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Occurrence of Multidrug-Resistant Bacteria in Selected Water Distribution Systems in Oyo State, Nigeria

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Abstract: In this study, water samples were collected from raw treated and two randomly selected municipal taps of water distribution systems of dams in Oyo State Nigeria. Physicochemical properties of water samples, microbial quality and antibiotic resistant profile of bacterial isolates was carried out while multidrug resistant bacteria were determined based on their resistance to over three classes of antibiotics. Results showed that pH of water samples ranged from 5.7 to 8.2 and BOD ranged between 1.12 and 12.30. The microbial quality of the water samples from some of the treated water and municipal tap were above the WHO recommended level (no coliform per 100ml of treated water). Antibiogram showed high resistance of bacteria to old generation antibiotic such as tetracycline (0-100%), streptomycin (17-100%) and sulfamethoxazole (33-100%) while MDR bacteria isolated from this study include *Proteus, Klebsiella, Alcaligenes, Aeromonas* etc. This work, therefore, showed that MDR bacteria of Public health significance were associated with water distribution systems in Oyo State Nigeria.

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

INTRODUCTION

The occurrence and spread of antibiotic-resistant bacteria are pressing public health problems worldwide and aquatic ecosystems are recognized reservoir for antibiotic resistant bacteria [1-3] Naturally occurring antibiotic resistant bacteria in the aquatic environment are selected and enriched by antibiotics found in sewage and agricultural runoff, which result from the increased and widespread use of antibiotics [1, 2, 4]. However, these bacteria may also inherit resistance or can develop resistance via spontaneous mutation or the acquisition of resistant genes [5]. The acquisition of a resistant gene via horizontal gene transfer is the most common and easiest way for bacteria to develop antibiotic resistance both in the environment and in a host [6].

Many bacteria transmit antibiotic resistance genes and these antibiotic resistance genes were recently proposed to be emerging contaminants because of their widespread occurrence in aquatic ecosystems [7, 8]. However, insufficient treatment of surface waters for the drinking water supply, malfunctioning of sewage collection systems and defective water distribution pipelines have led to contamination of potable water from water distribution systems by fecal coliform and other pathogenic bacteria [3, 9, 10]. These bacteria may be carrying antibiotic resistance gene which may be transfer to other bacteria in the water distribution system and via the food chain.

Moreover, there is no available information to the best of our knowledge on the occurrence of antibiotic resistant bacteria in water distribution systems in Oyo state Nigeria. This study therefore, determines the physicochemical parameter, microbial quality, molecular characterization of bacteria using 16S rDNA and antibiotic resistant profiles of bacteria from selected water distribution systems of two drinking water treatment dams in Oyo state Nigeria.

MATERIALS AND METHODS

Sites Description: Two study sites selected for in this study were the water distribution systems of Eleyele dam and Asejire dam both . The descriptions of the dams are as follows:

Eleyele Water Works was constructed in 1942 in a quest to create a modern water supply system to meet the challenge of water supply system, to meet the challenge

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of water scarcity for the emerging Ibadan metropolis and it was constructed on the main River Ona with a reservoir storage capacity of 29.5 million litres. The dam is located along Eleyele wetland in North eastern part of Ibadan, southwestern Nigeria within longitude N07°25'00" and N07°27'00" and latitude E03°50'00" and E03°53'00". The wetland and the associated Eleyele dam receive water from River Alapata and the head Stream of River Ona. The catchment of Eleyele wetland is relatively well drained with network of River Ona and its tributaries (such as Ogbere, Alapata and Ogunpa). River Ona flows roughly in North South direction [11]. Asejire Water Works is a reservoirthat is located in Asejireon the Osun River, about 30 kilometers east ofIbadan. The reservoir was built in the late 1960s. Farming is totally banned in the catchment area and trees have been planted on the banks prevent erosion. With the volume of water supply, the reservoir remains full throughout the year [12]. The reservoir provides raw water to the Asejire and Osegere water treatment plants inIbadan [13]. The water supply project was completed in 1972 and has a capacity of about 80 million liters per day, of which 80% is used for domestic purposes [14].

