World Applied Sciences Journal 32 (3): 403-414, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.32.03.83237

Drinking Water Distribution Systems of Dams in Ondo State, Nigeria as Reservoir of Multi-Drug Resistant Bacteria

[1,2]*A.T. Adesoji,* [2]*A.A. Ogunjobi and* [3,4]*I.O. Olatoye*

[1]Department of Biological Sciences, Federal University Dutsin-Ma, Katsina State, Nigeria
[2]Department of Microbiology, University of Ibadan, Nigeria,
[3]Department of Veterinary, Public Health and Preventive Medicine, University of Ibadan, Nigeria
[4]Paul G. Allen School for Global Animal Health, Washington State University, USA

Abstract: The occurrence of multidrug resistant (MDR) bacteria across food, water and environment is a major global public health challenge. Drinking water distribution systems of selected dams in Ondo State Nigeria was investigated for their physicochemical properties, microbial quality and presence of MDR bacteria. It was observed that the pH of the water ranged between 5.20 to 7.40 while DO ranged between 1.45 to 6.82mg/l and the residual chlorine of the water was between 0.00 to 0.09mg/l. Coliform count of the water samples range from 0 to 9.33 x10^2 CFU/ml. Gram negative bacteria recovered from the water include *Proteus*, *klebsiella*, *Alcaligenes*, *Pseudomonas* etc. and they were observed to show multiple resistant to various antibiotics which include tetracycline, streptomycin, ampicillin, sulfamethoxazole. This study revealed that MDR bacteria of public health significance are present in the drinking water distribution system of Ondo State, Nigeria.

Key words: Multidrug resistant bacteria · Antibiotics · Dams · Water distribution systems

INTRODUCTION

Water is an essential natural resource for sustainability of life on earth [1]. One of the targets of the millennium development goals (MDG) in terms of healthy living for the masses can be achieved through the supply of safe and convenient water [2]. The presence of pathogenic (disease-causing) organisms (bacteria) is a concern when considering the safety of drinking water. [3]. However, many authors have reported the isolation of bacteria like *Aeromonas*, *Methlobacterium*, *Bacillus* and *Pseudomonas* from diverse aquatic environments like well water and heavily polluted waters [4-6].

However, studies have shown increased level of bacteria resistance to various antibiotics among these water bodies bacteria due to misuse of antibiotics in human health, veterinary, aquaculture, agriculture and household products [7,8]. Whenever antimicrobials are used, bacteria inevitably develop resistance mechanisms either through spontaneous mutations or by acquiring genes from other bacteria. The later may occur by transduction (mediated by bacteriophages); conjugation (which involves direct cell-to-cell contact and transfer of plasmids or transposons); or transformation, involving the uptake of free DNA that results from bacterial lysis [10, 11].

Water distribution system can therefore, be a source of transfer of antibiotic resistance genes between bacteria isolates which eventually can result into public health threat which can result into high cost and long period of treatment. However, to the best of our knowledge no study has been carried out on the occurrence of antibiotic resistant bacteria in water distribution systems of dams in Ondo State, Nigeria. This study therefore, aims at determining if drinking water distribution systems of selected dams located in Ondo State, Nigeria would be a source of multidrug resistance bacteria.

MATERIALS AND METHODS

Study Areas: Two water distribution systems which include Owena-Ondo and Owena-Ijesha were selected in Ondo State, Nigeria for this study. Both dams were constructed and owed by Ondo State government.

Corresponding Author: A.T. Adesoji, Department of Biological Sciences, Federal University Dutsin-Ma, Katsina State, Nigeria.

Owena-Ijesha dam was commissioned in 1965 with an installed capacity of 19600 m^3/day and to supply water to a population of 787867 people. Owena-Ondo dam was commissions in 1971 and installed with a capacity of 5450 m^3/day and designed for an estimated populace of 192340. The dam is supplied with water from the Owena River and it covers an appropriate surface area of 7.8km^2.

Sample Collection and Analysis: Water samples for microbiological and physicochemical analysis were obtained two times each between December, 2010 and January, 2011 to represent onset of dry season and June, 2011 and July, 2011 to represent the start of raining season from raw, treated and two municipal taps of the water distribution systems. Raw untreated water and treated water of each of the dams were only analyzed for physicochemical analysis. The following steps were followed when sampling water for microbial analysis as previously described by Rice [12]. Plastic sample bottles (50ml) were used for the sampling. Samples for the treated water i.e. dams final output and municipal taps was taken by opening of the taps and allowing the water to run for 3-4min before collection. Collected samples were then kept at 4°C in the cooler box packed with ice and transported to the laboratory for analysis within six hours. Samples from the raw water were taken from the dam output before getting to the treatment plants. Samples for chemical analysis were taken with 500ml sample bottles from the raw water and treated water of the dams and then transported into the laboratory for chemical analysis.

Microbial: Serial dilution of the water samples was carried out aseptically up to 10^{-4} in order to obtain countable bacteria colonies on the agar plate. The samples were then mixed by shaking before plating on appropriate media. Total plate counts was determined by plating out with a sterile pipette 1ml of the diluted samples from 10^{-2} and 10^{-4} into sterile petri dish and Nutrient agar that has been sterilized and kept at 45°C in a water bath was aseptically poured into the petri dish and allowed to solidify on the flat surface. The plates were then incubated in an inverted position in an incubator set at 37°C overnight. Colonies developed on the agar plates were then counted with a colony counter. For all treated water samples i.e. the final dam water samplings and the municipal samplings, undiluted samples of the water and samples diluted to 10^{-1} was plated out on the Nutrient agar (NA) plates. Similar steps were also carried out for coliform on Eosin methylene blue (EMB) agar and deoxcholate agar which was used as selective agar for isolation of *E. coli* and other coliform. Colonies with different morphologies were picked and streaked out on Nutrient Agar plate for purification. Cultures were then stored at 4°C on Nutrient Agar (NA) slant [13].

Physicochemical: The pH of the water samples was determined by the use of pH meter while Biological Oxygen demand (BOD), Chemical Oxygen demand (COD), Dissolve Oxygen (DO) and Total Organic carbon (TOC) was determined by the method of Skoog and West [14] and Radojeric and Baskin [15]. DO was determined by measuring 200cm^3 of water sample into a beaker with measuring cylinder. A probe of the DO was then inserted. The DO meter was switched on and the DO value (mg/l) was recorded after 2 minutes of automated value adjustment. Conductivity of the water was determined by inserting a conductivity electrode in water samples while residual chlorine of water samples were determined by the use of a chlorine comparator with N,N-diethylparaphenylenediamine (DPD)

Total solid (TS) was determined by keeping the water samples at 103°C in a clear dry glass beaker of 150ml capacity in an oven for 1 hr. The capacity and appropriate identification mark was then place on it. After, 100ml of the thoroughly mixed sample was measured into the beaker. The beaker was then placed in an oven maintained at 103°C for 24 hrs. After 24 hours, the beaker was then cooled and weigh. The weight of the solid in the beaker was determined by subtracting the weight of the clean beaker from the weight determined after addition and drying of the sample in the beaker. Total solid (TS) was then determined as follows:

$$\text{Total solid, TS (mg/l)} = \frac{\text{mg of Solids in the beaker x 1000}}{\text{Volume of sample}}$$

