Assessment of Antibiotic Resistance in Groundwater Bacterial Strains: A Community-based Study

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Authors' contributions

This work was carried out in collaboration among all authors. Author ENF conceptualized and designed the study and wrote the initial draft of the manuscript, Author SMO collected and managed the literature searches and author SOF performed the data analysis. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: The microbial quality of private and community water supply from groundwater is mostly not monitored nor assessed by any established government agencies in Nigeria. Contamination by pathogenic and antimicrobial resistant microbial agent, and the potential of resistant genes transfer from environmental bacteria to human pathogens is a major risk for public health in these water systems.
Aim: This study was aimed at evaluating the microbiological quality and safety in terms of antibiotic resistance bacterial profile of groundwater sources in a typical peri-urban settlement in Nigeria.

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Methodology: Bacterial contaminants were isolated by membrane filtration technique, characterized and analyzed using standard microbiological approach.

Results: A total of 22 and 8 Gram negative and positive bacteria respectively were isolated. The isolates comprised 7 bacterial genera with *Klebsiella* and *Enterobacter* species having the highest incidence of 20% respectively, followed by *Bacillus* (17%) and least being *Shigella* with 7% incidence. All the isolates were 100% resistant to cefotaxime, followed by 76.7% and 73.3% resistant to vancomycin and meropenem respectively. The least resistance of 3.3% was against ciprofloxacin. Resistance in borehole storage tank water was highest (80%) against vancomycin followed by 70% for meropenem, while 75% resistance was against vancomycin and meropenem respectively in the well water, Seventy-three percent (73%) of the isolates showed multidrug resistance (MDR) pattern, and a multiple antibiotic resistant index (MARI) ≥ 0.3.

Conclusion: The results of this study suggest that groundwater source in the studied community is contaminated with antibiotic resistant bacteria and underscores the public health implication and concern for monitoring of groundwater supply in Nigerian communities.

1. INTRODUCTION

Water supply to world populations especially in Africa, remains a critical public health concern. This has been attributed to factors such as rapid rise in population and its increased pressure on the limited fresh water resources; urbanization, and industrialization and the resultant pollution (Kaiser RA et al, 2022). Lack of accessibility to safe drinking water and sanitation in developing world, particularly in Africa, continues to be a major challenge in the attainment of UN Sustainable Development Goal (SDG) number six which is aimed at ensuring the availability and sustainable management of water and sanitation for all (Chitonge H et al, 2020).

Groundwater is considered as the major source of freshwater to billions of populations globally and is the most vital source of public water supply in many regions of the world (Szekeres E et al, 2018, Zainab SM et al, 2020). Domestic water is generally supplied to homes through private or public water supply companies. Water supplied by public water companies is considered safe to drink and does not pose a health risk; since the quality of the water supplied is periodically checked. Private water source on the other hand, comprising hand dug wells and boreholes, is also usually safe to drink but can be contaminated and is not usually monitored. Groundwater quality is threatened by anthropogenic activities, mainly from industrial and agricultural sources, because there is the possibility of several pollutants getting to the aquifers (Kaiser RA et al, 2022, Zainab SM et al, 2020). Contamination can be by pathogenic bacteria resulting from leakages in faulty septic tanks and cesspools, agricultural soil run offs or runoffs from hospital environment (WHO, 2017, Correa-Martinez CL et al, 2020). In peri-urban communities in particular, groundwater contamination by pathogenic and antibiotic resistant bacteria is mostly by certain practices such as use of pit latrines and sometimes septic tanks onsite sanitation system for the management of human waste onsite, as well as the 'bush method' (Twinomucunguzi FRB et al, 2020). This problem is exacerbated by proximity and unlined or semi-lined cesspits which increases the probability of contamination by leakages to the groundwater aquifer (Twinomucunguzi FRB et al, 2020).

In Nigeria, 71 million of the over 203 million population lack access to clean water and 130 million lack access to basic sanitation (RTI International). Public water supply in Nigeria is through the State Water Board (SWB) which has been moribund or hampered by nonexistent or weak incentives for better performance, inadequate or ineffective operations and maintenance, aging infrastructure, weak institutional capacity, and little accountability to consumers (RTI International). Hence private water source has been most prevalent. Individual households and communities have resorted to hand dug wells and boreholes for water provision; the main difference between both being the vertical depth (Ighalo JO and Adeniyi AG, 2020).

