

ORIGINAL ARTICLE

Genotypic Characterization of Aminoglycoside Resistance Genes from Bacteria Isolates in Selected Municipal Drinking Water Distribution Sources in Southwestern Nigeria

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ABSTRACT

BACKGROUND: Multi-drug Resistant (MDR) bacteria could lead to treatment failure of infectious diseases and could be transferred by non-potable water. Few studies have investigated occurrence of Antibiotic Resistance Genes (ARGs) among bacteria including Aminoglycoside Modifying Genes (AMGs) from Drinking Water Distribution Systems (DWDS) in Nigeria. Here, we aimed at characterization of AMGs from DWDS from selected states in southwestern Nigeria.

METHODS: One hundred and eighty one (181) MDR bacteria that had been previously characterized using 16S rDNA and showed resistance to at least one aminoglycoside antibiotic were selected from treated and untreated six water distribution systems in southwestern Nigeria. MDR bacteria were PCR genotyped for three AMGs: *aph*(3'')^c, *ant*(3'')^b and *aph*(6)-1a^d.

RESULTS: Out of 181 MDR bacteria genotyped, 69(38.12%) tested positive for at least one of the genotyped AMGs. Highest (50, 27.62%) detected gene was *ant*(3'')^c followed by *aph*(3'')^c(33, 18.23%). Combination of *aph*(3'')^c and *ant*(3'')^b in a single bacteria was observed as the highest (14, 7.73%) among the detected gene combination. *Alcaligenes sp* showed the highest (10/20) occurrence of *ant*(3'')^b while *aph*(3'')^c was the highest detected among *Proteus sp* (11/22). Other bacteria that showed the presence of AMGs include: *Acinetobacter*, *Aeromonas*, *Bordetella*, *Brevundimonas*, *Chromobacterium*, *Klebsiella*, *Leucobacter*, *Morganella*, *Pantoea*, *Proteus*, *Providencia*, *Psychrobacter* and *Serratia*.

CONCLUSIONS: High occurrence of *ant*(3'')^c and *aph*(3'')^c among these bacteria call for urgent attention among public health workers, because these genes can be easily disseminated to consumers of these water samples if present on mobile genetic elements like plasmids, integrons and transposons.

KEYWORDS: Aminoglycoside-modifying gene, antibiotic resistance, treated water and untreated water

INTRODUCTION

Aminoglycosides (Ams) are potent, broad-spectrum antibiotics with many desirable properties for the treatment of human infections (1). This class of antibiotics exerts their antibacterial activity by inhibiting the protein synthesis via binding to the 16S rRNA and by disrupting the bacterial cell membrane integrity (2). Over the years, the emergence of resistant strains has reduced the potential of aminoglycoside in empiric therapies (3).

Safe potable water is essential for people and animals. Safe drinking water is, however, required to be devoid of fecal coliform bacteria, thereby, reducing the risk of waterborne diseases. In developing countries, poor water quality from untreated or ineffective and inefficient water treatment contributes to the risk of child mortalities resulting from waterborne infectious diarrhea (4). The most common and widespread health risks associated with drinking water in developing countries are of biological origin (5). Municipal water distribution systems have been found to support growth of certain antibiotic-resistant bacteria populations and it is also an important reservoir for the spread of antibiotic resistance to opportunistic pathogens (6). It is well documented that horizontal gene transfer is a key route to the acquisition of aminoglycoside resistance genes (7). The genes that encode the modifying enzymes can reside on the chromosome or on mobile genetic elements such as plasmids and transposons (8) which are readily transferable from bacteria of similar species and even between different bacterial species (9).

A number of mechanisms of aminoglycoside resistance known includes reduced uptake or decreased cell permeability (10), alteration of the ribosomal binding site by rRNA methylases (11-13), overexpression of efflux pump (14, 15) and production of aminoglycoside-modifying enzymes (AMEs). However, among resistance mechanisms to aminoglycoside, inactivation by aminoglycoside-modifying enzymes is the most important, both in terms of level and frequency of resistance conferred to the bacterium (1). There are three families of aminoglycoside-modifying enzymes including aminoglycoside acetyltransferases (AACs),

phosphotransferases (APHs), and nucleotidyltransferases (ANTs).

