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PLASMID PROFILE OF ANTIBIOTIC RESISTANT BACTERIA IN SACHET WATER SAMPLES SOLD IN ABEOKUTA, SOUTHWEST, NIGERIA.

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ABSTRACT

Antibiotic sensitivity pattern of bacterial isolates obtained from sachet water from Abeokuta, Ogun State, Nigeria was investigated. A total of 300 sachet water samples were collected during the dry and rainy season of 2011 to 2012. Selected physicochemical analysis of the sachet water showed a pH range of 5.5-6.5, turbidity of 1.0 –1.5 NTU and temperature of 28-30^{o}C. The mean aerobic mesophilic bacterial count of the sachet water samples ranged from 13.4 x 10^3 – 18.7 x 10^3 CFU/mL, while the mean total coliform count ranged from 0.153 - 0.163 x 10^3 CFU/mL. The physicochemical parameters of the sachet water samples were within the WHO standard for potable water. Bacteria isolated from sachet water samples include *Escherichia coli, Klebsiella* sp, *Enterobacter* sp and *Pseudomonas aeruginosa*. Most of the isolates were highly sensitive to Levoxin and Ceftazidime. Plasmid analyses revealed that there were detectable plasmids in 7.1% of the 14 multi-drug resistant isolates. Cured plasmid encoded resistant isolates were susceptible to Ceftazidime, Ceftriaxone, Gentamycin, Augmentin and Streptomycin.

Keywords: plasmids, antibiotic resistant bacteria, multiple drug resistance, potable water, ceftazidine

INTRODUCTION

Good quality water is colourless, odourless, tasteless and free from faecal pollution (Shilklomanov, 2000). Coliform bacteria are indicator organisms mostly used in bacterial water analysis. They are easily found in animal faeces and most especially from human coliform bacterial contaminates; soil and raw surface water (Shilklomanov, 2000). Coliforms are rod shaped Gram negative organisms which ferments lactose with production of acid and gas when incubated at 37^{o}C (Edberg *et. al*, 2000). Faecal coliform is a smaller group within the total coliform family. It inhabits the intestine of mammal and has a relatively short life span. This serves as an indication for contamination by sewage (Edberg *et al.*, 2000). *Escherichia coli* is the most preferred faecal coliform used in water analysis because it gives indication of faecal contamination. It also does not grow and reproduce in the environment consequently, it is considered to be the species of coliform bacteria that is the best indicator of faecal pollution and the presence of pathogens (Shilklomanov, 2000; Edberg *et. al.*, 2000). The need for having potable water is of great public health significance because of water borne infections. Outbreaks of major epidemics throughout the world have implicated water as major sources of infection. Recently, the emergence of bacterial resis-

tance to most of the commonly used antibiotics is of considerable medical significance. Non contamination with faecal matter is the most important parameter of water quality because human faecal matter is generally considered to be a great risk of human enteric pathogens (Scott *et al*., 2003). Water has played a significance role in the transmission of human diseases. Potential health problems may exist due to microbial content of sachet water since water is one of the vehicles for transmission of pathogenic organisms (Brock, 1991; Prescott *et al.,* 2005). However, the type of organisms present depends on a number of factors such as the type of soil over which the water flows, contamination by animals, sewage and agricultural waste (Hunter, 1993).Water borne diseases are associated with improper provision of water and sanitary services and the effect of these diseases vary in severity from stomach upsets to even death (Sonu *et al*., 2007). Most of the victims are young children especially from the developing world, an estimated number of more than 34 million people die as a result of these water related diseases making it the leading cause of diseases and death around the world (WHO, 2005).

