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Physicochemical Properties and Occurrence of Antibiotic-Resistant Bacteria in Ife and Ede Water Distribution Systems of Southwestern Nigeria

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Abstract: This study investigated the physicochemical properties, microbial qualities and occurrence of antibiotic-resistant bacteria in raw, treated and municipal taps water samples of two dams in Ife and Ede respectively in southwestern Nigeria during December, 2010 and January, 2011 and June and July, 2011 period. It was observed that pH of all water samples were within the WHO limits 6.5 -8.5. No residual chlorine was observed at the raw and treated water of both dams during June/July sampling while residual chlorine ranging between 1.00 – 1.71 mg/l was observed at the treated water during December/January period. Coliform count of all treated and municipal water samples were all above the united state Environmental protection Agency maximum contamination level. Bacteria identified from the treated and municipal taps included*Proteus, Alcaligene, Brevundimonas, Pseudomonas, Klebsiella, Morganella, Acinetobacter, Providencia, Chromobacterium, Stenotrophomonas*, *Camamonas, Lysinibacillus* Antibiotic resistant profiles of Gram-negative bacteria from Ife water samples revealed high percentage resistance to sulfamethoxazole (81-100%), tetracycline (33-73%), amoxicillin/clavulanic acid (33-73%) compared to low resistance to nalidixic acid (0-10%) and chloramphenicol (0-27%). All Gram-positive bacteria from water samples were observed to be sensitive to ciprofloxacin. These results revealed that treated water samples from this studyareunfit for human consumption and also arereservoirs of antibiotic-resistant bacteria.

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

INTRODUCTION

Water meant for human consumption should be free from pollution and should be safe and acceptable. Indeed the microbial quality of potable water should not exceed limits specified in the water quality guideline [1]. However, the microbial quality of water in several rural Nigerian communities has been reported to be poor, unsafe and not acceptable for human consumption [2] and several studies have detected antibiotic-resistant bacteria (ARB) in drinking water systems worldwide [3-5].

However, the emergence of bacteria resistance to antibiotics is common in areas where antibiotics are used, but antibiotic-resistant bacteria also increasingly occur in aquatic environments [6, 7]. The widespread use of antibiotics in medicine and in intensive animal husbandry is indicative of the selection pressure exerted on bacteria [6]. Intensive animal husbandry causes resistant bacteria to enter the environment directly from liquid manure and muck [7]. Although the manner of acquisition of resistance may vary among bacteria species, resistance is created by only a few mechanisms (i) Antibiotic inactivation-direct inactivation of the active antibiotic molecule [8]; (ii) Target modification-alteration of the sensitivity to antibiotic by modification of the target [9] (iii) Efflux pumps and outer membrane (OM) permeability changes- reduction of the concentration of drug without modification of the compound itself[10]or (iv) Target bypass- some bacteria become refractory to specific antibiotics by bypassing the inactivation of a given

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enzyme. This mode of resistance is observed in many trimethoprim and sulfonamide- resistant bacteria [11]. Many resistance plasmids (R factors/plasmids) can confer multiple-drug resistance, due to single R plamids containing several different genes [12]. Genes assembled in plasmids protect bacterial populations against antibiotics. It is the resistant plasmid that plays a substantial role in bacterial resistance to antibiotics[13]. The resistant plasmid can be transferred between various strains of bacteria through conjugation and transformation processes [14].

The interactions of both the physical and chemical properties of water play a significant role in composition, distribution and abundance of aquatic organisms [15]. Sangpal *et al*. [16]reported that water pollution is an acute problem in major rivers and dams in india. Water for human consumption must be free from pathogenic organisms, toxic elements and chemical substances in concentration large enough to affect health [17, 18]. The addition of various kinds of pollutants through sewage, industrial effluents, agricultural runoffetc., into the water main stream brings about series of changes in the physicochemical characteristics of the water, which have been the subject of several investigations [19]. A number of chemical contaminants have been shown to cause adverse health effects in humans as a consequence of prolonged exposure through drinking water from various sources. Much of ill health whichaffects humanity, especially in the developing countries can be traced to lack of safe and wholesome water supply [20-22].

Historically, concerns about the microbial quality of drinking water have focused on the occurrence of pathogens in drinking water distribution systems [23, 24]. However, the presence of ARB and chemical contaminants in source water and finished drinking water may also greatly affect public health and is an emerging issue for the general public and the drinking water industry [3]. Therefore, the aims of this study were to determine the physicochemical property, microbial quality and occurrence of antibiotic-resistant bacteria in water distribution systems of Ife and Ede dams located in south-western part of Nigeria.

MATERIALS AND METHODS

Study Areas: Ife dam is located in Obafemi Awolowo Universitycampusin Ile-Ife, Osun State Nigeriaand equipped with mechanical and auxiliary spillways. The Dam supplies water to the University community.