In Gram-negative organisms, resistance to aminoglycosides such as amikacin, tobramycin and gentamycin was reported to vary from 32.6% to 83.6% which is mediated by AAC (6) and APH (2) activity (16-18). Furthermore, resistance to tobramycin and amikacin is also mediated by an ANT(4) enzyme encoded by *ant* (4) gene (19). An earlier study from India reported the prevalence of *aac* (6')-I and *aac* (3)-II was 42.8 % and 20.4 %, respectively (16). Odumosu et al. (20) was the only Nigerian author found to have reported *aac* (6') and *ant* (2)-I" among *Pseudomonas aeruginosa*. Therefore, surveillance study on aminoglycoside resistance due to AMEs is very scanty among Nigerian isolates. However, in combatting the spread of antibiotic resistance, monitoring of phenotypic resistance rates is essential and the availability of information about the epidemiology of the genetic elements responsible for the expressed phenotype is crucial (21). We previously reported the isolation of several opportunistic bacteria like *Acinetobacter* spp, *Aeromonas* spp, *Brevundimonas* spp, *Morganella* sp, *Psychrobacter*, *Pantoea* spp etc that phenotypically exhibited resistance to gentamicin, kanamycin and streptomycin in municipal drinking water distribution systems from south Western Nigeria. Studies on the physicochemical parameters and microbial qualities of these water samples were also conducted (22-24). Therefore, this study aimed at detection of AMEs such as *aph* (3'')^c, *aph* (6)-I^d and *ant* (3'')^b from these isolates and determination of plasmid profiles.

MATERIALS AND METHODS

Sample collection, sample sites, bacteria isolates, molecular characterization and antibiotic resistant profiles: Sampling methods, dam description, antibiotic resistant profiles and molecular characterization used in this study were properly elucidated in our previous studies (22-26). However, for readers' clarity, six dams from three southwestern Nigeria states (i.e. two dams per state) that include Osun, Oyo and Ondo State were selected for this study. Thereafter, ninety-six water samples (100 ml per sample) were purposively collected aseptically into sterile screw cap bottles

across the Drinking Water Distribution Systems (DWDS) of these dams that make use of Water Treatment Plants (WTPs) for treatment of their raw water. These samples were collected four times between December 2010 and July 2011 from raw, treated and two randomly selected municipal distribution taps of these sampled DWDS. These WTPs employ conventional methodologies for water purification, which include filtration, flocculation, sedimentation and disinfection. These dams include Ife and Ede Erinle dams located in Obafemi Awolowo University, Ile-Ife and Ede respectively in Osun state. Eleyele and Asejire dam sited in Ibadan and Asejire, Oyo State respectively.

Owena-Ondo dam located near Akure town, Ondo State and Owena-Ijesha dam located near Ilesha, Osun State were also included in the study. Maps of these sites are presented on Figure 1. Ife dam is the smallest (0.95 km²) while Ede Erinle Dam extends approximately 14.0 km² at the normal water level. The Eleyele Dam (1.5 km²) serves the Ibadan metropolis in Oyo State Nigeria. The Asejire Dam (7.5 km²) is located in Asejire, approximately 30 km east of Ibadan. The Owena-Ondo Dam is 7.8 km² while the Owena-Ijesha Dam is 1.7 km² and is located near Ilesha, in Osun State.