Efforts are being taken by all technological advancement including antibiotic usage to control transmission of water-borne diseases, but multi-drug resistance by some organisms such as *Escherichia coli*, warrants the beginning of steps to prevent public health hazards (Tambekar *et al.,* 2006; Pandey and Musrat, 1993). The use, misuse and under use of antibiotics are responsible for resistance development to bacterial antimicrobials worldwide. Antibiotic resistance has been reported in *Acinectobacter*, *Alkaligenes*, *Citrobacter*, *Enterobacter*, *Pseudomonas*, *Serratia sp*. isolated from river (Praven *et al.,* 1997). Antibiotics used in poultry or agriculture practice to prevent disease can contaminate surface and underground water (Ash, 2002) from where they can be transported to humans in drinking water thereby adding to resistance problems. Drinking water quality has always been a major issue in many countries, especially in developing countries (Assembly of Life Sciences, 1977). The World Health Organization in its "Guidelines for Drinking water quality" publication highlighted at least seventeen different and major genera of bacteria that may be found in water which are capable of seriously affecting human health (WHO, 2006). The proportion of water borne disease outbreaks associated with the distribution system failures has been increasing over the years (Moe and Rheingans, 2006). Antimicrobial resistance take place when bacteria adjust or adapt in a way that permits them to stay alive in the presence of antibiotics designed to kill them, bacteria evolve resistance to these drugs, typically by acquiring chromosomal mutations and multidrug resistant plasmid (Finch *et al*., 2003; Nichol *et al*., 2003; Sheng, 2002). Plamids are self – replicating, circular extra-chromosomal DNA elements. Most plasmids contain genes that give a selective advantage to an organism specifically in limiting or competitive environments such as genes encoding antibiotic resistance. Self-mobilisable plasmids that carry genes required for conjugation (tra genes) as well as antibiotic resistance genes are the main mobile genetic elements involved in antibiotic resistance dissemination via Horizontal Genetic Transfer (HGT) (Liebert *et al*, 1999). The trend of producing sachet water is now on the increase with Abeokuta, south west Nigeria, having lots of these manufacturers. This research is aimed at determine the antibiotic resistant pattern of bacterial isolates obtained from sachet water against some of

the commonly used antibiotics and determine the plasmid profile of the multiple antibiotic resistant strains.

MATERIALS AND METHODS

Samples Collection

A total of three hundred sachet water samples were collected randomly from various parts of Abeokuta metropolis.. Three brands of sachet water namely SB, GM and LD were used for the study. Samples were collected during the onset and peak of the rainy and dry season of 2011 and 2012.. The samples were taken to the laboratory in insulated containers with ice packs.

Total Aerobic Mesophilic Bacterial Count in Sachet water

Total aerobic mesophilic bacterial count in sachet water was determined using spread plate method. In this method, serial dilutions of the respective water samples were made as follows: A row of sterile bottles containing 9ml of peptone water, labelled 1-5, was set up for each water sample. The sachet water container (nylon) were surface sterilized with 70% Alcohol, after which a sterile needle and syringe was used to withdraw 1 ml of the water sample. One mililitre of the test sample was added to the first bottles on each row containing 9ml of diluents, to give 1:10 dilution. This was thoroughly mixed, and 1ml volume was transferred from the first bottle on the same row to the second bottle (1:100). This process was carried out up to the fifth bottle and for the respective samples using different sterile pipettes for each sample. Thereafter, 0.1mL of the diluted sample was inoculated in triplicates unto already sterilized solidified Plate Count Agar (PCA), MacConkey Agar and Eosin Methylene Blue (EMB) agar using a fresh sterile 1ml pipette for each dilution. Using a sterile glass spreader, the inoculum was spread on the surface of the agar medium. The inoculated plates were incubated at 37^{o}C for 24 hours. The viable organisms were counted after incubation. The Colony Forming Unit (CFU/ml) was determined for each sample. Pure cultures of the colonies of the isolates were obtained by inoculating them onto fresh sterile Nutrient and MacConkey agar plates.

Physico-Chemical Assessment of Sachet Water

This involved the assessment of the odour, colour, temperature and turbidity of the samples. Acidity was determined as described by Kegley and Andrews (1998).The pH of the water sample was determined using a pH meter (Jenway, USA). A two part calibrated turbidity tube was used, with calibrations from 5-25 nephlometry turbidity units. The joined tubes were held over a white paper, while slowly pouring the water sample into the tube until the black cross at the bottom was no longer visible. At this point the reading was taken from the side of the tube as the turbidity value of the water sample. The colour of the formation was determined using solution of potassium Chloro-Platinate (K_2PtCl_6) tinted with small amount of hydrated Cobalt Chloride ($CoCl_2.6H_2O$) as described by AOAC (1996). The standard was prepared by dissolving 0.5g Platinum (1.246g K_2PtCl_6) in distilled water. It was subjected to repeated evaporation to dryness in a water bath after the addition of excess HCl. After the addition of 1g crystalline Cobalt (I) Chloride, the residue was dissolved in 100ml conc HCl. The solution was warmed to obtain a bright solution and make up to 1 litre with deionized water. A set of permanent colour standards were then prepared by dilution: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0. 6.0 and 7.0ml .The operations were carried out in a fume cupboard. Colour was measured in

Pt. Co with HACH DR/2000 spectrometer. Temperature was determined using a digital thermometer.

