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ENUMERATION AND DETERMINATION OF ANTIBIOGRAM OF BACTERIA ISOLATES FROM HAND-DUG WELL WATER IN BOSSO METROPOLIS, NIGERIA

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ABSTRACT

Regular water supply surveillance is crucial in low-income nations like Nigeria, where households often rely on alternative sources, predisposing preventable water-borne diseases. Therefore, this study determined the occurrence and antibiogram of bacteria in hand-dug well water in Bosso metropolis, Nigeria. Exactly 10 well water samples were collected and processed for bacteria isolation and identification using standard microbiological techniques. The antibiotic sensitivity was determined via Kirby-Bauer disc diffusion techniques. The total viable bacteria count ranged from 0.45×10^3 cfu/mL to 2.15×10^4 cfu/mL, while the total coliform bacteria count ranged from 0.27×10^3 cfu/mL to 8.91×10^4 cfu/mL. Out of the 18 different bacteria isolated, *Pseudomonas* species had the highest occurrence (26.9%), and the least (3.9%) was observed for each of *Shigella* sp., *Klebsiella* sp., and *Staphylococcus aureus*. Also, 7 out 26 bacterial isolates isolated were multidrug-resistant. *Escherichia coli*, *Shigella* sp., *Salmonella* sp., *Pseudomonas* sp., and *Klebsiella* sp. were susceptible (100%) to ciprofloxacin and ofloxacin, though resistant (100%) to ampicillin and amoxycillin/clavulanate. All the Gram-negative bacteria isolates except *Salmonella* sp. were susceptible to gentamycin. Similarly, *Pseudomonas* sp., *Proteus* sp., and *Salmonella* sp. exhibited varied resistance to nitrofurantoin (71–100%), cefuroxime (50–100%), and ceftazidime (42.9–100%). *Bacillus* sp., *Enterococcus* sp., *Staphylococcus* sp., and *Streptococcus* sp. were resistant to cloxicillin and amoxycillin/clavulanate. In addition, *Bacillus* sp. (100%) and *Enterococcus* sp. (100%) were resistant to ciprofloxacin while remaining susceptible to ofloxacin and amoxycillin/clavulanate. Our findings highlight the urgent need for enhanced water quality and public health measures in the study areas. The presence of multidrug-resistant bacteria in hand-dug well water, underscores the critical need for infrastructure, more surveillance, and awareness on safe water practices.

Keywords: persistent**,** hand-dug, metropolis, coliform, pathogenic

INTRODUCTION

Water serves as the most valuable component of every living organism (Oludairo and Aiyedun, 2016; Idowu *et al*., 2011). However, the quality of limited amount of fresh water available on earth is under constant pressure (Oyedum *et al*., 2016). Conserving the quality of fresh water is essential for the food production, recreational water use and drinking-water supply.

Hand-dug wells remain a very important source of portable water in developing areas of the world including Nigeria where they are routinely dug (Idowu *et al*., 2011). Many rely on this untreated surface and ground water for domestic use, which is often contaminated with fecal matter of plants and/or animal origin (Reyes-López *et al*., 2008; Oluyege *et al*., 2009). Such water bodies do contain pathogenic bacteria capable of causing infectious diseases (Gandaseca *et al*., 2011; Oyedum *et al*., 2016).

Undoubtedly, treated pipe borne water supply is scarce and epileptic in Nigeria particular in the semi-urban and rural setting. Consequently, majority of the populace that resides in these areas depends on hand-dug well as their ultimate source of water for domestic uses (Ibrahim *et al*., 2018).

One of the major threat to public health in the recent time is antibiotic resistance and the role of the environments in the spread of pathogenic bacteria implicated in life threatening diseases is gaining unprecedented attention (Bengtsson-Palme *et al*., 2018; Larsson *et al*., 2019). This is partly due to improper sewage and fecal disposal alongside pollution arising from agricultural activities (Huijbers *et al*., 2015).

Previous studies by Eseoghene *et al*. (2013) and Oyedum *et al*. (2016) conducted in Gidan Kwano and Bosso parts of Minna, Niger State, Nigeria demonstrated the occurrence of bacteria isolates of public health concern in sampled well water, therefore, necessitating further study. Hence, this study investigated the antibiogram of bacteria isolates from hand-dug well water in Bosso Metropolis in order to ascertain the safe use of this water.

