Incidence of Multiple Antibiotic-Resistant Gram-Negative Bacteria Isolated from Drinking Water Sources in Ado-Ekiti, Ekiti State, Nigeria

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Abstract: This study investigated the incidence of multiple antibiotic-resistant Gram-negative bacteria isolated from drinking water sources in Ado-Ekiti, Nigeria. Fifty samples of drinking water from wells, boreholes, streams, sachet and bottled water were randomly collected from Ado-Ekiti metropolis, Nigeria for microbiological analyses. Isolation, identification, antibiotic sensitivity testing of Gram-negative bacteria were carried out using standard methods. The mean total viable and coliform counts of water samples were considerably high. A total of eighty-two (82) Gram-negative bacteria comprising *Klebsiella* spp, *Pseuddmonas* spp, *Proteus* spp, *Shigella* Spp, *Escherichia coli*, *Salmonella* spp, *Alcaligens* spp and *Citobacter* spp were isolated. The percentage resistance of the isolates to the antibiotics ranged from 0.6%, in ofloxacin and gentamicin, to 31.5%, in amoxicillin with multiple antibiotic resistance (MAR) to 3 to 6 classes of antibiotics. Fifteen MAR Gram-negative bacteria examined for the presence of plasmid revealed that all except *Klebsiella* sp, haboured a conjugative plasmid of >lKb. The investigation on the incidence of water-borne diseases in Ado-Ekiti, Nigeria revealed that 26.2% respondents claimed to have experienced water-borne diseases in the last three years which is statistically significant (p-value = 0.00). Therefore, adequate drinking water sanitation and disinfection program must be put in place to ensure safety against water-borne antibiotic-resistant pathogens in Ekiti state and by extension Nigeria.

Key Words: Drinking water sources, Gram-negative bacteria, Multiple antibiotic-resistance, Plasmid, Water-borne diseases

Introduction

In Nigeria, despite the abundant water sources, portable drinkable water development has not been able to match the feet with phenomenal population increase (Oteze, 2006). Water is a vital environmental factor to all forms of life and plays a great role in socio-economic development of human population (Aderibigbe *et al*., 2008). Many of the sources of water available for drinking include surface water (streams, rivers and lakes), ground water (wells, boreholes and springs) and rain water. Unfortunately, these sources of drinking water are contaminated through human activities (Al-Dulaimi and Younes, 2017). For instance, rivers are used as site for the disposal of refuse, human sewage, domestic and industrial waste waters. Streams and rivers running through areas of significant human influence such as farms, cities and industrial locations, are therefore prone to the pollution, especially in Nigeria where environmental protection regulations are not strictly enforced (Figueras and Borrego, 2010).

The pathogenic organisms spread by polluted water include parasites, protozoans, viruses and bacteria of public health importance (Ashbolt, 2015).

Bacteria species commonly isolated from the polluted water bodies include *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Vibrio* species, *Salmonella* species among others (Dziuban *et al*., 2006). Water pollution is a major cause of illnesses (Nwachukwu, 2001; Omololu-Aso *et al*., 2010; Raji and Ibrahim, 2011) and potential health problems may exist due to the microbial content of the drinking water since water is one of the vehicles for the transmission of pathogenic and antibiotic-resistant organisms (Nwidu *et al*., 2008; Allen *et al*., 2010).

The emergence of bacteria that are resistant to most of the commonly used antibiotics is of considerable medical significance because of public health implications (Khan and Maliki, 2001; Allen *et al*., 2010). The presence of antibiotic-resistant bacteria in different water sources throughout the world has been documented (Ronald *et al*., 2002; Allen *et al*., 2010; Oluyege *et al*., 2011; Tagoe *et al*., 2011; Olowe *et al*., 2015). Selection of antibiotic-resistant bacteria in nature may result from natural production of antibiotics by soil organisms, runoff from animal feed or crops, or waste products from treated animals or humans. Natural reservoirs of resistance genes may provide a source of transferable traits for emerging pathogens (Ronald *et al*., 2002).

Water is one of the components of the earth which serves as a usual habitat for Gram-negative

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bacteria (Ronald *et al.*, 2002; Habi and Daba, 2009; Olaoye and Onilude, 2009). The importance of Gram-negative bacteria has increased since the advent of broad spectrum antibiotics because these organisms often carry multiple antibiotic-resistant genes. Therefore, their presence in drinking water sources is of public health significance. This study was carried out to determine the incidence of multiple antibiotic-resistant Gram-negative bacteria isolated from drinking water sources in Ado-Ekiti, Nigeria.