Ede dam is located in Ede town also in Osun state (southwestern Nigeria).

Sample Collection: Water samples for microbiological and physicochemical analysis were obtained two times each between December, 2010 and January, 2011to represent the beginning of dry season and June, 2011 and July, 2011 to represent the beginning of raining season from raw and treatedwater and two municipal taps of the water distribution systems. Only raw untreated water and treated water of each of the dams was tested for physicochemical analysis. The following steps were followed when sampling water for microbial analysis as previously described byRice [25]. 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 Quality Determination: 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 werethen mixed by shaking before plating on appropriate media. Total plate counts [26] was determined by plating out with a sterile pipette 1ml of the diluted samples from 10^{-2} and 10^{-4} in to sterile Nutrient agar plates. The plates were then incubated in an inverted position in an incubator set at 37°C for 24 hrs. 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 deoxycholate agar as selective agar for isolation of *E. coli* and other coliform. Colonies with different morphologies were observed on the plates and streaked out on Nutrient Agar plate for purification. Colonies were then stored at 4°C on Nutrient Agar (NA) slants.

Determination of Physicochemical Properties of Water Samples: 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.[27]andRadojeric and Baskin [28].

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	Amoxicillin/Clavulanic Acid (32/16)	LIN	Lincomycin (4)
CEF	Ceftiofur (12)	CIP	Ciprofloxacin (4)
SU	Sulfamethoxazole (512)		
SXT	Sulfamethoxazole/Trimethoprim (76/4)		

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) [1].

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 xg for 1min. Extracted DNA supernatant (5µl) was used as template with 2mM $MgCl_2$, 0.8 mMdNTPs, 0.2 µM of primer 1 and primer 2 and 1X PCR buffer. Reaction conditions 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 [29, 30]. PCR products were sequenced (Eurofins MWG, USA) and manual base calls and sequence trimming was completed by *sequencher* (5.0). BLASTn(Altschul *et al*., [31] was used to identify closes sequence matches (www.ncbi.nlm.nib.gov/BLAST/blast)

Assessing Antibiotic Resistance Properties of Bacteria: The antibiotic resistance profile of the bacteria was determined using breakpoint assays on LB agar platesAdesoji and Ogunjobi, [32]. 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 and '0' for no growth on each antibiotic plate.

RESULTS

Physicochemical Properties of Water Samples: The results of the physicochemical properties of the raw water were shown in Table 2 while that of the treated water from the dams were shown in Table 3. The results showed that the pH of the raw water ranges from 6.9 to 7.4 while the lowest BOD (1.21mg/l) was observed at Ede during December/January sampling representing the beginning of dry seasons while the highest (5.28mg/l) was observed during June/July sampling at Ife representing the raining season samples. Total dissolve solids (TDS) of water samples were all lower than the WHO recommended limit of 500mg/l. The highest TSS (769.00 mg/l) was observed during June/July samples at Ede while lowest (23.40mg/l) was from Ife Dam. The residual chlorine of the raw water ranged from 0 mg/l to 0.43 mg/l.

Table 2: Physicochemical properties of raw water sample of Ife and Ede dams in South Western Nigeria during dry and raining season of December, 2010 and June, 2011

Sampled Dam		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)
IFE	June/July	6.90	5.38	64.70	3.40	2.65	100.00	260.00	158.00	65.00	0.00
	December/January	7.40	4.36	28.20	5.61	2.17	97.00	124.00	23.40	55.30	0.43
EDE	June/July	7.10	3.89	37.60	4.21	5.21	60.00	840.00	769.00	48.00	0.00
	December/January	7.4	1.21	11.00	5.39	2.08	42.00	81.00	38.40	24.80	0.39
	WHO limit	6.5-8.5	6-9	-	-	-	500	-	500	500	0.5

Table 3: Physicochemical properties of treated water samples of Ife and Ede dams South Western Nigeria during dry and raining season of December, 2010 and June, 2011

Sampled Dam		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)
IFE	June/July	6.90	1.28	43.10	5.32	1.32	120.00	182.00	61.00	67.00	0.00
	December/January	7.60	1.33	34.10	4.20	2.76	129.00	138.00	6.29	71.00	1.71
EDE	June/July	7.30	1.68	41.30	5.81	1.22	70.00	110.00	39.00	37.10	0.00
	December/January	7.90	0.43	21.70	5.66	1.54	61.00	83.00	19.20	34.20	1.00
	WHO limit	6.5-8.5	6-9	-	-	-	500	-	500	500	0.5