Sample Collection, Determination of Colony Forming Unit (Cfu) and Isolation of Bacteria: Water samples were obtained twice each during the raining and dry season between December, 2010 and July 2011. Samples for microbiological analysis were taken aseptically from raw, treated and two water distribution taps in the municipals in screw cap bottles and transferred to the Laboratory in ice pack containers. Samples for chemical analysis were taken with 500ml bottles only from the raw and treated water from the water treatment plant and transported to the Laboratory for chemical analysis.

Serial dilution of the water samples were then made to enumerate bacteria load by pour plate technique on Nutrient agar (NA) and Eosin Methylene blue agar (EMB) followed by incubation at 37°C for 24hr. Treated water samples were plated directly on NA and EMB for bacteria enumeration. Bacteria were then selected based on morphological differences and then re-isolated on NA by streaking. Gram staining was then performed and isolates were then stored.

Determinationof the Physicochemical Properties of Water Samples: pH of the water samples was determined by the use of pH meter of glass electrode 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. [15] and Radojeric and Baskin [16]. DO was determined by measuring 200cm^3 of water sample into a beaker with measuring cylinder and the DO meter was switched on and the DO value (mg/l) was recorded.

Total solid (TS) was determined by keeping at 103°C a clear dry glass beaker of 150ml capacity in an oven for 1 hr. The capacity and appropriate identification mark was then placed on it. After, 100ml of the thoroughly mixed sample was measured with measuring cylinder 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 weighed. 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:

Total solid,

$$TS\left(mg/I\right) = \frac{mg\ of\ in\ thebea\ker \times 1000}{Volume\ of\ sample}$$

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)

DNA Isolation and 16s rDNA Sequencing: Total genomic DNA was extracted from isolates after culturing in Luria Betani (LB) broth overnight followed by addition of 200 µl of 5% chelex. Mixture was then boiled at 100°C for 10 min and centrifuged at 13k x g for 1min. The reaction mixture for the PCR include 5µl DNA used as template together with 2mM $MgCl_2$, 0.8 mM dNTPs, 1.5u platinum taq 0.2 µM of each primer 1 and primer 2 and 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 [17, 18]. PCR products were sequenced (Eurofins MWG, USA) and manual base calls were sequenced trimming was completed by sequencher (5.0). BLASTn was used to identify closes sequence matches (www.ncbi.nlm.nib.gov/BLAST/blast)

Table 1: Antibiotic concentration used for breakpoints.

Antibioticsforgramnegativeswithconcentration (ug/ml)		Antibioticsforgrampositiveswithconcentration (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/ClavulanicAcid (32/16)	LIN	Lincomycin (4)
CEF	Ceftiofur (12)	CIP	Ciprofloxacin (4)
SU	Sulfamethoxazole (512)		
SXT	Sulfamethoxazole/Trimethoprime (76/4)		

Assessing Antibiotic Resistance: The antibiotic resistance profile of the bacteria was determined using breakpoint assays on LB agar plates. Agar was autoclaved, cooled to 55°C and then antibiotics were added at specific concentration (Table 1) before agar was poured into petri dishes (150 x 15mm). Bacteria was retrieved from freezer stocks and stabbed with a 96-well pin replicator in sterile LB broth in another 96 well plate and cultured overnight at 37°C in an incubator. 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 and '0' for no growth on each antibiotics plate.

RESULTS AND DISCUSSION

Physicochemical Properties of Water: These results are shown on Table 2a and 2b. The pH of water samples ranges from 5.70 and 8.20. It was observed that the lowest value was recorded at the treated water sample of Asejire dam during the dry season. This value was below the WHO limit of 6.5-8.5 [19]. It is therefore suggested that this water sample was acidic and could lead to dissolution of copper pipes which could in turn lead to copper poisoning and resulting into abdominal pains, nausea, vomiting, diarrhea, headache and dizziness as reported by Chinwe *et al.* [20]. Copper poisoning could also influences formation of liver cirrhosis know as non-indian childhood cirrhosis [21].