Materials and Methods

Collection of Water Samples

Fifty samples of drinking water from underground water (wells and bore holes), surface water (stream), and commercially produced water (sachet and bottled water) were randomly collected from Ado-Ekiti metropolis, Nigeria for microbiological analysis as described by the Standing Committee of Analysts (2002). All the samples were taken to the laboratory on ice for bacteriological examination within 6 hours of collection.

Bacteriological Analysis of the Water Samples.

Total viable count and total coliform counts were determined using standard methods employed by Oyedeji *et al.* (2010). The total coliform count was determined by using membrane filtration technique. One hundred ml of each water sample was filtered through a sterile membrane Whatman filter paper (0.45 µm), after which the membrane filter was inoculated with its face retaining bacteria upward on Eosin Methylene Blue agar. The plates were incubated at 37°C for 24 hours. After 24 hours of incubation, the plates were examined for growth and discrete colonies developed were counted and recorded.

Isolation and Identification of Gram-Negative Bacteria from the Water Samples.

Isolation and identification of Gram negative bacteria were carried out according to the standard methods of Standing Committee of Analyst (2006). Pure cultures of isolated organisms were Gram differentiated and then biochemically identified according to Cowan and Steel (1991). The biochemical tests included citrate utilization test, oxidase test, indole test, methyl red test, motility test and triple sugar iron test.

The antibiotic sensitivity testing was carried out using the techniques of Clinical Laboratory Standards Institute (CLSI) (2015). Sensitivity testing of the bacterial isolates was carried out against eight classes of antibiotics (Biotec Lab. United Kingdom) which comprised of Tetracycline: Tetracycline (TET, 30µg); Betalactam: Augmentine (AUG, 30 µg); Fluoroquinolone: Ofloxacin (OFL, 30 µg); Quinolone: Nalidixic acid (NAL, 30 µg); Aminoglycoside: Gentamicin (GEN, 10 µg); Nitrofurans: Nitrofurantoin (NIT, 30 µg); Sulfonamide: Cotrimoxazole (COT, 25 µg) and Penicillin: Amoxicillin (AMX, 25 µg).

Incidence of Water-Borne Diseases in Ado-Ekiti, Nigeria

The incidence of water-borne diseases in Ado-Ekiti was investigated by administering questionnaire to 1000 residents of the community from where the water samples were collected. The data collected from the respondents were analyzed descriptively, using SPSS version 16 (SPSS Inc. Chicago, IL)

Statistical Analysis of Data

The data obtained in this study were compiled and analyzed descriptively using Ms Excel (Microsoft corp., USA) and SPSS 16.0 statistical package (SPSS Inc. Chicago, IL). The significant level at $p \leq 0.05$ was used.

Results

Bacterial Population of Drinking Water Sources in Ado-Ekiti, Nigeria

The estimation of the mean total viable count (TVC) and the mean total coliform count (TCC) of the different drinking water samples in Ado-Ekiti, Nigeria are presented in Table 1. It was observed that the mean TVC and TCC of stream water samples were much higher than that of other water samples. It was also observed that the mean TVC and the mean TCC increase in the same order of stream water, well water, borehole water, sachet water and bottled water. Therefore, it can be deduced from the table that the higher the mean TVC, the higher the mean TCC. Statistically, the mean TVC and TCC of the water samples are not significant. (*P*-value = 0.33 and 0.36 for mean TVC and mean TCC respectively).

Table 1. Mean Total Viable Bacterial Count (TVC) and Mean Total Coliform Count (TCC) of Different Drinking Water Sources in Ado-Ekiti, Nigeria

Drinking water sources examined (n)	Mean TVC ± SD ($\log_{10}$ cfu/ml)	Mean (TCC) ± SD ($\log_{10}$ cfu/ml)
Well (10)	4.57 ±0.35	3.84 ± 0.38
Borehole (10)	4.49 ± 0.29	2.59 ± 1.82
Stream (10)	5.39 ± 0.24	4.05 ± 0.22
Sachet (10)	4.14 ± 0.36	0.73 ± 1.56
Bottled (10)	3.50 ± 0.33	0.31 ± 0.97
***P*-value**	0.33	0.36

Identification and Occurrence of Gram-Negative Bacteria Recovered from Drinking Water Sources in Ado-Ekiti, Nigeria

A total of 82 isolates were recovered from all the samples of drinking water collected. The Gram staining test carried out on all the isolates showed that100% of the isolates were Gram-negative organisms. The biochemical characterization (Table 2) performed on all the isolates grouped the isolates into various genus; 20.7% were identified as *Klebsiella* spp., 15.9% as *Pseudomonas* spp., 19.5% as *Proteus* spp., 6.1% as *Shigella* spp., 11.0% as *Escherichia coli*, 17.1% as *Salmonella* spp., 6.1% as *Alcaligenes* spp. and 3.7% as *Citrobacter* spp.