Table 4: Coliform and total bacteria countsof Ife and Ede 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	
Water sample	Ife	Ede	Ife	Ede	Ife	Ede	Ife	Ede
Raw water	3.0×10^3	1.4×10^3	6.2×10^3	1.5×10^4	1×10^3	3.0×10^4	8.9×10^3	8.7×10^4
Treated water	1.0×10^2	4.78×10^2	1.28×10^2	2.82×10^3	1.2 x10	9.1×10^2	1.5×10^2	1.4×10^3
Municipal Tap 1	1.4×10^3	2.8×10^3	1.99×10^2	1.51×10^3	2.0 x 10	7.0×10^2	3.4×10^2	9.1×10^2
Municipal Tap 2	3.4×10^3	4.0×10^2	1 x 10°	1 x 10°	1.9 x 10	5.9×10^2	9.7 x 10	9.1×10^2

In the treated samples, the pH ranged from 6.90 to 7.90 in all water sampled while lowest DO (4.20 mg/l) was found during December/January sample of Ife dam and the highest (5.81 mg/l) during June/July sampleof Ede dam. The residual chlorine of both dams during June/July was found to be 0.0 mg/l while during December/January sample the highest (1.71 mg/l) was observed from Ife dam while the lowest (1.00 mg/l) was recorded at Edetreated water which was higher than recommended values. COD of these water samples ranged from lowest of 21.70 mg/l during December/January samples at Ede dam to highest of 43.10 mg/l during June/July samplestaken from Ife dam.

Microbial Quality of Water Samples: Table 4 shows the results of microbial quality of the water samples. It was observed that the coliform count at the raw water samples during June/July sampling of Ife was 3.0×10^3cfu/ml while that of Ede was 1.4×10^3cfu/ml. During December/ January sample, coliform count of Ife raw water went up to 6.2×10^3 while that of Ede was 1.5×10^4cfu/ml. Coliform count of the treated water ranged from 1.0×10^2 cfu/ml at Ife during June/July period to 4.78×10^2cfu/ml during the same period at Ede. Municipal tap water samples revealed that, all were above no coliform count for portable water recommended by WHO.

The total bacteria count of the treated water of Ede dam during June/July samples was 9.1×10^2 cfu/ml while 7.0×10^2cfu/ml was recorded during the same period at the first municipal water distribution tap sampled. At Ife dam, it was observed that the total bacteria count for the raw water was 8.9×10^3cfu/ml. At both municipal tap samplings of Ede during December/January period, 9.1×10^2 was found for each of the samples.

Bacteria recovered and identified after blasting the 16DS rDNA sequence of the bacteria at the raw water of Ife dam included*Eschericchia*, *Klebsiella*, *Providencia,Proteus*, *Aeromonas*, *Acinetobacter*, *Pseudomonas*, *Stenotrophomonas*, *Camamonas* and *Bacillus* (Table 5). Bacteria identified at the treated water included *Proteus, Alcaligenes, Brevundimonas, Chromobacterium*, *Peudomonas, Bacillus* and *Lysinibacillus*. However, it was found that highest percentage (31.25%) of bacteria at each of the raw and treated water was *Bacillus.* It was observed that 12.5% of

Table 5: Bacteria Identified in Ife and Ede water samples

Sampled Dam	Location	bacteria	No of Isolates	Percentage total bacteria from location (%)
Ife	Raw water	*Escherichia*	2	12.5
		Klebsiella	1	6.25
		Providencia	1	6.25
		Proteus	1	6.25
		Aeromonas	2	12.5
		Acinetobacter	1	6.25
		Pseudomonas	1	6.25
		Stenotrophomonas	1	6.25
		Camamonas	1	6.25
		Bacillus	5	31.25
		Total	16	
	Treated water	*Proteus*	4	23.53
		Alcaligenes	2	11.76
		Brevundimonas	1	5.88
		Chromobacterium	1	5.88
		Pseudomonas	2	11.76
		Uncultured bacteria clone	1	5.88
		Bacillus	5	31.25
		Lysinibacillus	1	5.88
		Total	17	
	Municipal Tap 1	*Klebsiella*	1	16.67
		Chromobacterium	1	16.67
		Uncultured bacterium clone	1	16.67
		Bacillus	3	50.00
		Total	6	
Ede	Raw water	*Proteus*	2	9.52
		Bordetella	1	4.76
		Camamonas	1	4.76
		Chromobacterium	1	4.76
		Acinetobacter	2	9.52
		Aeromonas	1	4.76
		Pseudomonas	3	14.29
		Stenotrophomonas	1	4.76
		Sphingobacterium	1	4.76
		Bacillus	7	33.33
		Staphylococcus	1	4.76
		Total	21	
	Treated water	*Morganella*	1	25.00
		Alcaligenes	2	50.00
		Bacillus	1	25.00
		Total	4	
	Municipal Tap 1	*Acinetobacter*	3	21.43
		Pseudomonas	6	42.86
		Bacillus	5	35.71
		Total	14	
	Municipal Tap 2	Gram negative		
		Escherichia	2	14.29
		Providencia	1	7.14
		Psychrobacter	1	7.14
		Alcaligenes	2	14.29
		Chromobacterium	2	14.29
		Bacillus	5	35.71
		Lysinibacillus	1	7.14
		Total	14	

