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Microbiological, Physiochemical and Antibiotic Sensitivity Analysis of Bacterial Consortia Associated with Packaged Water Vended in Akure, Ondo State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Packaged water, most especially sachet and bottled water, is one of the major sources of drinking water in Nigeria, especially for commuters, whose major way of quenching thirst is to buy from road side vendors and previous studies has shown that packaged water does not always measure up to standard.

Aim: Investigations were done to determine adherence to physiochemical, microbiological standards. Antibiotic susceptibility profile and plasmid profiling of enumerated bacteria using disc diffusion method were carried out on vended packaged water samples.

Location of Study: Eleven brands of sachet water and seven brands of bottled water samples vended in Akure, Ondo State were randomly selected.

Methodology: Physiochemical analysis was carried out and the parameters were checked in line with standard organization of Nigeria (SON) specifications. Isolation of bacteria was carried out using standard procedures and isolates were identified by various biochemical tests. Plasmid analysis and curing was conducted following standard protocols

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Results: Total bacterial counts of sachet water brands ranged from 0.2 × 102 to 4.5 × 102 CFU/ml while that of bottled water brands ranged from 0.1 × 102 to 4.2 × 102 CFU/ml. *Bacillus subtilis, Corynebacterium diphtheriae, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus, S. epidermidis and Shigella dysentariae. Streptococcus pneumoniae* was isolated from the samples.
Conclusion: Most packaged water (Bottled water, sachet water) samples are improperly handled and produced, which could serve as a source for spread of antibiotic-resistant potential pathogens, a risk to public health. better surveillance should be done on packaged water sale and production, public health education is encouraged for safety of residents in the Metropolis.

Keywords: Antibiotics; bacteria; bottled water; physicochemical; resistance; sachet water.

1. INTRODUCTION

Safe drinking-water, sanitation and hygiene are crucial to human health and wellbeing [1]. It is also one of the most important and most abundant compounds on earth, vital to any organism's survival [2]. Water is one of the most important needs of all forms of life and it's unavoidable in man's daily life, constituting a sizeable percentage of man's daily food intake because human bodies do not have reserve supply [3]. Quality water is colorless, tasteless, odourless, as well as free from faecal contamination [4]. And if water does not follow any of the standards stated above, then it needs to be treated, cleaned or filtered to meet establ**i**shed drinking standards [5,6].

Water is not only essential for life; it also remains an important source of disease transmission [7] and infant mortality in many developing countries [8]. The World Health Organization (WHO) generates international water quality guidelines that are used in setting standards and regulations for water quality world-wide [9]. Water-related diseases mainly include those due to drinking unsafe water or exposure to contaminated recreational water like swimming pools [10].

Packaged water is water that meets all federal and provincial regulations for potable water; it is sealed in a sanitary container, and is sold for human consumption [11]. Potable means that the water is safe for human consumption. Sachet water is any commercially treated water, manufactured, packaged and distributed for sale in sealed food grade containers and is intended for human consumption [12]. Packaged water or as it is commonly called "pure water "is classified as food under the guidelines for inspection and requirements for packaged water facility in Nigeria (fresh and renewal applications) by National Agency for Food and Drug Administration and control in Nigeria. Deficiency in water supply could be because of increase in human and animal population [13]. Coliforms are a group of lactose-fermenting *Enterobacteriaceae,* which most likely acquired the resistance by horizontal gene transfer and therefore constitute the same taxon [14].

These bacteria mostly do not cause any disease, but their presence in drinking water gives an indication that the water supply may be vulnerable to contamination from more harmful bacteria and other microorganisms (Washington State Department of Health, 2019).

The integrity of these packaged waters is doubtful, in fact, there are unconfirmed reports that most of the vendors do not treat their packaged waters before selling to the public. Although also unconfirmed with cogent data the rate of corruption of the regulating bodies of this packaged water there has been a high surge of packaged water producing companies with improper monitoring. These packaged water products commonly termed "Pure Water" are usually not free of physical, chemical and microbial contaminants [15]. The major source of contamination of the packaged water occurs during the products' processing and storage, its improper handling by hawkers and it could become contaminated by improper storage and length of storage [15]

Water sources like hand-dug wells and boreholes used for the production of packaged water are vulnerable to microbial contamination through rainfall runoffs and unhealthy environments situated close to the sachet water industry [16]. By their very nature, antibiotics must exhibit selective toxicity because they are produced by one microorganism and exert varying degrees of toxicity against others [17]. Plasmids are extra chromosomal and self-replicating elements in the cell wall of bacteria cells [18].