A major risk for public health in water systems is the contamination by pathogenic and antimicrobial resistant microbial agent, and the transfer of resistant genes from environmental

bacteria to human pathogens (Correa-Martinez CL et al, 2020, Oliveira M et al, 2021). The potential of drinking water to transport microbial pathogens to community populations, and the consequent illness, is well documented (Carmen MED et al, 2016, Serwecińska L, 2020). Water acts as the most important mode for bacterial dissemination and spreading of antibiotic resistance between man and environment (Serwecińska L, 2020, Hossain MZ et al, 2017, Tangwa B et al, 2019). Antibiotic resistant bacteria (ARB) in water are potential sources of antibiotic resistance gene (ARG) transfer to humans through direct consumption and indirectly through food chain (Hossain MZ et al, 2017). The human population are vulnerable to harmful infection with antibiotic resistant pathogens, and/or ARG if in contact with ARB contaminated water, through water related activities including drinking, swimming, and washing. The resistance carried by commensal or environmental bacteria could be transmitted through horizontal gene transfer (HGT) (Oliveira M et al, 2021). This undermines the ability to prevent and control infectious diseases, failure in the disease treatment, prolonged hospital stay, and thus pose a great threat to public health (Pereira AL et al, 2022).

Potability of water can only be determined by monitoring and assessing the water, including the microbiological quality (WHO 2017). However, in Nigeria the quality of community water supply is poorly monitored while that of private water supply is not monitored nor assessed by any established government agencies, despite a standing policy to that effect. This is further compounded by poor policy making and implementation in Nigeria, in connecting hygiene, water insecurity, security and wellbeing (Shehu B and Nazim F, 2022). This study aimed at evaluating the microbiological quality and safety in terms of antibiotic resistance bacterial profile of groundwater sources in a typical peri-urban settlement in Nigeria. The findings would underscore the necessity or otherwise of establishing and implementing effective private water supply monitoring system in the country.

2. MATERIALS AND METHODS

2.1 Study Area

Ipetumodu is an ancient Yoruba city in the Southern-western Nigeria with a population size of over 135,000. Ipetumodu is the headquarters of the Ife North local government, in Osun State, Nigeria. The community is mostly populated by farmers, some civil servants and students of tertiary institutions.

2.2 Samples Collection

The water samples were collected from different hand dug wells and overhead storage plastic tanks supplied by boreholes in Ipetumodu town (Supplementary file), between the period of April to July 2021. The samples were collected using sterile plastic containers of 2.5 liters each. Collecting water sample from the borehole was performed by opening the outlet tap fully to allow the water flow for few minutes, sampling bottles were rinsed with some of the water from the storage tank and then completely filled and covered. The well water was drawn out using the bucket with rope found at the sampling locations. The bucket and sampling bottles were first rinsed with the well water, before filling up and covering the latter. The filled sampling bottles were stored in an ice-packed cooler boxes and transported to the microbiology laboratory unit of Oduduwa University, Ipetumodu, Osun State for Microbiological analysis. Description of the sampling points is presented in Table 1.

2.3 Preparation and Sterilization of Culture Media

Culture media comprising eosin methylene blue agar (EMBA), *Salmonella-Shigella* agar (SSA), Nutrient agar (NA) and Mueller Hinton agar (MHA) were prepared according to manufacturer's instruction and used for the cultivation of microorganism associated with the sample. The NA was for isolation of heterotrophic bacteria, EMBA plate was used for the isolation of *Escherichia coli* and other enteric bacteria while SSA was for the isolation of *Shigella* and *Salmonella* species. MHA was used for antibiogram testing. Media were sterilized by autoclaving at 121°C for 15 minutes, cooled to 45-50°C, and, then poured into sterile petri plates to solidify, excepting the SSA which was heated to boiling point before dispensing into sterile petri dishes (Fatokun EN et al, 2021).

2.4 Bacterial Isolation

Isolation of bacteria from water sources was carried out using the membrane filtration technique. A sterile membrane filter paper (0.45μm pore size) was inserted into a vacuum filter funnel on a catchment vessel, in order to trap the microbes. Then 100ml of the sample

