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Bacterial Antibiogram and Physicochemical Parameters of Well Water in Iworoko-Ekiti, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AGO and FOO designed the study and wrote the protocol. Author AGO carried out the laboratory work, data analysis and wrote the first draft of the manuscript. Author FOO monitored the experimental procedures and handled the critical revision of the manuscript. Author KJA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Water is very essential for human survival. In fact, water defines some countries; it is so important that nations which do not have high quality and enough quantity have gone to war over it. However, as important as water is, not all water is drinkable.
Objective: The objective of the study is to determine the potability of some selected raw well water sample in Iworoko-Ekiti, Nigeria.
Methodology: Routine bacteriological analyses of the water samples were carried out to identify and quantify the bacteria present in them. Antibiotic sensitivity pattern of the isolated bacteria was also determined using pour plate method. Assessment of physicochemical parameters (pH, temperature, total dissolved solids, conductivity, turbidity, biochemical oxygen demand, dissolved oxygen) and mineral constituents (Ca^{2+}, Na^{+}, Mg^{2+}, Pb^{2+}, Cu^{2+} and Cr^{+}) were carried out on the water samples using standard chemical methods.
Results: The results of bacterial water analyses revealed that the bacteria with highest percentage of occurrence were *Klebsiella pneumoniae* (41.40%) followed by *Escherichia coli* (19.54%) while the least value (5.75%) was recorded against *Staphylococcus aureus.* Antibiotics susceptibility test

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showed zones of inhibition that ranged from 3.33±0.33 mm to 28.67+1.15 mm against *Bacillis subtilis* and *Proteus mirabilis* respectively. Physicochemical tests revealed that the pH of the water samples ranged from 5.4 to 6.3. Also, the conductivity ranged from 54 us/cm -743 us/cm. Highest biochemical oxygen demand (BOD) recorded was (2.40 mg/L) whereas lowest value was 0.80 mg/L. Sulphate concentrations of the well water samples ranged from 777.92 mg/L to 1078.28 mg/L. Highest value recorded in sodium content was 130.00 mg/L while the least was 70.24 mg/L. Potassium also had highest value of 162.10 mg/L against 71.30 mg/L which was the least.

Conclusion: This study revealed that the well waters are not fit for drinking purpose due to the presence of some pathogenic bacteria that could cause illness.

Keywords: Wells; water; physicochemicals; antibiotics; pathogens.

1. INTRODUCTION

Water is indispensable to all lifeforms even though it provides no calories or organic nutrients [1]. Apart from its usefulness in food preparation, drinking and cleaning purposes such as washing and bathing, it is equally useful for irrigation, sport and cultural purposes. In fact, water is believed to be the second most essential nature-endowed resources after air [1]. About 70% of the earth surfaces are covered by water and it can be tapped from different sources; from atmosphere inform of precipitates [2], on earth surfaces such as rivers, springs, lakes [3] or the underground: wells and tube wells [4]. As important as water is, not all waters are drinkable. Therefore, the quality of drinking water supply has critical impact on the health of the people that depend on it as it plays a vital role in the spreading of various diseases when the source is faecally polluted. It can be an important vehicle in spreading various dangerous diseases like hepatitis, cholera, dysentery, typhoid and diarrhea [5]. This makes it imperative that thorough microbiological and physiochemical examinations should be conducted on the raw water samples to determine its potability. Potable water is water that is free from disease causing microorganisms and chemical substances that are dangerous to health [6].

In Nigeria, providing potable water for all the citizens at this present time is seemed to be unrealizable. State owned drinking water reservoirs that distribute water are limited to some urban cities and the supply is usually epileptic [7]. Majority of the rural populace do not have access to potable water and therefore, depend on well, stream and river waters for domestic uses. Consequently, people who could not access pipe borne water and depended on well waters as their source of water supply are still becoming ill of gastrointestinal infections. In such case, they resort to antibiotics for treatment, which are fast losing their effectiveness. Ayandiran et al. [8] had reported the antibiotic resistance of bacterial isolates from drinking water source in Igbokoda, Nigeria.