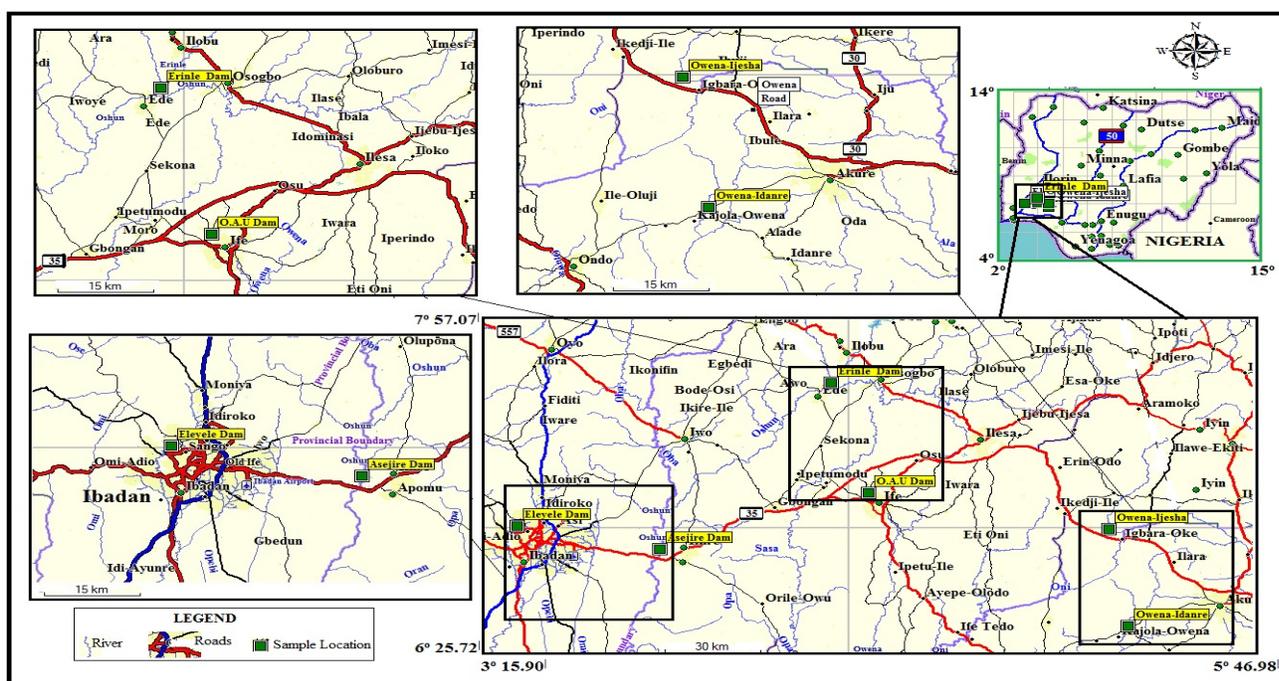


Figure 1: Location of sampled dams, indicated by green squares (The map was created by Dr. Akinola Eluwole)

After samples were collected, they were serially diluted and plated on Nutrient agar, eosin methylene blue agar (EMB), and Deoxycholate agar (DCA). Thereafter, bacteria were picked with the aim of maximizing the diversity of colony morphology represented from each sample. Picked colonies were re-streaked on Nutrient agar to obtain pure cultures. These were subsequently transferred to Nutrient agar slants and also stored in phosphate buffer glycerol at -80°C . Molecular characterization of bacteria using 16S rDNA

sequencing was also determined. Afterwards, antibiotic susceptibility of isolates with various concentration of antibiotics was determined using a point inoculation method as previously described (25). Thereafter, we selected 181 Multi-Drug Resistant (MDR) bacteria for AMEs genotyping based on resistance to over three classes of antibiotics and at least one aminoglycoside antibiotic tested for. The aminoglycoside antibiotics include gentamycin, kanamycin and streptomycin.

Genotyping isolates for aminoglycoside resistance genes: The diversity of aminoglycoside resistance genes encoded in the genome of 181 aminoglycoside and multidrug resistant isolates was assessed by testing for the presence of three selected AMEs belonging to the family phosphotransferases (*aph(3'')*^c and *aph(6)-1d*^d) and adenylases (*ant(3'')*^b). Total genomic extraction of isolates was carried out using the Chelex extraction method as previously described (25, 26). The PCR amplification reaction include 5µl of the chelex

extracted DNA as template, with 2 mM MgCl₂, 0.8 mM dNTPs, 0.2 µl of each of the forward and reverse primer and 1X PCR buffer. Reaction conditions included 1 min denaturation (95°C) followed by 30 cycles of 96°C for 30s, 55°C for 30s and 72°C for 3s and a final extension of 72°C for 10min. However, primer sequences used for the reaction for the AMEs amplifications are shown in Table 1. The PCR products were separated and visualized by gel electrophoresis (1 %) to confirm amplification.

Table 1: Primers used in this study for amplification of Extended beta lactamase (ESBL) resistant genes

Primer pair	Target	Sequence (5'-3')	Annealing temperature (°C)	Amplicon size (bp)	Reference
<i>aph(3'')</i> ^c -F <i>aph(3'')</i> ^c -R	<i>aph(3'')</i> ^c	GCTCAAAGGTCGAGGTGTGG CCAGTTCTCTCGGCGTTAG	55 55	515 515	[31] "
<i>ant(3'')</i> ^b -F <i>ant(3'')</i> ^b -R	<i>ant(3'')</i> ^b	CAGCGCAATGACATTCTTGC GTCGGCAGCGACA(C/T)CCTTCG	55 55	295 295	" "
<i>aph(6)-1d</i> ^d -F <i>aph(6)-1d</i> ^d -R	<i>aph(6)-1d</i> ^d	GACTCCTGCAATCGTCAAGG GCAATGCGTCTAGGATCGAG	55 55	560 560	" "

Determination of presence of plasmid on bacteria isolates: This was carried out based on the method described in our previous study (26). This involves picking and inoculating a single colony from pure overnight bacteria culture grown on Luria Bertani (LB) agar into LB Broth, thereafter incubating the broth overnight. One hundred and fifty microliter of the culture was later pelleted by centrifugation for 10 minutes at 1000xg. The supernatant was removed and re-suspended in 100µl of lysis buffer (3% SDS, 50mM Tris pH 12.6 with 50mM Tris adjusted by 1.6ml 2N NaOH up to 100ml final volume). The mixture was incubated at 55°C for 1 hour. The resulting plasmid was extracted with 150µl of Phenol: chloroform (1:1, v/v, pH 7.9) and mixed by inversion several times before spinning at highest speed for 10 minutes. Fifty microliter of the supernatant was transferred into a new tube and mixed with 10µl of a loading dye. The mixture containing the plasmid was run on 1% agarose gel electrophoresis in 1X TAE buffer for more than 3 hours at a voltage of 8v/cm.