Table 1. Description of sites for wells and borehole water samples collection

S/N	Sample source	Code	Observed surrounding features
1	Well	THW	The well was constructed in within an enclosed residence. It was covered with a rusted metal and the body was well plastered with cement. No form of activity is done around the area.
2	Well	HTW	The well was neatly constructed and secured in a private residence and covered to prevent impurities.
3	Well	CIW	The well was constructed with blocks only and located at the middle of the local community. The top edges of the well were filled with algal growth and the area was filled with grasses. The mouth of the well is usually covered with wooden plank.
4	Well	CW	The well was constructed with blocks and has growths of weeds around the well and was covered with planks.
5	Borehole	RAB[a]	The borehole is located at the central community and was channeled through a rusty looking tap situated in public. area. Domestic activities are carried out around the tap area, there was also growth of weeds and scrap metals dump around the site of the tap.
6	Borehole	BCB[b]	The borehole water was channeled from a pumping machine to a pipe through a wall fence to the exterior of the building. Domestic activities are carried out around the tap area such as washing of cloths and dishes etc., are usually carried out at the tap area.
7	Borehole	PHB[b]	The borehole was connected by a pipe from the pumping machine into a storage tank from which a tap is connected for water outlet. Domestic activities such as washing of cloths and dishes are usually carried out at the tap area.
8	Borehole	SDHB[b]	The borehole water connects through the wall and into a tank. The tank was open. Domestic activities are carried out around the tap area.

Footnote: [a] *Borehole comprises water samples directly from borehole*
[b] *Borehole water samples from borehole storage tanks.*

was measured into the funnel that was tightly fixed to a vacuum pump and turned on to allow the water flow through the filter. Thereafter, the membrane filter paper was gently picked using sterile forceps and placed immediately on the surface of a sterile plate medium. This procedure was repeated in duplicate for each sample and for different culture media. The plates were incubated at 37℃ for 24hrs after which distinct colonies on the agar were counted and sub cultured on nutrient agar and the respective selective media plates to get pure colonies (Forster B and Arango-Pinedo, C, 2016). Pure isolates were then stored in nutrient agar slants at 4℃ till further use.

2.5 Characterization and Identification of Isolates

Characterization and identification of the isolates were carried out by Gram staining, cultural, morphological and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology (Salton MRJ, 1994 and Noel KR,

John HG, 1984). Biochemical characteristics were determined using conventional biochemical reactions for indole, methyl red-voges-Proskauer, citrate (IMViC), catalase and coagulase tests (Fatokun EN and Fakorede CN , 2021).

2.6 Antibiogram Tests

The antibiogram of all the bacterial isolates was determined by the disk diffusion method according to the Bauer - Kirby technique (CLSI, 2017). The following antibiotics were used for testing: GN=Gentamycin (30µg), CIP=Ciprofloxacin (5µg), TET=Tetracycline (75µg), CTX=Cefotaxime (30µg), MEM= Meropenem (10µg), VAN=Vancomycin (30µg), AMK=Amikacin (30µg). Colony of each pure bacterial isolates was emulsified into a test tube containing 2ml of sterile normal saline (85%NaCl) using sterilizing flexible loop and homogenized to give a turbidity that is equivalent to 0.5 McFarland standards. A sterile cotton swab stick was dipped into the suspension, drained to remove excess culture and then

streaked on the entire surface of the Mueller Hinton agar (MHA) plate. The inoculated plates were allowed for 3-5 minutes to dry. The antibiotics disks were properly placed aseptically on the surface of the inoculated plates using sterile forceps and gently to ensure even contact with the medium. Thereafter, the plates were left on the bench for about 5 minutes to allow the antibiotics diffuse into the medium, before incubating at 37℃ for 24 hours. The zone of inhibition around the disc was measured and the result was interpreted as resistance, susceptible or intermediate, based on the interpretative standard of the CLSI manual (Noel KR and John HG,1984). The multidrug resistant (MDR) bacteria were determined as resistance to at least one agent among three or more classes of the antibiotics tested (Sweney MT et al, 2018). The multiple antibiotic resistance index (MARI), is defined as a/b where; a' represents the number of antibiotics to which an isolate was resistant, and b' represents the number of antibiotics to which the isolate was exposed (Krumperman PH, 1983).

2.7 Data Analysis

Data generated were analyzed by entering into Microsoft Excel, and the use of descriptive statistics to examine the susceptibility pattern of bacteria isolated from groundwater.

3. RESULTS

3.1 Total Heterotrophic Bacterial count

The total heterotrophic bacterial population recorded during the study is presented in Table 2. The total heterotrophic bacterial mean count of water samples from well water ranged from 60CFU/100ml to 64CFU/100ml while the value for total heterotrophic mean count for the borehole water samples ranged from 56CFU/100ml to 62CFU/100ml.

3.2 Total Coliform Bacterial Count

The total coliform bacteria mean count of water samples from well water and borehole water samples ranged from 61CFU/100ml to 66CFU/ml, and 52CFU/100ml to 63CFU/100ml respectively. The total coliform bacterial count is presented in Table 2.