Comparing the DO values of raw and treated water samples of Eleyele dam. It was observed that treated water has higher values of 5.34 mg/L and 5.72 mg/L during raining and dry seasons respectively compared to 2.66 mg/L and 3.28 mg/L observed for raw water sample during raining and dry season respectively. However, DO is an important parameter of water quality and index of physical and biological process going on in the water [22]. It is very vital for all living organisms and it may be present in water as a result of direct diffusion from air or as a result of photosynthetic activity of autotrophs. Concentration of DO as reported by Ashok and Govind [22] may be one of the most important parameters to indicate water purity and to determine the distribution and abundance of various algal groups. A high COD value of 65.20 and 88.60 mg/l was observed for raw samples of Eleyele dam during raining season and dry season respectively. Similar value of 74.10 mg/l was also observed during sampling of the treated water during the raining season compared to 19.80 mg/l observed during the dry season. Asejire samples of the treated water showed COD values of 28.70 and 23.00 mg/l during the raining and dry

Table 2a: Physicochemical properties of raw water obtained from Asejire and Eleyele Dams.

		pH	BOD (mg/l)	COD (mg/l)	DO(mg/l)	TOC (mg/l)	TDS (mg/l)	TS (mg/l)	TSS (mg/l)
Asejire	Raining season	7.10	2.38	13.60	4.82	3.03	80.00	130.00	47.00
	Dry season	8.20	4.82	28.30	3.84	4.31	64.00	120.00	50.00
Eleyele	Raining Season	6.70	7.32	65.20	2.66	2.65	120.00	362.00	240.00
	Dry Season	6.90	12.30	88.60	3.28	5.32	76.00	280.00	200.00
	WHO limit	6.5-8.5	6-9	-	-	-	500	-	500

Table 2b: Physicochemical properties of treated water obtained from Asejire and Eleyele treatment plants.

		pH	BOD (mg/l)	COD (mg/l)	DO(mg/l)	TOC (mg/l)	TDS(mg/l)	TS(mg/l)	TSS(mg/l)
Asejire	Raining season	7.20	3.99	28.70	3.11	2.11	110.00	150.00	37.00
	Dry season	5.70	2.10	23.00	5.18	0.83	82.00	111.00	27.00
Eleyele	Raining Season	6.10	2.66	74.10	5.34	1.22	160.00	384.00	220.00
	Dry Season	7.10	1.12	19.80	5.72	0.49	103.00	140.00	30.00
	WHO limit	6.5-8.5	6-9	-	-	-	500	-	500

Table 3: Coliform and Total plate counts of water samples from Asejire and Eleyele water distribution systems during raining and dry seasons.

	Enterobacteriaceaecount(cfu/ml)				Total bacteria count (cfu/ml)			
	Raining Season		Dry season		Raining Season		Dry season	
	Asejire	Eleyele	Asejire	Eleyele	Asejire	Eleyele	Asejire	Eleyele
Raw water	1.9×10^3	2.5×10^5	3×10^5	6.45×10^2	3.23×10^2	3.98×10^3	1 x 106	2.0×10^5
Final water	1.2×10^2	ND	ND	ND	2.69×10^4	ND	1.4 x 10	ND
MunicipalTap 1	1.6×10^2	ND	ND	ND	3.47×10^3	ND	7.9 x 10	3.98
Municipal Tap 2	3.6×10^3	ND	ND	ND	2.63×10^3	ND	ND	3.98