Total dissolved solid (TDS) was determined as TDS (mg/l) = mg of solid in the beaker x 1000 (volume of sample) while

Total suspended solid (TSS) was determined as TSS (mg/l) = TS (mg/l) – TDS (mg/l)

Molecular Characterization of Bacteria Using 16s rDNA Sequencing: Total genomic DNA was extracted from isolates after streaking stock culture on Luria Betani (LB) agar overnight followed by dispensing 200 µl of 5% chelex in a tube and then taking a loopful of a colony of bacterium into the chelex solution. Mixture of the bacteria and chelex solution was then boiled at 100°C for 10 min and centrifuged at 13k x g for 1min. Extracted DNA supernatant (5µl) was used as template with 2mM $MgCl_2$, 0.8 mM dNTPs, 0.2 µM of each primer 1 and primer 2 and

Table 1: Antibiotic concentration used for breakpoints

Antibiotics for gram negatives with concentration (ug/ml)		Antibiotics for gram positives with concentration (ug/ml)	
FF	Florfenicol (16)	SU	Sulfamethoxazole (512)
T	Tetracycline (16)	AM	Ampicillin (0.5)
S	Streptomycin (16)	T	Tetracycline (16)
G	Gentamycin (16)	SXT	Sulfamethoxazole/Trimethoprim (76/4)
K	Kanamycin (64)	G	Gentamycin (16)
C	Chloramphenicol (32)	E	Erythromycin (8)
N	Nalidixic Acid (30)	RIF	Rifampin (4)
AMC	Amoxillin/Clavulanic Acid (32/16)	LIN	Lincomycin (4)
CEF	Ceftiofur (12)	CIP	Ciprofloxacin (4)
SU	Sulfamethoxazole (512)		
SXT	Sulfamethoxazole/Trimethoprime (76/4)		

1X PCR buffer. Reaction condition included 1min denaturation (95°C) followed by 30 cycles of 96°C for 30s, 60°C for 30s and 72°C for 30s and a final extension of 72°C for 10min. PCR products were then separated and visualized on 1% agarose gel electrophoresis to confirm amplification. The 16s rDNA sequence was amplified using 16s-8F (AGAGTTTGATCMTGGCTCAG) and 16s-517R (ATTACCGCGGCTGCTGG) primers [16, 17]. PCR products were sequenced (Eurofins MWG, USA) and manual base calls were sequence trimming was completed by sequencher (5.0). BLASTn was used to identify closes sequence matches (www.ncbi.nlm.nib.gov/BLAST/blast)

Antibiotic Breakpoint Susceptibility Test: The antibiotic resistance profile of the bacteria was determined using breakpoint assays on LB agar plates. The Agar medium was autoclaved, cooled to 45°C and then antibiotics were added to specific concentration (Table 1) before pouring the medium into petri dishes (150 x 15mm). Overnight cultures were then 'stabbed' from the 96-well plate onto agar plates using 96-well pin replicator and incubated overnight at 37°C. Isolates were scored as '1'for growth of '0' for no growth on each antibiotic plate.

RESULTS AND DISCUSSION

Physicochemical Properties of Water: The results of the physicochemical properties of the raw and treated water are shown on Table 2 and 3 respectively. The results obtained showed that the pH of the raw water during the two seasons of sampling ranged between 6.50 and 7.50 in raw water and 5.20 and 7.70 in treated water. The pH of the raw water was similar to the result of Pranavam *et al.* [18]. Ravisankar and Poongothai. [19] described such water pH as either sharply acidic or alkaline in nature. However, the minimum range of the pH obtained for the treated water during the wet and dry seasons sampling in both locations- (Owena-Ondo and Owena-Ijesha) was observed to be slightly acidic in nature. Total dissolved solid (TDS) of both the raw and untreated water was observed not to be above the 500 mg/l limit recommended by WHO. [20]. Dissolved oxygen (DO) of both the raw and untreated water ranged between 1.45 to 3.11 mg/l and 4.33 to 6.82 mg/l respectively. These values exceed the range of 2.5 to 3.8 mg/l observed by Fatombi *et al.* [21] in their study with surface water in Lagbe town. But these values obtained in this study were below WHO recommendation. As suggested by WHO [22] the conductivity of portable water should not exceed 300 µs/cm. In this study, we obtained the conductivity range of 32.50 to 42.00 µs/cm and 5.6 to 59.50 µs/cm in raw and treated water respectively during both sampling periods. Also in this study, no residual chlorine was detected in the raw water samples, but the treated water has values of 0.03 mg/l and 0.09 mg/l at Owena-Ondo dam during dry and wet season sampling periods respectively. These values were below 0.5 mg/l maximum limit recommended by WHO. In this study, we observed that the Total solid (TS) and Total suspended solid of the raw water sampling ranged between 190 to 430 mg/l and 120 to 370 mg/l respectively. These values were observed to be higher than values obtained for the treated water which ranges between 93.00 mg/l to 320 mg/l and 9.20 to 210 mg/l respectively. This showed that the treatment process was effective in reduction in the solid material in the water as passed through the treatment plant. Also, we observed that the Total dissolve solid (TDS) of the raw water was between 50 mg/l to 66 mg/l compared to higher range observed for the treated water samples (83.00 to 100 mg/l). The values of TDS and TSS recorded in this study are below WHO recommendation.

Microbial Quality of the Water Samples: The results of the coliform and total plate counts of the water samples from the water distribution system of these dams are shown on Table 4. We observed that the coliform and total bacteria count was higher in raw water compared to the treated water in some of the water distribution system

Table 2: Physicochemical properties of raw water sampling of Owena-Ondo and Owena-Ijesha dams in Ondo State, Nigeria during dry and raining season of December, 2010 and June, 2011

		pH	BOD (mg/l)	COD (mg/l)	DO (mg/l)	TOC (mg/l)	TDS (mg/l)	TS (mg/l)	TSS (mg/l)	Conductivity (µs/cm)	Residual Chlorine(mg/l)
Owena-Ondo	June/July	6.50	4.83	53.60	3.11	6.22	66.00	420.00	350.00	42.00	0.00
	December/January	7.40	6.83	49.80	1.45	1.34	62.00	190.00	120.00	34.00	0.00
Owena-Ijesha	June/July	7.10	6.62	64.60	3.21	6.43	50.00	430.00	370.00	32.50	0.00
	December/January	7.30	5.72	56.10	2.34	1.43	58.00	360.00	287.00	34.80	0.00
	WHO limit	6.5-8.5	6-9	-	-	-	500	-	500	300	0.5