Table 2: Biochemical Characterization of Isolates from Drinking Water Sources

Organisms	Biochemical Tests								
	Methyl red	Oxidase	Citrate	Indole	Motility	Triple sugar iron			
						Slant	Butt	G. P.	H_2S
Klebsiella spp.	+	_	+	_	_	Yellow	Yellow	+	_
Pseudomonas spp.	_	+	+	_	+	Red	Red	_	+
Proteus spp.	+	_	+	+	+	Yellow	Yellow	+	+
Shigella spp.	+	_	_	+	_	Red	Yellow	_	_
Escherichia coli	+	_	_	+	+	Yellow	Yellow	+	_
Salmonella spp.	+	_	_	_	+	Red	Yellow	_	+
Alcaligens spp.	-	+	+	_	_	Red	Red	_	_
Citrobacter spp.	+	_	+	_	+	Yellow	Yellow	+	+

Key: +: positive -: negative G.P : Gas Production

The evaluation of occurrence of Gram-negative bacteria in the different drinking water sources revealed that among the five sources of drinking water sampled, stream water was found to have the highest bacterial composition of 39%, while bottled water had the lowest of 4.9% (Figure 1).

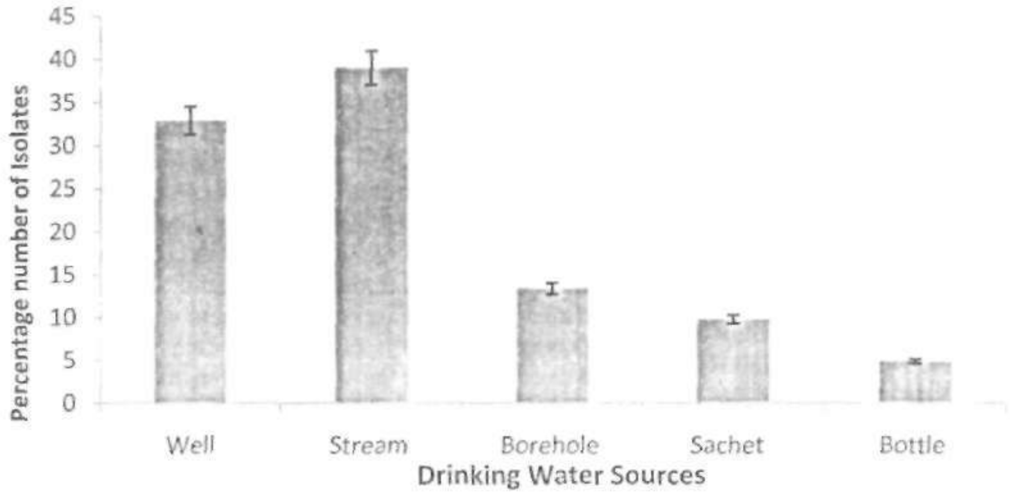

Figure 1: Occurence of Isolates in Different Drinking Water Sources in Ado - Ekiti, Nigeria

Antibiotic Susceptibility of the Isolates

The overall antibiotic resistance profile of the bacterial isolates is presented in Figure 2. Of all the antibiotics tested, resistance to amoxicillin was estimated as 31.5% which was the highest followed by 25.8% resistance to augmentin, 16.9% to tetracycline,11.2% to cotrimoxazole,7.3% to nitrofurantoin, 6.2% to nalidixic acid and 0.6% to ofloxacin and gentamicin. Table 3 summarizes the antibiotic resistance patterns of the bacterial isolates. It was observed that 100% *Alcaligenes* spp. and *E. coli* showed resistance to amoxicillin and augmentin respectively. Also, among the isolates, *Pseudomonas* spp. (30.0%) and *E. coli* (20.0%) were more resistant to tetracycline and cotrimoxazole respectively. Relatively, 18.2% and 23.1% *Alcaligenes* spp. were more resistant to nalidixic acid and nitrofurantoin respectively compared with other isolates. Also, it was observed from the table, that all the isolates showed 100% susceptibility to ofloxacin and gentamicin except *Pseudomonas* spp. (100%) which showed resistance to these antibiotics.