3.3 Incidence of Bacterial Isolates

A total of thirty (30) bacterial isolates were recovered from the water samples of borehole and well water, of which 22 and 8 were Gram negative and positive bacteria respectively. The

isolates consisted of the following 7 genera with their respective incidence; *Klebsiella* (20%), *Enterobacter* (20%), *Bacillus* (17%), *Pseudomonas* (13%), *Salmonella* (13%) *Staphylococcus* (10%), and *Shigella* (7%). The percentage incidence is presented in Fig. 1.

3.4 Antibiogram of Bacteria Isolated from Water Samples

3.4.1 Susceptibility pattern

The susceptibility pattern of the bacterial isolates depicted in Fig. 2, showed a highest susceptibility of 63.3% to ciprofloxacin followed by 50% susceptibility to tetracycline. The isolates were least susceptible to gentamicin by 13.3%. All the isolates were 100% resistant to cefotaxime, followed by 76.7% resistant to vancomycin which was however very close to 73.3% of the isolates being resistant to meropenem. The least resistance of 3.3% was against ciprofloxacin which also had the highest intermediate antibiotic reaction of 33.3%.

Apart from complete resistance to cefotaxime, antibiotic resistance pattern differed in isolates from the two water sources, as presented in Fig. 3. Isolates from borehole storage tank water were highly resistant (80%) to vancomycin followed by 70% resistant to meropenem. However, those from well water has similar resistance of 75% to vancomycin and meropenem respectively, and 70% resistant to amikacin.

3.4.2 Multiple antibiotics resistant pattern of bacterial isolates

Seventy-three percent (73%) of the isolates from both the hand dug wells and borehole storage tank water samples showed multidrug resistance (MDR) pattern (Fig. 4). *Bacillus* and *Shigella* species showed 100% resistance to cefotaxime, meropenem and vancomycin respectively, while all *Enterobacter* spp. were 100% resistant to both cefotaxime and vancomycin, in addition to 83% resistance against meropenem. All the isolates except the *Shigella* spp. showed some level of resistance to amikacin (Fig. 4).

Multiple antibiotic resistant index (MARI) by isolates from both water sources range from 0.3 to 1, the highest being a *Klebsiella* sp. (2SDH1) from a storage tank. Two *Enterobacter* spp., each from well and storage tank, a *Salmonella* and *Staphylococcus* species both from different storage tanks all had a MARI value of 0.9. All the *Bacillus* spp. have MARI value of of 0.7,

except one (isolate 2RA2) from the borehole in central community with 0.6. The multiple antibiotics resistant pattern is shown in Table 3.

Table 2. Bacterial counts from well water and borehole water samples

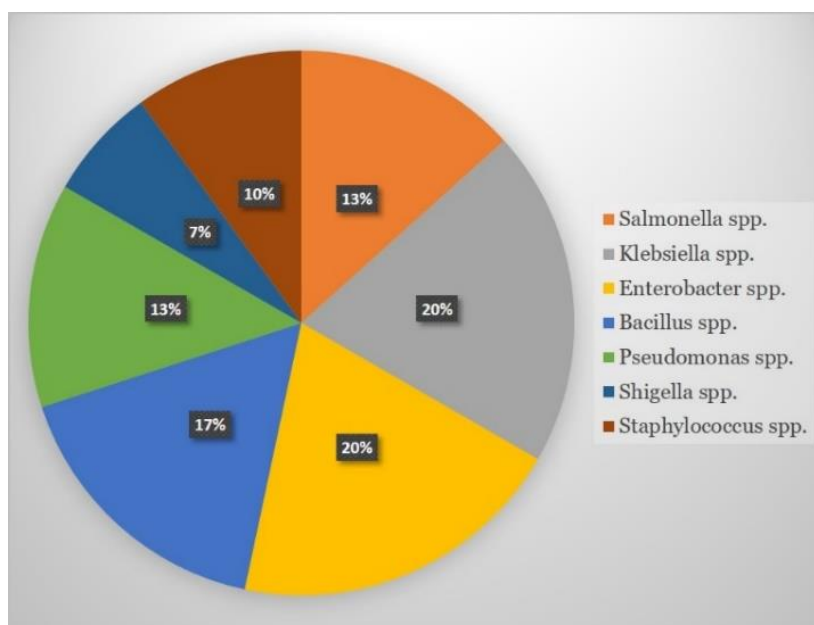| Water source	Codes	Total heterotrophic count (CFU/100ml)	Total coliform count (CFU/100ml)
Well Water	THW	62	62
	HTW	64	64
	CIW	61	66
	CW	60	61
Borehole water	RAB	62	63
	BCB	58	59
	PHB	59	52
	SDHB	56	53