Table 3: Physicochemical properties of treated water sampling of selected dams in Ondo State, Nigeria during dry and raining season of December, 2010 and June, 2011

		pH	BOD (mg/l)	COD (mg/l)	DO (mg/l)	TOC (mg/l)	TDS (mg/l)	TS (mg/l)	TSS (mg/l)	Conductivity (µs/cm)	Residual Chlorine(mg/l)
Owena-Ondo	June/July	5.40	1.32	47.40	5.21	1.65	80.00	220.00	130.00	40.00	0.09
	December/January	7.70	2.11	81.30	4.83	1.90	96.00	130.00	32.80	59.50	0.03
Owena-Ijesha	June/July	7.40	3.11	34.80	4.33	2.45	100.00	320.0	210.00	52.00	0.04
	December/January	5.20	1.87	78.00	6.82	1.75	83.00	93.00	9.20	5.60	0.05
	WHO limit	6.5-8.5	6-9	-	-	-	500	-	500	300	0.5

Table 4: Coliform and Total plate counts of Owena-Ondo and Owena-Ijesha water distribution systems during raining and dry seasons sampling

	Coliform count (CFU/ml)				Total bacteria count (CFU/ml)			
	June/July		December/January		June/July		December/January	
	Owena-Ondo	Owena-Ijesha	Owena-Ondo	Owena-Ijesha	Owena-Ondo	Owena-Ijesha	Owena-Ondo	Owena-Ijesha
Raw water	5.6 x 10	3.02×10^2	3.2×10^5	4.0×10^5	3.2×10^3	3.3×10^3	3.2×10^5	6.0×10^5
Treated water	3.0 x 10	2.0×10^2	4.8 x 10	3.0	6.2×10^2	6.4 x 10	6.8 x 10	0
Municipal Tap 1	0	8.91	9.33×10^2	0.0	0	4.2 x 10	2.0×10^5	0
Municipal Tap 2	0	8.91	2.13×10^2	4.0×10^2	0	1.4×10^2	4.0×10^2	2.0 x 10

sampled (e.g. Owena-Ijesha). However, the count in some of the treated water exceeded what was suggested by WHO [22] For drinking water of no coliform per 100ml. For example, treated water and the water from municipal taps of Owena-Ijesha during June/July sampling has coliform count ranging between 8.91 to 2 x 10^2 CFU/ml. During dry season, samples obtained at the same dam showed values ranging from 3.0 to 4.0 x 10^2 CFU/ml while no coliform count was obtained during wet season sampling of both municipal taps of Owena-Ondo dam. During December/ January sampling at Owena-Ondo coliform bacteria count range between 4.8 x 10 CFU/ml at their treated water to 9.33 x 10^2 at the first municipal tap sampled while its total plate count ranges between 6.8 x 10 CFU/ml at the treated water to 2.2 x 10^5 CFU/ml at the raw water sample. However, EPA [23] reported that isolation of coliform from water is of great importance in different fields of microbiology as is used as a continuous standard when water is under study. They are described as important indicator of water quality and along with other organism; they make up an important part of water standard. In this study, the range of coliform count obtained in some of the water samples correlate with the coliform with the coliform count range of up to 4.7 x 10 to 8.0 x10 CFU/ml reported by Oluyege *et al.* [24], in treated and untreated water samples from Ero dam in Ekiti State Nigeria.

Bacteria Identified, There Susceptibility to Antibiotics and MDR Bacteria: In this study, a total of 72 and 61 bacterial isolate were recovered from the sample locations of Owena-Ondo and Owena-Ijesha respectively (Table 5). It was observed that *Bacillus* was the highest occurred bacteria in the entire sample points of Owena-Ondo dam. *Bacillus* species are Gram positive aerobic spore-forming and most members of the genus are saprophytic prevalent in the soil, water and air and on vegetation [25]. However, high occurrence of this *Bacillus* particularly in the treated municipal water could be as a result of spores formation that makes them of very high resistance to chlorine disinfectant used in the water treatment plant. At Owena-Ijesha *Proteus*, *Pseudomonas* and *Kebsiella* showed the highest percentage of bacteria from the raw water and each constitute 5% of total bacteria while *Bacillus* was the highest at the treated water and municipal 2 tap constituting 60% and 41.67% of the total bacteria. Other bacteria isolated in these treated and municipal water include *Ralstonia, Proteus, Alcaligenes, Acinetobacter, Citrobacter, Enterobacter, klebsiella, Pseudomonas* and *Enterococcus* which were similar to bacteria reported by Suthar *et al.* [26]. These authors isolated a wide range of pathogenic bacteria from potable water samples from some rural habitation of Northern Rajasthan, India. The presence of some of these coliforms is an indication of fecal pollution and a consequent hazard of contracting water-borne disease from the use of such water [27]. Such polluted water could be as a result of leakage of pipes conveying this water to the final consumers and ineffectiveness of water treatment plant processing. Some of these bacteria like *Proteus*, *Klebsiella* and *Pseudomonas* etc. have been described as opportunistic pathogens [28]. This occurrence of these bacteria in some

Table 5: Bacteria isolated from Owena-Ondo (Dam 5) and Owena-Ijesha (Dam 6) water samples as Identified by 16S rDNA sequencing

Sampled Dam	Location	Bacteria	No of Isolates	Percentage total bacteria from location (%)
Owena Ondo	Raw water	*Bacillus sp*	7	53.85
		Escherichia sp	1	7.69
		Klebsiella sp	3	23.08
		Leucobacter sp	1	7.69
		Uncultured bacteria clone	1	7.69
		Total bacteria (Raw water)	13	
	Treated water	*Alcaligenes sp*	2	18.18
		Aquitalea sp	1	9.09
		Bacillus sp	3	27.27
		Klebsiella sp	1	9.09
		Morganella sp	1	9.09
		Pseudomonas sp	1	9.09
		Proteus sp	1	9.09
		Staphylococcus sp	1	9.09
		Total (Treated water)	11	
	Municipal Tap 1	*Alcaligenes sp*	2	9.09
		Bacillus sp	10	45.45
		Escherichia sp	2	9.09
		Lysinibacillus sp	2	9.09
		Proteus sp	2	9.09
		Pseudomonas sp	2	9.09
		Providencia sp	2	9.09
		Total (Municipal Tap 2)	22	
	Municipal Tap 2	*Acinetobacter sp*	1	3.85
		Bacillus sp	13	50
		Lysinibacillus sp	1	3.85
		Morganella sp	4	15.38
		Myroides sp	1	3.85
		Proteus sp	5	19.23
		Serratia sp	1	3.85
		Total	26	

Total bacteria from Owena-Ondo (72)

Table 5 (Cont'd): Bacteria from Owena-Ondo (Dam 5) and Owena-Ijesha (Dam 6) water samples as Identified by 16S rDNA sequencing