Multiple Antibiotic-Resistances among the Isolates

Multiple antibiotic-resistances were observed among the bacterial isolates. These ranged from three classes of antibiotics to six classes of antibiotics (Table 4). The table reveals that a total of six isolates showed multiple resistances to six classes of antibiotics, three isolates were resistant to five classes of antibiotics, six isolates to four classes of antibiotics and twelve isolates to three classes of antibiotics. Resistance to three antibiotics was common among the isolates from all the water samples with the majority having the resistotype AUG/TET/AMX. Also, multiple resistance to four and six classes of antibiotics was also common among these isolates. Only three isolates from stream and sachet water showed multiple resistance to five classes of antibiotics. Also, all the isolates with multiple resistance to six antibiotics has the same resistotypes, AUG/TET/AMX/COT/NIT/NAL irrespective of their sources. From among all the isolates that showed multiple antibiotic-resistance, *Pseudomonas* spp. were more predominant.

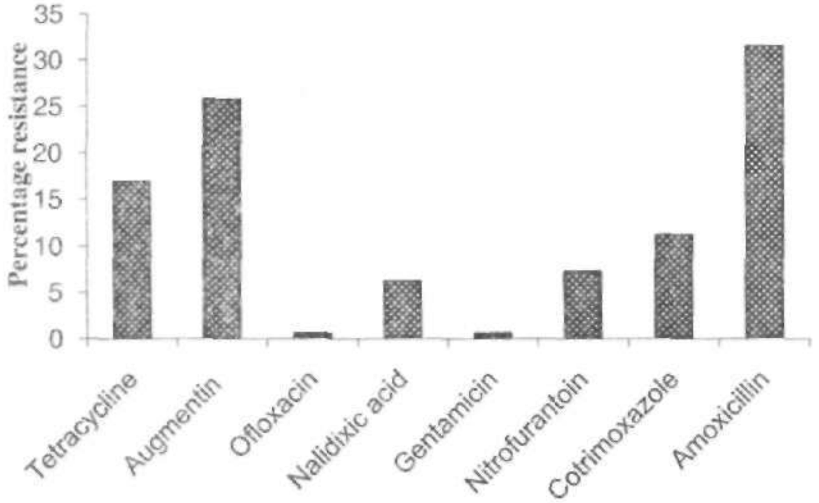

Figure 2. Overall Antibiotic Resistance Profile of the Bacterial Isolates from Drinking Water Sources in Ado-Ekiti, Nigeria

Table 3. The Antibiotic Resistance Pattern of the Gram-Negative Bacteria Isolated from Drinking Water Sources in Ado-Ekiti, Nigeria

Gram-Negative Bacteria (n)	Resistance Pattern (%)							
	TET	AUG	OFL	NAL	GEN	NIT	COT	AMX
Klebsiella spp. (17)	5 (16.7)	11(23.9)	0	2(18.2)	0	1(7.7)	4 (20.0)	14 (25.0)
Pseudomonas spp. (13)	9 (30.0)	10(21.7)	1(100)	3(27.3)	1(100)	5(38.5)	5 (25.0)	10 (17.9)
Proteus spp. (16)	7 (23.3)	8(17.4)	0	2(18.2)	0	1 (7.7)	3 (15.0)	13 (23.2)
Shigella spp. (5)	0	1(2.2)	0	0	0	0	0	1 (1.8)
Escherichiacoli (9)	2 (6.7)	9(19.6)	0	1(9.1)	0	2(15.4)	4 (20.0)	7 (12.5)
Salmonella spp. (14)	3 (10.0)	2(4.3)	0	1(9.1)	0	1 (7.7)	2 (10.0)	5 (8.9)
Alcaligenes spp. (5)	3 (10.0)	4(8.7)	0	2(18.2)	0	3(23.1)	2 (10.0)	5 (8.9)
Citrobacter spp. (3)	1 (3.3)	1(2.2)	0	0	0	0	0	1 (1.8)

Key: TET-tetracycline, AUG-augmentin, OFL-ofloxacin, NAL-Nalidixic acid, GEN-gentamicin, NIT-nitrofurantoin, COT-cotrimoxazole and AMX-amoxicillin

Table 4. Multiple Antibiotic Resistance among Isolates from Different Drinking Water Sources in Ado-Ekiti, Nigeria