Statistical analysis: Principal component analysis (PCA) biplot of relationship between the AMGs, and the sample locations was determined based on

correlation matrix of association using PAST statistical software. In the PCA biplot, each AMGs is represented by a line, the line direction indicates that the values of the variables increase in that direction. However, the length of the line is related to the rate of increase. Longer lines are more gradual in increment while shorter lines are faster in increment.

RESULTS

Selected bacteria, AME positive strains and plasmid profiles: Bacteria (n=181) used in this study were selected from our previous study (22-24). It should be noted that results of the physicochemical parameters and microbial qualities of these water samples had also been reported in these previous studies (22-24). However, among these bacteria, those that tested positive for at least one AME gene are presented in Table 2. Combination of *aph(3'')*^c and *ant(3'')*^b in a single bacteria was observed as the highest (14, 7.73%) among the detected gene combination in a single bacteria (Table 2). *Alcaligenes* sp showed the

highest (10/20) occurrence of *ant(3'')^b* while highest occurrence of *aph(3'')^c* was detected among *Proteus* sp (11/22) as shown in Table 2. Out of a total of 181 MDR and aminoglycoside resistant bacteria genotyped 69 (38.12%) tested positive for at least one of the selected AME genes. The highest (50, 27.62%) detected gene was *ant(3'')^c* followed by *aph(3'')^c* (33, 18.23%) as shown in supplementary Table 1. Supplementary Table 1 also shows the frequency of distributions of these genes across the sample locations. Moreover, from Ife Dam, 11 isolates were PCR genotyped from the finished water out of which 3 tested positive for AMEs. Principal Component Analysis (PCA) showed that the most important samples collected (eight) were restricted to: Eleyele raw water, Ife raw water, Asejire raw water, Ife finished water,

Owena Ijesha municipal tap I, Ede raw water, Owena Ondo municipal tap water 2 and Owena Ijesha raw water (Figure 2). The highest eigenvalue (5.62) was observed in the first principal component with percentage variance (70.21%). This present study revealed that aminoglycoside resistant positive gene sites (AmRG) and *ant(3'')^b* genes were correlated to raw water from Ife Dam and raw water from Asejire Dam. Moreover, the genes and gene combinations: *aph(3'')^c*, *aph(6)-1d^d*, *aph(3'')^c+ant(3'')^b*, *aph(3'')^c+aph(6)-1d^d*, *ant(3'')^b+aph(6)-1d^d*, *aph(3'')^c+ant(3'')^b+aph(6)-1d^d* were strongly correlated with the collected samples from sites viz: finish water from Ife, tap water from Owena-Ijesha, raw water from Ede and tap water from Owena-oundo.

