load in this study are higher than the values (1.8×10^4-6.8×10^4) reported by [4] of well water in Khana Local Government Area of Rivers State. The total coliform in this study are above the acceptable/ permissible limits recommended by the world health organization (WHO). The WHO has recommended that the acceptable limit of coliform in drinking water (underground water) should be between 0-10 CFU100/mL, while total faecal coliform should be 0 CFU/100mL [25]. Thus, the well water from the various stations are not suitable for drinking since the counts exceeds the recommended limits.

Microbial Types

The bacterial isolates identified from the various well water samples include; *Klebsiella* sp *Enterobacter sp, Pseudomonas sp,* and *Serratia sp.* Amongst the identified isolates, *Klebsiella* sp *Enterobacter sp,* and *Serratia* sp were the most prevalent organisms in the well water recording frequency of 26.32%. *Pseudomonas* sp were the least predominant isolates with frequency of 21. 05%. Many factors could be responsible for the presence of these microbial types. Wells could be contaminated with fetching buckets (scoop). For instance, out of carelessness, some fetchers drop the buckets on the ground instead of on the concrete floor. Thus, introducing the buckets into the well along with the rope could have contributed to presence of these microorganisms.

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More, so, contamination could arise when leachates sips down into the underground aquifer or when water in dumpsites sips into the underground. Species of, *Enterobacter*, *Serratia* and *Pseudomonas* which are present in this study have been reported by previous studies [4]; [3]; [14]). *E. coli*, *Salmonella* species, and *Klebsiella* sp have been identified in spring water which is a source of drinking water in Ihitte/Uboma of Imo State, Nigeria [11]. With the exception of *Salmonella* which was not identified in this current study, all bacterial isolates in their study are similar to those present in this current study. Thus, the prevalence of gram negative microbes in drinking water especially underground water is well documented. Most of the bacteria identified in this study are of public health importance since they are associated with different types of diseases ranging from food poisoning, boils, skin infections, and urinary tract infections [13]. Due to their prevalence in antimicrobial resistance, they are referred to as emerging problems in the health care (Chelsie *et al.*, 2014).

Antimicrobial Profile

The response to the antibiotics by *Pseudomonas* sp showed that they were highly resistant to Ceftazidime, Nitrofurantoin and Ceftriaxone. They were only susceptible to Gentamycin (Table 2). Also, resistance to Ofloxacin, Augmentin and Cefixime was recorded and were in the order of 50%, 75% and 75%, respectively. Out of the five *Enterobacter* sp subjected to determine their antimicrobial susceptibility, five were completely (100%) resistant to Ceftazidime, Augmentin and Ceftriaxone, while four (80%) were resistant to Gentamycin and Ofloxacin (Table 3). The result also showed that while some of the *Enterobacter* isolates had intermediate response to the antibiotics, none was susceptible to any of the antibiotics (Table 3). The antibiotics susceptibility pattern of *Klebsiella* sp showed that out of the five isolates of *Klebsiella*, five were completely (100%) resistant to Ceftazidime, while four (80%) were resistant to Augmentin and Cefixime, respectively (Table 4). The result also showed that 80% of the isolates were susceptible to ofloxacin, nitrofurantoin and ceftriaxone, while 60% were susceptible to Gentamycin. It is worthy to note that though there was no resistance recorded against ofloxacin, nitrofurantoin and ceftriaxone, 20% had intermediate response. Intermediate response could mean that the *Klebsiella* isolates are developing some sort of resistance towards these antibiotic agents. The susceptibility pattern of *Serratia* sp showed that all the isolates were 100% resistant to Ceftazidime, Augmentin and Ceftriaxone. While only 80% resistance was recorded for Gentamycin. Sixty percent (60%) resistance was recorded for Cefuroxime and Cefixime (Table 5). Also, despite 20% of the isolates being exhibiting intermediate response to Ofloxacin and Nitrofurantoin, 80% of the *Serratia* isolates were completely sensitive (Table 5). The susceptibility pattern of *Klebsiella*, *Serratia*, and *Enterobacter* sp showed that they were all resistant to Ceftazidime and Cefuroxime. As a result of indiscriminate disposal of antimicrobial agents, bacterial isolates could develop or synthesize substances or routes which would confer immunity to antimicrobial agents and they could transmit the resistance to other bacteria in the environment via conduction, transformation or conjunction. This statement agreed [10] and [27]. All the bacterial isolates were resistant to more than two antibiotics. The MAR index of all the isolates were greater than 0.2 (Table 7). Thus, we could posit that greater proportion of these isolates could have resulted from high risk source of environments with high use of antibiotics. The level of resistance in this study could also be drawn from the indiscriminate use of antibiotics, alteration of antibiotic target sites by the bacterial isolates, use of antibiotics in livestock feeds and self-medication. Also, the activities surrounding an environment could be responsible for the level of resistance. For instance, environments were wastes especially wastes