seasons respectively. However, as defined by Ashok and Govind. [22] COD is the measure of pollution in aquatic ecosystem. They also went further to report that it estimates carbonaceous factor of organic matter. Variation in these COD values of these water samples showed the degree of chemical pollution in these water samples. The aim of BOD test is to determine the amount of biochemically oxidisable carbonaceous matter [23]. Higher concentration of 12.30 mg/L was observed for raw water sample of dry season of Eleyele dam compared to 7.32 mg/L observed during raining season. Higher value (4.82 mg/l) was also observed during the dry season of raw water of Asejire dam compared to raining season sample (2.38 mg/L). At the treated water samplings, BOD values ranges from the lowest value of 1.12 mg/L during dry season sample of Eleyele dam to the highest value of 3.99 mg/L during raining season of Asejire dam. These values were lower than that observed in raw water samples. This probably could be because the water has been treated and hence, organic pollutants have been removed from the water. However, the BOD values of the treated water were within the recommended value by WHO. Conductivity of raw water samples obtained at Asejire during the raining season was the lowest (41.60µs/cm) while the highest value (138 µs/cm) was observed at Eleyele during dry season. Treated water sample showed lowest conductivity for Asejire raining season sample (78.10 µs/cm) and highest value at Eleyele dry season (194.00 µs/cm). These values are lower than that observed by Sangpal *et al.* [24] who observed between 288- 962 µs/cm in their report on Ujjanni reservoir in indian. High conductivity value indicated a large quantity of dissolved mineral salt thereby making the water sour and unsuitable for drinking [24]. These results showed that the conductivity of all the water samples were all within the recommended WHO limits. TDS values of raw water samples ranges from 64 to 120 mg/L while TDS values for treated water samples ranges from 82 to 160 mg/L. From these results it was observed that the TDS values were within the permissible limits of WHO [19]. No residual chlorine was observed for treated water samples during the raining season of Asejire dams while 0.61 and 2.32 mg/L was observed at Asejire and Eleyele dry season samples respectively. These values were observed to be above the permissible recommended level by WHO. [19] which is 0.5 mg/L.

Microbial Quality of Water Samples: Results obtained from this study showed that water sampled from the two water distribution systems showed variable level of microbial quality (Table 3). It was observed that the coliform count of raw water of Asejire dam during raining and dry season were 1.9×10^3 cfu/ml and 3×10^5 cfu/ml respectively. It was also observed that no coliform count was observed in all the treated water and municipal taps of both dams during the dry seasons. During raining season, it was only at Eleyele water distribution system that had no coliform in the treated and the two municipal tap sampled. In Asejire raining season samples, coliform count range between 1.2×10^2 cfu/ml and 3.6×10^3 cfu/ml between the treated water sample and the two municipal tap. This did not meet the WHO drinking water standard of no coliform per 100 ml water. Thus this treated water samples are unsuitable for human consumption. Total heterotrophic bacteria count was not detected in Eleyele treated water during the raining season while values between 2.63 x10^3 and 3.47 x 10^3, cfu/ml was observed between the treated and two municipal taps of Asejire dam. However, according to WHO [25] report high total bacteria concentration does not itself present a risk to human health. Nevertheless, total bacteria count can be used in assessing the cleanliness of different treated water pumped to consumers from these dams. Moreover, [26] reported that the microbial contaminations of drinking water could be influenced by factors such as their raw water source, treatment process employed and hygienic practices observed in production especially at the treatment plant. However, it was observed that the residual chlorine (Table 2b) of Asejire treated water samples during the raining season was 0.00mg/l which is below limit of 0.5 mg/l by WHO [25], which probably could be the reason why the microbial load in the sample was high. The dry season sample, 0.61ml/L of residual chlorine was observed while no coliform count was observed at this sample (Table 3). Similar results were also observed at Eleyele treated water samples. A value of 2.32 mg/L was observed as residual chlorine during the dry season sample of it treated water (Table 2b) however, no coliform count was observed in the samples, for treated and municipal taps. But a value of 3.98 cfu/ml total bacteria count was observed at each of its municipal tap sample. However, it should be noted that no treatment process or method used in mass production of drinking water yield a sterile product; it only produces a safe product devoid of pathogenic bacteria [27]. This could be the reason why bacteria were still found in the treated water from this dam.

Bacteria Identified and Susceptibility to Antibiotics: A total of 44 and 23 bacteria were isolated from all sample points of Asejire and Eleyele water distribution systems respectively as shown on Table 4. *Alcaligenes*, *Proteus*, *Klebsiella* and *Bacillus* were isolated at both raw and treated water of Asejire dam while *Pseudomonas* and *Staphylococcus* were found only at its distribution taps. This probably could be that the treatment process of these dams was not effective to remove these bacteria. However, WHO [25] suggested reasons for this ineffectiveness as high source water contamination, inefficient coagulation during treatment, inefficient filtration methods (e.g. failure in filtration, backwash recycling and poor maturation of filters) and poor disinfection techniques (e.g. no free-residual disinfectant and short contact times). It could also be that the bacteria were resistant to chlorine concentration in these water samples. However, experience has shown that maintenance of residual chlorine cannot be relied upon to totally eliminate the occurrence of bacteria from drinking water [28]. Most of these bacteria found especially in treated and municipal taps were opportunistic pathogens

