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Antibiotic Susceptibility Profile of Bacteria Isolated from Drinking Water Sources in Amai Kingdom, Delta State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GIO, IHM and AAO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GIO, IHM and IAT managed the analyses of the study. Authors AAO and IAT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the susceptibility profile of bacterial isolates from hand dug wells and borehole waters within the Amai Kingdom, in Ukwuani Local Government Area of Delta State.

Place and Duration of Study: Department of Microbiology, Novena University, between April 2016 and November 2016.

Methodology: A total of 30 boreholes and 30 well water samples were randomly sourced using sterile tightly locked plastic containers and transported to the laboratory within 2 hours. The water

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samples were cultured using spread plate method and the pure bacterial isolates tested for susceptibility to some commercial antibiotic discs using the Kirby-Bauer method.

Results: Bacteria were isolated respectively from 50% and 100% of the borehole and well water samples collected. A total of 45 bacterial isolates, distributed among the genera *Staphylococcus* (6.7%), *Bacillus* (10.0%), *Escherichia* (16.7%), *Pseudomonas* (11.7%) *Enterobacter* (8.3%), *Proteus* (10.0%), and *Klebsiella* (11.7%), was encountered. Majority of the isolates were sensitive to Amoxicillin-clavulanic acid, Ciprofloxacin, Ceftriaxone, Gentamicin, Ofloxacin, and resistant to Ampicillin, Cotrimoxazole and Tetracycline. *Bacillus* sp (56%) was more resistant ($p=.05$) than *S. aureus* (33%), while the most resistant Gram-negative bacteria was *Pseudomonas* sp. (54%), followed by both *E. coli* (44%) and *Klebsiella* sp (44%), and both *Proteus* sp., (33%) and *Enterobacter* sp. (33%). Drug resistance was observed in at least three of the tested antibiotic disks.

Conclusion: Findings from this study suggest that the drinking water sources in the Amai Kingdom are bacteriologically poor, with multiple-drug resistant bacteria, and thus of public health importance. Public education on dangers associated with indiscriminate dumping of waste and drug abuse is strongly recommended.

Keywords: Water; antibiotics; coliforms; multi-drug resistance; Amai Kingdom.

1. INTRODUCTION

The biosphere is composed of air, land, water and its living components. Of all these components, water is generally known to occupy over 70% of the earth's surface. Water is a very basic natural requirement for life. It is so vital that humans cannot exist without it. However, access to potable water has been an age old challenge because of the rising human populations and activities, which are in no small measure impacting negatively on the chemical and microbiological qualities of available water supplies.

Over the past decades, much progress has been achieved on water supply, sanitation and hygiene according to World Health Organization report [1]. From the report, about 2.3 billion people gained access to improved drinking water between 1990 - 2012, and also, a decline in the mortality rate of children due to water-related diarrheal disease from approximately 1.5 million deaths in 1990 to over 600,000 in 2012. However, As the world turns its attention to the formulation of the post-2015 Sustainable Development Goals (SDGs) much remains to be done particularly in areas of improving more access to potable water, sanitation, improved sewage management systems and public enlightenment especially in developing countries that still have high percentage of water crisis [1].

Finding solutions to these problems have been challenged by the current waves in multiple-drug resistance and production of extended spectrum

beta-lactamases reportedly demonstrated by enteric bacteria [2-4]. The presence of extended spectrum beta-lactamases (ESBLs) genes confers on them multi-drug resistance abilities up to the third and fourth generations of cephalosporins, monobactams and carbapenems [5]. It is of great public health importance because of the possible transmission of their plasmid-encoded resistant genes to humans when they consistently drink contaminated water [4,6]. Currently, the incidence of human infections caused by extended-spectrum cephalosporin (ESC) resistant enterobacteria has risen unprecedentedly because they are becoming more resistant to multiple clinically important antibiotics [7,8]. It's therefore, pertinent to investigate the qualities and antibiotic sensitivity patterns of bacterial isolates from drinking water sources as an essential step in ascertaining their portability and drug resistant patterns.

There is a paucity of such information on the bacterial isolates from the major drinking water sources used in the Amai Kingdom; which is a fast growing community in Ukwuani Local Government Area of Delta State, South-Southern Nigeria. This study was therefore undertaken to augment the currently available information and with a view to bridging the knowledge gap.