MATERIALS AND METHODS

Description of the sample site

Well waters (10) were obtained from different locations in Bosso metropolis, Minna, Niger State, Nigeria (Figure 1). Minna is a developing metropolitan in the North-central, Nigeria situated within the latitude (8º20º N; 11º30º N) and longitude (3º30º E; 7º20ºN). Minna is the capital of Niger state and has 74,344km^2 wide landmark with an estimated population of 286,838 in 2016 (Ademola *et al.*, 2018).

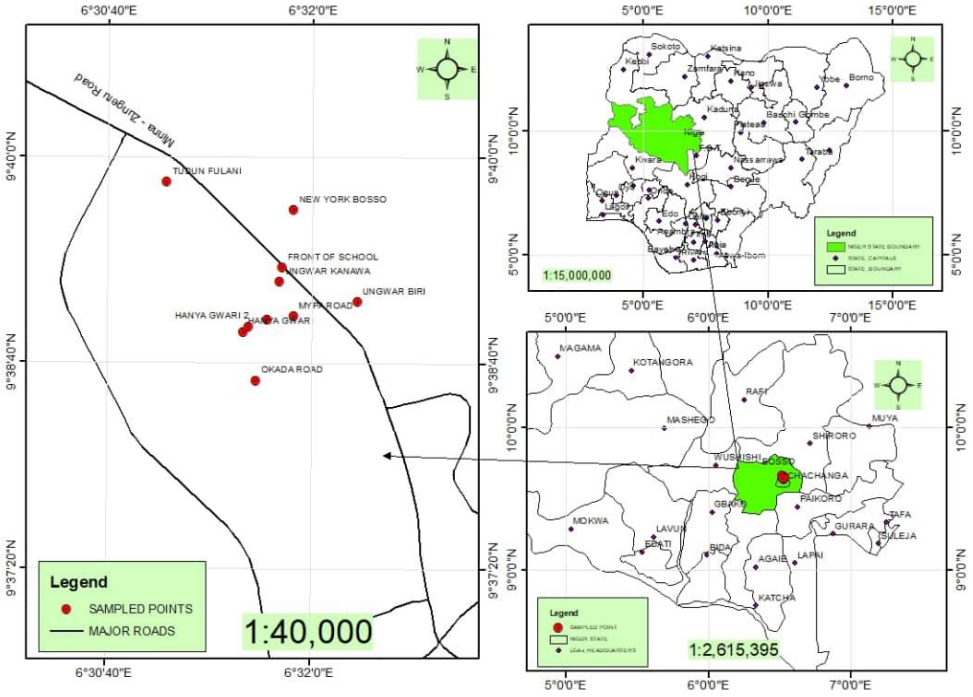

Figure 1: Study area and distribution of hand-dug well water included in the study

Sample Collection and enumeration of bacteria in hand-dug well water samples

One hundred and fifty millilitre (150 mL) water were collected in duplicate into sterile sampling bottles from the sampled hand-dug wells. One milliliter of each sample was serially diluted to 10^{-6}. Twenty milliliter of sterile molten Nutrient agar (NA) and MacConkey agar (MCA) was dispensed in duplicate into sterile petri dishes, each containing 1 mL of 10^{-4} of the serially diluted water sample. The mixtures were swirled gently, allowed to set at 28 ±4°C and incubated invertedly for 24 hours at 37°C in an incubator. The colonies that emerged were enumerated and presented as colony forming unit per milliliter (cfu/mL).

Bacterial isolation

A loopful of the well water samples enriched in 9 mL sterile peptone broth at 37ºC for 24 hours was inoculated on blood agar (BA), Salmonella Shigella Agar (SSA), Eosin Mthylene Blue Agar (EMB) and Mannitol Salt Agar (MSA). The inoculated plates were incubated at 37ºC for 24 hours as described by Jannatul *et al*. (2016). Discrete bacteria colonies on the 24 hours old culture plates were sub-cultured by streaking onto fresh corresponding medium for pure isolates. The pure isolates were sub-cultured on double strength NA slants and kept at 4ºC for further use.

Identification of the iolated bacteria

Standard microbiological methods were used for the identification of the isolated bacteria. The biochemical tests carried out include: Gram staining catalase, coagulase and oxidase**,** indole, hydrogen sulphide, methyl red, citrate and urease test (Arya *et al*., 2019; Cheesebrough, 2006).

