Iranian Journal of Microbiology
Volume 14 Number 3 (June 2022) 351-361

ORIGINAL ARTICLE

Assessment of well water as a reservoir for extended-spectrum β-lactamases (ESBL) and carbapenem resistant *Enterobacteriaceae* from Iwo, Osun state, Nigeria

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Received: June 2021, **Accepted:** March 2022

ABSTRACT

Background and Objectives: Unsafe water supplies are of public health concern, especially in developing countries. This article aims to investigate the microbiological quality of water from eight Wells in Iwo and to explore for the extended-spectrum β-lactamase (ESBL) and carbapenemase genes contained in isolated enteric bacteria from in the water samples.
Materials and Methods: Bacterial isolation and identification were done using standard conventional methods. Antibiotic susceptibility testing was conducted using the Kirby–Bauer method. Ten phenotypically carbapenem-resistant isolates were further subjected to genotypic analysis (PCR amplification) for the detection of ESBL and carbapenemase gene.
Results: A total of 148 *Enterobacteriaceae* isolates belonging to seven (7) genera were isolated and identified which included *E. coli, Enterobacter* spp., *Klebsiella* spp., *Salmonella, Citrobacter* sp, *Proteus*, and *Shigella*. Results showed that 55% of isolates were resistant to tetracycline, 28% to cefepime, the least resistance was shown in moxifloxacin and gentamicin which had 6% and 9%, respectively, of the total isolates. For the two carbapenems used, results showed meropenem and imipenem had resistant values of 14% each, respectively. Two isolates carried the bla_{CTX-M} gene while the carbapenemase gene (bla_{KPC}, bla_{NDM}, and bla_{OXA}) was not detected in all the ten isolates.
Conclusion: There was also negative chromosomal detection of carbapenemase in MDR isolates from well waters in Iwo town. Consequently, resistance to carbapenem antibiotics in these isolates may not be mediated by carbapenemase but by the production of extended-spectrum β-lactamases and through other mechanisms of resistance.

Keywords: Antibiotic resistance; Carbapenem; *Enterobacteriaceae*; Extended-spectrum β-lactamases; Nigeria; Water quality

INTRODUCTION

Water is a vital resource needed for the sustenance of life (1). An adequate, safe, and accessible supply must be available to all. One of the main aim of WHO is improving on access to safe drinking water which is essential to human life through a system assessment, management and communication measures, as well as monitoring that can result in significant benefits to health which is a basic human right (2). The quality of water is usually examined to assess its hygienic level as well as its suitability for general use. Different types of waterborne diseases such as dysentery, cholera, diarrhea, typhoid, are caused by drinking contaminated water and can lead to untimely loss of lives, especially in developing countries. A

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combination of both poor quality health care system and insufficiency in the supply of pure potable water in developing countries, have been adjudged to be responsible for the alarming lists of waterborne disease outbreaks (3). It is reported that 80% of the diseases worldwide occur due to contaminated water and water borne pathogens (4).

The family, *Enterobacteriaceae*, are Gram-negative rod shaped bacteria commonly known as an enteric organisms or enterobacteria. *Enterobacteriaceae* are normal floral of the gastrointestinal tracts of humans and animals, where they thrive without causing infection in their hosts; they also serve as microbiological indicators of water quality and fecal contamination (5, 6). Some common fecal coliform genera include *Escherichia, Klebsiella, Citrobacter, Enterobacter, Morganella,* and *Hafnia* (5). During rainfalls, enteric organism may be washed into rivers, streams and underground waters; the untreated water coming from these sources may contain coliforms, and their subsequent consumption by the human populace, could pose a health-risk. Hence, water quality assessment is a needed tool, to predict and minimize the future risks of water-borne diseases, especially in the developing countries.

Antibiotics have been invaluable in the management of bacterial infections. Antibiotic resistance is responsible for thousands of death annually and is also projected to increase dramatically as a global health hazard (7, 8). It is estimated that antibiotic resistance might lead to ten million deaths annually by 2050 (9, 10). Drug-resistant organisms are bacteria that have acquired or developed resistance mechanisms to render one or more antibiotics, inactive. The development of resistance to various antibiotics can be attributed to the inappropriate use of antibiotics and worsened by the slow pace at which new antibiotics are produced (11). It is a general knowledge that antibiotic resistant organisms and genes are produced from the release of unused antibiotics into the environment (12). Several scientific documents have given evidences to the presence of antibiotic resistant organisms in potable waters, surface waters, and wastewaters (12).