2. MATERIALS AND METHODS

2.1 Study Area

The study site is Amai kingdom, which is one of the fastest developing rural communities in

Ukwuani local Government Area of Delta state (Fig. 1). It lies within latitude: 05° 45' N and longitude 06° 50' E in the Niger Delta region of Southern Nigeria. The surrounding communities include Obiaruku (North), Ogume (South), Umuebu (East), and Ezionum (West). Novena

University campus is located in the central part of the community (Fig. 2). The major occupation of the aborigines is peasant farming. Their major and easily accessible sources of drinking water in the community are hand dug wells and borehole.

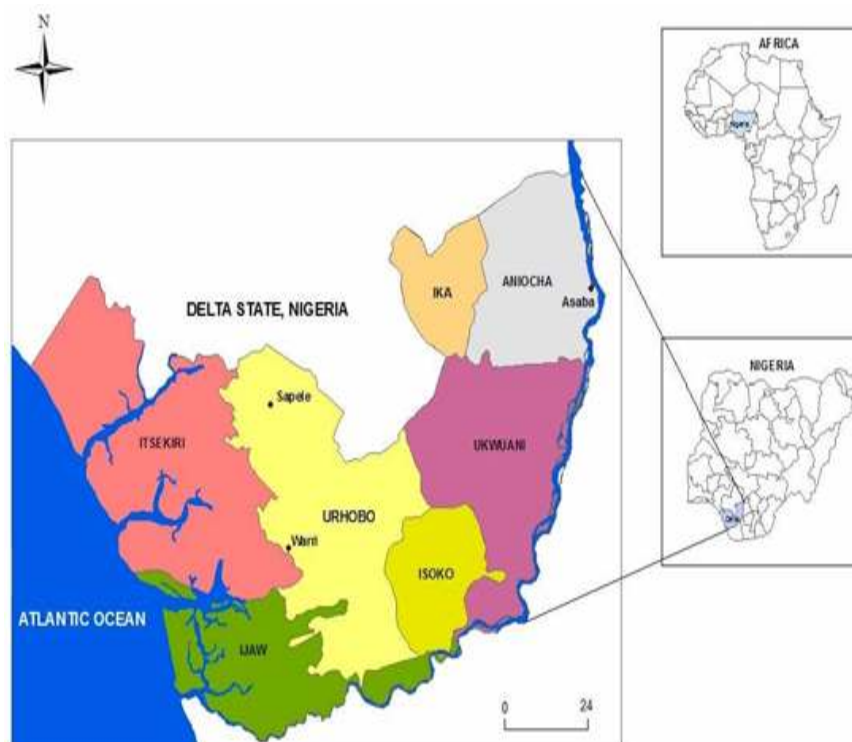


Fig. 1. Map of Delta State showing Major LGA/Ethnic groups [9]

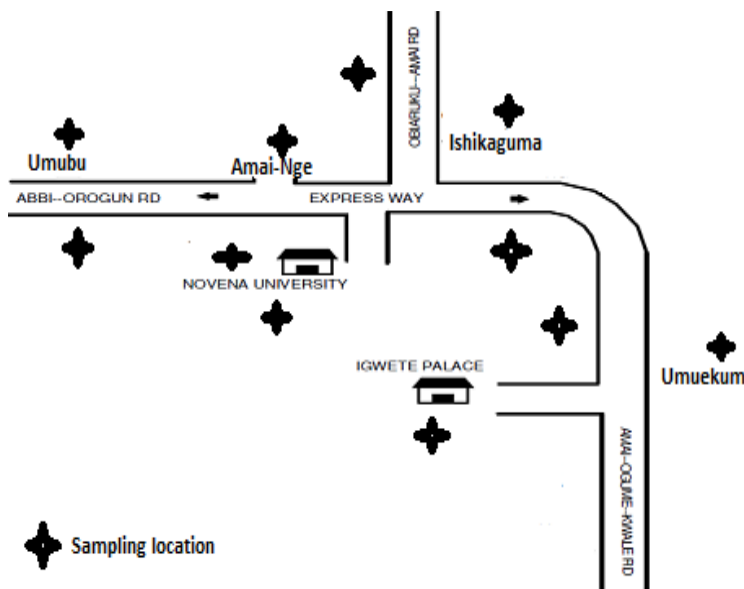


Fig. 2. Map of Amai showing sampling areas

2.2 Sample Collection

Water samples were collected from boreholes and hand dug wells used by Amai indigenes and dwellers as a source of drinking, cooking and bathing purposes. A total of 60 water samples, comprising 30 wells and 30 boreholes, were randomly collected. Samples from the wells were collected in duplicate by lowering a clean plastic container tied to a synthetic rope down the well. Samples from borehole water were collected by first opening the tap to flow out for about 2 min., before putting the container to collect. All the water samples were well labeled and transported in black polyethylene bags within 2hrs to the Department of Microbiology Laboratory, Novena University, for bacteriological study between April 2016 and November 2016.