Antibiotics susceptibility tests

Commercially available antibiotics discs (Rapid Lab: LOT SK03/P) of antibiotics in use for the treatment of both Gram-negative and Gram- positive infections were tested against isolated bacteria. The antibiotics cloxicillin, ampicillin, amoxycillin/clavulate, ofloxacin ciprofloxacin gentamycin nitrofurantoin erythromycin ceftazidime, cefuroxime, and ceftriaxone (Table 1). Individual colonies of the isolated bacteria were adjusted to 0.5 McFarland standards in normal saline and inoculated on Muller Hinton agar (MHA) using ~~a~~ sterile swab sticks. Thereafter, antibiotics discs were placed at 25 mm spacing apart on the inoculated MHA plate while 6 mm sterile whatman filter paper No. 3 (Germany), impregnated with sterile distilled water was used as control. The plate was then incubated at 37°C for 18 hours and observed were classified as susceptible, intermediate, or resistant based on Clinical Laboratory Standard Institutes' interpretation (CLSI, 2018). Multidrug resistance index (MARI) per individual isolate was determined based on the method described by Tumbarello *et al*., (2012).

Table 1: Classes of antibiotics and antibiotics used in this study

Classes of antibiotics	Antibiotics
Penicillin	cloxicillin (CXC), ampicillin (AMP) and amoxycillin/clavulanate (AUG)
Quinolones	ofloxacin (OFX) and ciprofloxacin (CPR)
Aminoglycoside	gentamycin (GEN)
Nitrofurans	nitrofurantoin (NIT)
Macrolides	erythromycin (ERY)
Cephalosporins	ceftazidime (CAZ), and cefuroxime (CRX)

Data analysis

The statistical package for social sciences (SPSS) version 24 was used to analyse the study's data. In assessing the association between the rate of resistance, intermediate, and susceptibility patterns of the isolated bacteria to the antibiotics, cross-tabulation was used. ANOVA and Duncan's Multiple Range Test (DMRT) were used to assess whether there were significant changes in the bacteria load of hand-dug well water from Bosso city, Minna, Nigeria ($p > 0.05$).

RESULTS AND DISCUSSION

Microbial load of hand-dug well water in Bosso metropolis, Minna, Nigeria

The microbial count of hand-dug well water sampled were presented in Table 2. The total viable bacteria count ranges 4.5×10^3 to 2.15×10^4 cfu/mL while, the total coliform bacteria counts were between 2.7×10^3 and 8.91×10^4 cfu/mL

Table 2: Microbial load of hand-dug well water in Bosso metropolis, Minna, Nigeria

Sample codes	TVBC ($\times10^4$)	TCBC ($\times10^4$)
1	0.81^{c}	1.22^{ab}
2	0.53^{d}	1.01^{ab}
3	2.15^{a}	8.91^{a}
4	0.91^{c}	2.02^{ab}
5	0.74^{c}	1.65^{ab}
6	2.12^{a}	1.0^{ab}
7	0.57^{d}	0.33^{b}
8	0.46^{d}	0.72^{b}
9	1.2^{b}	0.27^{b}
10	1.1^{b}	0.30^{b}

Values are means of duplicate determinations. Means with dissimilar letter (s) differ significantly according to the Duncan Multiple Range Test (DMRT) $\mathbf{p \leq 0.05}$

Keys: TVBC = Total Viable Bacteria Count and TCC = (Total Coliform Count)

Morphological and biochemical characteristics of bacteria isolated from hand-dug well water in Bosso metropolis, Minna, Nigeria

Twenty-six (26) bacteria were isolated and identified as *Staphylococcus* sp. (3.9%), *Klebsiella* sp. (3.9%), *Bacillus* sp. (11.5%), *Proteus* sp. (7.7%), *Escherichia* coli (11.5%), *Streptococcus* sp. (7.7%), *Enterococcus* sp. (7.7%), and *Pseudomonas* sp. (26.8%) (Table 3 and Figure 1).

Antibiogram of bacterial isolates from hand-dug well water in Bosso metropolis, Minna, Nigeria

Escherichia coli, *Shigella* sp., *Salmonella* sp., *Pseudomonas* sp. and *Klebsiella* sp. were susceptible (100%) to ciprofloxacin and ofloxacin. However, they were not susceptible to ampicillin (100%) (Table 4). Similarly, all the isolated gram-negative bacteria isolated except *Salmonella* sp. were susceptible to gentamycin.