In Gram-negative bacteria, the β-lactams antibiotics are being rendered inactive by the presence of β-lactamases (encoded by *bla* genes), a major driving force of resistance (13).

Carbapenems such as imipenem, meropenem, and ertapenem are a β-lactam class of highly effective antibiotics considered to be one of the last lines of treatment for serious infections caused by multidrug-resistant organisms, especially *Enterobacteriaceae*; nevertheless, their potency has been jeopardized by the emergence of carbapenemase-resistant bacteria (14, 15). Carbapenemases have previously been identified as a cause of carbapenem resistance in Gram negative bacteria (GNB) from Nigeria (16). Also, extended-spectrum β-lactamase (ESBL) bacteria constitute a significant public health threat since they make infections due to *Enterobacteriaceae* difficult to treat, even with the last generations of β-lactams. In water environment, the presence of ESBL- producing *Enterobacteriaceae* and carbapenems, such as *K. pneumoniae* and *E. coli*, harboring a wide variety of genes, have been reported globally (17).

Recently, the World Health Organization's (WHO's) has listed some pathogens of Global Priority, and carbapenem-resistant *Enterobacteriaceae* (CRE) was listed among the critical priority pathogen group (18). CRE confer resistance to penicillins and cephalosporins (12). Presently, bla_{KPC}, bla_{OXA-48}, bla_{NDM}, bla_{VIM}, and bla_{IMP} are among the most commonly reported carbapenemase encoding genes in *Enterobacteriaceae* around the world (11, 12).

However, there remained carbapenem-resistant isolates without the production of a carbapenemase. Other pathways, through which resistance in CRE are expressed include such activities as enzymatic degradation, efflux pumps, decreased permeability via porin, and inhibition of transpeptidases (i.e penicillin-binding proteins) (19). These block the penetration of the antibiotic within the bacterial cell wall. The resistant organisms thus generated through this mechanism have the ready capacity to transfer their resistance gene to other organisms in the environment, using the genetic mobile elements contained in their cells, and consequently, limit therapeutic options.

Therefore, this study aimed to determine the occurrence and antibiotic resistance pattern of extended-spectrum β-lactamases and carbapenem-resistant *Enterobacteriaceae* from well waters in Iwo, Osun State, Nigeria, and consequently, assess the potential public health impact.

MATERIALS AND METHODS

Study area. This study was carried out in Iwo township, the headquarters of Iwo Local Government Area

(LGA) in Osun State, Southwestern Nigeria, (Fig. 1).

Collection of well water sample. Eight locations of wells were selected for the collection of water samples. From each location, the samples were collected from 3 different wells for each season. The readings of every sample site were taken by Global Positioning System (GPS) (Fig. 1) in the Iwo area between August 2019 and February 2020. Using aseptic techniques, different well waters were fetched using a clean plastic bucket tied to a plastic rope before transferring into a labelled sterile bottle. The collected water samples were immediately transported to the Laboratory for analysis.

Isolation of target microorganisms. Isolation and identification of members of the family *Enterobacteriaceae* was according to conventional procedures such as cultural characterization and biochemical tests. One milliliter (1ml) of each well water sample was transferred into a test tube containing 9 ml of sterile Maximum Recovery Diluent (MRD). The tube and content were incubated for 24 hours and afterward 10-fold serial dilution was carried out; subsequently, 0.5ml from 10^{-2} and 10^{-4} diluents were spread on sterile Lab M product of Eosin methylene blue (EMB), Xylose lysine deoxychocolate (XLD) and MacConkey plates. The spreading procedure was done in duplicates. The inoculated plates were incubated at 37°C for 24 hours. After incubation, colony growths with the characteristic mucoid pink, purple and green metallic sheen colours were presumed to be members of *Enterobacteriaceae*. For isolation of *Salmonella* spp, a black colouration on XLD was presumed for *Salmonella* sp. Distinct colonies were selected; these were further purified by repeated streaking on sterile fresh EMB and XLD plates and the plates incubated at 37°C for 24 hours in an inverted position. Pure colonies obtained were morphologically and biochemically characterized, using the methods of Cheesbrough (21). The bacterial isolates were viewed under microscope and characterized using morphological and biochemical tests that were further identified using the keys provided in Bergey's