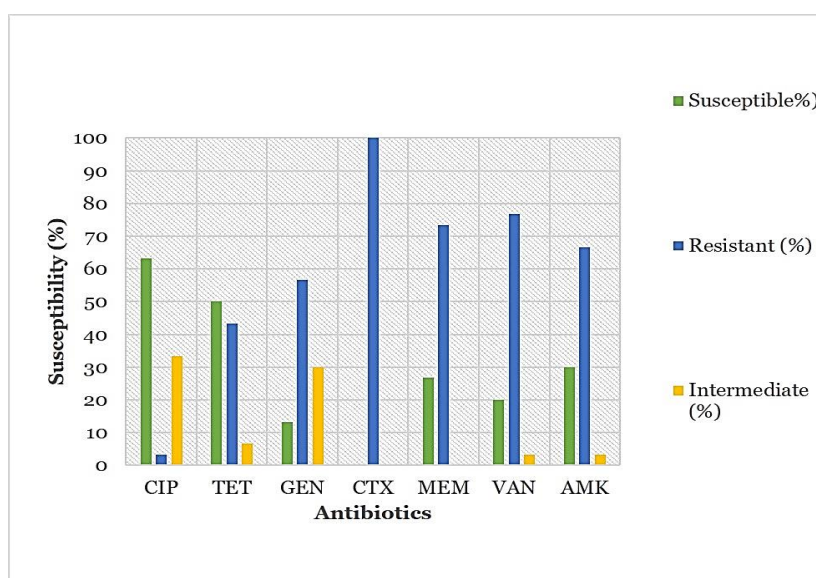**Fig. 1. Percentage incidence of bacterial isolate**

Fig. 2. Susceptibility pattern of bacteria isolated from water samples
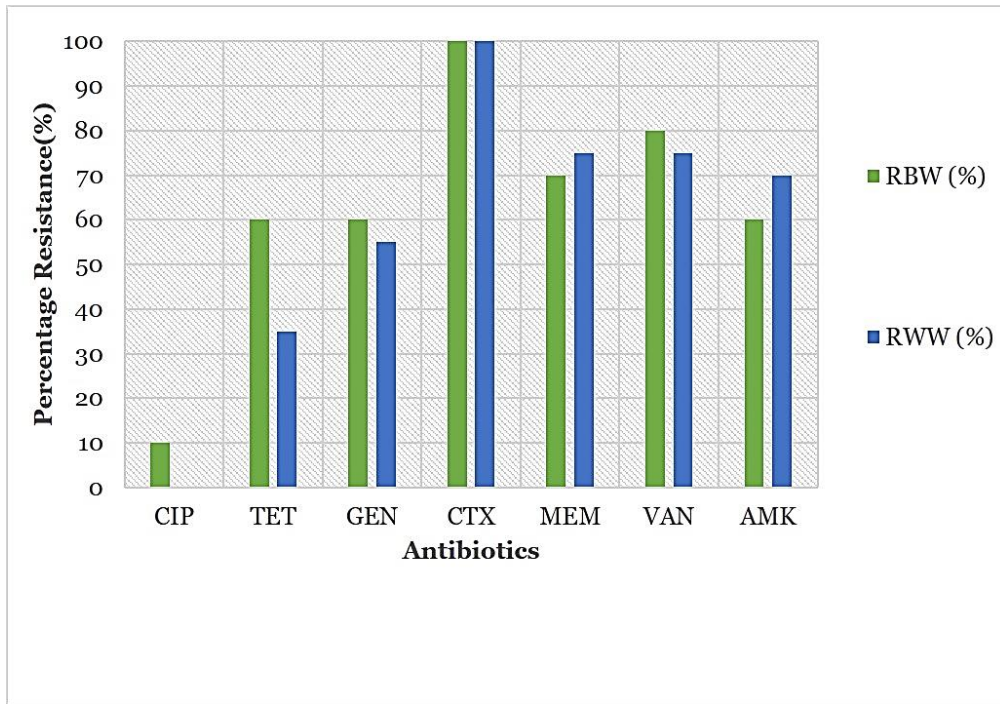
Fig. 3. Resistant pattern of bacterial isolates from well water and water from borehole storage tank
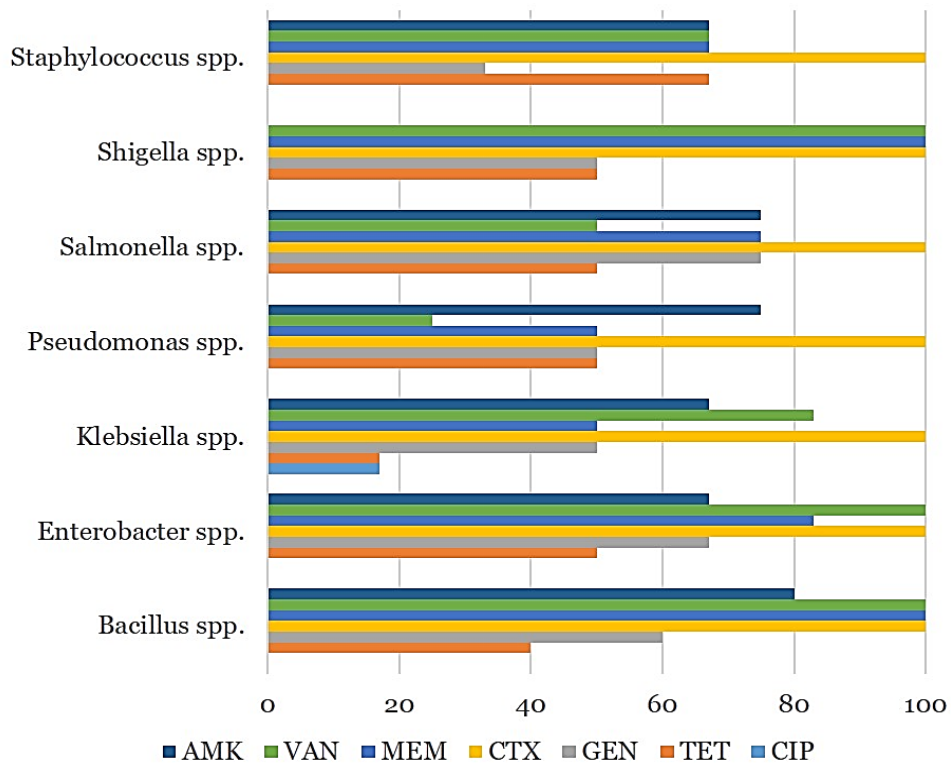