Furthermore, 100% of the *Proteus* species isolated was resistant to ampicillin, augmentin and nitrofurantoin while the *Pseudomonas* sp., and *Salmonella* sp. displayed a range of resistance levels to nitrofurantoin (71-100%), cefuroxime (50-100%) and ceftazidime (42.9-100%) (Table 4).

Bacillus sp., *Enterococcus* sp., *Staphylococcus* sp. and *Streptococcus* sp. demonstrated resistance to the activity of cloxicillin and amoxycillin/clavulanate. Similarly, Bacillus sp. (100%), Enterococcus sp. (100%) and Streptococcus sp. (62.5%) were resistant to ciprofloxacin. However, all the gram-positive bacteria isolates from the hand-dug well water sampled in Bosso metropolis were susceptible to ofloxacin and amoxycillin/clavulanate (Table 5).

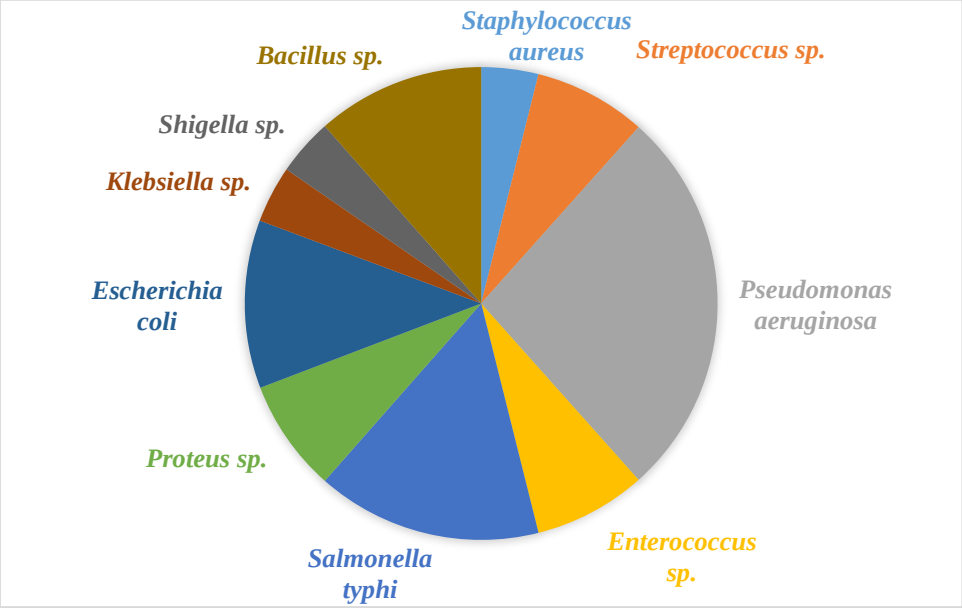

Figure 2: Occurrence of bacterial isolates from hand-dug well water in Bosso metropolis, Minna, Nigeria

Table 3: Morphological and biochemical characteristics of bacterial isolates from hand-dug well water in Bosso metropolis, Minna, Nigeria

Ic	Sh	GR	Cat	Cit	Ure	Coa	Glu	Oxi	H_2S	Lac	Ind	Mot	VP	MR	Man	Suspected Bacteria
1	C	+	+	+	-	+	+	-	-	+	-	-	+	-	+	*Staphylococcus* species
2	R	-	+	+	+	NA	+		-	+	-	-	+	-	+	*Klebsiella* species
3	R	-	+	+	+	NA	+	-	+	-	-	-	-	+	-	*Proteus* species
4	R	-	+	-	-	-	+		-	+	+	+	-	+	+	*Escherichia coli*
5	R	-	+	+	-	NA	+	-	-	-	-	+	-	+	+	*Salmonella* species
6	C	+	-	-	-	NA	+	-	NA	+	-	-	-	-	-	*Streptococcus* species
7	R	+	+	+	-	NA	-	-	NA	-	-	+	+	-	+	*Bacillus* species
8	C	+	-	-	-	NA	+	-	-	+	-	-	+	NA	+	*Enterococcus* species
9	R	-	+	+	-	-	-	+	-	-	-	+	-	-	+	*Pseudomonas* species
10	R	-	+	-	-	NA	NA	-	-	-	+	-	-	+	+	*Shigella* species