However, World Health Organization recommended that before human consumption, drinking water should be treated, free from toxic chemicals and pathogenic microbes [9]. But in Nigeria, drinking water treatment is almost rarely done before consumption especially for the private wells and no such international or national standards are followed for physiochemical and bacteriological analysis of this drinking water. Federal Ministry of Water Resources reported that only about sixty percent of Nigerians have access to potable water [10]. The supply and sanitation coverage rates in Nigeria are amongst the lowest in the world. The country was said to be characterized by low levels of access to an improved water source and limited access to improved sanitation (Water supply and sanitation in Nigeria, Wikipedia). Therefore, a lot of people still become sick as result of drinking contaminated water and all these factors are applicable to Iworoko-Ekiti, a suburb of Ado-Ekiti where the environment is under study. The aim of this study is to determine the antibiotic susceptibility pattern of bacterial pathogens and physicochemical parameters of selected well water from Iworoko-Ekiti, Nigeria.

2. METHODOLOGY

2.1 Study Area and Sampling

Iworoko-Ekiti is located at the fringe of Ado-Ekiti, beside Ekiti State University and inside it are privately owned students' hostels. Its geographical coordinates are 7° 44' 0" North, 5° 16' 0" East and falls under rain forest vegetation belt. The community was divided into four zones; Are-Ekiti road area (AR), Idogun street area (DG), Abebi area (BB) and Odo-odo street area

(DD). Four wells were randomly selected with one from each zone. Well water forms the major source of drinking water in these areas and most of the wells under study were privately owned which are usually open to general public. All the studied wells were covered however; they were all close to one source of contaminant or the other such as surface water, refuse dumpsite or septic tank. The four-litre containers that are used as fetcher to draw water from these well are usually left on the wells. All the wells are not less than 10 years old and depth are between 1.83 m to 2.74 m.

(maplandia.com)

2.2 Collection of Well Water Samples

The water samples were collected using the same method as inhabitant normally used. For bacteriological water analysis, contamination of the water samples was avoided before and after sampling by collecting the samples in clean, sterile 100ml screw-cap bottles. The samples were labeled to indicate the sources from which they were collected. They were transported to Akure in an ice-pack container to the Microbiology Postgraduate laboratory Obanla Campus, Federal University of Technology Akure. The bacterial examination of the well water samples and the identification of the different species of the bacteria in the analyzed water samples were carried out as promptly as possible in the laboratory.

2.3 Bacteriology

The bacteriological enumeration of the well water samples were done after serial dilution using pour-plate method. Bacterial plate counts were carried out using Nutrient agar (NA), MacConkey agar and Eosin Methylene Blue agar (EMB) for the isolation and enumeration of enteric coliforms as described by Barrow and Feltham [11]. All the media were prepared according to the manufacturer's instruction. All plates were incubated at 37℃ for 24 hrs and plates were counted with colony counter to obtain the colonies counts of the viable bacteria.

2.4 Characterization and Identification of Isolates

Pure cultures of the bacterial isolates were observed for morphological characteristics, gram-stained and were subjected to various biochemical tests; catalase, oxidase, coagulase, citrate, Methyl Red, Voges-Proskaeur, motility and indole tests as well as sugar fermentation test as described by Olutiola et al. [12].

2.5 Antibiotic Sensitivity Pattern

The antibiotic sensitivity test was carried out in order to compare the sensitivity of the microorganisms to the different commercially available antibiotics. Sterile Petri dishes were seeded aseptically with 1 ml each of the standardized broth cultures of the test isolates from water samples. A volume 15 ml of sterilized Mueller Hinton agar was poured aseptically on the seeded plates. The plates were swirled carefully for even distribution and allowed to gel. With the aid of sterile forceps the antibiotic discs were placed firmly on solidified plates and incubated for 24 hours at 37℃. After incubation, clear areas around the discs were measured in millimeter (mm). Unseeded agar plate with antibiotics served as the control experiment.