Fig. 4. Multiple antibiotics resistance pattern of bacterial isolates

Table 3. Multiple antibiotics resistant index of isolates from well water and borehole storage water tanks

Serial No.	Suspected organism	Source	Isolate code	CIP (30ug)	TET (30ug)	GEN (30ug)	CTX (30ug)	MEM (10ug)	VAN (30ug)	AMK (30ug)	Susceptibility percentage	MARI
1	*Bacillus* spp.	Borehole	(2RA2)	I	S	I	R	R	R	R	R=57, I=29, S=14	0.6
			(PH1)	I	S	R	R	R	R	R	R=71, I=14, S=14	0.7
		Well water	(HT2)	S	S	R	R	R	R	R	R=71, S=29	0.7
			(C2)	I	R	R	R	R	R	S	R=71, I=14, S=14	0.7
			(CI1)	I	R	I	R	R	R	R	R=71, I=29	0.7
2	*Enterobacter* spp.	Borehole	(2BC1)	S	I	R	R	R	R	R	R=71, I=14, S=14	0.7
			(2RA1)	S	R	R	R	R	R	R	R=86, S=14	0.9
			(BC2)	S	S	I	R	S	R	S	R=29, I=14, S=57	0.3
		Well water	(2HT1)	S	R	I	R	R	R	R	R=71, I=14, S=14	0.9
			(2TH1b)	S	S	R	R	R	R	S	R=57, S=43	0.6
			(2HT2)	I	R	R	R	R	R	R	R=86, I=14	0.7
3	*Klebsiella* spp.	Borehole	(2SDH1)	R	R	R	R	R	R	R	R=100	1
			(2SDH2)	S	I	I	R	S	R	S	R=29, I=29, S=42	0.3
		Well water	(2CI1)	I	S	S	R	S	R	S	R=29, I=14, S=57	0.3
			(2TH2)	I	S	I	R	S	S	R	R=29, I=29, S=43	0.3
			(HT2b)	S	S	R	R	R	R	R	R=71, S=29	0.7
			(HT1)	S	S	R	R	R	R	R	R=71, S=29	0.7
4	*Pseudomonas* spp.	Borehole	(2BC2)	S	S	R	R	R	I	R	R=57, I=14, S=29	0.6
			(2PH2)	S	R	S	R	S	S	R	R=43, S=57	0.4
			(RA1)	S	S	I	R	S	S	R	R=29, I=14, S=57	0.3
		Well water	(2C2)	S	R	R	R	R	R	S	R=71, S=29	0.7
5	*Salmonella* spp.	Borehole	(2BC1)	S	R	R	R	R	R	S	R=71, S=29	0.7
			(2PH2)	I	R	R	R	R	R	R	R=86, I=14	0.9
		Well water	(2TH1)	S	S	I	R	S	S	R	R=29, I=14, S=57	0.3
			(CI2)	S	S	R	R	R	S	R	R=57, S=43	0.6
6	*Shigella* spp.	Borehole	(BC1)	S	R	S	R	R	R	S	R=57, S=43	0.6
			(RA1)	I	S	R	R	R	R	S	R=57, I=14, S=29	0.6
7	*Staphylococcus* spp.	Borehole	(2SDH1)	S	R	R	R	R	R	R	R=86, S=14	0.9
		Well water	(2CI2)	I	S	I	R	S	S	R	R=29, I=29, S=43	0.3
			(TH2)	S	R	S	R	R	R	I	R=57, I=14, S=29	0.6