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Comment [A68]: ditto

Comment [A69]: ???

of pharmaceutical products or livestock feeds are carelessly dumped could habour more resistant microorganisms than those environments were such activities are minimized or not practiced. More so, excretory products of live stocks which are fed with feeds containing antibiotics in the environment could be decomposed by a particular organism which in turn could use such substances in building itself against similar agents. [1] reported that the continuous inclusion of antimicrobial agents in feeds for animals could result to the proliferation of zoonotic pathogens which could be selectively resistant to some antibiotics and could be transferred to humans. It is well documented that in other to adapt in an environment, microorganisms try to synthesize substance or modifications that could aid them and most of them are able to pick up resistant DNA in the environment and incorporate it in their DNA, while other bacteria could receive resistance gene from a donor [10]; [13). Furthermore, the bacterial isolates showed varying level of resistance to Ofloxacin. Ofloxacin is considered to be a fluroquinolone antibiotic which possess broad spectrum activities and is used in treatment of bacterial infections of skin, urinary tract, bronchitis, pneumonia, chlamydia and gonorrhea [10]. Resistance of bacterial isolates to fluroquinolones is not new as some studies have reported higher resistance. [11] in a study of the Detection of Vancomycin Resistance among *Enterococcus faecalis* and *Staphylococcus aureus* reported that 79.03% of *Enterococcus faecalis* were resistant to Ciprofloxacin (a fluroquinolone) while 57.7% *Staphylococcus aureus* were resistant to Ciprofloxacin. High resistance to Gentamycin by isolates in this study were also recorded. Gentamycin is an aminoglycoside and carries out its antimicrobial effects by attaching to the 30S ribosomal subunit of the bacteria; thus, altering the proof-reading function which leads to the synthesis of toxic proteins caused by wrong interpretation of the mRNA [28]. Resistance of the bacterial isolates in this study agreed with previous studies [11] and [12].

Physicochemical Parameters

The pH of all the well water across the stations varied from acidic to slight acidity and they ranged between 4.66-6.17. With the exception of the SISA well water station which is within the acceptable limit, all the pH values of the other well water are below the 6.5 – 8.5 and 6.50-7.50 permissible limits of the WHO and NIS, respectively [29]. The pH of the different well stations which were acidic could corrode pipes and iron buckets, produce bad odour in food and drinks and also stain fabrics. This statement agreed with Mwekaven *et al*. [29]. The range of pH in this current study, though slightly acidic are lower than those reported by Obire and Osigwe [12] of spring water, and Mwekaven *et al.* [29] in different well water. The temperature of the well water varied respectively. A study by Charkhabi and Sakizadeh [30] reported a correlation between the pH and temperature of water body. Thus, an increase in temperature causes an increase in the pH and the effect on the pH also affects the dissolved oxygen which affects the amount of BOD available in the water. In this current study, no correlation of temperature and pH was made but the result showed that the temperature varied across the various well water with variations also observed in the pH. The temperature ranges in this current study (Table 6) are less than those reported by previous studies [29, 31; 32]. More so, the increase of the physico-chemical parameters of water above the required limits or out of the range required have been reported to have detrimental effects on health [25]. Thus, all the physico chemical parameters are within the WHO recommended limits. According to Mwekaven *et al.* [29] there are no recommended standards for DO and BOD for water. However, the DO in this study are

higher than the 2.00-4.00Mg/L reported by [29] and lower than the 9.24 mg/L to 9.34 mg/L reported by Ajit and Padmakar [33].