Owena-Ijesha	Raw water	*Alcaligenes sp*	4	12.50
		Acinetobacter sp	2	6.25
		Aeromonas sp	1	3.12
		Morganella sp	1	3.12
		Proteus sp	5	15.63
		Providencia sp	2	6.25
		Pseudomonas sp	5	15.63
		Klebsiella sp	5	15.63
		Serratia sp	1	3.12
		Myroides sp	1	3.12
		Bacillus sp	3	9.38
		Lysinibacillus sp	1	3.12
		Uncultured bacterium clone	1	3.12
		Total (Raw water)	32	
	Treated water	*Ralstonia sp*	1	20.00
		Proteus sp	1	20.00
		Bacillus sp	3	60.00
		Total (Treated water)	5	
	Municipal Tap 1	*Alcaligenes sp*	2	16.67
		Acinetobacter sp	1	8.33
		Bacillus sp	2	16.67
		Citrobacter sp	2	16.67
		Enterobacter sp	1	8.33
		Klebsiella sp	1	8.33
		Pseudomonas sp	1	8.33
		Enterococcus sp	2	16.67
		Total	12	
	Municipal Tap 2	*Alcaligenes sp*	2	16.67
		Bacillus sp	5	41.67
		Brevundimonas sp	1	8.33
		Chromobacterium sp	1	8.33
		Proteus sp	1	8.33
		Pseudomonas sp	1	8.33
		Lysinibacillus sp	1	8.33
		Total	12	

Total bacteria from Owena-Ijesha (61)

Table 6: Multidrug Resistant bacteria from Owena-ondo water samples and their resistance phenotypes (Dam 5)

Sources	Bacteria/Strain ID	Resistant Phenotypes
Raw water (Gram negatives)		
OWODRW	*Uncultured bacterium clone* (230A)	AM, SU, GEN
OWODRW	*Klebsiella pneumoniae* (335)	AM, SU, SXT, AMC, C, CEF, FF
Raw water (Gram positives)		
OWODRW	*Bacillus cereus* (338)	SU, E, RIF, LIN
OWODRW	*Bacillus* sp. (K)	AM, SU, SXT, LIN
OWODRW	*Bacillus cereus* (232)	AM, SU, SXT, LIN
OWODRW	*Leucobacter komagatae* (230B)	AM, SU, SXT, GEN
Treated water (Gram negatives)		
OWODFW	*Pseudomonas otitidis* (350)	AM, SU, SXT
OWODFW	*Proteus mirabilis* (201)	AM, SU, SXT, S, CEF
OWODFW	*Alcaligenes* sp (198)	T, AM, SU, SXT, S, K
OWODFW	*Morganella morganii* (199)	AM, SU, AMC, S, CEF
OWODFW	*Alcaligenes faecalis* (197)	T, AM, SU, SXT, S, K, CEF
OWODFW	*Klebsiella* sp (386B)	T, AM, SU, SXT, AMC, S, C, FF
Treated water (Gram positives)		
OWODFW	*Bacillus* sp. (200A)	AM, SU, SXT, LIN
OWODFW	*Bacillus* sp. (202B)	AM, SU, SXT, LIN
OWODFW	*Staphylococcus* sp (202)	AM, SU, SXT, T, GEN
Municipal Tap 1 (Gram negatives)		
OWODM1	*Proteus mirabilis* (273)	T, SU, SXT, S
OWODM1	*Pantoea agglomerans* (214)	T, AM, SU, AMC, S
OWODM1	*Alcaligenes* sp. (272B)	T, AM, SU, SXT, S, GEN, K
OWODM1	*Morganella morganii* (215A)	T, AM, SU, SXT, AMC, S, CEF
OWODM3	*Proteus vulgaris* (372)	T, AM, SU, SXT, S, C, N, CEF, FF
OWODM1	*Pseudomonas* sp (260)	T, AM, SU, SXT, AMC, C, N, CEF, FF

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)OWODFW: Owena-ondo treated water, OWODM1: Owena-ondo municipal 1

Table 6 (Cont'd): Multidrug Resistant bacteria from Owena-ondo water samples and their resistance phenotypes (Dam 5)

Sources	Bacteria/Strain ID	Resistant Phenotypes
Municipal Tap 1 (Gram positives)		
OWODM1	*Bacillus altitudinis* (271A)	AM, T, RIF, LIN
OWODM3	*Bacillus* sp. (269A)	AM, SU, SXT, LIN
OWODM3	*Bacillus* sp. (188)	AM, SU, SXT, LIN
OWODM3	*Bacillus thuringiensis* (270)	AM, SU, SXT, LIN
OWODM1	*Bacillus cereus* (215B)	AM, SU, SXT, E, LIN
OWODM1	*Bacillus* sp. (245A1)	AM, SU, T, RIF, LIN, CIP, GEN
Municipal Tap 2 (Gram negatives)		
OWODM2	*Escherichia coli* (210A)	T, AM, SU, AMC
OWODM2	*Providencia rettgeri* (253B1)	S, SU, SXT, AMC
OWODM2	*Pseudomonas putida* (251B)	T, AM, SU, SXT, S, C,
OWODM3	*Serratia marcescens* (218A)	T, AM, SU, AMC, CEF
OWODM3	*Morganella morganii* (218B)	T, AM, SU, AMC, CEF
OWODM2	*Escherichia coli* (210B)	T, AM, SU, SXT, AMC
OWODM3	*Morganella morganii* (169)	T, SU, SXT, AMC, S, K, CEF
OWODM2	*Alcaligenes faecalis* (253A)	T, AM, SU, SXT, S, GEN, K, C
OWODM3	*Proteus vulgaris* (190)	T, AM, SXT, S, K, C, N, CEF, FF
OWODM2	*Providencia rettgeri* (209)	T, AM, SU, SXT, S, C, N, CEF, AMC
OWODM2	*Proteus vulgaris* (184B)	T, AM, SU, SXT, AMC, S, C, N, CEF, FF
OWODM3	*Proteus vulgaris* (171B)	T, AM, SU, SXT, AMC, S, C, N, CEF, FF

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)
Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT) OWODM2: Owena-ondo municipal 2 OWODM3: Owena-ondo municipal 3

Table 6 (Cont'd): Multidrug Resistant bacteria from Owena-ondo water samples and their resistance phenotypes (Dam 5)

Sources	Bacteria/Strain ID	Resistant Phenotypes
Municipal Tap 2 (Gram positives)		
OWODM2	*Bacillus* sp (371)	AM, SU, SXT, LIN
OWODM2	*Bacillus* sp (187)	SU, SXT, AM, LIN
OWODM2	*Bacillus* sp. (245A2)	AM, SU, SXT, LIN
OWODM2	*Bacillus* sp. (252A)	AM, SU, SXT, LIN
OWODM2	*Bacillus cereus* (245B)	AM, SU, SXT, LIN
OWODM2	*Lysinibacillus* sp (219*B)	AM, SU, T, LIN, GEN
OWODM2	*Bacillus pumilus* (184A)	SU, SXT, T, E, RIF, LIN
OWODM2	*Bacillus cereus* (186)	AM, SU, SXT, T, E, RIF, LIN
OWODM2	*Myroides odoratus* (269B)	T, AM, SU, SXT, AMC, S, GEN, K, CEF

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)OWODM2: Owena-ondo municipal 2OWODM3: Owena-ondo municipal 3

Table 7: Multidrug Resistant bacteria from Owena-Ijesha water samples and their resistance phenotypes (Dam 6)