Key: Ic (Isolate code), GR (Gram Reaction), Sh (Shape), Ct (catalase), Cit (Citrate), H_2S (Hydrogen Sulphide), Mot (Motility), Nit (Nitrate), MR (Methyl Red), VP (Vorges Proskauer), Ure (Urease), Oxi (Oxidase), Ind (Indole), Glu (Glucose), Coa (Coagulase), Lac (Lactose) and Man (Manitol), C (Cocci), R (Rod), + (Positive), - (Negative) , NA (Not applicable), Sc (Sample code)

Table 4: Antibiotics resistant patterns of Gram-negative bacteria from hand-dug well water in Bosso metropolis, Minna, Nigeria

Bacteria isolates	No. of isolates	Patterns	CPR (%)	GEN (%)	AMP (%)	AUG (%)	NIT (%)	CRX (%)	CAZ (%)	OFL (%)
		I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Escherichia coli	3	R	0(0)	0(0)	3(100)	3(100)	0(0)	0(0)	0(0)	0(0)
		S	3(100)	3(10)	0(0)	0(0)	3(100)	3(100)	3(100)	3(100)
		I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Klebsiella sp.	1	R	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)
		S	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)	1(100)
		I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2(28.6)	0(0)
Pseudomonas sp.	7	R	0(0)	0(0)	7(100)	7(100)	5(71.4)	6(85.7)	3(42.9)	0(0)
		S	7(100)	7(100)	0(0)	0(0)	2(28.6)	1(14.3)	2(28.6)	7(100)
		I	1(50)	0(0)	0(0)	0(0)	0(0)	1(50)	2(100)	1(50)
Proteus sp.	2	R	0(0)	0(0)	2(100)	2(100)	2(100)	1(50)	0(0)	0(0)
		S	1(50)	2(100)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)
		I	0(0)	1(25)	0(0)	0(0)	0(0)	0(0)	2(50)	0(0)
Salmonella sp.	4	R	0(0)	1(25)	4(100)	4(100)	4(100)	4(100)	2(50)	0(0)
		S	4(100)	2(50)	0(0)	0(0)	0(0)	0(0)	0(0)	4(100)
		I	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)
Shigella sp.	1	R	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)
		S	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	1(100)
p-value			0.188	0.739	0	0.007	0.007	0.406	0.249	0.188

Key: I (Intermidiate), R (Resistant), S (Susceptible); *For other abbreviations see Table 1.

Table 5: Antibiotics resistant patterns of Gram-positive bacteria from hand-dug well water in Bosso metropolis, Minna, Nigeria

Bacteria isolate	No. of isolates	Pattern	CXC (%)	AUG (%)	GEN (%)	ERY (%)	OFL (%)	CAZ (%)	CRX (%)	CPR (%)
		I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Bacillus sp.	3	R	3(100)	2(66.7)	0(0)	2(66.7)	0(0)	2(66.7)	1(33.3)	3(100)
		S	0(0)	1(33.3)	3(100)	1(33.3)	3(100)	1(33.3)	2(66.7)	0(0)
		I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Enterococcus sp.	2	R	2(100)	2(100)	0(0)	1(50)	0(0)	2(100)	0(0)	2(100)
		S	0(0)	0(0)	2(100)	1(50)	2(100)	0(0)	2(100)	0(0)
		I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)
Staphylococcus sp.	1	R	1(100)	1(100)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)
		S	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	1(100)
		I	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	1(12.5)
Streptococcus sp.	2	R	2(100)	2(100)	0(0)	2(100)	0(0)	1(50)	2(100)	5(62.5)
		S	0(0)	0(0)	2(100)	0(0)	2(100)	0(0)	0(0)	2(25.0)
p-value			0.000	0.592	---	0.620	----	0.28	0.045	0.125

Keys: I (Intermidiate), R (Resistant), S (Susceptible); *For other abbreviations see Table 1.

Staphylococcus sp. and *Enterococcus* sp. were susceptible to ceftazidime and cefuroxime respectively to which *Bacillus* sp. and *Streptococcus* sp. exhibited varying degree of resistance (≥ 33.3%) (Table 6). In addition, all the 7 out 26 bacterial isolates from hand-dug well water in Bosso metropolis, Minna, Nigeria were multidrug resistant with MARI greater than 0.3 (Table 6).