2.3 Sterilization of Glassware and Other Materials

All glassware used were thoroughly washed with detergent, rinsed and allowed to dry. The glassware was then wrapped with aluminium foil and sterilized in the hot air oven at 170°C for 60 mins. The distilled water used for serial dilutions, was autoclaved at 121°C for 15 min. The work bench was swabbed with 70% alcohol before and after every experiment.

2.4 Bacteriological Analysis of Water Sample

One milliliter of the water samples from each location was added to 9 ml sterile peptone water, and serially diluted up to 10^{-4} . Thereafter, 0.1 ml was aseptically collected from each dilution of 10^{-2} and 10^{-4} , and cultured on sterile Nutrient agar, MacConkey agar and Eosin methylene blue agar plates using spread plate techniques, before incubation at 37°C for 24 hrs. Total viable counts of the colonies were calculated for the colonies examined on Nutrient agar, while total coliform counts and Faecal coliforms count were calculated from typical colonies growing on MacConkey agar and Eosin methylene blue agar incubated at 44.5°C. All the colony counts were expressed as cfu/100 ml of water sample.

2.5 Characterization and Identification of Bacterial Isolates

The colonies were subcultured to obtain pure isolates. The pure isolates were then characterized by Gram's Staining and Biochemical tests such as indole test, Voges-

Proskauer test, MethyRed test, citrate test, catalase tests, coagulase test, oxidase test, motility test and sugar fermentation test. Identity of the isolates was matched with the Bergey's Manual of Determinative Bacteriology for confirmation (Holt et al. 2002).

2.6 Antimicrobial Susceptibility Test

Antimicrobial susceptibility pattern of each isolate was done using conventional disc diffusion method according to National Committee Laboratory Standards (NCCLS) recommendation [10]. This was carried out using commercial multiple antibiotic discs. The discs used included Gentamicin (10 µg), Ampicillin (30 µg), Ofloxacin (5 µg), Chloramphenicol (25 µg), Ciprofloxacin (5 µg), Tetracycline (30 µg), Norfloxacin (30 µg), Cefuroxime (30 µg), and Amoxicillin (30 µg) for Gram-negative and Gentamycin (10µg), Cephalexin (30 µg), Cloxacilin (5 µg), Ceftriaxone (30 µg), Amoxicillin-clavulanic acid (augumentin) (30 µg), Cotrimoxazole (25 µg), Erythromycin (10 µg), Clindamycin (10 µg), and Ciprofloxacin (5 µg) for Gram-positive bacteria. A turbid suspension of the isolates was made in distilled water using 0.5 McFarland Standard, prepared as a comparator. A sterile swab was dipped into the bacteria suspension, pressed on the side of the bottles to allow excess drip-off, and then used to evenly streak the entire surface of the Mueller-Hinton agar. Sterile forceps were then used to place the multiple antibiotic discs in a circular pattern on the media. The process was carried out for all the identified isolates, and the plates incubated at 37°C for 24 h. After incubation, the zone of inhibition for each antibiotic was measured from the centre of the disc to the point where clearing stopped.

2.7 Statistical Analysis

Data was analyzed using the descriptive statistic SPSS (version 20).

3. RESULTS

A total number of 45 bacterial species distributed among two Gram-positive genera *Staphylococcus* and *Bacillus*, and five Gram-negative genera *Escherichia*, *Pseudomonas*, *Enterobacter*, *Proteus* and *Klebsiella*, were isolated from the water samples in the study locations (Table 1). Further analysis showed that 50% of the isolates occurred in all borehole water samples, while 100% of them occurred in well water samples. For the well water samples, *E. coli* (26.7%) appeared the most, while the

least frequent isolates were *S. aureus* (10.0%), *Proteus* sp. (10.0%), and *Enterobacter* sp. (10.0%). On the other hand, both *Pseudomonas* sp. (10.0%) and *Proteus* sp. (10.0%) occurred most in borehole water as against *S. aureus* (3.3%) that was the least isolate. The average mean total viable counts, total coliform and faecal coliform counts were 2.5×10^2 cfu/100 ml, 2.4×10^1 cfu/100 ml and 0 cfu/100 ml for borehole water and 6.4×10^6 cfu/100 ml, 2.4×10^3 cfu/100 ml and 0.7×10^2 cfu/100ml for well water samples (Table 1).