2.6 Physiochemical Parameters of the Well Water Samples

<u>2.6.1 Determination of physiochemical parameters of the well water</u>

The physiochemical parameters were carried out using HANNA multi-parameter H19828. The parameters determined include: pH, temperature, conductivity, turbidity, total dissolved solid (TDS), biochemical oxygen demand (BOD), total suspended solid (TSS), dissolved oxygen (DO) [13]. For the ion concentrations determination, the water samples were digested with concentrated nitric acid prior to use and were quantified using Atomic Absorption Spectrometer according to Ademoroti [14]. The ion and heavy metals determined include Cl^{-} , Na^{+}, Ca^{2+}, Mg^{2+}, SO_4^{2-} , Cr^{1}, Cd^{+2}, Pb^{+2}, Fe^{+2} and Cu^{+2} metals were all analysis using atomic absorption spectrophotometer.

2.6.2 Determination of Minerals and Heavy Metals in the Water

All the water samples were first digested with concentrated Nitric acid (HNO_3) and pre concentrated before analysis using Atomic Absorption spectrophotometer (AAS) Alpha 4 model. About 100 ml of well-mixed water sample was measured into a 250 ml beaker and 10ml conc. HNO_3 was added. The solution was evaporating to near dryness on hot plate under a medium heat (solution not allowed to boil). The beaker with the content was allowed to cool to room temperature after which another 10ml portion of conc. HNO_3 and 5 ml H_2O_2 were added. The beaker was immediately covered with a watch glass and returned to the hot plate and heated under a gentle reflux action. This was continued until a whitish residue was obtained. The residue was dissolved with 5 ml conc. HNO_3 and some quantity of distilled water. The solution was then filtered after cooling through Whatman paper N0 42 into a 25 ml volumetric flask and made to the mark with distilled water. The solution was then transferred into a polythene bottle prior AAS analysis [14].

2.7 Statistical Analysis

Data obtained were presented as mean ± SE (standard error). Significance of difference between different treatment groups was tested using one-way analysis of variance (ANOVA) and significant results were compared with Duncan's multiple range tests using SPSS window 7 version 1.6 software. For all the tests, the significance was determined at the level of $P<0.05$.

3. RESULTS

The quantity of frequency of occurrence of each bacterium isolated is shown in Table 1. *Klebsiella pneumoniae* and *Escherichia coli* were the most frequently encountered pathogens present in all the water samples with 41.40% and 19.54% respectively. *Pseudomonas aeruginosa* occurrence totaled 9 (10.34%) of the isolates and *Salmonella typhi* had 8.33%. *Staphylococcus aureus* recorded 5.75% while both *Proteus mirabilis* and *Bacillus substilis* have percentage quantity of 6.90% each. The antibiotics susceptibility profiles of the bacterial isolates are shown in Fig. 1. All the bacterial isolates were susceptible to ciprofloxacin (CPX) with *Proteus mirabilis* being the most susceptible (28.67±1.15 mm) followed by *K. pneumoniae* 20.00±0.00 mm. *Bacillus substilis, P. aeruginosa, S. typhi*, and *S. aureus* were all resistance to ampiclox (APX) but *E. coli, K. pneumoniae* and *P. mirabilis* were only susceptible with mean value of 10.67±1.15 mm inhibition. With zinnacef (Z), only *P. mirabilis* and *E. coli* were sensitive to the antibiotic (12.00±0.00 mm and 8.00±0.00 mm respectively) while other isolates; *P. aeruginosa, K. pneumoniae, S. typhi, S. aureus* and *B. substilis* were resistant. Rocephin(R) recorded 6.00±0.00 mm and 11.67±0.58 mm inhibition against *B. substilis* and *S. aureus* respectively. Pefloxacin (PEF) demonstrated good antibacterial activity against the bacterial isolates with inhibition ranging from 12.33±0.58 mm to 27.33±1.53 mm in *K. pneumoniae* and *E. coli* respectively. Erythromycin (E) exhibited mild activity with zone of inhibition that ranged from 4.00±0.00 mm to 14.33±0.58 mm correspondingly with *P. aeruginosa* and *K. pneumoniae*.