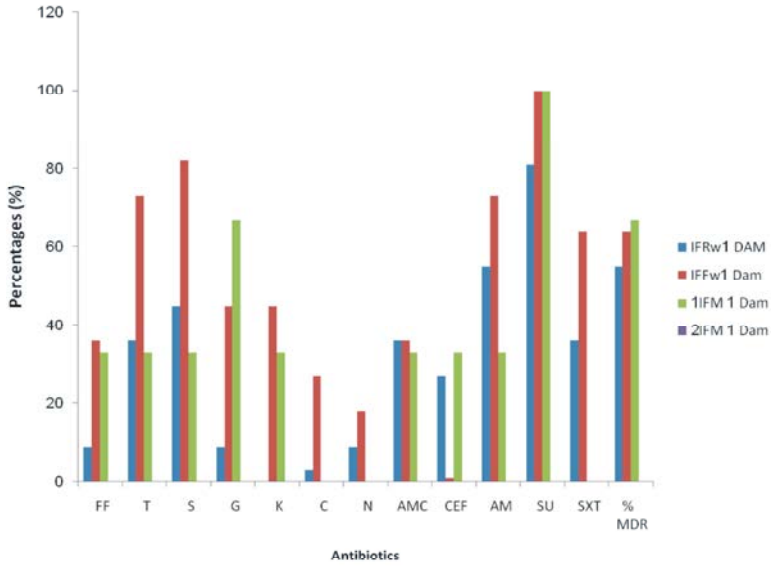

Fig. 1: Percentage of Gram negative bacteria that were resistant to antibiotics from Ife water samples
(IFM1= Awo Hall, IFM2= Fajuyi Hall)
(Rw= 11, Fw= 11, M1= 3, M2= 0, Total no of Bacteria= 25)
Rw= Raw water, Fw= Treated water, M1 and M2= Municipal tap 1 and 2
Ampicillin (AM);Ceftiofur (CEF); Chloramphenicol (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)

the total bacteria from the raw water were *Escherichia* while 23.53% of total bacteria from the treated water were *Proteus*sp. At Ife municipal distribution tap 1 water, it was observed that the highest occurring bacteria (50%) was *Bacillus*out of the total bacteria from this sample point. At Ede raw water sample, it was observed that 9.52% of total bacteria isolated were *Proteus*while other bacteria identified by 16S rDNA included *Bordetella*, *Camamonas*, *Chromobacterium*, *Acinetobacter*, *Aeromonas*, *Pseudomonas*, *Stenotrophomonas* and *Shingobacterium*. At Ede treated water,bacteria identified included *Morganella*, *Alcaligenes*and *Bacillus* while the highest occurring was *Alcaligene* (50%). Higher bacteria genera was identified at municipal tap 2 compared to municipal tap 1 which included *Escherichia*, *Providencia*, *Psychrobacter, Alcaligene* and *Chromobacterium*, *Bacillus* and *Lysinibacillus* while *Acinetobacter*, *Pseudomonas* and *Bacillus* were the three bacteria genera identified at the municipal tap 1 and the *Pseudomonas* showed the highest occurrence (42.86%) of the total bacteria recovered from the sample point.

Antibioticssusceptibility of the Isolates: The results of the antibiotic susceptibility tests of the isolateswere shown in Fig. 1 to 4. It was observed that Gram-negative bacteria from Ife water samples (Fig 1) showed higher percentageof resistance to sulfamethoxazole (81-100%), tetracycline (33-73%), amoxicillin/clavulanic Acid (33-36%), gentamycin (9-65%), ampicillin (33-73) compared to low resistance to nalidixic acid (0-18%) and chloramphenicol (0-27%). At Ede water samples high percentage resistance to these antibiotics was also observed among Gram-negative bacteria which were shown in Fig. 3.

Results of percentage resistance to antibiotic among Gram-positive bacteria from Ife (Fig 2) showed that no bacteria isolated display resistance to ciprofloxacin while high resistance to sulfamethoxazole (40-67%), ampicillin (20-33%), tetracycline (33-50%), erythromycin (20-67%), sulfamethoxazole/trimethoprim (20-67%), rifampin (20-33%), lincomycin (83-100%), gentamicin (33-40%) were observed. At Ede water samples (Fig 4) Gram-positive bacteria also showed that all bacteria from all water sampled were sensitive to ciprofloxacin while high resistance to other antibiotics was also observed as shown in Figure 4. Figures 1 to 4 also showed high percentage of bacteria from water samples to be multidrug-resistant (MDR). Phenotypic expression of bacteria to these antibiotics as shown in Tables 6 and 7 and showed a wide diversity of bacteria including