Characterization and Identification of Isolates

Pure cultures of bacterial isolates were subjected to various morphological and biochemical tests. After which they were identified using Bergey's Manual of Systematic Bacteriology. The following tests were carried out: Gram stain, Motility, Spore staining, Oxidase test, Urease test, Indole, Methyl red test, Citrate test, Vogues Proskauer test, Catalase test, Coagulase test, Fermentation of glucose, lactose and sucrose.

Antimicrobial Sensitivity Testing

Commercially available antibiotic impregnated 8mm sensitivity discs (Abtek Biological Ltd, UK) were used to determine the drug sensitivity profile of the isolates. Fifteen different antibiotic discs comprising of Gentamycin (Gen), Erythromycin (Ery), Levoxin (Lev), Ampicillin (Amp), Augmentin (Aug), Ceftriaxone (Cef), Cotrimoxazole (Cot), Ofloxacin (Ofl), Tetracycline (Tet), Streptomycin (Str), Ciprofloxacin (Cip), Cloxacillin (Cxc), Amoxicillin (Amx), Cefuroxime (Cxm) and Ceftazidime (Caz) were used in this study. The antimicrobial sensitivity test of each isolate was carried out as described by the Kirby – Bauer disc diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2003). The turbidity of the bacterial suspensions was compared with 0.5 Macfarland's barium sulfate standard solution. The standardized bacterial suspension was then swabbed and inoculated on to Muller Hinton Agar (Lab M Limited, UK) using sterile cotton swabs and left to dry for 10minutes, before placing the antimicrobial sensitivity discs. After incubation, the diameter of the zone of inhibition were measured and compared with zone diameter of interpretative chart (CLSI, 2003 & NCCLS, 2007) to determine the sensitivity of the isolates to antibiotics. Standard strains of *Escherichia coli ATCC*25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 17853 were used as control.

Plasmid Extraction

Plasmid isolation was done using alkaline lysis method using Zymogen, UK plasmid isolation kit (Sambrook *et al.*, 1989). Pure isolates of *Pseudomonas aeruginosa* strains were inoculated on Nutrient agar and incubated overnight. Six hundred microlitre of grown bacterial culture was transferred into a 1.5ml microcentrifuge tube. This was centrifuge for 30 seconds and the supernatant discarded, then 600 µl of sterile distilled water was added to the bacterial cell pellet and resuspended completely. 100µl of the lysis buffer was also added. This was mixed by inversion of the tube 6 times within 2 minutes. The lysis buffer changed from opaque to clear blue indicating complete lysis. Three hundred and fifty microlitre (350 µl) of the neutralizing buffer was then added and mixed thoroughly. The sample turned yellow forming a yellowish precipitate indicating complete neutralization and was centrifuge for 3minutes. The supernatant was transferred into column provided and placed into a collection tube and centrifuged for 15seconds. The flow through was discarded. Two hundred microlitre (200 µl) of the washing buffer was added to the column and centrifuged for 30seconds. Also 400µl of the washing buffer was added and centrifuged for 1minute and transferred into a clean 1.5ml microcentrifuge tube. Thirty microlitre (30 µl) of elution buffer was added directly into the column matrix and allowed to stand

for one minute at room temperature after which it was centrifuge for 30 seconds to elute the plasmid DNA. Powdered agarose (0.8% w/v) was boiled in Tris acetic, EDTA (TAE) buffer intermittently until the solution becomes a clear gel. The Agarose solution was allowed to cool to 45°C, then7µl of ethidium bromide was added using a micropipette for 3minutes. The clear gel solution was poured into the gel tray with comb in place and allowed to solidify. Thereafter, the comb and the gel tray were removed . The gel was placed into the tank containing the gel buffer. Then, 20µl of bromophenol blue with 20µl of the sample was mixed and loaded into the wells. Thereafter, 2µl of the tracking dye (bromophenol blue) was mixed with 1µl of the marker, and load into the last well. bromophenol blue helps to track the distance moved by the sample on the gel. The cover of the tank was carefully placed on it, and then plugged to the power source to run from negative to positive direction making sure it does not run distance far more than ¾ of the gel for approximately 1hour. Then, the gel was viewed with a U-V transilluminator as described by Maniatis *et al*., 1982).