Table 4: Distribution of isolates from the two selected water distribution systems

Sampling Table Dam	Location	bacteria	No. of Isolates
Asejire	Raw water	*Pseudochrobactrum sp*	1
		Alcaligenes sp	4
		Proteus spp	3
		Klebsiella sp	1
		Leucobacter sp	1
		Bacillus sp.	3
		Lysinibacillus sp	1
		Uncultured bacterium clone	1
	Treated water	*Alcaligenes sp*	1
		Proteus spp	2
		Pseudomonas sp	2
		Klebsiella sp	1
		Bacillus sp	3
		Myroides sp	1
		Uncultured bacterium clone	1
	MunicipalTap 1	*Pseudochrobactrum sp*	1
		Chromobacterium sp	2
		Pseudomonas sp	1
		Bacillus sp	5
		Staphylococcus sp	1
		Uncultured bacterium clone	1
	Municipal Tap 2	*Acinetobacter sp*	3
		Klebsiella sp	1
		Proteus sp	1
		Pseudomonas sp	1
		Staphylococcus sp	1
		Total	44
Eleyele	Raw water	*Alcaligenes sp*	4
		Aeromonas sp	3
		Klebsiella sp	5
		Morganella sp	1
		Proteus sp	2
		Providencia sp	1
		Trabulsiella sp	1
		Bacillus sp	1
		Staphylococcus sp	1
	Treated water	*Klebsiella sp*	1
		Proteus sp	1
		Alcaligenes sp	1
		Staphylococcus sp	1
		Total	23

Table 5a: Phenotypic patterns of drug resistance among isolates from Asejire water distribution systems

Source	Bacteria	Resistance pattern
Raw water	Gram-negative, fermentative rod	
	Proteus mirabilis	T, SU, SXT, S, N
	Proteus vulgaris	T, AM, SU, SXT, S, C, N, CEF, FF
	Klebsiella pneumoniae	T, AM, SU, SXT, AMC, S, C, CEF, FF
	Gram-negative, nonfermentative rod	
	Acinetobacter calcoaceticus	SU, T, SXT, S, N
	Alcaligenes faecalis	AM, SU, SXT, S, CEF
	Leucobacter komagatae	SU, SXT, T, GEN, K, N
	Pseudochrobactrum glaciei	T, AM, SU, SXT, AMC, S, K, CEF
	Gram-positive rod	
	Bacillus sp	AM, SU, SXT, E, RIF, LIN
	Lysinibacillus sp	T, AM, SU, SXT, LIN
	Uncultured bacterium clone	T, AM, SU, SXT, AMC, GEN, K, C, N, CEF
Treated water	Gram-negative, fermentative rod	
	Klebsiella pneumoniae	T, AM, SU, SXT, S, C
	Proteus mirabilis	T, AM, SU, AMC, S, CEF
	Proteus vulgaris	T, AM, SU, SXT, AMC, S, C, N, CEF, FF
	Gram-negative, nonfermentative rod	
	Alcaligenes sp	AM, SU, SXT, S
	Pseudomonas sp	T, AM, SXT, S, GEN, K, CEF
	Pseudomonas sp	T, AM, SU, SXT, AMC, S, GEN, K, C, CEF, FF
	Gram-positive rod	
	Bacillus cereus	AM, SU, SXT, LIN
	Bacillus pumilus	AM, SU, SXT, T, E, RIF, LIN
	Myroides odoratus	T, AM, SU, SXT, AMC, S, GEN, K, C, FF
	Uncultured bacterium clone	T, AM, SU, S, GEN, CEF, AMC
Municipal 1 Tap	Gram positive rod	
	Bacillus sp.	AM, SU, SXT, LIN
	Bacillus sp	AM, SU, SXT, T, RIF, LIN, GEN
	Gram positive cocci	
	Staphylococcus sp	AM, SU, SXT, T, GEN
	Uncultured bacterium clone	T, AM, SU, SXT, AMC, S, GEN, K, N, CEF
	Chromobacterium sp	T, AM, SU, SXT, AMC, S, CEF
Municipal 2 Tap	Gram negative rod	
	Klebsiella pneumoniae	AM, SU, SXT, AMC, C, CEF, FF
	Proteus vulgaris	T, AM, SU, SXT, S, GEN, C, N, CEF