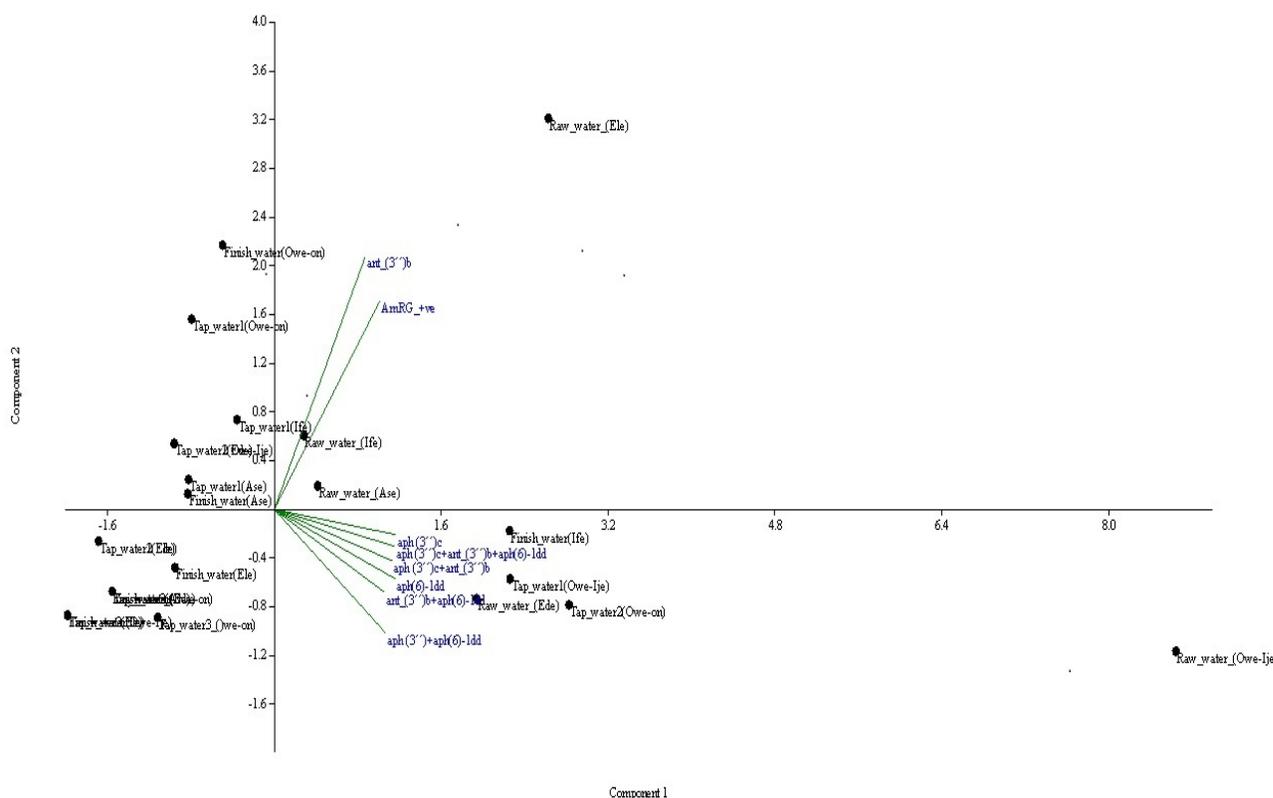


Figure 2: Biplot showing correlation between samples location and amplified genes using principal component analysis

Table 2: Summary of prevalence and total number of aminoglycoside-resistant bacteria species and genotypes

Genus	No of all isolated MDR	No (percentages) of MDR testing positive for AmRG	Sources AmRG isolates	AmR genotypes	No of Genus positive for AmRG
<i>Acinetobacter</i>	6	2 (2.90)	EDM1	<i>aph</i> (3'') ^c <i>ant</i> (3'') ^b	1 1
<i>Aeromonas</i>	5	3 (4.35)	IFRW, ERW	<i>aph</i> (3'') ^c <i>ant</i> (3'') ^b <i>aph</i> (6)-1d ^d <i>aph</i> (3'') ^c + <i>ant</i> (3'') ^b ₊ <i>aph</i> (6)-1d ^d	1 3 1 1
<i>Alcaligenes</i>	20	11 (15.92)	IFFW, ERW, EM1, OWODFW, OWODM2, OWIRW, OWIM1, OWIM2	<i>aph</i> (3'') ^c <i>ant</i> (3'') ^b <i>aph</i> (6)-1d ^d <i>aph</i> (3'') ^c + <i>ant</i> (3'') ^b ₊ <i>aph</i> (6)-1d ^d	3 10 2 2
<i>Bacillus</i>	45	6 (8.70)	IFW, IFM1, EDRW, EDM1, OWODFW, OWODM1, OWIRW	<i>aph</i> (3'') ^c <i>ant</i> (3'') ^b <i>aph</i> (6)-1d ^d <i>aph</i> (3'') ^c + <i>ant</i> (3'') ^b <i>aph</i> (3'') ^c + <i>ant</i> (3'') ^b ₊ <i>aph</i> (6)-1d ^d	2 6 1 1 1
<i>Bordetella</i>	1	1 (1.45)	EDRW	<i>aph</i> (3'') ^c <i>aph</i> (6)-1d ^d <i>aph</i> (3'') ^c + <i>aph</i> (6)-1d ^d	1 1 1
<i>Brevundimonas</i>	2	1 (1.45)	IFFW	<i>aph</i> (3'') ^c <i>ant</i> (3'') ^b <i>aph</i> (6)-1d ^d <i>aph</i> (3'') ^c + <i>ant</i> (3'') ^b ₊ <i>aph</i> (6)-1d ^d	1 1 1 1 1
<i>Chromobacterium</i>	5	3 (4.35)	IFM1, EDRW, AM1	<i>aph</i> (3'') ^c <i>ant</i> (3'') ^b <i>aph</i> (3'') ^c + <i>ant</i> (3'') ^b	1 3 1
<i>Klebsiella</i>	14	8 (11.59)	AFW, ERW, OWODFW, OWIRW, OWIM1	<i>aph</i> (3'') ^c <i>ant</i> (3'') ^b	1 7
<i>Leucobacter</i>	2	2 (2.90)	ARW, OWODRW	<i>aph</i> (3'') ^c	2
<i>Morganella</i>	7	2 (2.90)	EDFW, ERW	<i>aph</i> (3'') ^c <i>ant</i> (3'') ^b	1 1
<i>Pantoea</i>	1	1 (1.45)	OWODM1	<i>ant</i> (3'') ^b	1
<i>Proteus</i>	22	16 (23.19)	EDRW, ARW, AFW, AM2, ERW, EFW, OWODFW, OWODM1, OWODM3, OWIRW, OWIM2, IFFW	<i>aph</i> (3'') ^c <i>ant</i> (3'') ^b <i>aph</i> (6)-1d ^d <i>aph</i> (3'') ^c + <i>ant</i> (3'') ^b <i>aph</i> (3'') ^c + <i>ant</i> (3'') ^b ₊ <i>aph</i> (6)-1d ^d	11 7 4 1 2
<i>Providencia</i>	5	3 (4.35)	IFRW, OWODM2,	<i>aph</i> (3'') ^c <i>ant</i> (3'') ^b <i>aph</i> (6)-1d ^d	2 1 1