Curing of Plasmid

Curing of plasmid was done to establish and confirm plasmid encoded features among the selected multi drug resistant bacteria. The modified methods of Ahrne *et al.* (1989) and Bhalakia (2006) were used. A stock solution of 10 % Sodium Dodecyl Sulphate (SDS) was prepared by the addition of 10g SDS to 100ml of nutrient broth. The pH was adjusted to 7.8-8.0. The mixture was steamed for 1hour and kept aside as stock. The overnight cultures of selected isolates in nutrient broth were diluted 100-fold and 0.5ml volume of each of the isolates were transferred aseptically into fresh 30ml volume of nutrient broth. The cultures were incubated with shaking for 3 hours and SDS stock solution was added to give the required final concentration of 1% (w/v). The cultures were incubated at room temperature with mild agitation for 72hours. The cultures were serially diluted and plated on Muller Hinton agar plates respectively. The cells were then tested for antibiotic susceptibility as described by the Kirby–Bauer disc diffusion method (Bauer *et al*., 1966). The plasmid content was determined by carrying out the plasmid isolation procedure described above using the Agarose gel electrophoresis.

RESULTS

The physicochemical properties of the sachet water showed a pH range of 5.5-6.5, turbidity of 1.0 - 1.5 NTU and temperature of 28 - 30°C (Table 1).

The aerobic mesophilic bacteria count of SB, GM and LD sachet water samples ranged from 15.9 - 17.5 × 10^3 CFU/mL, 12.5 - 13.3 × 10^3 CFU/mL and 15.5 - 21.9 × 10^3 CFU/mL respectively while the total coliform count ranged from 0.150 – 0.156×10^3 CFU/mL, 0.154 – 0.162 ×10^3CFU/mL and 0.160 – 0.166 ×10^3CFU/mL respectively. The mean bacterial loads of SB, GM and LD sachet water samples in that order are as follows 16.7 × 10^3, 13.4 × $10^3$and 18.7 × 10^3CFU/mL respectively while the mean of total coliform counts are; SB 1.53×10^3CFU/mL, GM 0.158 × 10^3CFU/mL and LD 0.163 × 10^3CFU/mL (Table 2).

All the sachet water brands showed presence of bacterial contamination with a total of 4 species of bacteria detected. *Escherichia coli* accounted for (34.15%) of the bacteria isolated.

Table 1: Physicochemical profile of the three different Sachet water brands used in this study.

SEASON	BATCH	APPEARANCE	ODOUR	COLOUR (TCU)	TEMPERATURE OC	pH Range (Mean)	TURBIDITY (NTU)
DRY	SB	Clear	Odourless	0.00	28	6.0-6.5 (6.25)	1.0
	GM	Clear	Odourless	0.00	30	5.5-6.0 (5.75)	1.0
	LD	Clear	Odourless	0.00	28.5	5.5-6.0 (5.75)	1.5
RAINY	SB	Clear	Odourless	0.00	28.5	6.0-6.5 (6.25)	1.0
	GM	Clear	Odourless	0.00	28	6.0-6.5 (6.25)	1.0
	LD	Clear	Odourless	0.00	29	5.5-6.0 (5.75)	1.5
	WHO STANDARD	Clear	Odourless	15	-	6.5 - 8.0	0.5

Keys: ^{0}C: Degree Celsius, pH: Potenz Hydrogen, TCU: True Colour Units NTU: Nephlometry Turbidity Unit

Table 2: Bacterial aerobic mesophilic count and total coliform count of sachet water samples from Abeokuta.

Sachet water Code	Aerobic Mesophilic Count (103 x CFU / mL) Range (Mean)	Total Coliform counts (103 x CFU / mL) Range (Mean)
SB	15.9 -17.5	0.150 – 0.156
	(16.7)	(0.153)
GM	12.5-13.3	0.154 – 0.162
	(13.4)	(0.158)
LD	15.5- 21.9	0.160 –0.166
	(18.7)	(0.163)

while *Pseudomonas aeruginosa* (31.71%), *Enterobacter sp. and* (21.95%) and *Klebsiella sp* (12.19%) respectively (Table 3).