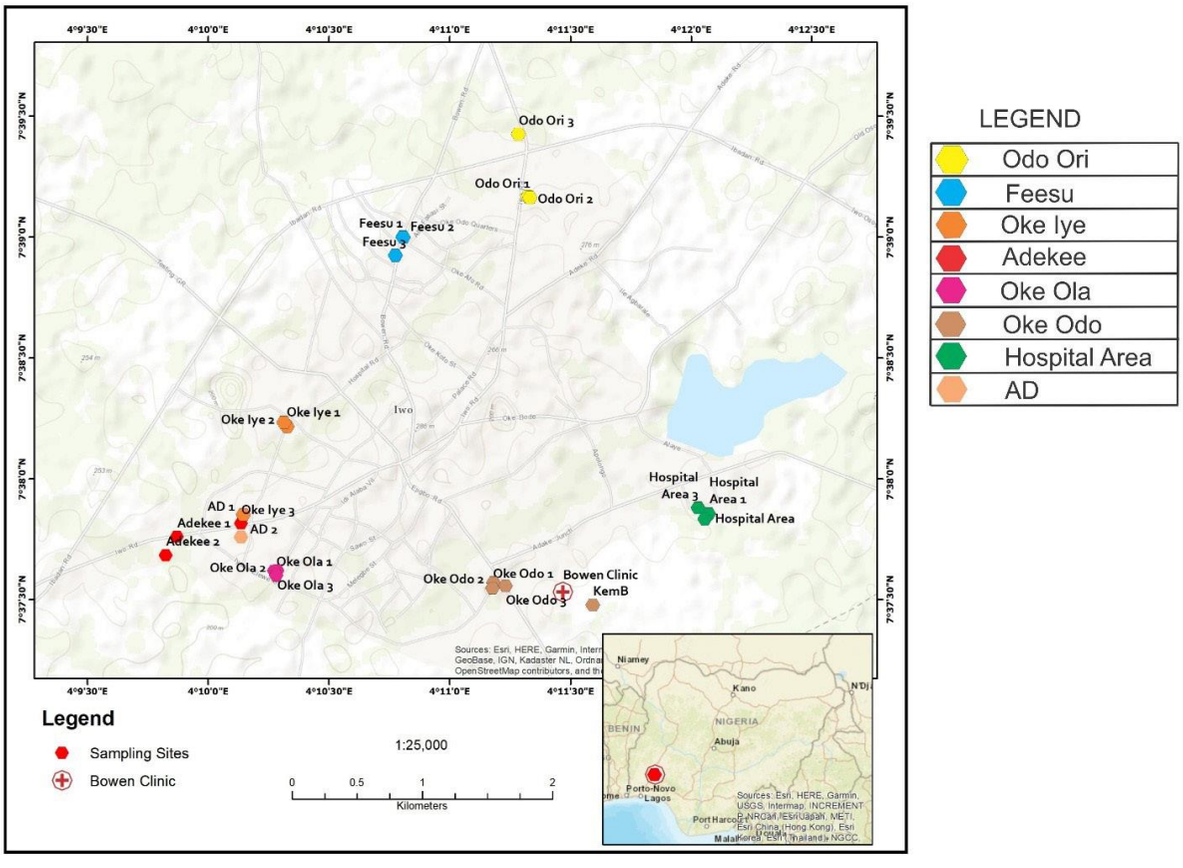

Fig. 1. Map view of Iwo township showing sampling site

Manual of Determinative Bacteriology.

Antibiotic susceptibility test. The susceptibility of all the obtained isolates to common antibiotics and carbapenem drugs was determined by disc diffusion technique, in accordance with the Kirby-Bauer method (21). Each bacterial isolate suspension was prepared and standardized by adjusting it to 0.5 McFarland standard of inoculum size of 10^8 CFU/ml. A sterile cotton swab was dipped into each inoculum; the excessive fluid was removed by rotating the head of the cotton swab with firm pressure against the wall of the tube. The cotton swab was then swabbed uniformly over the entire surface of Mueller Hinton agar contained in a sterile Petri-dish by rotating the plate approximately 60°C each time to ensure even distribution, and confluent growth. The plates were left to dry for 5 minutes and antibiotic discs were placed centrally using sterile forceps. The antibiotics (Oxoid) used were ceftazidime (30 µg), cefepime (30 µg), aztreonam (30 µg), gentamicin (30 µg), moxifloxacin (75 µg), tetracyclin (30 µg), ampicillin (10 µg) and ticarcilin (5 µg).