Water sources	Bacterial isolates (n)	Number of bacteria with multiple resistance			
		6 antibiotics (%)	**5 antibiotics (%)**	**4 antibiotics (%)**	**3 antibiotics (%)**
Well	*Klebsiella* spp (7)	0	0	1(14.29) *AUG/AMX/NIT/NAL	3 (42.86) *AUG/AMX/COT, AUG/TET/AMX
	Pseudomonas spp (2)	1 (50.0) *AUG/TET/AMX/COT/NIT/NAL	0	0	0
	Alcaligenes spp (2)	1 (50.0) *AUG/TET/AMX/COT/NIT/NAL	0	0	1 (50.0) *AUG/AMX/NIT
Stream	*Klebsiella* spp (4)	0	0	0	
	Pseudomonas spp (8)	1 (12.5) *AUG/TET/AMX/COT/NIT/NAL	0	1 (12.5) *AUG/TET/AMX/COT	2(25.0) *AUG/TET/AMX
	Proteus spp (7)	0	0	1 (14.3) *AUG/TET/AMX/COT	0
	Escherichiacoli (3)	0	1 (33.3) *AUG/AMX/COT/NIT/NAL	1 (33.3) *AUG/TET/AMX/COT	0
Borehole	*Pseudomonas* spp (3)	1 (33.3) *AUG/TET/AMX/COT/NIT/NAL	0	2 (66.7) *AUG/TET/AMX/GEN, AUG/TET/COT/NIT	0
	Proteus spp (3)	1 (33.3) *AUG/TET/AMX/COT/NIT/NAL	0	0	1 (33.3) *AUG/AMX/NAL
	Citrobacter spp (1)	0	0	0	1 (100) *AUG/TET/AMX
Sachet	*Klebsiella* spp (3)	0	1 (33.3) *AUG/TET/AMX/COT/NAL	0	1 (33.3) *AUG/TET/AMX
	Proteus spp (1)	0	0	0	1 (100) *AUG/TET/AMX
	Salmonella spp (2)	0	1 (50.0) *AUG/TET/AMX/NIT/NAL	0	0
	Alcaligenes spp (1)	1 (100) *AUG/TET/AMX/COT/NIT/NAL	0	0	0
Bottled	*Escherichiacoli* (2)	0	0	0	1 (50.0) *AUG/TET/AMX

n= number of isolates, *= resistotypes, TET-tetracycline, AUG-augmentin, OFL-ofloxacin, NAL-Nalidixic acid, GEN-gentamicin, NIT-nitrofurantoin, COT-cotrimoxazole and AMX-amoxicillin

Plasmid Profile of Isolates

The plasmid profile of fifteen organisms from those that showed multiple resistance patterns is illustrated in Figure 3. It was observed that all the organisms carried single plasmid of >1Kb each except organism in lane 15. It was observed that the organisms have their plasmids at the same band (1Kb). Table 5 reveals the different resistance patterns of the plasmid-borne isolates. This table shows that the resistance patterns are of varying degrees. Therefore, organisms in lanes 1, 2, 6, 7, 9 and 10 have the same resistance pattern (AUG,TET,AMX,COT,NIT,NAL), lanes 11 and 12 were resistant to AUG,TET,AMX, Lanes 8 and 14 displayed resistance to AUG,TET,AMX,COT while the other lanes have their peculiar resistance pattern. The organism in Lane 15 did not carry plasmid and its resistance pattern was different from others.

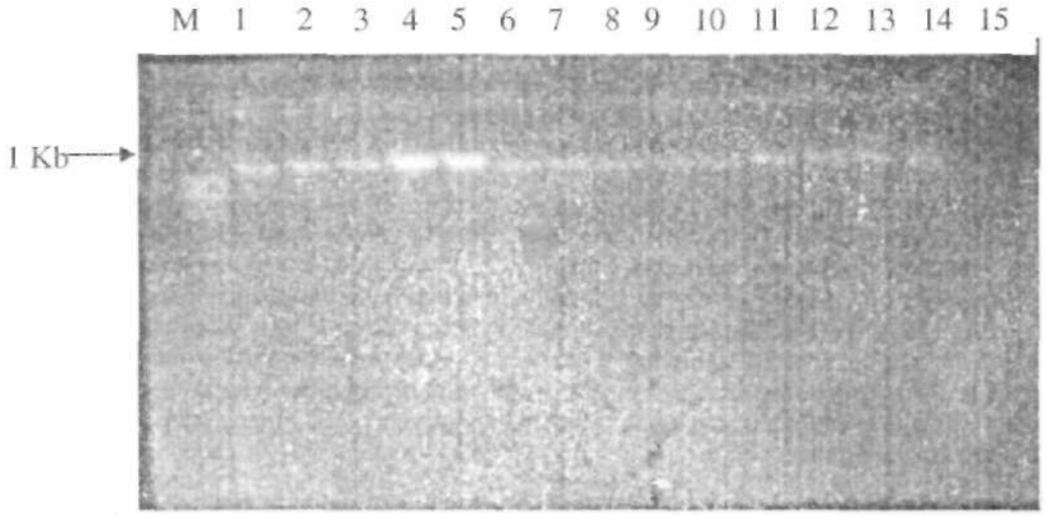

Figure 3. The Plasmid Profile of Selected Multiple Antibiotic-Resistant Gram-Negative Bacteria. Plasmid DNA on 0.8% agarose. M= 1Kb molecular weight size marker (Lambda Hind III), Lane 1- 15 represents the Gram-negative isolates from different drinking water sources in Ado-Ekiti, Nigeria.