Tables 2 and 3 show the antibiotic susceptibility patterns of each isolate. Among the Gram-positive bacteria, *Staphylococcus* and *Bacillus* spp were highly susceptible to

Amoxicillin-clavulanic acid, Ciprofloxacin, Ceftriaxone, Gentamicin, Ofloxacin, and resistant to Ampicillin and Cotrimoxazole and (Table 3). Among the Gram-negative bacteria, majority of the strains displayed significant sensitivity to Ciprofloxacin, Ofloxacin and Gentamicin, and resistant to, Ampicillin, Tetracycline and Norfloxacin (Table 3). All the isolates displayed antimicrobial resistance to at least three of the tested antibiotics. Among the Gram-positive bacteria, *Bacillus* sp (56%) was more resistant to the antibiotics tested than *S. aureus* (33%) (Fig. 3). The most resistant Gram-negative bacteria was *Pseudomonas* sp. (54%), followed by both *E. coli* (44%) and *Klebsiella* sp (44%), and both *Proteus* sp., (33%) and *Enterobacter* sp. (33%) (Fig. 4).

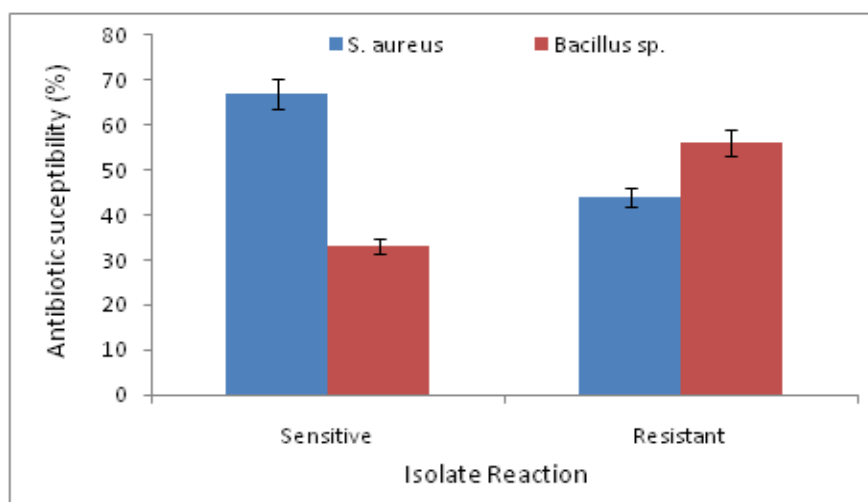


Fig. 3. Mean Percentage antibiotic susceptibility pattern of gram-positive bacteria

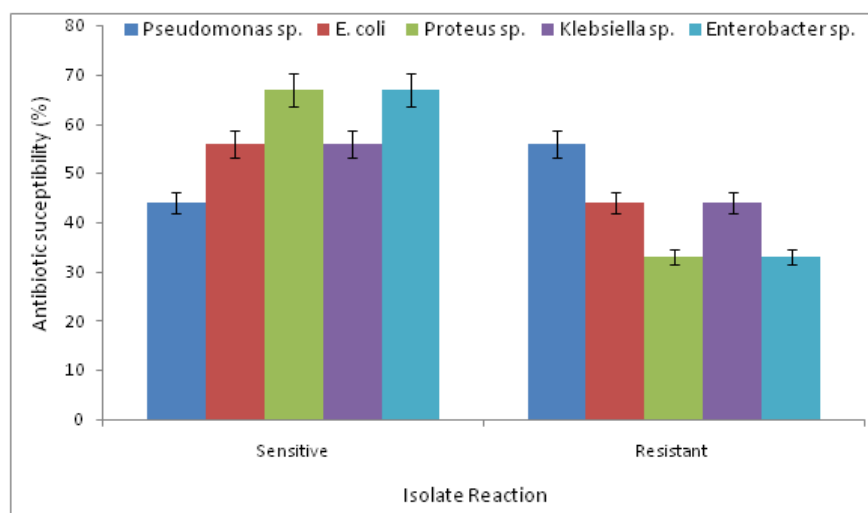


Fig. 4. Mean percentage antibiotic susceptibility pattern of gram-negative bacteria