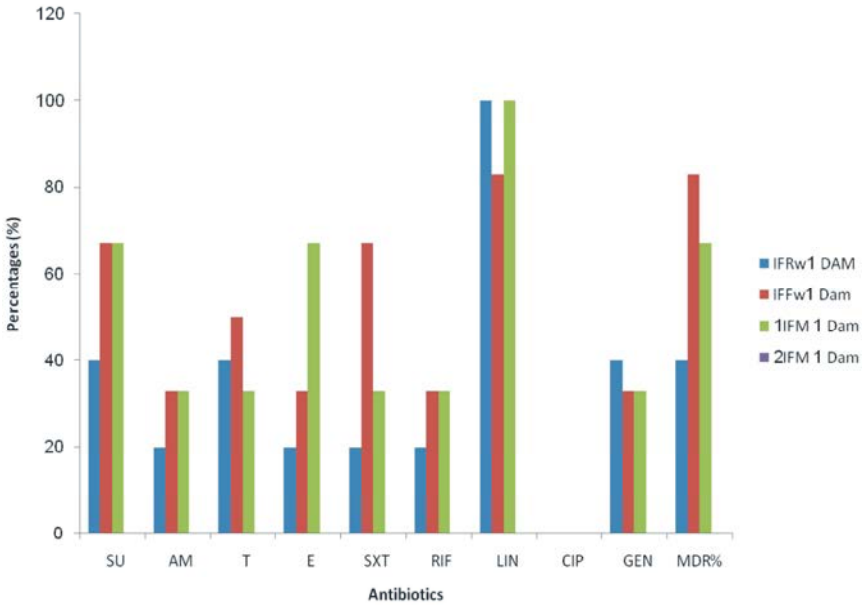

Fig. 2: Percentage of Gram positive bacteria that were resistant to antibiotics from Ife water samples
(IFM1= Awo Hall, IFM2= Fajuyi Hall)
Rw= 5, Fw= 6, M1= 3, M2= 0, Total no of Bacteria= 14)
Rw= Raw water, Fw= Treated water, M1 and M2= Municipal tap 1 and 2
Sulfamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin (E); Rifampin (RIF);Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprim (SXT)

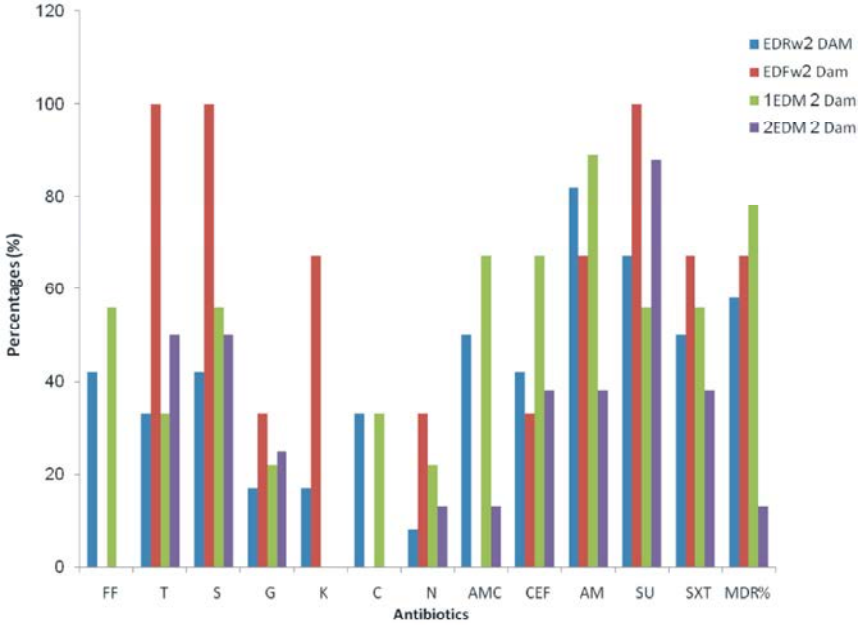

Fig. 3: Percentage of Gram negative bacteria that were resistant to antibiotics from Ede water samples
(EDM1= Muslim Grammar School, EDM2= Obada)
(Rw= 13, Fw= 3, M1= 9, M2= 8, Total no of Bacteria= 33)
Rw= Raw water, Fw= Treated water, M1 and M2= Municipal 1 tap 1 and 2
Ampicillin (AM);Ceftiofur (CEF); 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)