Table 5. Plasmid Profile of the Gram-Negative Bacteria and their Resistance Pattern.

Gram-negative bacteria	Water source	Resistance pattern	Presence of plasmid (1Kb)
Lane 1- *Alcaligenes*	Sachet water	AUG, TET, AMX, COT, NIT, NAL	+
Lane 2- *Proteus*	Borehole	AUG, TET, AMX, COT, NIT, NAL	+
Lane 3- *E. coli*	Bottled water	AUG, AMX, COT, NIT	+
Lane 4– *Pseudomonas*	Borehole	AUG, TET, AMX, GEN	+
Lane 5 – *E. coli*	Stream	AUG, AMX	+
Lane 6- *Pseudomonas*	Borehole	AUG, TET, AMX, COT, NIT, NAL	+
Lane 7- *E. coli*	Well	AUG, TET, AMX, COT, NIT, NAL	+
Lane 8- *E. coli*	Stream	AUG, TET, AMX, COT	+
Lane 9– *Pseudomonas*	Well	AUG, TET, AMX, COT, NIT, NAL	+
Lane 10 - *Alcaligenes*	Well	AUG, TET, AMX, COT, NIT, NAL	+
Lane 11-*Pseudomonas*	Stream	AUG, TET, AMX	+
Lane 12–*Pseudomonas*	Stream	AUG, TET, AMX	+
Lane 13- *Proteus*	Sachet	AUG, TET, AMX, NIT, NAL	+
Lane 14- *Proteus*	Stream	AUG, TET, AMX, COT	+
Lane 15- *Klebsiella*	Stream	AMX, COT, NAL	-

The Incidence of Water-Borne Diseases in Ado-Ekiti, Nigeria

The socio-demographic characteristics of the 1000 respondents are summarized in Table 6. The respondents were found to use different water sources, 247 (24.7%) of the respondents use well water, 410 (41%) use borehole water, 230 (23%) depend on pipe-borne water while 61 (6.1%) use stream water. Fifty-two (5.2%), of the respondents claim to use other sources of water such as sachet and bottled water. It was found that these water sources are statistically significant ($p< 0.05$) in respect of water-borne diseases.

Data on various activities which take place near or around the water sources are presented in Table 7. It was observed that farming activity was predominant (24.4%) among all the activities that took place near the water sources. The p-value of these activities that took place near the water sources was < 0.05 which indicated that there is a relationship between these activities and the water-borne diseases. The data collected on those who have experienced water-borne diseases within the last three years revealed that 262 (26.2%) respondents have experienced water-borne disease within the last three years, while 738 (73.8%) claimed not to have experienced it. This incidence of water-borne disease is statistically significant ($p< 0.05$). Also, data on sources of drinking water and experience of water-borne diseases are presented in Table 8. Analysis of data showed that those who sourced drinking water from these water sources had experienced water-borne diseases within the last three years.

Table 6: Socio-Demographic Profile of the Respondents

Variables	Frequency	Percentage (%)
Age of respondents		
0 to 10	24	2.4
11-20	315	31.5
21-30	609	60.9
31 -40	40	4.0
50 and above	12	1.2
Sex		
Male	457	45.7
Female	543	54.3
Educational status		
Primary	74	7.4
Secondary	123	12.3
ND	566	56.6
NCE	91	9.1
HND	111	11.1
B. Sc	29	2.9
Other Higher Degrees	6	0.6
Occupation		
Artisan	172	17.2
Non-Artisan	828	82.8
Residential Status		
Indigenes	339	33.9
Non-indigenes	661	66.1

Table 7. The Activities that Take Place Near or Around the Water Sources of Drinking Water

Activities	Frequency	Percentage
Grave	48	4.8
Buying and selling	40	4.8
Construction	24	2.4
Decomposition of plants	20	2.0
Dumping	88	8.8
Farming	244	24.4
Football	48	4.8
Health centres	92	9.2
Industrial activities	10	1.0
Palm oil production	20	2.0
Mechanic workshop	40	4.0
Panel beating	24	2.4
Religious centre	24	2.4
Schools	44	4.4
Washing	204	20.4
None	30	3.0
Total	1000	100

p value < 0.05

Table 8: The Sources of Drinking Water and Experiences of Water-Borne Diseases

Source of water	Yes	No	Indifferent	Total	X^2	df	p-value
Well	97 (39.3)	150 (60.7)	0	247	1.814e^{2}	12	0.000
Borehole	88 (21.5)	322 (78.5)	0	410			
Stream	43 (70.5)	18 (29.5)	0	61			
Pipe-borne	18 (7.8)	206 (89.8)	6 (2.5)	230			
Others	16 (30.8)	36 (69.2)	0	52			
Total	262	732	6	1000			