Table 1. Percentage occurrence and bacterial loads of water samples

Isolates	Borehole water	Well water	Total
Gram-positive bacteria:			
<i>Bacillus</i> sp.	2 (6.7%)	4(13.3%)	6 (10.0%)
<i>Staphylococcus aureus</i>	1 (3.3%)	3 (10.0%)	4 (6.7%)
Gram negative bacteria:			
<i>Escherichia coli</i>	2 (6.7%)	8 (26.7%)	10 (16.7%)
<i>Proteus</i> sp.	3 (10.0%)	3 (10.0%)	6 (10.0%)
<i>Enterobacter</i> sp.	2 (6.7%)	3(10.0%)	5 (8.3%)
<i>Klebsiella</i> sp.	2 (6.7%)	5(16.7%)	7 (11.7%)
<i>Pseudomonas</i> sp.	3(10.0%)	4 (13.3%)	7 (11.7%)
Total	15 (50.0%)	30 (100%)	45 (75.0%)
Bacterial load			
Mean total viable count (cfu/ml)	2.5 x 10 ²	6.4 x 10 ⁶	
Mean total coliform count (cfu/ml)	2.4 x 10 ¹	1.4 x 10 ³	
Mean faecal coliform count (cfu/ml)	0	0.7 x 10 ²	

Table 2. Antibiotic susceptibility patterns of Gram positive bacterial isolates (No. of R or S isolates/ n)

Bacteria	AU	FX	AP	AM	CO	CX	GN	CD	E
<i>S. aureus</i> (n=4)	S (4/4)	S (4/4)	S (3/4)	NA	R (3/4)	R (4/4)	S (4/4)	S (4/4)	S (4/4)
<i>Bacillus</i> sp. (n=6)	S (5/6)	S (6/6)	R (5/6)	R (6/6)	R (5/6)	S (6/6)	S (6/6)	R (5/6)	R (6/6)

S=Sensitive, R=Resistant, CX=Cephalexin, GN=Gentamicin, AP=Cloxacillin, AM=Ampicillin, CD=Clindamycin, CP=Ciprofloxacin, AMX= Amoxicillin-clavulanic acid FX=Ceftriaxone, CO=Cotrimoxazole, E=Erythromycin.
Zone of Inhibition: 0 -13 mm = Resistance; 14 -17 mm = Intermediate sensitivity;
18 mm and above = Sensitivity, NA=Not applicable

Table 3. Antibiotic susceptibility patterns of Gram negative bacterial isolates (No. of R or S / n)

Bacteria	CIP	TE	NF	AX	OF	C	CF	AM	GN
<i>Pseudomonas</i> sp. (n=7)	S (7/7)	NA	R (7/7)	S (5/7)	S* (4/7)	NA	NA	R (7/7)	S (7/7)
<i>E. coli</i> (n=10)	S (8/10)	R (5/10)	S* (6/10)	R (9/10)	S (10/10)	S (4/10)	R (5/10)	R (7/10)	S (10/10)
<i>Proteus</i> sp. (n=6)	S (6/6)	R (6/6)	R (4/6)	S (6/6)	S (6/6)	S (3/6)	S (4/6)	R (6/6)	S (6/6)
<i>Klebsiella</i> sp. (n=7)	S (7/7)	S (6/7)	R (7/7)	R (4/7)	S (7/7)	R (6/7)	S (5/7)	R (4/7)	S (4/7)
<i>Enterobacter</i> sp. (n=5)	S (5/5)	R (3/5)	S (5/5)	S (5/5)	R (2/5)	S (5/5)	S (3/5)	R (4/5)	S (5/5)

*S = Intermediate sensitivity, S=Sensitive, R=Resistant, AM=Ampicillin OF=Ofloxacin, C=Chloramphenicol, CF=Cefuroxime, TE=Tetracycline, AX= Amoxicillin, NF=Norfloxacin CIP=Ciprofloxacin, GN=Gentamicin.
Zone of Inhibition: 0-13 mm = resistance; 14 -17 mm = Intermediate sensitivity;
18 mm and above = Sensitivity, NA=Not applicable

4. DISCUSSION

Microorganisms are ubiquitous in nature. They exist in virtually all parts of the environment. The pathogenic microorganisms have been a major public health challenge. One of the major routes of human infections is through drinking of contaminated water. Water is consumed on daily

basis to maintain the normal physiological needs. The major indicators of pathogens in drinking water supplies are the coliform bacteria. Of the three major groups of coliform bacteria, the presence of faecal coliforms usually confirms recent faecal contamination of water supply, since these groups of bacteria naturally exist in the intestine of humans and animals [11]. A

typical example of public health important faecal coliforms is *E. coli*.