Table 5b: Phenotypic patterns of drug resistance among isolates from Eleyele water distribution systems

Source	Bacteria	Resistance pattern
Raw water	Gram negative, fermentative rods	
	Proteus vulgaris	T, AM, C, CEF
	Proteus mirabilis	T, AM, SU, SXT, AMC, S, K, N
	Providencia vermicola	T, AM, SU, GEN
	Trabulsiella guamensis	T, AM, SU, SXT, C, CEF
	Klebsiella pneumoniae	T, AM, SU, K, N
	Klebsiella pneumoniae	AM, SU, SXT, AMC, S, CEF
	Klebsiella pneumoniae	T, AM, SU, SXT, AMC, S, C, FF
	Gram negative, non-fermentative rods	
	Alcaligenes faecalis	T, AM, SU S, K
	Alcaligenes faecalis	T, AM, SU, S, K
	Alcaligenes sp	AM, SU, SXT, AMC, S, CEF
	Alcaligenes sp	T, AM, SU, SXT, AMC, S, GEN, K, N, CEF
	Aeromonas hydrophila	SU S, K, N
	Aeromonas punctata	AM, SU, SXT,CEF
	Aeromonas punctata	AM, SU, AMC,CEF
	Aeromonas caviae	T, AM, SU, SXT, AMC, S, N
	Morganella morganii	T, AM, SU, SXT, AMC, S, K, CEF
	Gram positive cocci	
	Staphylococcus arlettae	AM, SU, E, LIN
	Gram negative, fermentative rod	
Treated water	*Proteus vulgaris*	T, AM, SU, SXT, AMC, S, GEN, K, C, FF
	Gram negative, non-fermentative rod	
Municipal Tap 1	*Alcaligenes sp*	T, AM, SU, SXT, AMC, S, K

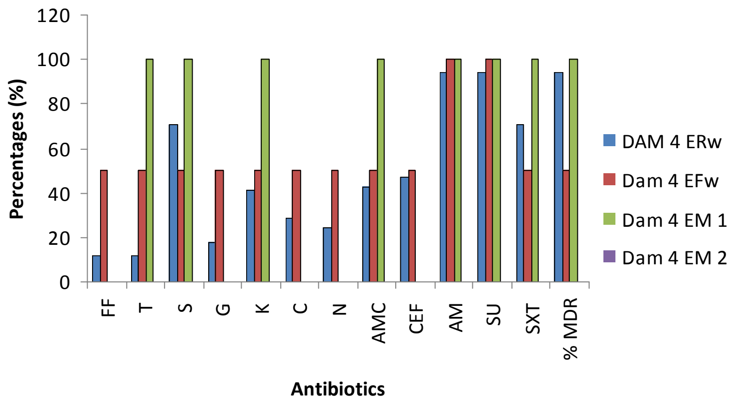

Fig. 1: Percentage of gram negative bacteria that are resistant to antibiotics from Eleyele water samples.
(EM1= Eleyele municipal Tap 1, EM2= Eleyele municipal Tap 2)
(Rw= 17, Fw= 2, M1= 1, M2= 0, Total no of Bacteria= 20)
(Codes: Rw= Raw water, Fw= Treated water, M= Municipal taps)