Discussion

The major sources of transmission of enteric pathogens in Nigeria and many other developing countries in the world is water which subsequently lead to water-borne diseases in humans (Begun *et al.*, 2003). The extent of microbial contamination of different drinking water sources in Ado-Ekiti, Nigeria was determined in order to ascertain their conformity with World Health Organization standard.

The determination of the mean total viable count (TVC) and mean total coliform count (TCC) of the drinking water sources revealed that all the water sources exhibited variable characteristics in terms of their microbiological quality. The water sampled from these sources failed to meet the World Health Organization drinking .water standard with the exception of some bottled water. World Health Organization recommends that number of bacteria capable of growing on standard agar plate incubated for 24 hrs at 37°C should not be more than 100 bacteria per *milliliter of water sample and that 100ml of water* sample for drinking should not contain more than one coliform bacteria (WHO, 2003). Similar results were obtained in the assessment of house-hold drinking water in Ado-Ekiti, Nigeria (Oluyege *et al.*, 2011). They reported that water samples collected from rain, tap water and wells had higher bacterial load than the WHO recommendation. Also, the low standard of packaged drinking water obtained in this study agreed with the submission of Onifade and Ilori (2008) and Oyedeji *et al.* (2010) that the microbiological quality of packaged water examined in Ondo state and Ile-Ife city respectively fell below the United States Environmental Protection Agency (USEPA) and WHO drinking water standard. However, the microbial load of the water samples is statistically insignificant. (p-value of mean TVC and mean TCC= 0.33 and 0.36 respectively). This implies that the TVC of these water sources is not enough to cause water-borne diseases and this is supported by WHO report (2002) which stated that a high heterotrophic bacteria plate count concentration does not itself present a risk to human health. Nevertheless, the heterotrophic bacteria plate counts are used as good indicators of the overall quality of these water sources (Obiri-Danso *et al.*, 2003). Also, exposure to faeçally contaminated water does not always translate into infection. However, the higher the faecal bacterial levels in water, the higher the chances of pathogens to be present in significant numbers too (Santo and Ashbolt, 2008).

Many factors could be responsible for the microbial load of the drinking water examined in this study. For instance, surface water and underground water are easily pruned to faecal contamination. Surface water such as streams and rivers are mostly used as dumping site for domestic wastes (Nwidu *et al.*, 2008). Also, wells that are not properly constructed or sited can be contaminated by surface run-off especially during raining season, underground leachates from leaking septic system, agricultural run-off or animal droppings (Adewoye, 2010; Imran, 2012). Most packaged water producers utilize some of these wells as raw water sources which may affect the quality of the packaged water. Although, other factors which can also affect the quality of the packaged water include treatment processes employed and hygienic practices observed in production (Geldreich, 1996). Howard *et al.* (2003) reported that properly constructed and maintained boreholes may provide a relatively safer source of water in terms of microbial load as compared to unprotected sources such as streams and wells. But on the contrary, the results from this study showed that some of the boreholes sampled are not safe for drinking purposes. This may probably be as a results of the topographic feature of the location of these boreholes. For instance, some of these boreholes sampled are sited in a sloppy environment. And in such an environment, there is possibility of leachates from improperly *constructed septic tanks around these boreholes.*

Many authors have reported the presence of these Gram-negative bacteria in various drinking water sources (Ronald *et al.*, 2002; Habi and Daba, 2009; Olaoye and Onilude, 2009). These bacteria can get into drinking water probably through faecal contamination from sewage discharge and livestock droppings. Their presence in drinking water is of public health importance as they have been implicated in causing water-borne diseases such as gastroenteritis, typhoid and paratyphoid fever, salmonellosis, cholera, among others (Santo and Ashbolt, 2008). Babaniyi (1991) reported that water-borne diseases in Nigeria were caused by viruses, bacteria, protozoans and helminthes and that 48.6% of the cases reported were traced to bacterial infections caused by ingestion of contaminated water.