The physicochemical analysis of the water samples are shown in Table 2, the pH of the water samples ranged from 5.4 to 6.3 while the turbidity ranged from 3.54 – 6.00 NTU. Also, conductivity ranged from 54-743 us/cm. Sample BB (Abebi area) has the lowest conductivity of 54 us/cm while sample AR (Are-Ekiti road area) has the highest conductivity of 743 us/cm. The total dissolved solids ranged from 30.00 – 328.00 mg/L, while total suspended solid ranged from 0.08-0.42 mg/l. Highest BOD was recorded in AR (2.40 mg/L) whereas lowest value was recorded in BB (0.80 mg/L). Dissolved oxygen of the water samples range from 6.20 to 8.60 mg/L. Higher sulphate concentrations observed in the water sample range from 777.92 to 1078.28 (mg/L) with the exception of water sample from Idogun street area (DG) where low value (403.37 mg/L) was recorded. The value for Total hardness was observed to be highest in sample from Are-Ekiti road area (AR) (250 mg/L) while that of Abebi area (BB) had the least (10 mg/L).

Table 1. Frequency of occurrence of the bacterial isolates

SN	Bacterial Isolate	AR	DD	DG	BB	Total	Percentage (%)	WHO
1	*Klebsiella pneumoniae*	12	8	10	6	36	41.40	0
2	*Escherichia coli*	5	5	4	3	17	19.54	0
3	*Salmonella typhi*	6	0	2	0	8	9.20	0
4	*Proteus mirabilis*	0	0	4	2	6	6.90	0
5	*Pseudomonas aeruginosa*	5	4	0	0	9	10.34	NES
6	*Bacillus substilis*	0	0	6	0	6	6.90	NES
7	*Staphylococcus aureus*	3	0	0	2	5	5.75	NES
	Total	31	17	26	13	87	100	