The result showed differences in numbers and types of organisms present in different brands (Fig 1). It showed that *Pseudomonas aeruginosa, Escherichia coli, and Escherichia coli* were the most predominant organism in SB, GM and LD respectively.

Overall susceptibility studies showed that Levoxin was the most active antibiotic with just two isolates showing resistance to it. Ampicillin, Amoxicillin and Cotrimoxazole were the least active, all isolates were resistant to them. *Escherichia coli* strains in the study were susceptible to Levoxin (85.7%), Ceftazidime (71.4%), Ciprofloxacin (71.4%) and Ceftriaxone and Gentamycin (57.2%) but were resistance to Ampicillin, Amoxicillin and Cotrimoxazole (100%). *Pseudomonas aeruginosa* was resistance to Ampicillin, Amoxicillin, Cefuroxime, Cloxacillin, Erythromycin, Ofloxacin and Tetracycline 100%, and was only sensitivity to Levoxin (76.9%).

Fourteen of the bacterial isolates obtained in this study showed resistance to more than two classes of antibiotics. Two of the multidrug resistance (MDR) isolates were *Klebsiella sp.,* five *Escherichia coli,* one *Enterobacter sp.* while the remaining six were *P. aeruginosa* (Table 5).

Plate 1, showed the Agarose gel electrophoretic analysis of plasmids extracted from multiple antibiotic resistant isolates. Lane M, is the standard molecular marker used. A single plasmid band was detected from one isolate of *Escherichia coli* isolated from sachet water sample (Lane, 6) with a weight of 254.9bp.

Table 3: Incidence of bacteria isolates from the sachet water produced in Abeokuta

Bacteria names	Sachet water n = 300	Incidence
E. coli		14 (34.15%)
Klebsiella sp.		5 (12.19%)
Enterobacter sp.		9 (21.95%)
Pseudomonas aeruginosa		13 (31.71%)
Total		41

Key: n= Total number of samples

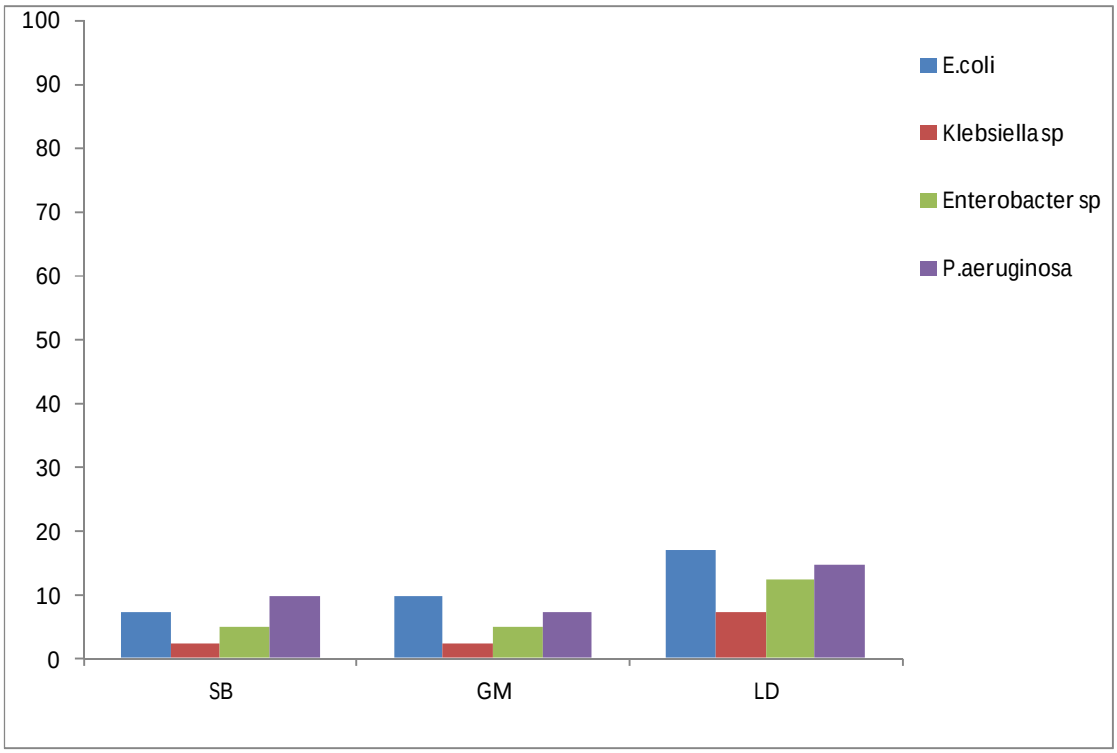

Figure 1: Percentage distribution of isolates in each sachet water brands produced in Abeokuta