Carbapenem-resistance *Enterobacteriaceae* (CRE) were identified phenotypically using carbapenem antibiotic (Oxoid) discs, meropenem (10 µg), and imipenem (10 µg). Results were interpreted according to the Clinical and Laboratory Standards Institute guideline and categorized as resistant, intermediate resistance, and susceptible (21). Isolates were regarded as multidrug-resistant (MDR) when they were resistant to at least three or more antimicrobial classes.

Detection of antibiotic resistance gene by PCR. Ten isolates from water samples that showed phenotypic resistance to the third-generation cephalosporin and carbapenem drugs (imipenem and meropenem) were screened for the presence of genes encoding ESBLs (bla_{CTX-M}, bla_{TEM}, bla_{SHV}) and carbapenemases (bla_{OXA-48}, bla_{VIM}, bla_{IMP}, and bla_{KPC}), using a multiplex primer. Chromosomal DNA of the isolates was extracted by boiling lysate method method as described by Queipo-Ortuno et al. (22).

PCR Assay. The PCR reaction was carried out using the Solis Biodyne 5× HOT FIREPol Blend Master mix. The DNA template was suspended in 25 µl of a reaction mixture, and the reaction concentration was brought down from 5× concentration to 1× concentration containing 1× Blend Master mix buffer Buffer (Solis Biodyne) with 1.5 mM $MgCl_2$, 200µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 25p Mol of each primer (BIOMERS, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne), Proofreading Enzyme, 5 µl of the extracted DNA, and sterile distilled water was used to make up the reaction mixture.

Thermal cycling was conducted in an Eppendorf Vapo protect thermal cycler (Nexus Series) for an initial denaturation of 95°C for 15 minutes followed by 35 amplification cycles of 30 seconds at 95°C; 1 minute at 56°C and 58°C and 1 minute 30 Seconds at 72°C. This was followed by a final extension step of 10 minutes at 72°C. The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100bp DNA ladder was used as DNA molecular weight standard. The primer sequences and corresponding temperature used in multiplex PCR reactions are listed in Table 1 (22-26).

RESULTS

A total of 148 isolates belonging to seven genera of the family *Enterobacteriaceae* were isolated from eight different sites in Iwo town. The distribution of the *Enterobacteriaceae* recovered from the different well water sampled showed that among all bacteria isolates obtained, *Klebsiella* sp. was the most dominant isolate at 20.9% of the total isolates followed by *E. coli* (20.3%), while the least isolated organism was *Shigella* (6.1%) of the total number of isolates obtained (Fig. 2).

Klebsiella sp., *E. coli,* and *Salmonella* sp. were prevalent in all the wells during the period of sampling. However, a relatively high prevalence of *E. coli* was recorded in Oke Ola and Odo ori samples, while *Shigella* sp. was scanty in all the wells at all locations.

Results of the antibiotic resistance showed that all the water-borne enteric bacterial isolates exhibited variable effects to all antibiotics tested, Fig. 3. revealed the antibiotic resistance profiles of the isolates. Resistance was observed for all antibiotics tested in this study, and the highest resistance recorded (100%) was against ticarillin and ampicillin (penicillin class) and ceftazidime (a 3rd generation cephalosporin) for all the isolates while 28% resistance was recorded against cefepime, (a 4th generation cephalosporin).

Table 1. PCR primers used in the study

S/N	Primer Name	Sequence (51-31)	Annealing Temperature	Base pair (bp)	References
1	Bla SHV-F	AAGATCCACTATCGCCAGCAG	56	200	(22)
	Bla SHV-R	ATTCAGTTCCGTTCCCAGCGG			
2	Bla TEM-F	AAACGCTGGTGAAAGTA	56	822	(23)
	Bla TEM-R	AGCGATCTGTCTAT			
3	Bla CTX-MF	TTTGCGATGTGCAGTACCAGTAA	56	590	(23)
	Bla CTX-MR	CGATATCGTTGGTGGTGCCATA			
4	VIM - F	TTTGGTCGCATATCGCAACG	58	500	(24)
	VIM- R	CCATTCAGCCAGATCGGGCAT			
5	IMP- F	GTTTATGTTCATACATCG	58	440	(24)
	IMP-R	GGTTTAACAAAACAACCAC			
6	KPC-F	GATACCACGTTCCGTCTGG	58	246	(25)
	KPC-R	GCAGGTTCCGGTTTTGTCTC			
7	Bla OXA- F	TAATGCTTTGATCGGCCTTG	58	353	(26)
	Bla OXA-R	TGGATTGCACTTCATCTTGG			