** NES- No established standard*

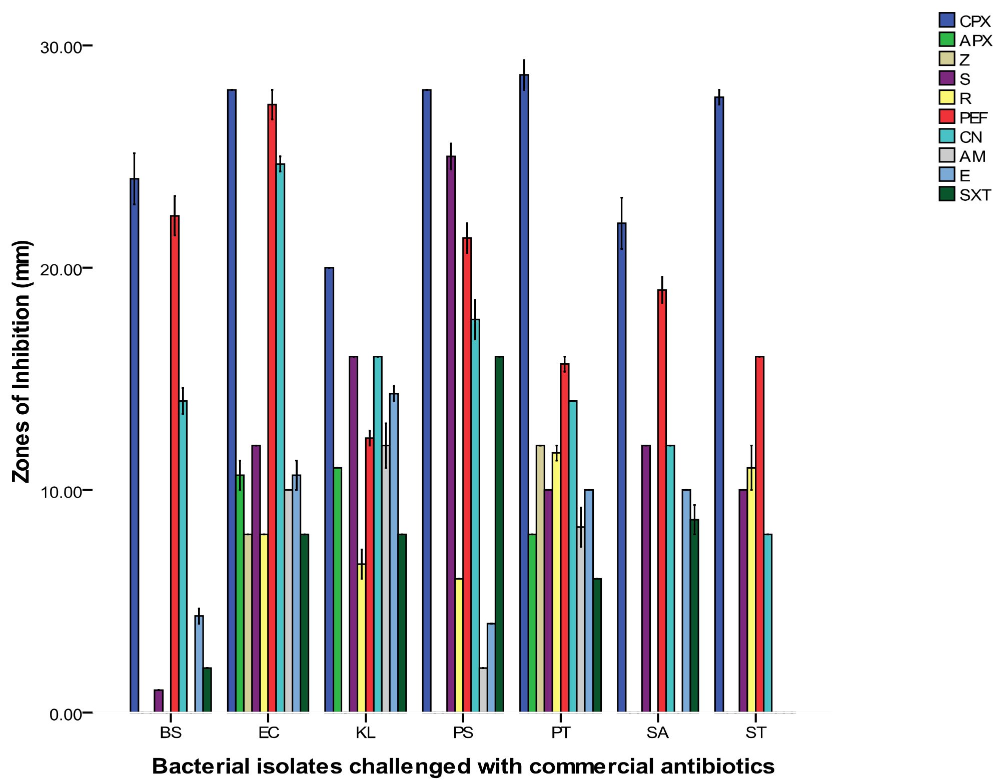

Fig. 1. Antibiotics susceptibility profile of the bacterial isolates (positive disc)

Legend: CPX-Ciprofloxacin; APX-Ampliclox; Z-Zinnacef;S-Streptomycin; R-Rocephin; PER-Pefloxacin; CN-Gentamycin; AM-Amoxacillin; E-Erythromycin; SXT-Septrin; BS-Bacillus subtilis; EC-Escherichia coli; KL-Klebsiella pneumoniae; PS-Pseudomonas aeruginosa; PT-Proteus mirabilis; SA-Staphylococcus aureus; ST-Salmonella typhi

Data presented in Table 3 revealed the mineral composition of the water samples. The sodium content of the water samples ranged from 70.24 mg/L to 130.00 mg/L. Highest value of potassium was recorded in DG (162.10 mg/L) and the least was 71.30 mg/L (AR). Phosphorous contents recorded in all the well water samples were exceptionally high, with (AR) containing about 50% of the total phosphate component (12.90 mg/ml) whereas Nigeria Standards for Drinking Water Quality (NSDWQ) limit is 0.1 mg/ml. Calcium was also observed to be highest in DG (48.8 mg/L) but lowest in AR (43.60 mg/L). Magnesium component of the water samples valued highest in BB (57.10 mg/L) whereas the lowest was 30.54 mg/L (AR). Highest iron content was found in BB (0.54 mg/L) but lowest was obtained in AR (0.12 mg/L). Heavy metals such as Zinc, Copper, Lead and Cadmium were not detected in all the water samples. Similarly, Chromium was not detected in all the water samples except in BB (0.01 mg/L).

Table 2. Physicochemical parameter of the well water samples

Parameter	AR	DG	DD	BB	NSDWQ/WHO
Colour	Unobjectionable	Unobjectionable	Unobjectionable	Unobjectionable	Unobjectionable
Odour	Unobjectionable	Unobjectionable	Unobjectionable	Unobjectionable	Unobjectionable
Temperature (℃)	30.50	32.50	33.60	28.00	Ambient
pH Range (mol/l)	6.10	6.30	6.20	5.40	6.50-8.50
Conductivity (ms/cm)	743.00	270.00	234.00	54.00	1000
Turbidity (NTU)	5.26	3.84	4.27	3.54	5.00
Total dissolved solid (mg/ml)	328.00	144.00	131.00	30.00	500
BOD (mg/ml)	2.40	2.20	1.60	0.80	4.00
Total suspended solid (mg/ml)	38.42	60.08	56.14	40.14	NS
Dissolved oxygen (mg/ml)	6.20	8.60	6.80	7.50	7.00
Hardness (mg/ml)	250.00	40.00	80.00	10.00	150
Sulphate (mg/L)	1078.28	403.37	1008.40	777.92	250
Chloride(mg/L)	200.13	186.32	160.08	102.07	250