Table 4: *In - vitro* susceptibility patterns of isolates from sachet water in Abeokuta

		Antibiotic (%)														
		Amp	Amx	Aug	Cef	Caz	Cxm	Cip	Cxc	Cot	Ery	Gen	Lev	Ofl	Str	Tet
E. coli	S	0(0)	0(0)	4(28.6)	6(57.2)	10(71.4)	7(50)	10(71.4)	3(21.4)	2(14.3)	5(35.7)	8(57.2)	12(85.7)	4(28.6)	4(28.6)	3(21.4)
(n= 14)	R	14(100)	14(100)	10(71.4)	8(42.8)	4(28.6)	7(50)	4(28.6)	11(87.5)	12(85.7)	9(65.3)	6(42.8)	2(14.3)	10(71.4)	10(71.4)	11(87.5)
Pseudo sp	S	0(0)	0(0)	6(46.1)	4(30.8)	10(76.9)	8(61.5)	7(53.8)	2(15.4)	2(15.4)	5(38.5)	9(69.2)	10(76.9)	8(61.5)	7(53.8)	5(38.5)
(n=13)	R	13(100)	13(100)	7(53.9)	9(69.2)	3(23.1)	5(38.5)	6(46.2)	11(84.6)	11(84.6)	8(61.5)	4(30.8)	3(23.1)	5(38.5)	6(46.2)	8(61.5)
Enterobacter sp	S	0(0)	0(0)	3(33.3)	6(66.7)	9(100)	3(100)	5(55.56)	2(22.2)	3(33.3)	5(55.6)	5(55.6)	8(88.9)	6(66.7)	6(66.7)	2(22.2)
(n=9)	R	3(100)	3(100)	6(66.7)	3(33.3)	0(0)	0(0)	2(44.4)	7(77.8)	6(66.7)	4(44.4)	4(44.4)	1(11.1)	3(33.3)	3(33.3)	7(77.8)
Kleb sp	S	0(0)	0(0)	1(20)	2(40)	2(40)	0(0)	1(20)	0(0)	0(0)	0(0)	1(20)	4(80)	0(0)	0(25)	0(0)
(n=5)	R	5(100)	5(100)	4(80)	3(60)	3(60)	5(100)	4(80)	5(100)	5(100)	5(100)	4(80)	1(20)	5(100)	5(75)	5(100)
Overall	R	41	41	27	23	10	17	16	34	34	26	18	7	23	24	31
(%)		(100)	(100)	(76.47)	(52.94)	(24.41)	(58.82)	(58.82)	(94.12)	(100)	(88.24)	(76.47)	(11.76)	(76.47)	(76.47)	(94.12)
(nt = 41)																

Keys: Gen = Gentamycin, Ery = Erythromycin, Lev = Levoxin, Amp = Ampicillin, Aug = Augmentin, Cef = Ceftriaxone, Cot = Cotrimoxazole, Ofl = Ofloxacin, Tet = Tetracycline, Str = Streptomycin, Cip = Ciprofloxacin, Cxc = Cloxacillin, Amx = Amoxicillin, Cxm = Cefuroxime, Caz = Ceftazidime.
S – Sensitive R – Resistant n - Number of bacterial isolates n_t- Total number of bacterial isolate

Table 5: Antibiotic resistance profile of bacterial isolates from Sachet water samples