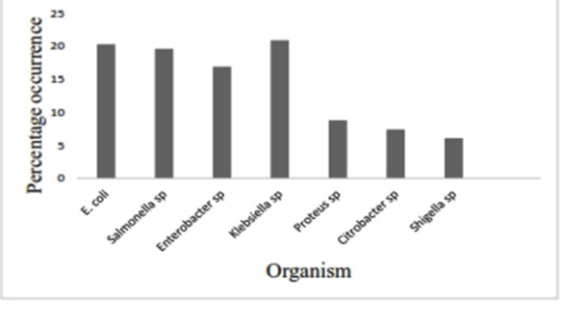

Fig. 2. Percentage occurrence of isolates across the well water samples in Iwo

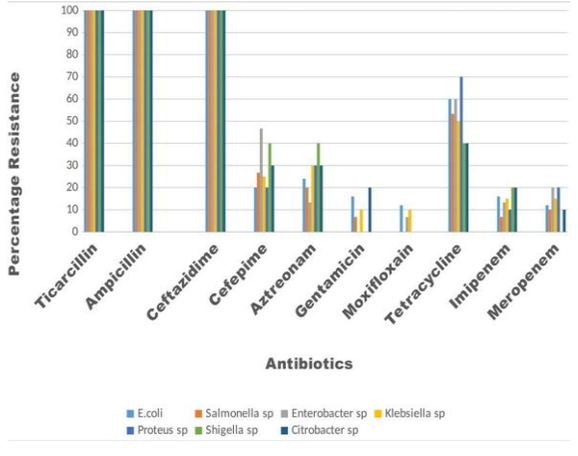

Fig. 3. Pooled antibiotic resistance pattern of obtained bacterial isolates from the various well

Fifty-five percent (55%) of the isolates showed resistance against tetracycline, followed by aztreonam recording 26% resistance. The lowest level of resistance was shown against moxifloxacin and gentamicin at 6% and 9% respectively. *Escherichia coli* isolates were the most resistant to tetracycline (15 isolates), followed by *Enterobacter* sp. which had 9 isolates and *Proteus* sp. had 7 isolates out of the 55 tetracycline-resistant isolates. The quinolone class of antibiotics (moxifloxacin) recorded 100% sensitivity against *Salmonella* sp, *Citrobacter* sp. and *Shigella* sp. and also the aminoglycoside class of antibiotic (gentamicin) showed 100% susceptibility to *Proteus* sp. and *Shigella* sp. Other isolates showed moderate sensitivity to the two classes of antibiotics.

For the two carbapenems used, results showed that 14% of the isolates were resistant to meropenem and imipenem each while their intermediate values were 19% and 16% respectively (Fig. 3). Three isolates each of *E. coli*, *Klebsiella*, and *Enterobacter* sp, respectively, showed high resistance to meropenem out of the 14 resistant isolates obtained, while 4 and 3 isolates of *E. coli* and *Klebsiella* sp, respectively, showed high resistance to imipenem out of the same 14 resistant isolates (Fig. 4).

The antibiotic susceptibility profile of the isolates showed a high sensitivity value in all the bacterial genera to a non-β-lactam antibiotic belonging to the class of aminoglycoside and quinolone (gentamicin