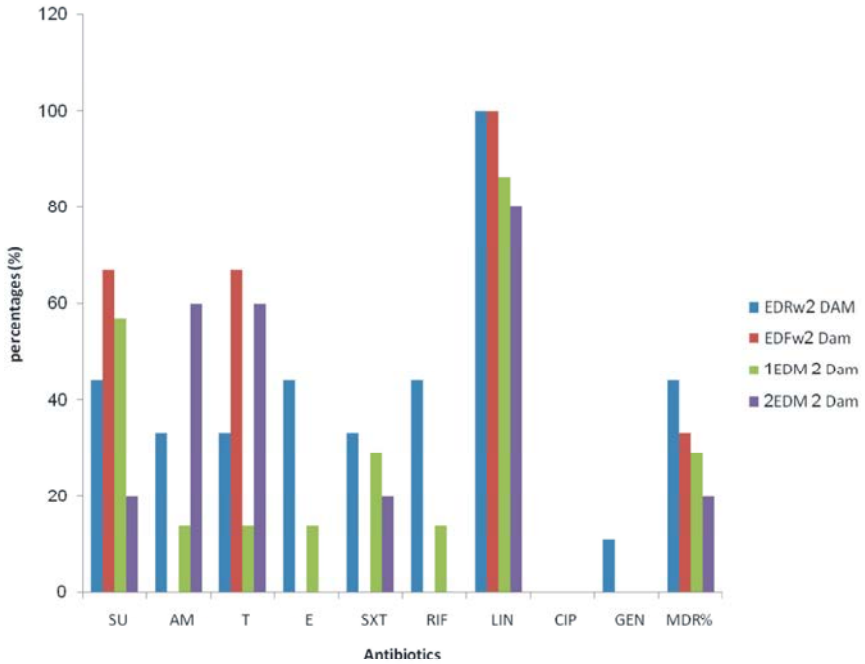

Fig. 4: Percentage of Gram positive bacteria that were resistant to antibiotics from Ede water samples (EDM1= Muslim grammar School, EDM2= Obada)
(Rw= 8, Fw= 1, M1= 5, M2= 6, Total no of Bacteria= 20)
Rw= Raw water, Fw= Treated water, M1 and M2= Municipal 1 tap 1 and 2
Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin (E); Rifampin (RIF);Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprim (SXT)

Table 6: Multidrug Resistant bacteria from Ife water samples and their resistance phenotypes (Dam 1)

Source	Bacteria/Strain ID	Resistant Phenotypes
Raw water (Gram negative)		
IFRW	*Escherichia coli* (319A)	T, SU, SXT, S, C, N
IFRW	*Stenotrophomonassp*(152A)	AM, SU, AMC, S, CEF
IFRW	*Aeromonascaviae* (321B)	T, AM, SU, SXT, AMC, S, C
IFRW	*Aeromonashydrophila*(321A)	T, AM, SU, SXT, AMC, S, C
IFRW	*Pseudomonas sp*(96B)	T, AM, SU, AMC, CEF, FF,
IFRW	*Providenciavermicola* (82)	T, AM, SU, SXT, S, GEN, CEF, FF,
Raw water (Gram positive)		
IFRW	*Bacillus thuringiensis*(116)	AM, SU, SXT, LIN
IFRW	*Bacillus pumilus*(117A)	SU, T, E, RIF, LIN, GEN
		Treated Water (Gram negative)
IFFW	*Brevundimonasdiminuta*(119B)	AM, SU, SXT, S, GEN, K, N
IFFW	*Chromobacteriumviolaceum*(86)	AM, SU, S, CEF
IFFW	*Alcaligenessp*(87A)	T, AM, SU, SXT, S
IFFW	*Proteus mirabilis* (316)	T, SU, SXT, S, N,
IFFW	*Pseudomonas sp* (89)	T, AM, SU, SXT, AMC, S, GEN, K, C
IFFW	*Proteus mirabilis* (122A)	T, AM, SU, SXT, AMC, S, GEN, K, C, FF
IFFW	*Uncultured bacterium clone* (44A)	T, SU, GEN, FF

Gram negative Antibiotics: Ampicillin (AM);Ceftiofur(CEF); Chloramphenicol (C) Florfenicol (FF); Kanamycin (K), Streptomycin (S) Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)
Gram positive Antibiotics: Sulfamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin (E); Rifampin (RIF);Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprim (SXT)
IFRW: Ife Raw water and IFFW: Ife Treated water

Table 6: (Cont'd): Multidrug Resistant bacteria from Ife water samples and their resistance phenotypes (Dam 1)

Source	Bacteria/Strain ID	Resistant Phenotypes
Treated Water (Cont'd) (Gram positive)		
IFFW	*Bacillus subtilis*(146A)	T, LIN, GEN
IFFW	*Bacillus thuringiensis*(120)	AM, SU, SXT, LIN
IFFW	*Bacillus pumilus*(146B)	AM, SU, SXT, LIN
IFFW	*Bacillus pumilus*(122B)	SU, T, E, SXT, RIF, LIN
IFFW	*Bacillus thuringiensis*(85A)	AM, SU, SXT, T, E, RIF, LIN, GEN
Municipal Tap 1 (Gram negative)		
IFM1	*Chromobacteriumviolaceum*(129)	SU, AMC, S, GEN, K, CEF, FF
IFM1	*Uncultured bacterium clone* (92A)	T, AM, SU, G
Municipal Tap 1 (Gram positives)		
IFM1	*Bacillus stratosphericus*(154B)	T, E, LIN, GEN
IFM1	*Bacillus thuringiensis*(329)	AM, SU, SXT, E, RIF, LIN