Sources	Bacteria/Strain ID	Resistant Phenotypes
Raw water (Gram negatives)		
OWIRW	*Klebsiella pneumoniae* (346)	T, AM, SXT, SU
OWIRW	*Klebsiella oxytoca* (175A)	AM, SU, SXT, K
OWIRW	*Proteus mirabilis* (173A)	T, SU, SXT, GEN
OWIRW	*Klebsiella* sp. (347)	T, AM, SU, SXT, S
OWIRW	*Providencia vermicola* (227B)	T, SU, S, GEN, CEF
OWIRW	*Morganella morganii* (206)	T, AM, SU, AMC, S
OWIRW	*Uncultured bacterium* (205A)	AM, SU, S, GEN, K
OWIRW	*Klebsiella* sp (389B2)	T, AM, SU, AMC, N
OWIRW	*Pseudomonas* sp. (342A)	AM, SU, AMC, CEF, FF
OWIRW	*Alcaligenes faecalis* (173B)	T, SU, SXT, AMC, S, CEF
OWIRW	*Serratia marcescens* (348)	T, AM, SU, AMC S, C, CEF
OWIRW	*Klebsiella pneumoniae* (345)	T, AM, SU, SXT, AMC, S, C
OWIRW	*Pseudomonas* sp (223)	SU, SXT, AMC, S, C, CEF, FF
OWIRW	*Acinetobacter baumannii* (222)	T, AM, SU, SXT, AMC, S, C, FF
OWIRW	*Pseudomonas* sp (343B)	T, AM, SU, AMC, S, N, CEF, FF
OWIRW	*Proteus vulgaris* (179)	T, SU, SXT, AMC, S, C, N, CEF, FF
OWIRW	*Pseudomonas* sp (175B)	T, SU, SXT, AMC, S, GEN, K, CEF
OWIRW	*Acinetobacter junii* (205B)	T, AM, SU, SXT, AMC, S, GEN, CEF
OWIRW	*Alcaligenes faecalis* (207)	T, AM, SU, SXT, AMC, S, K, N, CEF
OWIRW	*Myroides odoratus* (174B)	AM, SU, SXT, AMC, S, GEN, K, CEF
OWIRW	*Pseudomonas* sp (343A)	AM, SU, SXT, AMC, K, C, N, CEF, FF
OWIRW	*Proteus vulgaris* (178B)	T, AM, SU, SXT, AMC, S, C, N, CEF, FF
OWIRW	*Alcaligenes* sp (174A)	T, AM, SU, SXT, AMC, S, GEN, K, C, N, CEF

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)OWODM3: Owena-ondo municipal 3

of this drinking water rendered it unsafe for human consumption and can cause infections and diseases in immune-compromised individuals such as the old and infant which immune systems is still developing [29].

The antibiotic resistance profile of these bacteria revealed a high percentage of them showing multiple drug resistance to antibiotics like tetracycline, streptomycin, ampicillin, sulfamethoxazole, Lincomycin while susceptibility to antibiotics like ciprofloxacin, Nalidixic acid and gentamycin. We observed among gram negative bacteria from Owena-Ondo dam (Fig. 1) that out of the total bacteria from each of the sample location between 0-100%, 0-78%, 20-78%, 29-89%, 86-100%, 20-86% showed resistance to tetracycline, streptomycin, combination of amoxillin and clavulanic acid, ceftiofur, ampicillin, sulfamethoxazole and combination of sulfamethoxazole and trimethoprim respectively. Among the gram negative bacteria from Owena-Ijesha dam (Fig. 3). It was observed

Table 7 (Cont'd): Multidrug Resistant bacteria from Owena-Ijesha water samples and their resistance phenotypes (Dam 6)

Sources	Bacteria/Strain ID	Resistant Phenotypes
Treated water (Gram negatives)		
OWIFW	*Proteus vulgaris* (257B)	AM, SU, SXT, S, CEF
Treated water (Gram positives)		
OWIFW	*Bacillus pumilus* (357B)	AM, SU, SXT, E, LIN
OWIFW	*Bacillus altitudinis* (257A)	AM, SU, SXT, T, E, RIF, LIN
Municipal Tap 1 (Gram negatives)		
OWIM1	*Alcaligenes* sp (250B2)	T, SU, S, K, N, CEF
OWIM1	*Acinetobacter junii* (364)	AM, SU, AMC, CEF
OWIM1	*Klebsiella pneumoniae* (361)	T, AM, SU, S, CEF
OWIM1	*Enterobacter* sp (360)	AM, SU, SXT, AMC
OWIM1	*Citrobacter murliniae* (363)	T, AM, SU, AMC, S, CEF
OWIM1	*Citrobacter freundii* (362)	T, AM, SU, SXT, AMC S, N
OWIM1	*Pseudomonas* sp (196)	T, AM, SU, AMC, S, GEN, K, FF
OWIM1	*Alcaligenes faecalis* (250A2)	T, AM, SU, AMC, S, GEN, K, CEF
Municipal Tap 1 (Gram positives)		
OWIM1	*Bacillus cereus* (250)	AM, SU, SXT, LIN
Municipal Tap 2 (Gram negatives)		
OWIM2	*Brevundimonas naejangsanensis* (236B)	SXT, S, GEN, K, N
OWIM2	*Proteus mirabilis* (385B)	T, AM, SXT, S, C, N
OWIM2	*Chromobacterium* sp (366)	T, AM, SU, S, GEN, CEF
OWIM2	*Alcaligenes faecalis* (239)	T, AM, SU, SXT, S, K, CEF
OWIM2	*Alcaligenes* sp (238B)	T, AM, SU, S, GEN, K, N, CEF
OWIM2	*Pseudomonas* sp (244B)	T, AM, SU, SXT, AMC, S, GEN, K, C,FF

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)OWIFW: Owena-ondo treated water, OWIM1: Owena-ondo municipal 1

Table 7 (Cont'd): Multidrug Resistant bacteria from Owena-Ijesha water samples and their resistance phenotypes (Dam 6)

Sources	Bacteria/Strain ID	Resistant Phenotypes
Municipal Tap 2 (Gram positives)		
OWIM2	*Bacillus cereus* (370)	AM, SU, SXT, LIN
OWIM2	*Bacillus* sp. (203)	AM, SU, SXT, LIN
OWIM2	*Lysinibacillus* sp (244A)	AM, SU, SXT, LIN
OWIM2	*Bacillus thuringiensis* (238A)	AM, SU, SXT, LIN
OWIM2	*Bacillus pumilus* (244)	AM, SU, SXT, E, RIF, LIN