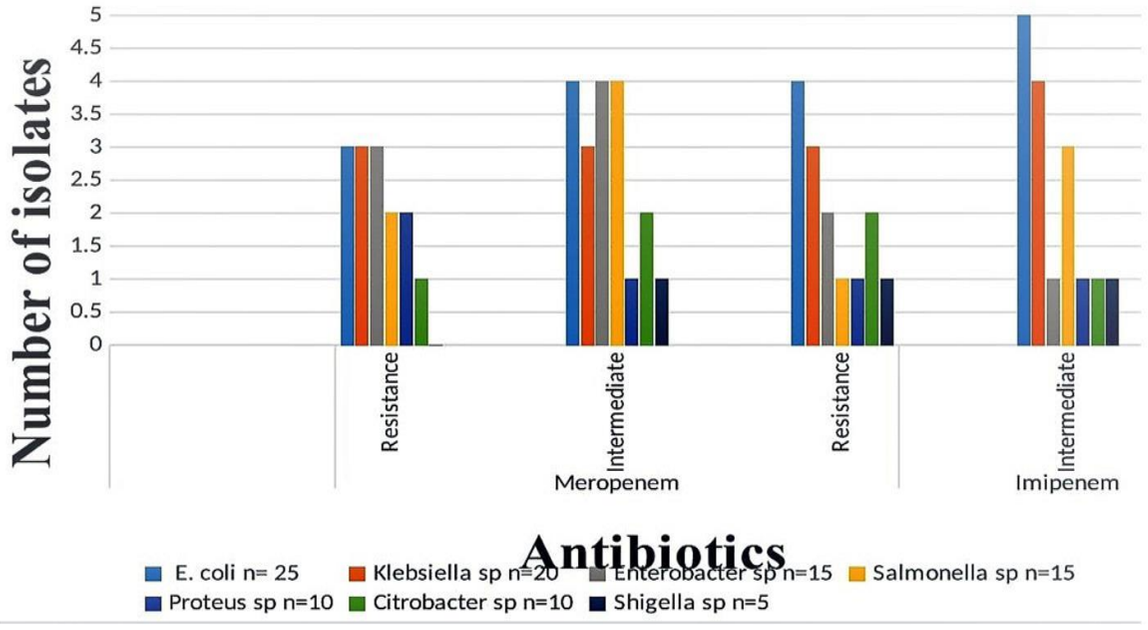

Fig. 4. Frequency of Carbapenem antibiotics resistant bacterial isolates

and moxifloxacin). Overall, the isolates showed a sensitivity of 88% and 86%, respectively, to these antibiotics.

PCR amplification using ESBL multiplex primers revealed that the two isolates (20%) from hospital area well water out of ten MDR harbored the ESBL gene bla_{CTX-M} with no gene indication for SHV and TEM (Fig. 5). Nevertheless, there were no carbapenemase gene detected within the spectrum range of bla_{OXA-48}, bla_{KPC}, bla_{VIM}, and bla_{IMP} genes, screened for among the ten MDR bacterial isolates subjected to PCR amplification (Fig. 6).

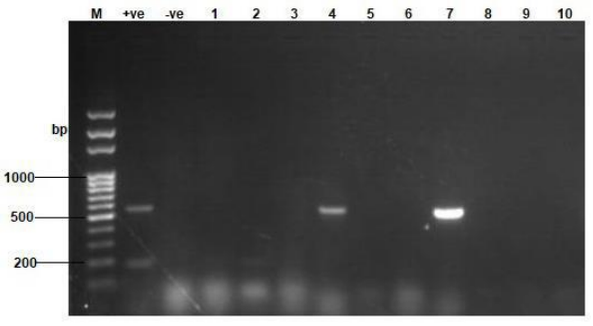

Fig. 5. Multiplex PCR for amplification of bla_{CTX}, bla_{SHV} and bla_{TEM} genes on 1.5% agarose gel electrophoresis: lane M: DNA ladder 100 bp; lanes 4 and 8 are positive for only bla_{CTX} showing a typical band size of 590 bp; lanes 1,2,3,45,6,7,8,9 and 10 shows negative for all the genes.

DISCUSSION

The high load of *Enterobacteriaceae* with varying frequencies was prominent in the different well waters investigated; this is an indication of the poor microbiology quality of the well waters. The result of this study conforms with the findings of several authors discovering the presence of coliforms such as *E. coli*, *Enterobacter* and non-coliforms such as *Salmonella* Typhi, *Proteus mirabilis*, in most water sources (27, 28) and Adesakin et al. (29) have also investigated the quality of domestic water sources in Samaru community in Zaria, Nigeria. Adequate treatment of water is of necessity as the detection of these enteric bacteria in potable water makes it

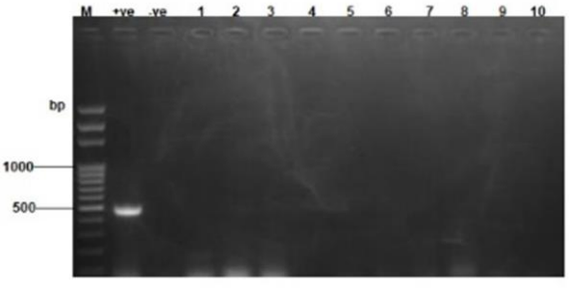

Fig. 6. Multiplex PCR for amplification of bla_{OXA-48}, bla_{VIM}, bla_{IMP} and bla_{KPC} genes on 1.5% agarose gel electrophoresis: lane M: DNA ladder 100 bp; all the lanes show negative for all the genes targeted for.

unsafe and quick attention is needed because these bacteria cause diseases that are chronic in human globally.