Table 2. continued....

<i>Psychrobacter</i>	2	2 (2.90)	EDM2, OWODM3	<i>aph</i> (3'') ^c <i>ant</i> (3'') ^b	1 1
<i>Serratia</i>	2	1 (1.45)	OWIRW	<i>aph</i> (3'') ^c	1
Uncultured bacteria Clone	7	3 (4.35)	IFM1, ARW, AM1	<i>aph</i> (3'') ^c <i>ant</i> (3'') ^b <i>aph</i> (6)-1 ^d <i>aph</i> (3'') ^c + <i>ant</i> (3'') ^b <i>aph</i> (3'') ^c + <i>ant</i> (3'') ^b + <i>aph</i> (6)-1 ^d	2 3 1 2 1

Codes: IRW = Ife raw water, IFFW = Ife treated water, IFM1 and IFM2 = Ife municipal tap 1 and 2, EDRW = Ede raw water, EDFW = Ede treated water, EDM1 and EDM2 = Ede municipal tap 1 and 2, ARW = Asejire raw water, AFW = Asejire treated water, AM1 and AM2 = Asejire municipal tap 1 and 2, ERW = Eleyele raw water, EFW = Eleyele treated water, EM1 and EM2 = Eleyele municipal 1 and 2, OWODRW = Owena Ondo raw water, OWODFW = Owena Ondo treated water, OWODM1 and OWODM2 = Owena-ondo municipal tap 1 and 2, OWIRW = Owena-Idanre raw water, OWIFW= Owena-Idanre treated water, OWIM1 and OWIM2 = Owena-Idanre municipal tap 1 and 2
Note: Bacteria was identified to the genus level by 16S rDNA partial Sequence
AmRG= Aminoglycoside resistance gene

However, bacteria such as *Aquitalae*, *Camamonas*, *Enterobacteria*, *Lysinibacillus*, *Myroids*, *Pseudochrobactrum*, *Sphingobacterium*, *Staphylococcus*, *Stenotrophomonas*, *Ralstonia* and *Trabusiella* were genotypes but none showed the presence of any of the AME amplified in this study (Data not shown). The plasmid profiling of

these MDR bacteria showed that they consist of plasmid size of between 28kb and 130kb. The majority of the bacteria carried one plasmid while few like *Proteus mirabilis* with strain ID 18B2 and *Proteus vulgaris* with strain ID 46 from Dam 3 and Dam 4 respectively both carried two plasmid each (Table 3).

Table 3: Plasmid carrying bacteria isolated from selected water samples from southwestern Nigeria