Table 6: **Multidrug resistance and MAR index of the isolated bacteria**

Isolates	MDR resistance pattern	MAR index
E. coli	AMP/AUG	0.2
Klebsiella sp.	AMP/AUG	0.2
Pseudomonas sp.	AMP/AUG/NIT/CPR	0.5
Proteus sp.	AMP/AUG/NIT/CRX	0.5
Bacillus sp.	CXC/AUG/ERY/CAZ/CPR	0.6
S. aureus	CXC/AUG/ERY	0.3
Streptococcus sp.	CXC/AUG/ERY/CAZ/CRX/CPR	0.7
Enterococcus sp.	CXC/AUG/ERY/CAZ/CPR	0.6
Salmonella sp.	AMP/AUG/NIT/CRX/CAZ	0.6
Shigella sp.	AMP/AUG/CAZ	0.3

*For abbreviations see Table 1.

Discussion

The findings of this study showed that hand-dug well water have variable microbiological characteristics. The TVBC and TCBC from the sampled hand--dug wells varied from 4.5×10^3 to 2.15×10^4 cfu/mL and 2.7×10^3 to 8.91×10^4 cfu/mL, respectively. This report is consistent with the earlier findings by Aboh *et al*. (2015) and Oyedum *et al*. (2016), where high coliform counts compared to the WHO standard (≥ 10 coliform/100 ml) was reported. The variation in TVBC and TCBC count suggest that the levels and/or types of pollution are different (Allamin *et al*., 2015). Therefore, well water is suspicious and unsuitable for drinking, as well as domestic purposes and so, requires treatments before consumption (Obiora, 2014). The lowest viable count and coliform counts observed in sample 7 and 8 may be due to the presence of a protective covering on the well as well as the greater distance between the well and any potential contamination source. Furthermore, good hygienic practices as observed among users of this well may be a contributing factor.

The well water samples were contaminated with one or more bacterial pathogens. The high prevalence of potential bacteria pathogens in the well water surveyed in this study suggest that water borne diseases in the studied may be casually related to the drinking water source.

The high occurrence of *Salmonella* sp. and *E. coli* (Figure 1) implies fecal contamination, and could be due to the fact that most of the wells sampled were not adequately covered or located close to drainage or sewage systems. Similarly, most of the sampled wells are shallow, therefore, their contamination may be as result of inflow from surface waters enhanced by rainfall (Efuntoye *et al*., 2010). The used of dirty containers for drawing water from the wells may also be responsible for the microbial contamination as observed among majority of the populace. Additionally, inadequate sanitation and unhygienic practices among the users may also facilitatesthe reduction of microbiological quality of the well water (Egbe *et al*. 2013). However, contrary to the findings of this study, Oyedum *et al*. (2016) and Efuntoye *et al*. (2016) found *E. coli* (24.4% and 36.5% respectively) as the most predominant specie isolated from well water.

Pseudomonas sp., *Proteus* sp., *Bacillus* sp., *S. aureus*, *Streptococcus* sp., *Salmonella* sp., *Shigella* sp. and *Enterococcus* sp. isolated in this study were multidrug resistant having ≥ 3 MRI. Therefore, the multidrug-resistant bacteria isolated from the hand-dug wells in Bosso, Nigeria are from a high-risk source with constant anthropogenic pressure responsible for antibiotic resistant selection. This report is similar to the report obtained by Efuntoye *et al*. in 2010 where isolated bacteria were found to be resistant to more than three 3 antibiotics from different antibiotic classes. Result analysis revealed a very ampicillin and augmentin high resistant (100%) by the isolated Gram negative bacteria which is in line with the report by Adejuwon *et al*. (2011), where ampicillin resistance bacteria were recovered from well water. Inadequate use and disposal of left-over antibiotic may have contributed greatly to the development of resistant to these first line drugs. This is dangerous to public health as the consumption of contaminated well water could facilitate the transfer of these bacteria to humans. The significant resistant observed to ceftazidime and cefuroxime in this study highlights a concerning trend. This observation undermined the use of cephalosporin as the last resort and may underscore the emergence, and spread of cephalosporin resistant mechanisms among bacteria in the study environment.

CONCLUSION

The presence of multidrug-resistant bacteria in hand-dug well water, underscores the critical need for infrastructure, more surveillance, and awareness on safe water practices.

RECOMMENDATIONS

i. Good environmental and personal hygiene must be ensured especially by users of well water to avoid their contamination with bacterial pathogens.
ii. Boiling of well water before consumption should be encouraged to prevent the incidence of waterborne diseases.
iii. The situation of siting wells near surface water run-off or soak away should be discouraged.

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