4. DISCUSSION

World Health Organization (WHO) recommends that potable water should have below 20 CFU/ml heterotrophic bacterial counts with no coliform (0 CFU/ml) bacteria or fecal coliforms (WHO 2017). The water sources investigated in this study had both high heterotrophic bacteria plate count as well as total coliform counts, some of which exceeded the WHO acceptable limits, and the maximum permissible level of 10CFU/ml for total coliform as specified by Standard Organisation of Nigeria (Nigerian Industrial Standard SON/SIN). In comparison, the observed counts were either higher than or similar to previous reports from other places within the state of the study area, within the country and also outside the country (Tangwa B et al, 2019, Bello OO et al, 2013, Olalemi AO et al, 2019). Similar result to this study with no incidence of *E. coli* but with highest THB in well water and borehole water samples has previously been documented (Ahmed SI et al, 2021).

Total coliform comprises bacteria of faecal origin, thermotolerant coliform, and some other bacteria from environmental sources. Hence, incidence of total coliforms may or may not suggest faecal contamination but may be due to contaminants from soil or organic matter (Akyala IA et al, 2013). Possible sources of total coliform occurrence include soil and water surrounding the wells or pipes, sediments, biofilms, and by improper covering or screen on the wells and storage tanks. Microorganisms may also gain access into storage tanks by surface runoff from the roof during heavy rain or wind, and also by re-growth of bacteria in stored water (Chalchisa D et al, 21018). Moreover, the retention time of water in storage tanks and the cleaning frequency of the tanks may also impact on the drinking water quality (Manga M et al, 2021).

Different bacterial genera of public health importance were identified in this study, all were isolated from the two water sources, except *Shigella* spp. which were all from borehole water samples. The most commonly occurring were *Klebsiella* and *Enterobacter* species, followed by *Bacillus* spp. This result agrees with previously reported 29.2%, 95% and 100 % highest incidence of *Klebsiella* spp. in well water from other communities in, Western Nigeria, Cameroon, and Eastern Nigeria respectively (Idowu AO et al, 2011, Ngwa N, Chrysanthus N, 2013, Nicodemus M et al, 2023). On the contrary, *S. aureus* was reported as highest incidence while the least was *Klebsiella* and *Enterococcus* spp. in Wukari, Northern Nigeria (Agwaranze DI et al, 2017).

Klebsiella spp. naturally occur in many water environments and may multiply to greater extent in nutrients rich waters. They may also colonize washers in taps of drinking-water distribution systems and grow in water distribution systems (WHO 2017). *Klebsiella* spp. may be found in many healthy humans and animals, and are excreted in faeces, hence, they are readily detected in sewage-polluted water (WHO 2017). Detection of *Klebsiella* spp. in drinking-water are generally as biofilm organisms and may not represent a health risk directly. However, the incidence of *Salmonella* spp. in this study is quite worrisome as this genus has been associated with several waterborne as well as foodborne-water related disease outbreaks (Franklin LJ et al, 2009, Beer KD et al, 2015, Liu H et al, 2018). Moreover, groundwater has been implicated as the source of an outbreak of *Salmonella Enteritidis* (Kovačić A et al, 2017). Salmonella infections are endemic in Nigeria, and *Salmonella* spp. are commonly associated with consumption of contaminated food and water (Akinyemi KO et al, 2021).