Conclusion

The well water from the different stations are not safe for drinking as microbial loads as well as coliform values exceeded the acceptable limits. More so, the bacterial isolates as presented in this study could be pathogenic especially when the water in this area are consumed without proper treatment. Diseases ranging from gastroenteritis to urinary tract infections and other cases of infections could be prevalent especially to consumers of untreated water from these locations. Furthermore, the level of antimicrobial resistance exhibited by bacterial isolates in this study is a cause for alarm.

Recommendation

We therefore recommend that well water should be properly treated, water could be boiled and stored in clean containers. Strict hygiene which would include covering of wells, not washing close to wells and spitting inside wells should be practiced. It would also be of immense help if treated pipe borne water sources are made available in this communities. After all, safe drinking water is the right of all.

REFFERENCES

1. Adeleke, E.O. and Omafuvbe, B.O. (2011). Antibiotic Resistance of Aerobic Mesophilic Bacteria Isolated from Poultry Faeces. *Research Journal of Microbiology*, 6 (4): 356-365.
2. Adogo L.Y., Anyanwu N.C.J., Ajiji M.A., and Bukola Ajide (January, 2016). Bacteriological and Physio-chemical analysis of Borehole Water in Auta Balifi Community, Nigeria, *British Microbiology Journal* 11(4):1-7.
3. Augustín, L., Adriana, L. S., Pedro, M. R. and María, M. S. (2014). Assessment of the Microbiological Quality of Groundwater in Three Regions of the Valencian Community (Spain). *International Journal of Environmental Research and Public Health;* 11: 5527-5540.
4. Azuonwu, O., Azuonwu, T. C. and Nwizug, W. L. (2017). Evaluation of Bacteriological Quality of Surface, Well, Borehole and River Water in Khana Local Government Area of Rivers State, Niger Delta. *Annals of Clinical and Laboratory Research;* 3: 183.
5. Fair, R. J and Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st Century. *Perspect Medicin Chem.* 6:25-64.
6. Guidelines for drinking-water quality: fourth edition incorporating the first addendum.Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO.
7. Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, S. T. (1994). Bergey's Manual of Determinative Bacteriology, Williams and Wilkins, Baltimore, Maryland, USA; 151 – 157.
8. Monica Cheesbrough(2006). Biochemical Test to Identify Bacteria. District Laboratory Practice in Tropical Countries, Second Edition;7;315-317
9. Obafemi O. Olubanjo, Alade E. Adebolu and Olubanjo M. Abosede (2018). Bacteriological. Assessment of Borehole and Wells Water in Akungba-Akoko, Nigeria, *International Journal of Agriculture, Environment and Bioresearch* 3(6):2456-8643.
10. Obioma A, Chikanka AT, Loveth NW (2017) Evaluation of Bacteriological Quality of Surface,
11. Well, Borehole and River Water in Khana Local Government Area of Rivers State, Niger Delta. Ann Clin Lab Res. Vol.5:No.3:183.
12. Obire, O and Osigwe, I.S. (2016). Bacterial Quality of Spring Water in Ihitte/Uboma LGA of Imo State, Nigeria. *Current Studies in Comparative Education, Science and Technology*, 3(2):149-155
13. Palamuleni, L. and Akoth, M. (2015). Physico-Chemical and Microbial Analysis of Selected Borehole Water in Mahikeng, South Africa. *International Journal of Environmental Research and Public Health*; 12: 8619-8630.
14. Prescott, L.M., Harley, J. and klein, D.A. (2011). Microbiology 8th.ed, McGraw-Hill New York. pp. 809-811.
15. Ramya, R., Shanthi, M., Uma, S. and Arunagiri, K. (2016). Detection of Vancomycin Resistance among *Enterococcus faecalis* and *Staphylococcus aureus. Journal of Clinical and Diagnostic Research*; 10(2): 4-6.