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)OWIM2: Owena-Ijesha municipal 2

that out of the total bacteria from each location between 0-100%, 75-100%, 0-75%, 48-63%, 50-88%, 88-100%, 38- 67% showed resistance to tetracycline, streptomycin, combination of amoxillin and clavulanic acid, ceftiofur, ampicillin and sulfamethoxazole and combination of sulfamethoxazole and trimethoprim respectively. From Fig. 1 it was observed that 20% of gram negative bacteria from the raw water of Owena-Ondo dam were multidrug resistance while the number increased to 88% at each of the treated water and first municipal tap respectively. From Fig. 3, it was observed that 81% of bacteria isolates from raw water of Owena-Ijesha were MDR while 50% of those isolated from the treated water were confirmed MDR and all bacteria isolated from each of the two municipal taps were MDR. Table 6 and 7 showed these MDR bacteria with their various resistance phenotypes. It was observed that these bacteria include *Klebsiella, Alcaligenes, Proteus, Pseudomonas, Myroides, Pseudomonas, Serratia, Providencia, Myroides* etc. Similarity was observed between the MDR bacteria isolated in our study and that isolated by Bolaji *et al.* [30] who also isolated MDR *klebsiella, Pseudomonas, Proteus* and *Enterobacteria* from hospital waste water from Ede in Southwestern Nigeria. High resistant to antibiotics like streptomycin, ampicillin, tetracycline, Amoxillin/clavulanic acid observed in this study have also

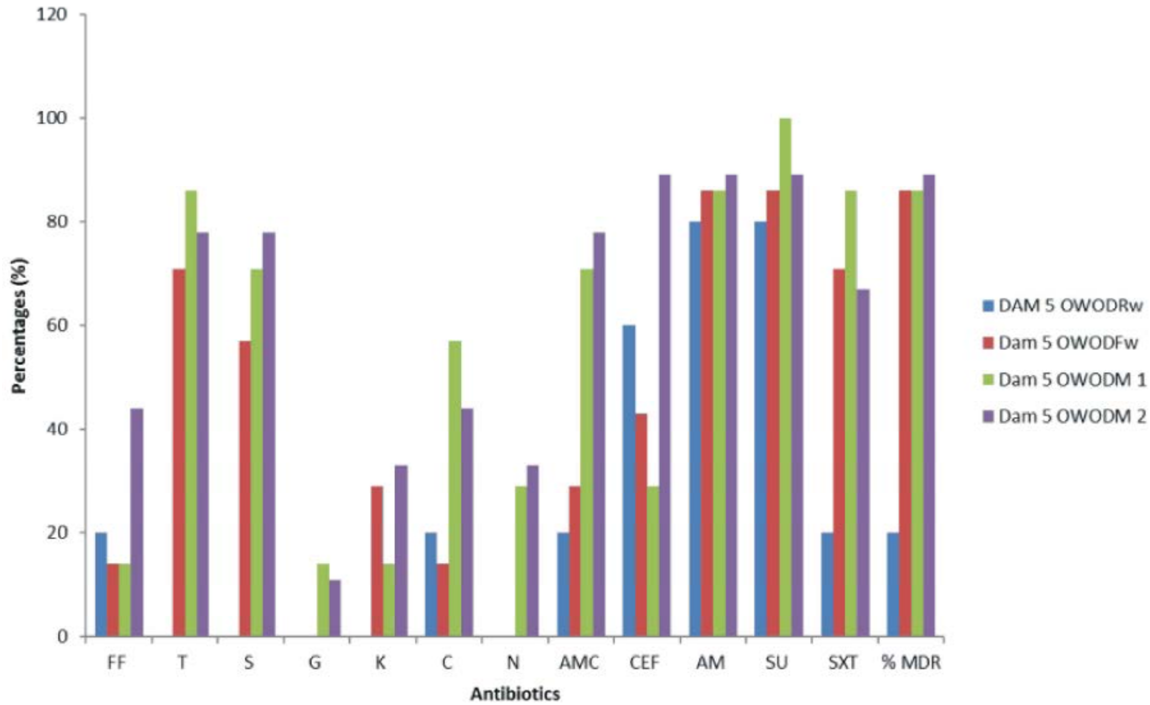

Fig. 1: Percentage of Gram negative bacteria that were resistant to antibiotics from Owena-ondo water samples OWODM1= NEPA, Akure OWODM2= Arakale, Akure)
(Rw= 4, Fw= 7, M1= 11, M2= 12, Total no of Bacteria= 34)
Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol (C) and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N);, Sulfamethoxazole (SU); Sulfamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

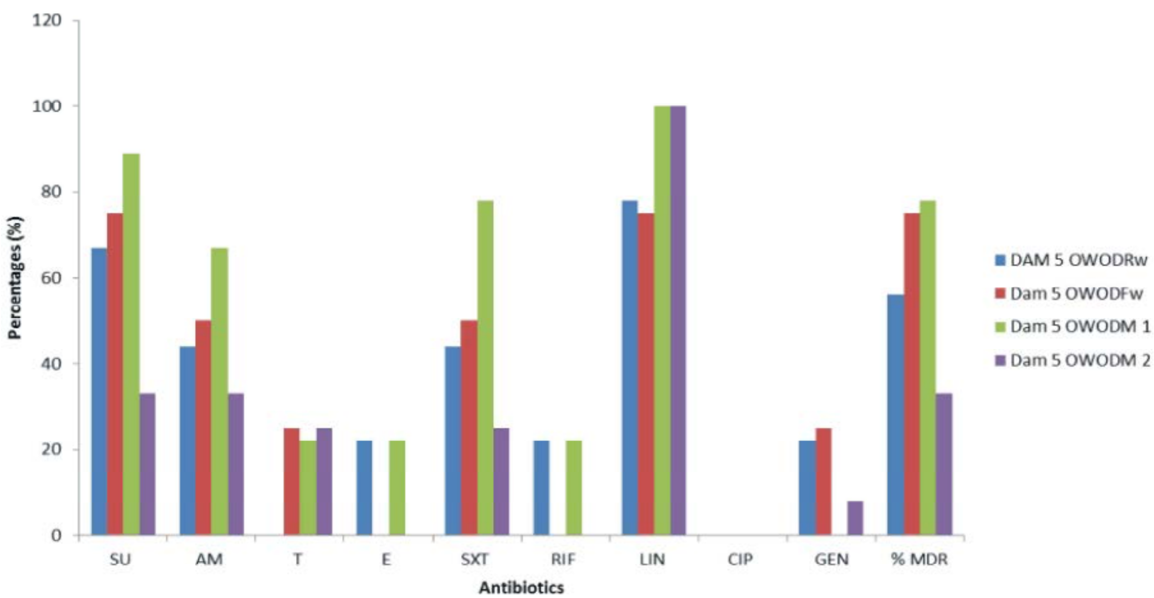

Fig. 2: Percentage of Gram positive bacteria that were resistant to antibiotics from Owena-ondo water samples (OWODM1= NEPA, Akure, OWODM2= Arakale, Akure)
(Rw= 9, Fw= 4, M1= 11, M2= 15, Total no of Bacteria= 39)
Sulfamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin (E); Rifampin (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/ Trimethoprim (SXT)