All the isolates in this study showed some level of resistance to all the antibiotics tested. In particular, there was complete resistance (100%) to cefotaxime, a third-generation cephalosporin, in addition to a very high resistance (73.3%) to meropenem, a carbapenem class of antibiotics. However, the isolates were mostly susceptible (63.3%) to ciprofloxacin. This is similar to a previous report on ESBL producing *E. coli* from potable water sources with resistance to carbapenem but showed high sensitivity to ciprofloxacin and gentamicin (Ekhosuehi A et al, 2018). Resistance to cefotaxime (cephalosporin) is often due to production of enzymes like extended-spectrum beta-lactamases (ESBLs). Although carbapenems are last-resort antibiotics for treating multidrug resistant Gramnegative bacterial infections, the spread of carbapenem-resistant strains has been of concern to physicians and primary health care providers; and an unprecedented threat to public and environmental health (Forster B, Arango-Pinedo, C, 2016).

Enterobacteriaceae resistant to third-generation cephalosporin and carbapenem antibiotics has been categorized by WHO to be priority pathogens for research and development (R&D) of new antibiotics, and of a critical threat to the

public health (WHO 2017, Adegoke AA et al, 2020). Third generation cephalosporins and carbapenems are adjudged to be the best available antibiotics for treating multi-drug resistant bacteria. Therefore, proliferation of enteric bacterial resistance to these classes of antibiotics is further indicative of the need for alternatives and fresh drug discovery.

Furthermore, this study also recorded a very high resistance to vancomycin. Vancomycin, a glycopeptide antibiotic, is effective against most Gram-positive cocci and bacilli, but is principally used against Gram-positive β-lactam-resistant bacteria and also in methicillin-resistant *Staphylococcus aureus* (MRSA) infections (Schneider F et al, 2022). Incidentally a very high percentage of resistance against vancomycin by isolates from both borehole storage tank and well water (80 and 75 % respectively) was recorded (Fig. 3). All the bacterial isolates including *Bacillus* and *Staphylococcus* species (100 and 67% respectively) had some level of resistance (Fig. 4). However, resistance by the gram negatives maybe intrinsic by reason of their outer membrane barrier. Similarly, in another study, isolates from untreated borehole and well water was most resistant to cefuroxime (91.5% and 81.8%, respectively (Ahmed SI et al, 2021).

Another high resistance recorded in this study was against amikacin the most widely used semisynthetic aminoglycoside class of antibiotic (Ramirez MS, Tolmasky Me, 2017). The drug is noted primarily for treatment of a broad spectrum of bacterial infections. Additionally, all the isolates showed some level of resistance to tetracycline, the highest being by *Staphylococcus* spp.

Of note also is the 73% recorded for MDR, and a MARI value above 0.2 for all the isolates in this study. This is similar to a minimum resistance index of 0.47 in boreholes and hand dug wells water and 0.625 in well and streams water reported elsewhere (Tangwa B et al, 2019, Okwelle AA et al, 2022); but higher than over 10% and 52.5% MDR from different water sources reported in some states of South Western Nigeria (Idowu AO et al, 2011, Oluyege JO et al, 2009).

Resistance to common antibiotics in Nigeria has been attributed to accessibility and affordability by the common Man (Adenaike O et al, 2016). Furthermore, prescription monitoring is not well conducted and prescription only medicines

(POM) including antimicrobials are routinely sold Over-The-Counter (OTC) in pharmacies and by patent proprietary medicines vendors (PPMVs). According to Nigeria's legislation, antimicrobials and other antibacterial should only be dispensed with prescription (Federal ministries of Agriculture, Environment and Health FMAEH, 2017). However, several factors including shortage of licensed prescribers and medicines in some areas, and the propagation of under-regulated patent medicine vendors and hawkers, have led to severe access problems as well as crisis of irrational drug use in Nigeria (Federal ministries of Agriculture, Environment and Health FMAEH, 2017).

Although the incidence of some potentially environmental isolates such as *Klebsiella* spp. may not pose a health risk directly, the potential for biofilms formation and antibiotics resistance, calls for concern. Resistance genes may be acquired by potentially pathogenic isolates from intrinsic resistant environmental bacteria, as has been previously documented (Oliveira M et al, 2021). Moreover, phenotypic resistance in bacteria is mostly associated with presence of one or more resistant genes which may be transferred among different bacterial groups (Kong M et al, 2023, Sparbrod M et al, 2023).

5. CONCLUSION

The results of this study suggest that groundwater source in Ipetumodu community in Nigeria is contaminated with bacterial counts exceeding the WHO acceptable limits, and the maximum permissible level specified by Standard Organisation of Nigeria. Moreover, the study also recorded a high incidence of multidrug resistant bacteria. These underscore the public health implication and an urgent need for monitoring of private water supply in Nigerian communities. Furthermore, effective dissemination of information on the need to safeguard groundwater sources and use of low cost water treatment method such as solar oxidation by the local communities is highly recommended.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ACKNOWLEDGEMENTS

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/115680