Table 3. Mineral composition of the well water samples

Minerals	AR	DG	DD	BB	NSDWQ/WHO
Sodium (mg/L)	110.00	130.00	100.40	70.24	200
Potassium (mg/L)	71.30	162.10	97.54	72.00	100
Calcium (mg/L)	43.60	48.80	45.30	46.45	200
Phosphorus (mg/L)	12.90	3.94	3.67	4.21	0.10
Magnesium (mg/L)	30.54	43.80	49.40	57.10	0.20
Zinc (mg/L)	0.00	0.00	0.00	0.00	3.00
Copper (mg/L)	0.00	0.00	0.00	0.00	1.00
Iron (mg/L)	0.12	0.15	0.18	0.54	0.30
Lead (mg/L)	0.00	0.00	0.00	0.00	0.01
Cadmium (mg/L)	0.00	0.00	0.00	0.00	0.03
Chromium (mg/L)	0.00	0.00	0.00	0.01	0.10

4. DISCUSSION

This study revealed that all well water samples from the locations under study were contaminated with two or more pathogenic bacteria. Bacterial pathogens isolated from the well water samples were *E. coli, Klebsiella pneumoniae, Proteus mirabilis*, *Pseudomonas aeruginosa, Bacillus substilis, Salmonella typhi* and *Staphylococcus aureus* According to World Health Organization, some of these pathogens are mainly of feacal origin and any water source that are being used for drinking or cleaning purpose should not contain any of the bacterial pathogens [9]. The fact that the coliform bacteria occurrence exceeded the zero total coliform per 100 ml of water for drinking water standard recommended by World Health Organization rendered the water unfit for consumption. Edema et al. [15] had earlier reported that the bacterial qualities of groundwater and pipe borne water in Nigeria were not satisfactory.

The conductivity, total solids, total dissolve solids, total suspended solids and the chloride contents as revealed by the physiochemical analysis of the well water samples showed that the parameters fell within the limits set by both the national and International standard regulatory bodies for drinking and domestic water uses. The biochemical oxygen demand (BOD) and dissolved oxygen (DO) of the well water samples as well complied with W.H.O's and NSDWQ standards. Heavy metals such as chromium, cadmium, copper and lead were absent, indicating that the water is wholesome. Wholesome water is defined as one not necessarily pure, but should not endanger health [16,17]. However, the pH of one of the well water fell within acidic range based on the general classification of water according to

Environmental Protection Agency [18]. This corroborates the report of Adediji and Ajibade [19] who documented low pH values in some well waters in Ede, Nigeria. The low pH could be as a result of leaching of metallic ions from the surrounding soil and dump sites to the aquifer [20]. Low pH of water can cause eye and gastrointestinal irritation, exacerbation of skin disorders as well as corrosion of metals [21]. The problem of low pH water can be treated by adding soda ash, a neutralizer to prevent the water from reacting with the house plumbing.

For the sulphate content, high concentrations were found in all the wells. The high sulphate contents found in the well water concur with the record of Loneragan et al. [22], who reported high sulphate level in well water. Minnesota Pollution Control Agency also reported that high solubility of sulphate in water was responsible for its elevated concentrations in many well waters [23], aquifers and surface water. High level of sulphate greater than 250 mg/L has been said to be associated with physiological effects such as diarrhoea, gastrointestinal irritation and dehydration in children and transients following consumption of drinking water with high sulphate concentration [24]. For a safety measure, water with sulphate level above 250 mg/L should not be used in preparation of baby food. Reverse osmosis (RO) can be used in treating sulphate problems in water [25].