Source	Bacteria/Strain ID	Resistance Phenotypes	No of Plasmid and Size
DAM 1 IRW	<i>Escherichia coli</i> (319A)	T,S, C, N, SXT, SU	1 (95kb)
DAM 1 IFW	<i>Alcaligenes sp</i> (87A)	T,S, AM, SXT, SU	1 (28kb)
DAM 1 IFW	<i>Proteus mirabilis</i> (122A)	FF, T, S, G, K, C, AMC, AM, SU, SXT	1 (28kb)
DAM 2 EDRW	<i>Chromobacterium violaceum</i> (382)	S, G, CEF, AM, SXT, AMC, SU	1 (95kb)
DAM 2 EDFW	<i>Morganella sp</i> (U)	T, S, AM, SXT, SU	1 (28kb)
DAM 2 EDFW	<i>Alcaligenes faecalis</i> (28A)	T, S, G, K, N, CEF, AM, SXT, SU	1 (28kb)
DAM 2 EDM1	<i>Klebsiella pneumoniae</i> (378)	S, CEF, AM, SXT, AMC, SU	1 (95)
DAM 2 EDM2	<i>Bacillus sp.</i> (110)	SU, AM, SXT, LIN	1 (120kb)
DAM 3 ARW	<i>Proteus mirabilis</i> (18B2)	T, S, N, SXT, SU	2 (130kb and 38kb)
DAM 4 ERW	<i>Proteus vulgaris</i> (33B)	T, C, CEF, AM	2 (120kb and 55kb)
DAM 4 EFW	<i>Proteus vulgaris</i> (46)	FF, T, S, G, K, C, AM, SXT, N, AMC, SU	1 (55kb)
DAM 5 OWODFW	<i>Alcaligenes faecalis</i> (197)	T, S, K, CEF, AM, SXT, SU	1 (55kb)
DAM 5 OWODFW	<i>Alcaligenes sp.</i> (198)	T, S, K, AM, SXT, SU	1 (28kb)
DAM 5 OWODFW	<i>Morganella morgani</i> (199)	S, CEF, AM, AMC, SU	1 (55kb)
DAM 5 OWODFW	<i>Bacillus sp</i> (202B)	SU, AM, T, SXT, GEN	1 (95kb)
DAM 5 OWODM1	<i>Morganella morgani</i> (215A)	T, S, CEF, AM, SXT, AMC, SU	2 (120kb and 28kb)
DAM 5 OWODM2	<i>Escherichia coli</i> (210A)	T, AM, SXT, AMC, SU	1 (55kb)
DAM 5 OWODM2	<i>Bacillus cereus</i> (245B)	SU, AM, SXT, LIN	1 (95kb)
DAM 5 OWODM2	<i>Alcaligenes faecalis</i> (253A)	T, S, G, K, C, AM, SXT, SU	1 (120kb)
DAM 5 OWODM2	<i>Escherichia coli</i> (210B)	T, AM, AMC, SU	1 (95kb)
DAM 6 OWIRW	<i>Alcaligenes faecalis</i> (173B)	T, S, CEF, SXT, AMC, SU	1 (55kb)

Table 3. Continued...

DAM 6 OWIM1	<i>Alcaligenes sp</i> (250B2)	Manu and Anurag. (27) detected <i>aac</i> (6) (43.5 to T, S, K, N, CEF, SU	2 (120kb and 22kb)
DAM 6 OWIM2	<i>Alcaligenes sp</i> (238B)	T, S, G, K, N, CEF, AM, SU	1 (55kb)
DAM 6 OWIM2	<i>Alcaligenes faecalis</i> (239)	T, S, K, CEF, AM, SXT, SU	1 (95kb)

Codes: IRW = Ife raw water, IFFW = Ife treated water, IFM1 and IFM2 = Ife municipal tap 1 and 2, EDRW = Ede raw water, EDFW = Ede treated water, EDM1 and EDM2 = Ede municipal tap 1 and 2, ARW = Asejire raw water, AFW = Asejire treated water, AM1 and AM2 = Asejire municipal tap 1 and 2, ERW = Eleyele raw water, EFW = Eleyele treated water, EM1 and EM2 = Eleyele municipal 1 and 2, OWODRW = Owena Ondo raw water, OWODFW = Owena Ondo treated water, OWODM1 and OWODM2 = Owena-ondo municipal tap 1 and 2, OWIRW = Owena-Idanre raw water, OWIFW = Owena-Idanre treated water, OWIM1 and OWIM2 = Owena-Idanre municipal tap 1 and 2

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); Sulfamethoxazole/Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sulfamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Rifampin (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprim (SXT)

DISCUSSION

In recent years, increasing occurrence of aminoglycoside resistant strains has posed a major threat not only because of their ability to cause serious infections but also because of their increasing resistance to several antimicrobial agents (27). The majority of epidemiological studies have focused on the spread of resistance in human pathogens while few studies have documented the occurrence of antibiotic resistance genes such as AMEs among environment bacteria. In this study, we detected *aph* (3'')^c, *ant* (3'')^b and *aph*(6)-*1d*^d among a wide range of environmentally significant bacteria from treated and untreated water from dams in Nigerian water distribution systems. Our previous work on these bacteria reported tetracycline resistance gene (25), beta-lactam resistance gene (28) and integron and gene cassette harboring other antibiotic resistance (29) genes among these bacteria. These genes could be circulating among humans and animals who depend on these water sources as well as in the environments and among the aquatic animals in these environments. The occurrence of AMEs among non-clinical bacteria from this study is similar to the results of Heuer *et al.* (30), who reported six clusters of genes such (*aac*(3)-*I*, *aac*(3)-*II/VI*, *aac*(3)-*III/IV*, *aac*(6P)-*II/Ib*, *ant*(2Q)-*I*, *aph*(2Q)-*I*) encoding gentamicin resistance gene among Enterobacteriaceae, *Pseudomonas* and *Acinetobacter* from soil, rhizospheres, piggery manure, faeces from cattle, laying and broiler chicken, municipal and hospital sewage water and coaster water.