Plasmids are pieces of usually circular, self-replicating DNA which can code for a variety of different functional gene groups [19]. Plasmid is a key factor that has led to the rise and global dissemination of multidrug-resistant (MDR) bacteria [20,21].

Hence, the essence of this study is to evaluate the microbiological, physicochemical profile and antibiotic sensitivity pattern of packaged water vended in Akure metropolis.

2. METHODOLOGY

2.1 Study Location

The area of study is Akure South is a Local Government Area in Ondo State, Nigeria. Its headquarters are in the town of Akure. It has an area of 331 km², it lies on latitude 7.25°N and longitude 5.19°E. The city is located on 396 meters high above sea level and a population of roughly 360, 200 at the 2006 census.

2.2 Collection of Samples

The water sample was purchased from road-side vendors who preserved their packaged water in buckets filled with water of unknown source around, Akure metropolis. The samples were placed in a plastic air-tight transparent bag, stored in ice packs and analyzed within 6hr of collection at the Microbiology laboratory of the Federal University of Technology Akure. The samples were coded as RC, PM, FT, FE, LL, JD, MP, FV, BN, FL, DC, EL, AQ, EV, BG, MV, FTT, BS to reflect respective brands.

2.3 Physiochemical Analysis

The physicochemical parameters analyzed were temperature, pH and turbidity, color, odor, total dissolved solids, total suspended solid, total solids, salinity, turbidity, and total hardness as described by Mohan et al*.,* [22] with little modifications.

2.4 Microbiological Analysis

2.4.1 Isolation of microorganisms

Nutrient Agar was used for the isolation of bacteria, while the Potato Dextrose Agar was used for isolating fungi. The agar (Nutrient agar (NA) and Potato Dextrose Agar (PDA) was prepared by measuring 2.8g, 3.9g in 100ml of distilled water in a conical flask respectively.

The mixtures were sterilized in the autoclave at a temperature of 121ºC for 15 minutes and then cooled to about 45^{o}C. After sterilization before being poured aseptically into sterile petri-dishes, 1ml inoculum of water sample was added aseptically in the Petri dishes, the Nutrient Agar plates were incubated aerobically at 37°C for 24h while, Potato Dextrose Agar was incubated at 28^{o}C for 48-72 h. Isolation of organisms was done by both swabbing the body of the sachet and tip area of the bottled water. A 0.1ml Aliquot of the content of both the sachet and bottled water sample were extrapolated using a micropipette (Microlit RBO, New Jersey, USA). A 5-fold serial dilution was made from the swab stick and 0.1ml of the 10^{-4} was inoculated through pour plate method on nutrient agar and potato dextrose agar.

2.5 Characterisation and Identification of Isolated Microorganisms

2.5.1 Total viable counts determination

Total viable count (TVC), is a quantitative estimate of the concentration of microorganisms such as bacteria, yeast or mould spores in a sample. The count represents the number of colony forming units (CFU) per g (or per ml) of the sample. Colony counting was carried out by counting the number of visible colonies that appeared on plates.

$$\text{CFU/ML} = \frac{\text{Number of colonies} \times \text{dilution factor}}{ml\ of\ sample\ suspension}$$

2.5.2 Identification of fungal isolates

Moulds were identified by staining with lactophenol cotton blue and viewed under microscope for characteristic features such as hyphae etc (Karabay et al*.,* 2007). while yeasts were subjected to sugar fermentation test. All these were carried out to characterize observed bacterial and fungal isolates and the comparison of these characteristics or observations with those in standard books such as Cheesborough [23].

2.5.3 Biochemical tests

Biochemical tests carried out include gram staining, oxidase test, Coagulase test, Catalase test, sugar fermentation test, motility test, spore staining test, urease test, citrate test, indole test,

starch hydrolysis test, Coliform test(most probable number) for bacterial isolates.