Majority of the Gram-negative bacteria isolated from the water sources in the present study were resistant to commonly used antibiotics such as amoxicillin, augmentin, tetracycline, cotrimoxazole, nitrofurantoin, nalidixic, ofloxacin and gentamicin. However, resistance to ofloxacin and gentamicin was very low (1.2%). This is similar to the study by Bhowmick *et al.* (2006) which reported that bacteria isolated from whey showed complete sensitivity to gentamicin. Also, *E. coli* isolated from drinking well water in Nigeria showed 100% sensitivity to gentamicin (Oyetayo *et al.*, 2007). Other findings that demonstrated the resistance of Gram-negative bacteria from drinking water sources to these antibiotics have been documented (Oyetayo *et al.*, 2007; Atif *et al.*, 2010; Tagoe *et al.*, 2011). The presence of antibiotic-resistant Gram-negative bacteria in drinking water is of health significance. This is because of the danger in promoting multiple antibiotic-resistant strains in humans. This is possible through colonization of the gastrointestinal tract and transfer of plasmids to the normal flora leading to more multiple antibiotic-resistant strains (Mckeon *et al*., 1995).

Investigations have shown that antibiotic-resistant bacteria from the environment haboured a conjugative R-Plasmid (Abdelatey *et al*., 2011; McArthur *et al*., 2011). These plasmids are mobile genetic elements and are self-replicating which might be ecologically stable. They have been found to encode resistance to multiple antibiotics, heavy metals and other toxic compounds (Karbasizaed *et al*., 2003). The proliferation of plasmid-borne antibiotic-resistant bacteria presents a potential health hazard because it represents therapeutic failure sources. The plasmid profile obtained in this study shows that the resistance pattern of the organisms which haboured plasmid are plasmid mediated resistance, while the organism, *Klebsiella* spp, that did not habour plasmid could probably have chromosomally mediated antibiotic resistance. The varying degrees of resistance exhibited by the isolates were probably not unexpected since the same antimicrobial resistance pattern can be encoded by unrelated plasmids, transposons, phages and chromosomal genes (Dombrovskii, 1990).

The socio-demographic characteristics of the respondents revealed that their ages ranged from >10 years to 50 years and above with the majority of them in the age group of 21-30 years (60.9%) which can be classified as the youthful age. This is similar to the findings by Aderibigbe *et al.* (2008) who reported that majority of their respondents fell within the productive age group (20-49 years). Majority of the respondents were females and it was observed from socio-demographic table that many of the respondents are educated even with majority having tertiary education. This also corroborates the findings of Aganaba and Osagbemi (2005) who found out that majority of the respondents in their study had tertiary level of education. It is worthy to note that the incidence of water-borne diseases in a community at times depends on the socio-demographic characteristics (age and educational status) of the respondents. For instance, water-borne diseases are common among children. Also, education enlightens individual because the more an individual is enlightened, the better will be his/her knowledge on how to ensure that water meant for domestic purposes meets the required standard. The age groups and educational status of the respondents in this study are statistically significant (p-value < 0.05) in relation to occurrence of water-borne diseases. World Health Organization reported that water-borne disease such as diarrhoea accounts for an estimated 4.1% of the total global burden of diseases attributable to unsafe water. This is responsible for the deaths of 1.8 million people every year and is mostly among children in the developing countries (http://www.who.int/csr/don/en/).

The respondents sourced their drinking water from well, boreholes, stream, pipe-borne water and other sources such as sachet and bottled water. Statistically, it was found out that the water sources in relation to the occurrence of water-borne diseases is significant (p value = 0.00) in Ado-Ekiti environs. Similar result was obtained in the findings by Nwidu *et al.* (2008). They randomly studied 100 case notes of water-borne diseases from general hospital, Amassoma, Niger Delta, Nigeria and reported that the water sources were implicated in the incidence of water-borne diseases in the community.

Studies have shown that different anthropogenic activities near drinking water sources can influence the quality of the water (Bannerman *et al*., 1993; Adewoye, 2010; Wolf-Rainer, 2011) and consequently pose danger of health risks to the community. From all the data collected, 262 (26.2%) respondents were reported to have experienced water-borne diseases. This is statistically significant (p value = 0.00) and therefore, the incidence of water-borne diseases in Ado- Ekiti, Ekiti State cannot be overlooked.

Conclusion

The findings from this study indicated that the incidence of water-borne diseases in Ado-Ekiti metropolis, Nigeria may probably be related to the sources of drinking water. Therefore, aggressive public health education in the study area should be put in place in order to maintain water sources and prevent out-breaks of water-borne diseases. This entails identifying several factors such as discharge of effluents into water bodies, disposal of refuse, inadequate septic system and human excretion that can contaminate water sources. Since majority of the members of Ekiti State community depends on private house hold water sources, there is need to educate them on how to maintain the quality of drinking water from their wells and/or water sources. This may be done through video, showing proper construction, maintenance and testing of water.

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