Gram negative Antibiotics: Ampicillin (AM);Ceftiofur (CEF); Chloramphenicol (C) Florfenicol (FF); Kanamycin (K), Streptomycin (S), Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)
Gram positive Antibiotics: Sulfamethoxazole(SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin (E); Rifampin(RIF);Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprim (SXT)
IFFW: Ife Treated water and IFM1: Ife municipal 1

Table 7: Multidrug Resistant bacteria from Ede water samples and their resistance phenotypes (Dam 2)

Source	Bacteria/Strain ID	Resistant Phenotypes
Raw water (Gram negative)		
EDRW	*Chromobacteriumviolaceum*(382)	AM, SU, SXT, AMC, S, GEN, CEF
EDRW	*Bordetellasp*(51)	T, AM, SU, SXT, S, C, N, CEF, FF
EDRW	*Proteus vulgaris* (58B)	T, AM, SU, SXT
EDRW	*Pseudomonas putida*(99A)	AM, SXT, AMC, FF
EDRW	*Pseudomonas sp.* (299B2)	AM, SU, AMC, CEF, FF
EDRW	*Pseudomonas sp*(299A)	AM, SU, SXT, AMC, C, FF
EDRW	*Stenotrophomonasmaltophilia*(58A)	T, AM, SU, AMC, S, K, CEF
Raw water (Gram positive)		
EDRW	*Bacillus altitudinis*(52B)	SU, E, RIF, LIN
EDRW	*Bacillus sp.* (57)	AM, SU, SXT, LIN
EDRW	*Staphylococcus sp* (98)	SU, SXT, T, E, RIF, LIN
EDRW	*Bacillus altitudinis*(24B)	AM, SU, SXT, T, E, RIF, LIN, GEN

Gram negative Antibiotics: 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)
Gram positive Antibiotics: Sulfamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin (E); Rifampin (RIF);Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)
EDRW: Ede Raw water

Table 7: (Cont'd) Multidrug Resistant bacteria from Ede water samples and their resistance phenotypes (Dam 2)

Source	Bacteria/Strain ID	Resistant Phenotypes
Treated water (Gram negative)		
EDFW	*Alcaligenesfaecalis*(28A)	T, AM, SU, SXT, S, GEN, K, CEF
EDFW	*Morganellasp*(U)	T, AM, SU, SXT, S
EDFW	*Bacillus cereus* (137A1)	AM, SU, SXT, T, E, RIF, LIN
Municipal Tap 1 (Gram negative)		
EDM1	*Pseudomonas sp*(306A)	AM, SU, AMC, CEF, FF
EDM1	*Pseudomonas putida*(85B)	AM, SXT, AMC, C, FF
EDM1	*Acinetobacterbaumannii*(107A)	AM, SU, AMC, S, C, CEF, FF
EDM1	*Pseudomonas sp* (159B)	T, AM, SXT, S, GEN, N, CEF
EDM1	*Pseudomonas sp* (106)	T, AM, SU, SXT, AMC, S, N
EDM1	*Pseudomonas sp*(304A)	T, AM, SU, SXT, AMC, S, GEN, CEF, FF
Municipal Tap 1 (Gram positive)		
EDM1	*Bacillus sp* (66A)	AM, SU, SXT, LIN
EDM1	*Bacillus sp* (110)	AM, SU, SXT, LIN

Table 7: Cont'd

Source	Bacteria/Strain ID	Resistant Phenotypes
Municipal Tap 2 (Gram negative)		
EDM2	*Acinetobacterbaumannii*(109)	AM, SU, SXT, AMC, S, C, CEF, FF,
EDM2	*Pseudomonas sp*(306B)	AM, SXT, AMC, C, FF
EDM2	*Psychrobactersp*(140)	T, AM, SU, SXT, S, CEF,

Gram negative Antibiotics: Ampicillin (AM);Ceftiofur (CEF); Chloramphenicol (C);Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sulfamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)
Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Rifampin (RIF);Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprim (SXT)
EDM 1: Ede municipal Tap 1 and EDM 2: Ede municipal Tap 2

Alcaligenes, *Morganella*, *Pseudomonas*, *Acinetobacter*, *Chromobacterium*, *Brevundimonas*, *Proteus* etc. from these water samples to be MDR bacteria. They showed resistance to commonly used antibiotics in Nigeria such as tetracycline, ampicillin, sulphamethoxazole, sulphamethoxazole/trimethoprim.