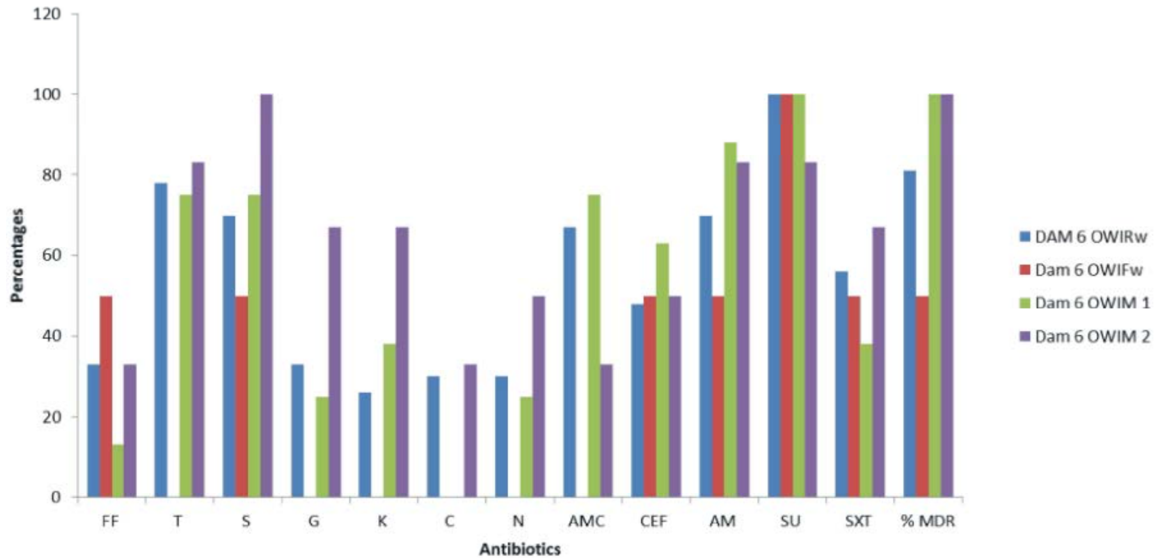

Fig. 3: Percentage of Gram-negative bacteria that were resistant to antibiotics from Owena-Ijesha water samples (OWIM1= Owena-Igbara oke Tap, OWIM2= Owena-Ijesha Tap).
(Rw= 28, Fw= 2, M1= 11, M2= 3, Total no of Bacteria= 44)

Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol (C) and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

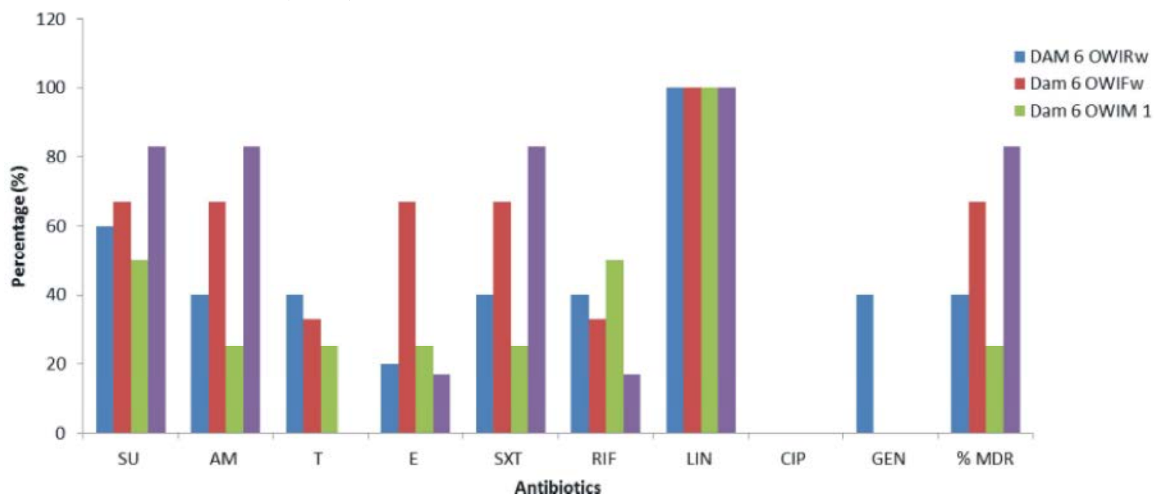

Fig. 4: Gram-positive bacteria that were resistant to antibiotics from Owena-Ijesha water samples (OWIM1= Owena-Igbara Oke Tap, OWODM2= Owena-Ijesha Tap)
(Rw= 4, Fw= 3, M1= 4, M2= 6, Total no of Bacteria= 17)

Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin (E); Rifampin (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprim (SXT)

been reported by Robert. [31]. Most of these antibiotics are commonly used or misused as feed additives or therapeutics agent as reported by Nyamboya *et al.,* [32] that reach ground water through their effluents. High prevalence of MDR *Pseudomonas* in municipal drinking water was also reported by Odjadjare *et al.* [33].

Among the gram positive bacteria, as shown on Fig. 2 and Fig. 4 showed resistance to ciprofloxacin while high percentage were observed to be resistant to lincomycin. At Owena-Ijesha dam (Fig. 4), it was observed that 100% of total bacteria from each of the sample location were resistant to this antibiotics while between

75-100% of total bacteria from each of the sample location of Owena-Ondo dam were resistant to lincomycin. Among the gram positive bacteria, it was observed that the high sensitivity observed to ciprofloxacin and high resistant to lincomycin was contrary to what was observed by Chikere *et al.* [34] among gram positive bacteria from clinical isolates from Nigeria. They observed a high resistant to ciprofloxacin but very high sensitivity to gentamycin, lincocin, riframpicin and streptomycin. However, studies have shown that high number of multidrug resistance bacteria in water bodies may be due to misuse of antibiotics in human health, veterinary medicine, aquaculture, agriculture and household products [7, 8]. And if these antibiotics are not eliminated they can reach surface water and ground water and potentially drinking water [9].

CONCLUSION

This study revealed that drinking water distribution systems of selected dams in Ondo State, Nigeria is reservoir of multidrug resistant bacteria which may be transferred to consumers of portable water through horizontal gene transfer. The presence of coliform bacteria also indicated fecal pollution of this water and is not safe and wholesome for human consumption. Therefore, there is the need for government and public health personal to tackle this problem in order to stop the spread.

ACKNOWLEDGEMENTS

We acknowledge Dr Call Douglas of Paul. G. Allen School of Global Animal Health, Washington state University, USA who allowed the Molecular Characterization of the bacteria isolates to be carried out in his Lab and also Lisa Lorfe who supplied the technical knowhow.