The unusual high phosphorus concentrations that were observed in all the samples is an indication of pollution from the environment which could be linked to the detergents used around the wells for cloth and dishes washing as observed during the water samples collection. Too much phosphorus consumption can cause health problems, such as kidney damage and osteoporosis [26]. Phosphorus found in the environment mostly occurs as phosphates. The concentration of iron ion recorded in Egunlusi villa was higher than permissible limit (0.3 mg/l). The reason for the high level of iron could be as a result of its ions that percolate from the deposit in the soil or rock into the well by the drainage action of rainfall. More also, it could be connected to corrosion of iron or steel well casing [27]. In most cases, high concentrations of iron in well water do not normally cause any health problem but lead to the browning of the water. However, the presence of this ion in well water may present health problems when iron reducing bacteria such as *Ferrobacillus feroxidans* bacteria gains access to the well [27].

Antibiotics sensitivity pattern determined showed that some of the bacterial isolates were susceptible while others were resistance. The most effective of all the antibiotics was ciprofloxacin. There was a sweeping susceptibility to it by all the bacterial isolates. Ciprofloxacin potency could be attributed to its broad spectrum activity against both gram-positive and gram-negative bacteria [28,29] Perfloxacin demonstrated similar antibacterial potency like unto ciprofloxacin, inhibiting all the isolates. This is in resonance with the work of Ait-Khaled et al. [30], Zahid et al. [31] who reported the efficacy and safety of Perfloxacin in the treatment of bacterial infections. Ciprofloxacin and Perfloxacin belong to the same group of floroquinolone antibiotic hence having the same mode of action [32,33]. With Ampicillin and zinnacef, *B. substilis, P. aeruginosa, S. typhi*, and *S. aureus* were resistant to the antibiotics. This is in line with the reports of documented by and Alam et al. [34,35] about the resistance of the aforementioned test isolates to zinnacef and ampicillin respectively. The two antibiotics belonging to the penicillin group of beta-lactam that inhibits the third and final stage of bacterial cell wall synthesis in binary fission, which ultimately leads to cell lysis [36,37]. However, this beta-lactam group of antibiotic is faced with challenge of resistance to some bacteria.

In this study, *Pseudomonas aeruginosa* and *Salmonella typhi* were not sensitive to chloramphenicol which concurs with the investigation of Li et al. [38] and Bolton et al. [39] who also reported antibiotic resistance of *P. aeruginosa* and *S. typhi* to chloramphenicol respectively. Streptomycin demonstrated good antibacterial activity against *E. coli*, *K. pneumoniae*, *Proteus* and *P. aeruginosa*. However, *Bacillus*, *S. aureus* and *S. typhi* were resistant to Streptomycin. Gentamicin displayed good antibacterial activity against all the test isolates. The antibiotic is of aminoglycoside group which are toxic to the sensory cells of the ear, and can cause permanent deafness as reported by Pandya et al. [40]. Shehab et al. [41] wrote that all antibiotics are not without their attending side effects. Moreover, this toxicity limits its clinical use according to Robert and Melanie [42]. With Septrin, *Staphylococcus aureus, Salmonella typhi* and *Bacillus substilis* were not susceptible to the antibiotic but showed gentle activity against other test bacteria. Septrin was once used as effective drug for the treatment of a variety of bacterial, fungal and protozoal infections such as urinary tract infections (UTIs) and acute uncomplicated bacterial cystitis (AUBC) in women [43]. Conversely, there are reported that problematic uropathogenic resistance to co-trimoxazole (Septrin) are on increasing worldwide [43].

The resistance of bacteria to the antibiotics could be blamed on misuse of drugs which is common in the developing countries due to poor access to qualified physicians. Besides this, drug abuse, inappropriate prescription by physician or bacterial genetic mutation/transduction of resistance gene from other microorganisms could also occasion the resistance of bacteria to antibiotic [44].

5. CONCLUSION

The bacteriological water quality of some privately owned student hostels in Iworoko-Ekiti that were examined revealed that the well waters are not fit for drinking purpose due to the presence of some pathogenic bacteria that could cause illness. However, most of the physiochemical parameters of the well waters fall within the recommended limits set by the national standard regulatory bodies for drinking water, with few exceptions in which higher concentration of sulphate and lower pH were recorded. This signals that the water is a potential potable water source for the people.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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