51.7%), *ant* (2) (17.4 to 20.2%) and *aph* (3) (5.6 to 10.1%) among a total of 255 Gram-negative clinical isolates recovered from clinical specimens that included *Acinetobacter sp* (9%), *Escherichia coli* (34.9%), *Pseudomonas sp* (29% and *Klebsiella sp* (27%). The most prevalent AMEs gene detected in their study was *aac* (6) which was contrary to our observation in this study where *ant* (3'')^b was the most prevalent gene detected. High occurrence of this gene was found among *Alcaligenes sp* (10/20) and *Klebsiella sp* (7/14). This is related to the work of Manu and Anurag. (2014) who reported the presence of *ant* (2) among 13 *Klebsiella sp* from clinical source. Furthermore, *Alcaligenes* was not isolated in their studies. Moreover, we did not find the presence of these genes in *Alcaligenes* in any literature; our study could be the first report of such occurrence. van Overbeek *et al.*, (31) reported the gene among *Proteus spp* from European habitat which include bulk and rhizosphere soils, manure from farm animals, activated sludge from waste water treatment plants and seawater. In this study, *aph* (3'')^c was the second most detected gene among the 3 AMEs amplified while it was mostly (11/22) detected among *Proteus spp*. Seven and four *Proteus spp* also showed the presence of *ant* (3'')^b and *aph*(6)-*1d*^d, respectively. Occurrence of AME in *Proteus* is similar to the studies of Wiczorek *et al.* (32) who also reported *aac*(6')-*1b* among *Proteus* from clinical samples from Poland.

Most of the bacteria observed in this study which seem to be carriers of these AMEs are opportunistic bacteria from aquatic environment. In

Nigeria, Igbiosa and Oviasogie (33) also reported the presence of AME such as *aacA4*, *aadA2*, *aadB*, *aacC4* and *aacA6'-1b* associated with class 1 integron among environmental *Stenotrophomonas maltophilia*. Meanwhile, from our previous studies on these same group of environmental isolates, where the integrase gene was genotype. We observed the presence of *aadA2*, *aadA1* and *aadB* associated with some of their class 1 integrons while *sat 2* was found associated with the class 2 integron of some of the isolated *Proteus* spp. Occurrence of these genes on mobile genetic element such as integrons showed that this gene could be transferred to other bacteria. Hence, there is a need for proper surveillance by public health workers.

Co-habitation of one or more AME genes in a single bacterium observed in many of these bacteria from this study is consistent with other previous studies in *P. aeruginosa* (34-36). *Aeromonas*, *Alcaligenes*, *Brevundimonas*, *Proteus* and an uncultured bacteria clone from this study showed the co-occurrence of *aph (3'')*^c, *ant (3'')*^b and *aph (6)-1d*^d in a single specie.

Plasmids carried by bacteria reported in this study were observed to be bigger in size than those reported by Aja *et al.* (37), Shafiani and Malik. (38) and Wang *et al.* (39). However, it is well known that plasmid is one of the most important mediators facilitating the fast spreading of antibiotic resistance in bacteria (40). Therefore, their occurrence among these bacteria from these water distribution systems suggest the occurrence of some of these genes on them, hence enhancing their shuttling to bacteria present in the Gastrointestinal tract (GIT) of the consumers. Thereby, causing greater havoc of antibiotic-resistant bacteria to the consumers of these drinking water.

In conclusion, this study observed high occurrence of aminoglycoside modifying genes such as *ant (3'')*^c (27.62%) and *aph (3'')*^c (18.23%) among MDR bacteria from southwestern Nigeria drinking water distribution systems. The most worrisome part is the detection of these genes among bacteria from drinking taps and the occurrence of plasmid of different sizes among these bacteria. This calls for urgent attention as monitoring of aminoglycoside modifying genes is necessary considering their co-selection and easy

dissemination among MDR bacteria that could be located in the consumers of the tap water. Hence, difficulty in treating human and animal infection among water consumers. A call for a functional antimicrobial resistance surveillance program among environmental clinical bacteria in Nigeria is very important.

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