16. Rajini Kuruf, Roland Persaud, John Ceaser and Vincent Raja (2010). Microbiological and Physiochemical Analysis of Drinking Water George Town, Guyana, *Nature and Science* 8(8):261-265.
17. Shittu, A.O. and Lin, J., (2006). Antimicrobial susceptibility patterns and characterization of clinical isolates of Staphylococcus aureus in KwaZulu-Natal province, South Africa. BioMed Central Infectious Diseases, 125–126.
18. Suely, A.P.F., Erica, M.D.S., Patricia, F.S., Paola, C.L., and Lúcia, M.T. (2007). Antimicrobial
19. resistance profiles of enterococci isolated from poultry meat and pasteurized milk in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz, Rio de Janeiro, 102(7): 853-859.
20. Sur D, Sarka BL, Dean J, Delta S, Niyogi SK, et al. (2006) Epidemiological, microbiological and electron microscopic study of a cholera outbreak in a Kolkata slum community. Indian J Med Res 123: 31-36.
21. Wemedo, S.A., Obire O. and Akani, N.P. (2012). Bacterial Population of an Oilfield Wastewater in Nigeria. *Asian Journal of Biological Sciences;* 5: 46-51.
22. WHO (2003) Guidelines for drinking water quality (3rd edn) WHO, Geneva, Switzerland.
23. Wemedo, S. A. and Robinson, V. K. (2018). Evaluation of Indoor Air for Bacteria Organisms and their Antimicrobial Susceptibility Profiles in a Government Health Institution. Journal of Advances in Microbiology; 11(3): 1-7.
24. EPA, (2002). US Environment Protection Agency, Safe Drinking Water Act Ammendment http:// www. epa. gov/safe water /mcl. Html
25. World Health Organization (WHO). (2011). Guideline for drinking water quality 4 Edition, WHO, Switzerland; 156.
26. Chelsie, E. A., Sara, N. S., Alejandra, Y. and Harry, L. T. M. (2014). Increased Incidence of Urolithiasis and Bacteremia During *Proteus mirabilis* and *Providencia stuartii* Coinfection Due to Synergistic Induction of Urease Activity. Journal of Infectious Diseases; 209(10): 1524–1532.
27. Dora, N., (2011). Methicillin Resistant *Staphylococcus aureus* (MRSA) A Global Threat.
28. Tormo, R., Gonzalo, M.A., Munoz, A.F. and Silva, I. (2012). Monitoring the occurrence of indoor fungi in a hospital. *Revista Iberoamericana de Micología* 29(4):227–234.
29. Mwekaven, S.S., Aorkwagh, M.T., Gundu, E.G. and Yange, T. (2017). Physico-Chemical and Microbiological Analysis of Well Water Stations In Settlements around Akperan Orshi College of Agriculture, Yandev. *International Journal of Science and Technology;* 6: 1.
30. Charkhabi, A.H. and Sakizadeh, M., (2006). Assessment of Spatial Variation of Water Quality

31. Aderibigbe, S.A., A.O.Awoyemi and Osagbami, G.K. (2008). Availability, Adequacy and Quality of water supply in Ilorin Metropolis, Nigeria. European. J. Sci. Res. 23 (4): 528-636.
32. Baird, C. and Cann, M. (2004). Environmental Chemistry (3rd edition). W.H. Freeman, USA.
33. Ajit, M. K. and Padmakar, A. S. (2012). Determination of Physico-Chemical Parameters of Deoli Bhorus Dam water. *Advances in Applied Science Research;* 3 (1):273-279.
34.