DISCUSSION

All the pH of both raw and treated water from all water sampledfrom this study were within the recommended limits suggested by WHO [33]. Mesner and Geiger [34] reported that low and high pH will kill most fish and very few animals can tolerate water with low or high pH. They also reported that heavy metals such as cadmium, lead and chromium dissolve more easily in more acidic water (lower pH). This is important because many heavy metals also become much more toxic when dissolved in water. Lower pH has also been implicated in causing corrosion of pipes [35] and exposure to extreme pH values also can results in irritation of eyes, skin and mucous membranes [35]. Residual chlorine above WHO recommended limit observed during December/January water samples at both dams treated water may lead to chlorination disinfection by-products [33]. Alsothe presence of some bacteria at this high concentration of chlorine suggested the development of resistant by these bacteria to this common chemical disinfectant.

In this study, COD of both the raw and treated water samples of both dams were all above 8.33 mg/l [36]. High COD recorded at the raw water could be because the water have not pass through any treatment process and thus, the dams were also polluted with organic materials. But high COD observed at the treated water samples could be because the treatment process used at both dams are not working effectively in removing organic materials from the dams. Hence, this process needs to be checked in order to improve the water's chemical quality.Rosalam and Duduku [37] in their studies on drinking water distribution system in Malaysia reported a COD value greater than 8.33 mg/l and therefore, concluded that the dam of this water distribution system as polluted.

At both sampling periods, higher COD were observed during June/July period compared to December/January period of both the raw and treated water. The high COD value at the raw watercould probably be because of high rainfall during June/July period which may be washing a lot of organic materials into the water compared to less or no rainfall during December/ January period. We observed the DO during June/July (raining season) to be lower than those recorded during December/January period (dry season). BOD, TDS, TSS and conductivity of the water of all treated samples were all observed to be within the WHO recommended limits for portable water.

It was observed that all the treated water from alltwo dams' treatment plants and those obtained from the municipal distribution taps show the presence of total coliforms (Table 3). High presence of total coliform during June/July water samples season may be because of the absence of residual chlorine in the water samples during this period as shown in Table 2. This might be because of treatment failure and therefore more chlorine should be added into the water. It might also be as a result of the season which is a period of rain in south western Nigeria.Report by Geldreish [38] showed that, heavy rainfall received every year at 1000-2000 mm may also affect the consistency of water treatment system operation and especially the residual chlorine. However, high residual chlorine observed during December/January period which is usually the dry season period in this part of Nigeria did not prevent the growth of this coliform. This means high chlorine concentration at the treatment plant does not guarantee the elimination of coliform and other bacteria suggesting resistant to chlorine. This therefore means these tapsat these points as of the period of sampling are not suitable for direct consumption

and additional treatments such as filtration and boiling are required as suggested by Rosalam and Duduku [37]. Lack of proper handling and hygienic behaviorof the taps could also influence the high occurrence of coliform in this water becauseactivities like washing of plates, clothes and so on were going on around the water distribution taps which could probably be introducing these bacteria into the water systems.

Bacteria identified in these treated water in particular include *Alcaligenes*, *Morganella*, *Pseudomonas*, *Acinetobacter*, *Chromobacterium*, *Brevundimonas*, *Proteus and Bacillus*. These bacteria could find their way into the water bodies as a result of leakages of pipes as reported by Issamand Hassan [39] because some of the pipes in the networks were observed to be old and cracked. High presence of *Bacillus* in this study could be because the bacteria have the tendency to form spore thereby resisting chlorine treatmentin the treated water [40].

Bacteria found in this study could also be of public health importance due to their high resistance to antibiotics and even multiple antibiotics. Therefore, presence of multiple antibiotic-resistant bacteria in the raw water could be as a result of run-off from agricultural farm land that are very close to these dams which may be making use of antibiotics as prophylactic in live-stock production or making use of organic fertilizers in their farmland which consist of un-metabolized antibiotics which eventually are washed into thewater system.

The relatively high level of resistance to antimicrobial agents could be a reflection of misuse or abuse of these agents in the environment [41, 42]. Olayemi *et al*. [43] also reported that most of the antibiotics that were reported for self-medication are ampicillin, tetracycline, co-trimoxazole and ciprofloxacin. The high percentage resistance observed in this study to some of the antibiotics could be because these antibiotics has been abused in clinical treatment.

This worked revealed that water samples obtained from Ife and Ede treated water dams were not good for human consumption as a result of the high presence of coliforms in these water samples. It was also observed that bacteria found in these treated samples were highly resistant to commonly used antibiotics in Nigeria. This could therefore, lead to the spread of antibiotic-resistance genes through food chain, hence, threat to public health. Therefore, health workers should enlighten the populace on the indiscriminate use of antibiotics in agriculture, veterinary medicine and human medicine.

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