All the wells sampled were positive for *Escherichia coli*. The high occurrence of *E. coli* is an indication of water pollution by either animal or human wastes (30). The detection of *E. coli*, a coliform bacterium in water, is considered not only an indicator of fecal pollution but also a public health risk. Though most *E. coli* are harmless, however, some are pathogenic and can cause disease. Isaacson et al. (31) and Chalmers et al. (32) have reported a verocytotoxin producing *E. coli* (VTEC) serotype 0157, as recognized group of enteric pathogens causing illness such as diarrhea and hemorrhagic colitis, when contaminated water is consumed. Many of the wells may probably have been located near septic tanks and other household drainage systems. *E. coli* is potentially life-threatening pathogen, to members of a community, regardless of the age group, and the immunocompromised, too; these individuals are at risk of diseases affecting the gastrointestinal tract (GIT), urinary tract (UTIs), and possible source of bacteremia, when the polluted water is consumed (33).

All the bacterial genera isolates obtained were highly sensitive to gentamicin and moxifloxacin, which are non-β-lactam antibiotics belonging to the class aminoglycoside and quinolone, respectively. Overall, the isolates showed a sensitivity of 88% and 86%, respectively, to these antibiotics. This indicates that these drugs are effective and efficient against enteric bacteria. The results of the study are in agreement with the previous report of Odonkor and Addo (34) who disclosed 74.2% and 90.7% susceptibility to moxifloxacin and gentamicin. This shows that these antibiotics are considered potent agents in the treatment of bacterial infection. It is worthwhile to note that fluoroquinolone and aminoglycoside group of antibiotics are very effective against the water-borne bacterial isolates and the use of fluoroquinolone has been found to be potent in the treatment of diarrhea (35).

However, resistance to frequently used antibiotics has escalated among Gram-negative bacteria, especially in the family of *Enterobacteriaceae*, as a result of acquisition and dissemination of ESBL gene (36). Organisms harboring extended-spectrum β-lactamases (ESBLs) can form resistance synergy with other antibiotics from different classes, become resistant to multiple antibiotics, hence, limits treatment options. The emergence of antibiotic-resistant organisms in the environment are difficult to combat with regular antibiotics; environmental compartments serving as reservoirs of these resistant strains, spread the organisms into drinking water sources and consumed by humans; hence, constitute a risk to public health risk (37).

All the isolates from the various well waters sampled showed 100% resistance to the antibiotic class of β-lactams (ampicillin, ticarcillin and ceftazidime) and 55% resistance to tetracycline. These resistances may be attributed to the over-the-counter ready availability of these antibiotics for use in the community without medical prescription.

The high-level resistance to β-lactam drug (penicillin and cephalosporin) observed might lead to the production of β-lactamases, an enzyme capable of hydrolyzing the β-lactam ring and these antibiotics could be a serious problem that should be looked into. Hamelin et al. (38) in their studies also had a similar record of resistance to the β-lactam drug. The resistance caused by third-generation cephalosporins has been found to spread fast within the environment through mobile genetic elements or could be chromosomally mediated and this is attributed to the presence of extended-spectrum β-lactamase which they have been acquired (39) ESBL enzymes can hydrolyze penicillins, third generation cephalosporins, and monobactams but habour modest or no activity against cephamycins and carbapenems. Nevertheless, the high resistance to β-lactam antibiotics as observed in this research work, invariably implies a higher cost in the treatment of antibacterial infections caused by the obtained pathogens. This is because β-lactam drugs are cheap and are the antibiotics of choice for first-line treatments.

This study has shown a low (14%) resistance occurrence of bacteria from well water to carbapenem. The result agrees with the similar reports of 33% and 13% imipenem resistance in water bodies from Khartoum, Sudan, and California, USA, respectively (40, 41). The low value of carbapenem resistance recorded was probably due to the high cost of purchase of carbapenems drug in Nigeria, which makes it less available for use, thus resulting in decreased selection pressure for the development of resistance. Nevertheless, in this same study, the intermediate susceptibility results of the isolates to meropenem and imipenem, were 19% and 16% respectively, and are of public health concern. This observation may

imply that these isolates have native ability to produce carbapenemase enzymes that may subsequently lead to therapeutic failure, if not controlled on time. This is because infection from these bacteria can become difficult to treat due to a high level of antibiotic resistance and could lead to high mortality.