Bacteria Isolates	Code of the isolates (n= 14)	Resistant antibiotic
Klebsiella sp.	*K 2*	Amp, Amx, Aug, Cxc, Cot, Ery, Gen, Lev, Ofl, Str, Tet
Klebsiella sp.	*K 5*	Amp, Amx, Cef, Caz, Cxm, Cip, Cxc, Cot, Ery, Str, Tet
Enterobacter sp.	*En 4*	Amp, Amx, Aug Cef, Caz, Cxm, Cip, Cxc, Cot, Ery, Ofl, Str,
P. aeruginosa	*P 2*	Amx, Amx, Aug, Cxm, Cip, Cxc, Cot, Ery, Ofl, Str, Tet
P. aeruginosa	*P 5*	Amp, Amx, Cef, Cxm, Cxc, Cot, Ery, Ofl, Str, Tet
P. aeruginosa	*P 8*	Amp, Amx, Aug, Cef, Caz, Cxm, Cip, Cxc, Cot, Ery,
P. aeruginosa	*P 9*	Amp, Amx, Aug, Cef, Cxm, Cip, Cxc, Cot, Ery, Gen, Ofl,
P. aeruginosa	*P11*	Amp, Amx, Aug, Cef, Caz, Cxm, Cip, Cxc, Cot, Ery,Str, Tet
P. aeruginosa	*P 12*	Amp, Amx, Caz, Cxm, Cip, Cxc, Cot, Ery, Gen, Ofl,
Escherichia coli	*E 1*	Amp, Aug, Cef, Cxm, Cip, Cxc, Cot, Ery, Gen, Lev, Ofl, Str,
Escherichia coli	*E 3*	Amp, Amx, Cxm, Cxc, Cot, Ery, Gen, Ofl, Str, Tet
Escherichia coli	*E 4*	Amp, Amx, Cxm, Cip, Cxc, Cot, Ery, Lev, Ofl, Str, Tet
Escherichia coli	*E 8*	Amp, Amx, Cxm, Cip, Cxc, Cot, Ery, Ofl, Str, Tet
Escherichia coli	*E 10*	Amp, Aug, Cef, Cxm, Cip, Cxc, Cot, Ery, Gen, Lev, Ofl, Str,

Keys: Gen = Gentamycin, Ery = Erythromycin, Lev = Levoxin, Amp = Ampicillin, Aug = Augmentin, Cef =Ceftriaxone, Cot = Cotrimoxazole, Ofl = Ofloxacin, Tet = Tetracycline, Str = Streptomycin, Cip = Ciprofloxacin, Cxc = Cloxacillin, Amx = Amoxicillin, Cxm = Cefuroxime, Caz = Ceftazidime.

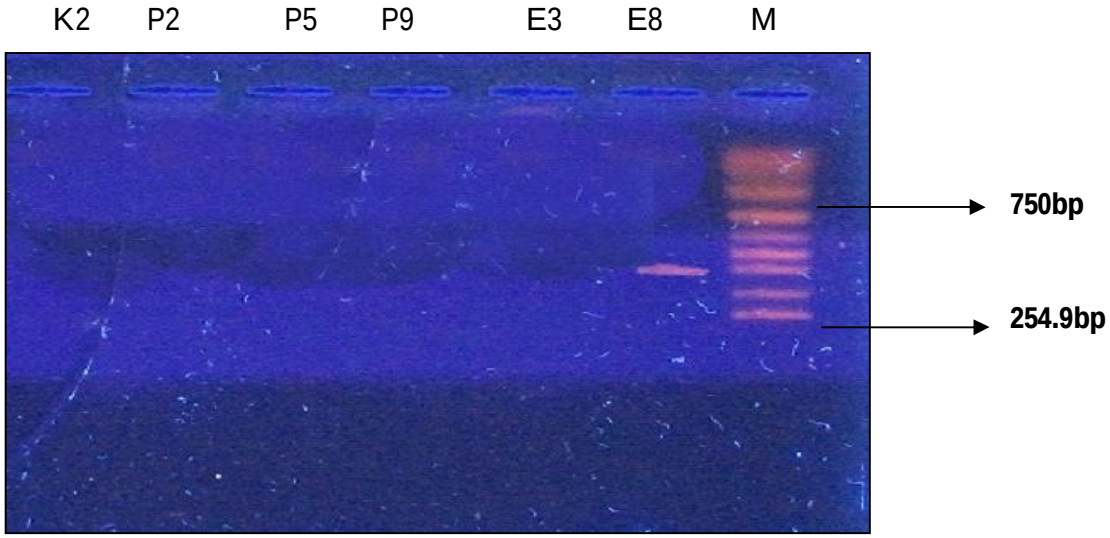

Plate 1: Agarose gel electrophoretic analysis of plasmids extracted from multiple antibiotic resistant isolates from sachet water. Lane 6. (*Escherichia coli* E8 254.9bp), Lane M, 0.1kbp DNA ladder