In this study, *E. coli* were the most frequent isolates, especially from the hand-dug well water samples. This was expected because majority of the wells in the study areas were shallow, uncovered, close to dump sites and not too high from the ground levels. So that human activities and their by-products easily find their way through runoff, wind, leakages among others, into the well waters on a daily basis. Besides, the users of the water make use of any available containers to fetch water from the wells.

Thus, the high occurrence of bacteria in the well water samples could be attributed to the above reasons. A similar suggestion had been submitted in the past by researchers who studied the bacteriological qualities of boreholes and hand dug well waters in several Nigerian communities [12-14]. Going by the WHO standard for drinking portable water, both water sources examined in this study are not safe for drinking because they contain higher than 100cfu/100ml total heterotrophic bacteria counts and non-zero coliform counts as recommended.

The presence of *Klebsiella* sp., *Pseudomonas* sp., *Proteus* sp, *Enterobacter* sp. and *S. aureus* in both water sources are worth-noting because they have been implicated in diverse disease of humans, such as skin infections, wound infections, urinary tract infections, gastroenteritis and respiratory tract infections in susceptible healthy and immunocompromised individuals [15].

Our study further revealed that the bacterial isolates displayed different resistance patterns to some commercial antibiotic disks. All the isolates were resistant to at least three of the antibiotics tested in this study. This is an indication of multiple-drug resistance, which is currently a major challenge in medicine. Additionally, majority of the Gram-positive isolates were found to be highly sensitive to Amoxicillin-clavulanic acid, Ceftriaxone and Gentamicin, and resistant to Ampicillin and Cotrimoxazole. It suggests that such drugs might not be effective in the treatment of ailments resulting from both bacteria. This observation is in concordance with the report of Ogunleye et al. [16] that *Bacillus* and *Staphylococcus* isolated from well waters used in Iworoko-Ekiti, Nigeria, were totally resistant to Ampicillin. However, this finding is in discordance with the submissions of Soge et al.

[17] and Samie et al. [18], who respectively reported 50% and 92% resistances to Ampicillin in Uganda and South Africa. Most of the Gram-negative bacteria were found to be highly resistant to Ampicillin. Again, these findings in agreement with previous studies that several Gram-negative bacteria isolated from drinking water sources were becoming resistant to Ampicillin [16,19,20].

It was also found that *Bacillus* sp were more resistant than *S. aureus*, while among the Gram-negative bacteria, *Pseudomonas* sp. were the most resistant, followed by both *E. coli*, and *Klebsiella* sp, and both *Proteus* sp., (33%) and *Enterobacter* sp. (33%). *Bacillus* sp., isolated from various water sources, has been reported to exhibit diverse antibiotic resistance [21], probably due to their enzymatic versatilities and stable endospore-forming capacities. In recent times, multiple-drug resistance has been reported among *E. coli*, *Klebsiella* sp., *Pseudomonas* sp. and *Proteus* sp. in various clinical and water samples [22-24]. Previous studies have also reported that most of the isolates carry antibiotic resistant plasmid genes that are transferable among species [25]. Additionally, the recent rising wave of production of extended-spectrum beta-lactamases among the enteric bacterial isolates from drinking water sources and clinical samples is becoming worrisome [4]. The presence of extended spectrum beta-lactamases (ESBLs) genes confers on them multi-drug resistance abilities up to the third and fourth generations of cephalosporins, monobactams and carbapenems leading to high incidence of human infections [4,7,8]. This study, therefore, confirms previous reports that most of the bacteria isolates from water sources are becoming resistant to important antibiotics used by clinicians.

5. CONCLUSION

This study has shown that the two major sources of water used for drinking and other domestic activities in Amai Kingdom did not meet the bacteriological standard of less than 100 cfu/100 ml and zero coliform content. The presence of multiple-drugs resistant bacteria, such as *E. coli*, *Klebsiella* sp., *Pseudomonas* sp. and *Proteus* sp. is of public health importance, because of the risk of infections and spreading of resistant genes, thereby worsening the efficacies of the currently available chemotherapeutic agents. Hence, public educations on dangers associated with indiscriminate dumping of waste,

construction of poor standard boreholes/well facilities, drug abuse, as well as on simple practices of water treatment before drinking are strongly recommended in the study location.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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