REFERENCES

1. Murray, R., J Stofan and E.R Eichner, 2003. Hyponatremia in athletes. Sports Science Exchange, 16: 1-6.
2. Orewole, M.O., O.W. Mkainde, K. Adekalu and K.A Shittu, 2007. Chemical Examination of piped water supply of Ile –Ife in South West Nigeria. Iranian Journal of Environmantal Health and Science Engineering, 4 (1): 51-56.
3. Skipton, S.O., B.I. Dvorak, W. Woldt and S. Wirth, 2007. Drinking water: Bacteria. Neb Guide. A publication of Univesity of Nebraska-Lincoln Extension, Institute of Agriculture and Natural Resources.
4. Lee, S.W., H.W. Oh, K.H. Lee and T.Y. Ahm, 2009. Methylobacterium dankookense sp. nov., isolated from drinking water. Journal of Microbiology, 47: 716-720.
5. Zhang, T., X. Fan, S. Hanadai, Y Kamagata and H.H. Fang, 2006. Bacillus macauensissp. nov., a long-chain bacterium isolated from a drinking water supply. International Journal of Systematic and Evolutionary Microbiology, 56: 349-353.
6. Pindi, P.K.and P.R. Yadau, 2010. Bacteria diversity in portable drinking water of Mahabubnagar district (A. P) India Bioscience Discovery, 2: 32-35.
7. Kapil, A., 2005. The challenge of antibiotic resistance: Need to contemplate. Indian Journal of Medical Research, 121: 83-91.
8. WHO, 2002. Use of antimicrobials outside human medicine and resultant antimicrobial resistance in humans. WHO fact sheet No. 268.
9. Kummerer, K., 2003. The significance of antibiotics in the environment. Journal of Antimicrobial Chemotherapy, 52: 5-7.
10. Barbosa, T. and S. Levy, 2000. The impact of antibiotic use on resistance development and persistence. Drug Resistant Update, 3: 303-311.
11. Livermore, D., 2003. Bacterial resistance: Origins, epidemiology and impact. Clinical Infection and Disease, 36: 11-23.
12. Rice, E.W., 1998. Samples. In: Standard Methods for the Examination of Water and Waste Water. Clesceri LS, Greenburg AE and Eation AD (eds). Published jointly by American Public Health Association, American Water Works Association and Water and Environmental Federation. United States of America (Part 9000), 9 of 19-9 of 21.
13. Adesoji, A.T. and A.A. Ogunjobi, 2013. Physicochemical Properties and Occurrence of Antibiotic-Resistant Bacteria in Ife and Ede Water Distribution Systems of Southwestern Nigeria. World Applied Sciences Journal, 27(9): 1098-1110.
14. Skoog, D.A. and D.M. West, 1975. Fundamentals of Analytical Chemistry. 2nd Edition, Holt Richard and Winston Inc. New York, pp: 112-26.
15. Radojeric, M. and V.N. Baskin, 1999. "Practical Environmental Analysis'' Royal Society of Chemistry, Cambridge, UK, pp: 140-150.

16. Turner, S., K.M. Pryer, V.P.W. Miao and J.D. Palmer, 1999. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. Journal of Eukaryotic Microbiology, 46: 327-338.
17. Muyzer, G., E.C. De Waal and A.G. Uitterlinden, 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology, 59: 695-700.
18. Pranavam, T.S., T.V. Rao, L. Punithavathi, S. Karunanithi and A. Bhaskara, 2011. Ground water Pollution in the Palar Riverbed near vellore, Tamil Nadu, India. Indian Journal of Science and Technology, 4(1): 19-21.
19. Ravisankar, N. and S. Poongothai, 2008. A study of groundwater quality in tsunami affected areas of Sirkazhi Taluk, Nagapattinam district, Tamilnadu, India, Science of Tsunami Hazards, 27(1): 47-55.
20. World Health Organisation (WHO), 2008. "Guidelines for Drinking-Water Quality," 3rd Edition, World Health Organisation, Geneva.
21. Fatombi, K.J., T.A. Ahoyo, O. Nonfodji and T. Aminou, 2012. Physicochemical and bacterial characteristics of groundwater and surface water quality in Lagbe Town: Treatment essays with Moringa oleifera seeds. Journal of water resource and Protection, 4: 1001-1008.
22. WHO, 2004 "International Standards of Drinking Water," World Health Organization, Geneva, pp: 55-79.
23. Environmental Protection Agency (EPA), 2002. Drinking water; national primary drinking water regulations: total coliforms (including fecal coliforms and *E.coli*). Federal Register, 54(124): 27544.
24. Oluyege, J.O., A.O. Oluyege, O.D. Dada, O. Ogunbanjo, O. Ilesanmi and O. Aregbesola, 2010. Incidence of drug resistant bacteria and physicochemical properties of Ero Dam, Nigeria. Report and Opinion, 2(12): 78-85.
25. Brooks, G.F., S.J. Butel and S.A. Morse, 2004. Medical Microbiology. (23rd ed). The McGraw-Hill Companies Inc. Singapore.
26. Suthar, S., S. Singh, V. Chhimpa, *et al*., 2008 "The problem of safe drinking water in Northern Rajasthan, India," in Proceedings of the 16th National Symposium on Environment, Puranik *et al*., Ed., pp: 491-496, Department of Environmental Science and Technology, Guru Jambheshwar University of Science and Technology, Hisar, India.
27. Pavan, K.P., P.R. Yadav and A.S. Shanker, 2013 "Identification of Opportunistic Pathogenic Bacteria in Drinking Water Samples of Different Rural Health Centers and Their Clinical Impacts on Humans," BioMed Research International, vol. Article ID 348250, 10 pages, doi:10.1155/2013/348250.
28. Awisan, J., V. Demicais, A. Badiangb, L. Carino, D. M. Ferrer, 2011. Aerobic pathogenic and opportunistic bacteria of public health significance in Irisan dumpsite soil. SLU Research, 42: 1.
29. Yi-Tsung, L., J. Yuan-Yu, C. Te-Li and F. Chang-Phone, 2010. Bacteremic community-acquired pneumonia due to Klebsiella pneumoniae: Clinical and microbiological characteristics in Taiwan, 2001-2008. BMC Infectious Diseases, 10307-313. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=s8h&AN=55563638&site=ehost-live on January 16, 2011.
30. Bolaji, A.S., I.O. Akande, F.A. Iromini, S.O. Adewoye and O. A Opasola, 2011. Antibiotic resistance pattern of bacteria spp isolated from hospital waste water in Ede South Western, Nigeria. European Journal of Experimental Biology, 1(4): 66-71.
31. Roberts, M.C., 2011. Environmental macrolide-lincosamide-streptogramin and tetracycline resistant bacteria. Frontiers in Microbiology, 2: 40.
32. Nyamboya, R.A., P.O. Okemo and O. Ombori, 2013. Isolation of High Antibiotic Resistant Fecal Bacteria Indicators, Salmonella and Vibrio Species from Raw Abattoirs Sewage in Peri-Urban Locations of Nairobi, Kenya. Greener Journal of Biological Sciences, 3(5): 172-178.
33. Odjadjare, E.E., E.O. Igbinosa, R. Mordi, B. Igere, C.L. Igeleke and A.I. Okoh, 2012. Prevalence of Multiple Antibiotics Resistant (MAR) Pseudomonas Species in the Final Effluents of Three Municipal Wastewater Treatment Facilities in South Africa. International Journal of Environmental Research and Public Health, 9: 2092-2107.
34. Chikere, C.B., B.O. Chikere and V.T. Omoni, 2008. Antibiogram of clinical isolates from a hospital in Nigeria. African Journal of Biotechnology, 7(24): 4359-4363.