DISCUSSION

The physicochemical analysis of sachet water presents values of parameters within the WHO benchmark standards. Turbidity was high in all the tested samples. The general WHO standard set for drinking water is < 0.1 NTU. A turbidity >0.5 NTU is considered unhealthy. Its importance is highlighted by the fact that suspended solids interfere with effective chlorination/ disinfection and helps to shield bacteria (Asano, 2007). The WHO guidelines for drinking water quality states that the pH range should fall between 6.5 and 8.0. The current study found sachet water with pH 5.5 and 6.5. Generally low pH values obtained in the water might be due to the high levels of free CO_2 which may consequently affect the bacterial counts (Edema *et al*., 2001).

Bacteria isolated from the sachet water samples include *E. coli, Klebsiella* sp, *Enterobacter* sp and *Pseudomonas aeruginosa*. Isolation of pathogenic and potentially pathogenic bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* are of importance and indicated that some of the tested sachet water is unsafe. This isolation showed water quality deterioration and that immunocompromised people are at risk (Yagoub and Ahmed, 2009). *Escherichia coli* is regarded as the most sensitive indicator of faecal pollution. Its presence in the sachet water samples is of major health concern and calls for urgent attention. The presence of this pathogen in the samples was an indication of the likely presence of other enteric pathogens (Petridis *et al*., 2002). *Pseudomonas sp* are very common in water systems due to their ease of colonization and they form thick biofilms which consequently have effect on turbidity, taste and odour of drinking water (WHO, 2006). Drinking contaminated water results in thousands of deaths every day, mostly in children under five years, in developing countries (WHO, 2004). In addition, diseases caused through consumption of contaminated water and poor hygiene practices are the leading cause of death among children worldwide, after respiratory diseases (WHO, 2003).

The resistance exhibited by *Pseudomonas aeruginosa and E. coli* to some of the antibiotics corroborates earlier report from South Eastern Nigeria (Nwachukwu and Emeruen, 2007).

Antibiotic resistant bacteria are a cause for concern because of possible colonization of the gastrointestinal tract and conjugal transfer of antibiotic resistance to the normal flora leading to more multiple antibiotic resistant organisms (Mckeon *et al*., 1995). The result from this study revealed a high level of bacterial contamination in LD 46.34%, GM 34.15% and SB 19.51%. This showed poor sanitary standard of operation in the production line of sachet-water. The presence of the same type of faecal bacteria in all brands shows common source of contamination. One *Escherichia coli* isolate and one *Pseudomonas sp* isolate were discovered to be resistant to the entire antibiotic spectrum in this study. These strains were isolated from LD sachet water. This is one significant finding from this study that is of serious public health concern. It is documented that bacteria habour series of antibiotic resistant genes which can be transferred to others horizontally (Piddock, 2006). In this study, plasmids were detected in 1(8.3%) out of the 14 multidrug isolates obtained from water samples. The 8.3% prevalence rate of plasmid encoded resistant isolates obtained from sachet water in this study was lower than the 18% prevalence rate of plasmid encoded resistant isolates present in sachet-water hawked in

Abakaliki town reported by Afiukwa *et al*, (2010). Plasmids have been found to confer drug resistance to their host bacteria by various mating processes such as conjugation, transduction and transformation (Tolmarby and Towner, 1990; Levis, 1993). Webb and Davies (1993) demonstrated that DNA encoding antibiotic resistance genes that are present in bacteria might be transferred to humans from the water consumed and thereby taken up by bacteria in the gastrointestinal tract of hosts, contributing to the rapid development of antibiotic resistance.

Majority of sachet-water distributed in this locality, are not of good sanitary quality for consumption as they harbor bacterial isolates with multiple resistance to different classes of antibiotics. Therefore, it is suggested that more effort is needed to limit the number of contaminated sachet-water in distribution by ensuring that manufacturers of such products are properly supervised by regulatory agencies to ensure that they apply the standard methods established by National Agency for Foods Drugs Administration and Control (NAFDAC) World Health Organization so as to meet the (standard) zero coliform presence in water. Regulatory activities that promote core hygiene values (e.g., hand washing, general cleanliness of storage environment and vendor containers) and a proper handling culture could produce the desired improvements rather than a tenacious focus on end-product monitoring, which does not always give a complete picture in terms of microbiological risk assessment.

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