which have be shown to possess few virulence factor [29, 30] and were therefore of no human health consequence. However, they can constitute a health risk for immunocompromised individuals, including children. For example some species of *Pseudomonas* can established itself in vulnerable patients, such as those with cystic fibrosis or hospitalized in intensive care units [31]. However, studies have also shown that opportunistic bacteria may be present in water distribution system pipeline as a result of their ability to occur in biofilm on the surface of pipelines and sloughing to the water is the explanation for the deterioration of water quality [27]. Felfoldi *et al.* [32] reported in their studies that hospital storage tanks represent a place for bacteria proliferation including a source for opportunistic pathogen contamination. Hence, the storage tank where the treated water from these dams was stored before being pumped to the municipal by high lift pump could be a place for the growth of these bacteria which are of public health significance. This study detected the presence of multi-drug resistant (MDR) bacteria among bacterial isolates which were shown on Table 5a and 5b. MDR bacteria which are Gram negative and fermentative rod that were commonly isolated from both the raw and treated water of Eleyele includes *Proteus* and *Klebsiella* while *Staphylococcus* was the only gram positive cocci isolated from the municipal taps (Table 5a). At Asejire the only gram negative MDR rod from its treated water was *Proteus* while *Providencia*, *Trabulsiella* were isolated from its raw water and gram negative, non-fermentative rod at this point includes *Alcaligene*, *Aeromonas* and *Morganella* spp (Table 5b). Percentage of MDR bacteria among gram negative bacteria at Eleyele ranges between 50% at the treated water to 100% at the municipal tap while that at Asejire varies between 25% at the raw water to 67% at the treated water. However, factors like inappropriate use of antibiotics [33] and uncontrolled availability of antibiotics and other drugs in several developing countries like Nigeria may have contributed to the prevalence of these MDR bacteria in water distribution systems of these dams. Several studies have reported that aquatic ecosystems were recognized reservoir of antibiotic-resistant bacteria and antibiotic resistance genes [34, 35, 36]. Hiraishi. [37] Shrivastava *et al.* [9] and Zellers. [38] also reported that antibiotic-resistant bacteria were common in drinking water. The authors also discovered that chlorine, an agent widely used for disinfection and which is used in dams selected for in this study selects for antibiotic-resistant bacteria. Hence, occurrence of these bacteria in these water distribution systems may be the disinfection process is selecting for antibiotic-resistant bacteria. However, it was observed that the bacteria showed more phenotypic resistance to old generation antibiotics like tetracycline, sulfamethoxazole, ampicillin, streptomycin and combination of sulfamethoxazole and trimethoprim compare to new generation antibiotic such as ciprofloxacin and gentamycin where low resistance was observed. This is similar to the report of Okesola and Oni [39] who worked with clinical bacterial isolates from South Western Nigeria. With respect to bacteria from each sample location. Fig. 2 and Fig. 3 showed that no gram positive bacteria from all the sampling points of the two dams showed resistance to ciprofloxacin.

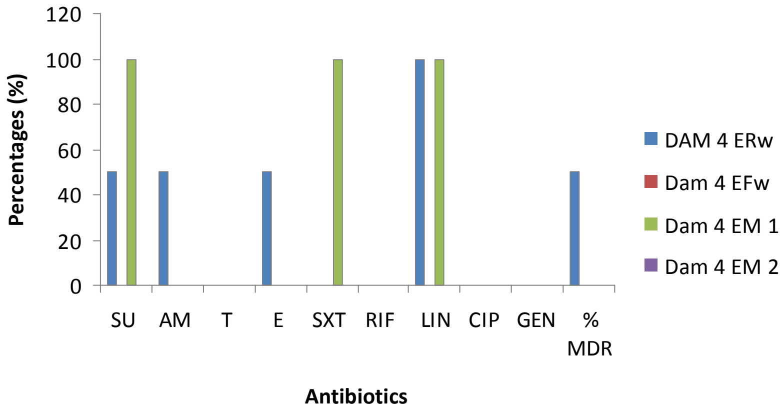

Fig. 2: Percentage of gram positive bacteria that is resistant to antibiotics from Eleyele water samples. (EM1= Eleyele Tanker Tap, EM2= Eleyele Tanker Tap) (Rw= 2, Fw= 0, M1= 1, M2= 0, Total no of Bacteria= 3) (Codes: Rw= Raw water, Fw= Treated water, M= Municipal taps)