The CRE isolates also have the potential for widespread transmission of resistance via mobile genetic elements. There are varieties of antibiotic mechanisms among carbapenemase resistant *Enterobacteriaceae* which widely varies (42) and can contribute to carbapenem resistance. These mechanisms include decreased outer membrane permeability or loss of porin channels, overexpression of efflux pumps, and production of β-lactamases and carbapenemase; enzymes that degrade β-lactam drug and carbapenem (43) Also, there could be a possibility of resistance genes transfer through plasmid acquisition between bacterial organisms which are resistant to any form of the β-lactam antibiotics that have dipeptide ring (44).

The observation (Fig. 5) that two *Klebsiella* isolates (20%), out of the ten molecularly screened MDR isolates, carried the CTX-M gene may imply that these genes could be responsible for ESBL production and carbapenem resistance. Infections caused by *K. pneumoniae* are known to be resistant to multiple drugs and are ESBL producers in humans (45).

Carbapenems (imipenem and meropenem) are potent in treating severe antibacterial. Nevertheless, it was noted from this study that the bacterial isolates were resistant to the carbapenem antibiotics when screened using the disk diffusion method but produced negative PCR result, at molecular level (Fig. 6). This observation may imply that the resistance may be due to other ESBL or carbapenemase genes not screened for by the PCR technique. Alternatively, resistance in these CRE may not be through the production of carbapenemase enzyme but rather to other resistance mechanisms such as β-lactamases, mutation, porin loss, or efflux pump resulting to their ability to become resistant to the carbapenems. Another factor that may be responsible is that the resistant gene may be plasmid-mediated and not chromosomal-mediated. Reports of carbapenemase genes in bacteria from environmental sources in Nigeria are scarcely available for a comparative assessment of our results. However, a 36.8% prevalence rate of carbapenemase gene detection has been reported in hospital-based studies carried out in Sokoto, Nigeria (46).

This study has also revealed resistance by *Enterobacteriaceae* to third generation cephalosporins which may be through ESBL production, and consequently lead to carbapenem resistance. Some authors (47) have professed that β-lactamases (ESBL and AmpC) are not by themselves strong carbapenemases, but could combine with other resistance mechanisms to precipitate carbapenem resistance.

The isolation of carbapenem resistant Enterobacteriaceae from environments may also be linked with the use of environmental antibiotics either for agriculture and livestock production or in the unintentional presence of pharmaceutical products in soils, organisms and ground water (47, 48). The geosphere and biosphere are regions for the detection of drugs and its metabolites; with the consequent effect that these chemical therapeutic agents could bioaccumulate in the general food chain and affect the health of millions of people (48-50). The persistence of antibiotics in the environment may bring about selective pressures on bacteria in the soils to generate new antibiotic resistant strains, via drafting antibiotic resistance genes from a whirlpool of diverse bacteria genes present in the soil environment (51).

CONCLUSION

The detection of potentially pathogenic microorganisms in well water, as well as their resistance to clinically relevant antibiotics, is an indication of potential microbiological pollution of the well water and a subsequent health risk to consumers. Therefore, pre-treatment of well water with the simple process of boiling is essentially advised before drinking.

Furthermore, the finding of negative chromosomal resistance to the carbapenemase gene, may indicate that resistance is not only mediated by the production carbapenemase gene but also by the carriage of the ESBL gene ($bla_{\text{CTX-M}}$) and other mechanisms of resistance.

The limitation of the present study includes the limited number (10 out of 148) of well-water sourced bacterial isolates utilized for molecular screening and also the current study did not use primers to target all known carbapenemase gene. Therefore, for further studies, more bacterial isolates should

be screened to establish a significant finding on the trend of carbapenem resistance in well-water isolates from Iwo, Nigeria.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Management of Bowen University, Iwo, Nigeria for their financial support in paying the full cost of the article processing fees. Also, Prof. Olatunde Dahunsi and Dr (Mrs) Abimbola Owoseni, for their support, expert advice and encouragement.

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