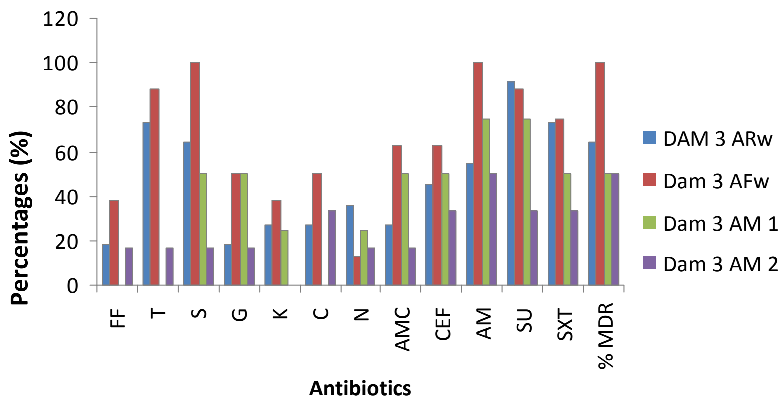

Fig. 3: Percentage of gram negative bacteria that are resistant to antibiotics from Asejire water samples (AM1= Asejire, AM2= Agodi gate boaster station)
(Rw= 11, Fw= 8, M1= 5, M2= 6, Total no of Bacteria= 30)

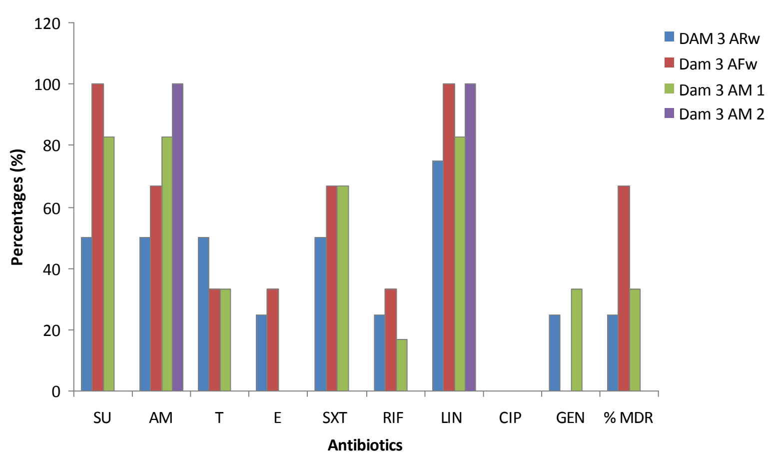

Fig. 4: Percentage of gram positive bacteria that are resistant to antibiotics from Asejire water samples (AM1= Asejire, AM2= Agodi boaster station)
(Rw= 4, Fw= 3, M1= 6, M2= 1, Total no of Bacteria= 14)

Also from Fig. 2 it was also observed that no gram positive bacteria from Eleyele dam were resistance to gentamicin. Fig. 1 showed that with respect to the number of bacteria from the sample points of Eleyele dam high percentage of the bacteria showed resistance ranging from 94 to 100% to ampicillin while all the bacteria from it municipal sample 1 showed 100% resistance to tetracycline, streptomycin, kanamycin amoxillin/clavulanic acid sulphamethoxazole and sulphamethoxazole/ trimethoprime. It was also observed (Fig. 3) that there were high levels of resistance to these antibiotics from bacteria isolated from Asejire dam. However, based on the findings of this study bacterial resistance to these antibiotics were high but comparison of the percentage resistance to antibiotics with previously published work is difficult because previous researchers have used different numbers and kind of antibiotics in their studies. However, our observation also corroborates the finding of Odeyemi *et al.* [40], who reported low incidence of antibiotic resistance to gentamycin. In a similar study Rice *et al.* [41] also collaborates this phenomenon by affirming that high-level gentamycin resistance are rarely detected among enterococci isolated from aquatic environment. High prevalence of resistance to tetracycline as found in this study was also demonstrated by previous studies [42, 43]. Work is presently going on to characterize the antibiotic resistance gene involved in the antibiotic-resistant phenotypes. This will help us to know the genes that are predominant among antibiotic-resistant bacteria found in this study.

CONCLUSIONS

The present result provided evidence that water distribution system in south western Nigeria is a source of multiple antibiotic resistant bacteria and hence as a potential route of entry of multidrug resistant zoonotic bacteria into human population through consumption of treated water. This high rate may not be unconnected with widespread indiscriminate and inappropriate use of antibiotics which is rampant in the environment. Hence, medical and paramedical staff should be educated on the irrational use of antibiotics. Furthermore, the public at large should also be enlightened through regular health education programmes on the dangers inherent in self-medication and inappropriate use of these drugs.

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