2.6 Standardization of Inoculum

The inoculum was standradized using 0.5 macfarland standard, the standard was prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate (BaCl2•2H2O), with 9.95 mL of 1% sulfuric acid (H2SO4) in a tube, a test suspension was prepared from a fresh, pure culture and inoculated in a broth and visually compared to the turbidity of the test suspension with the mcfarland standard and for test suspensions too light, inoculum with additional organisms were or test suspension was incubated till it matches the Mcfarland standard, and for cases where the test suspension is too heavy, sufficient broth or saline was added so that the turbidity of the test suspension matches the Mcfarland standard [24].

2.7 Antibiotic Sensitivity Test

Antibiotic sensitivity test was conducted by subjecting the bacterial isolates to ten selected antibiotics which include; Cefuroxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Augmentin (30 µg), Gentamycin (10 µg), Ofloxacin (5 µg), Ciprofloxacin (5 µg), and Nitrofurantoin (300 µg) (Oxoid, UK) [25]. Zones of inhibition (ZOI) displayed by bacterial organisms are compared with Clinical Laboratory Standard Institute [24] standard.

2.8 Plasmid Analysis

2.8.1 Plasmid extraction

The pelleted cells were re-suspended in 250µml of the Re-suspension Solution. The bacterial cells were re-suspended completely by vortexing or pipetting up and down until no cell clumps remain. 350µl of the neutralization solution was added and immediately mixed thoroughly by inverting the tube 4-6 times; it was centrifuged for 5mins to pellet cell debris and chromosomal DNA. The supernatant was transferred to the supplied gene JET spin column by decanting or pip petting. 500µl of the wash solution was added (diluted with ethanol) prior to first use the gene JET spin column for 30 – 60 seconds.

The flow – through solution was discarded and centrifuged for an additional 1 min to remove residual wash solution. This step is essential to avoid residual ethanol in plasmid preps. The Gene JET spin column was transferred into a fresh 1.5ml micro-centrifuge tube. 50µl of the Elution Buffer was added to the Centre of the Gene JET Spin column membrane to elute the plasmid DNA. It was incubated for 2 min at room temperature and centrifuged for 2 min. 1g of agarose was measured. It was placed in the microwave for 1-3 min until the agarose is completely dissolved.

The agarose solution was allowed to cool down to about 50°C. Ethidium bromide (EtBr) was added to a final concentration of approximately 0.2-0.5 µg/mL (usually about 2-3 µl of lab stock solution per 100 mL gel). Ethidium bromide (EtBr) binds to the DNA and allows one to visualize the DNA under ultraviolet (UV) light. The agarose was poured into a gel tray with the well comb in place. Newly poured gel was placed at 4°C for 10-15 minutes.

2.8.2 Plasmid curing

Plasmid curing was carried out in order to determine the location (plasmid-borne or chromosomal) of the drug resistance marker(s). The curing (elimination) of the resistant plasmids of the resistant isolates was done using sub inhibitory concentration of 10mg/ml of ethidium bromide as described by Sheikh et al. [26], Yah et al. [27]. Akortha and Filgona, [28] with slight modification as conducted by Onifade and Aiyenuro [29].

2.8.3 Post curing sensitivity test

Isolates were grown for 24 h at 37^{o}C in nutrient broth. After 24 h, the broth was agitated to homogenize the content and loopful of the broth medium were then sub-cultured onto Mueller Hinton Agar (MHA) plates and antibiotic sensitivity testing was carried out as previously described by Onifade and Bakare, [30]. Absence of zone of inhibition on Mueller Hinton agar was indicative of plasmids mediated resistance (plasmid cured), while presence of zone of inhibition on Mueller Hinton agar was indicative of chromosome-mediated (plasmid not cured).

2.9 Statistical Analysis of Data

Data obtained were subjected to analysis of variance (ANOVA) and means were compared using Duncan's New Range Test (DNMRT) with the aid of SPSS software version 20 at $p \leq 0.05$ level of significance.

3. RESULTS

3.1 Physicochemical Properties of Selected Brands of Packaged (Bottled) Water

The color, taste and odor of collected bottled water samples displayed a colorless, tasteless and odorless profile which are all deemed objectionable according to standard organization of Nigeria (SON) standard. The pH of the packaged bottled water samples ranged from 5.68 to 6.50 which is in line with SON standard. The temperature of the packaged bottled water samples ranged from 22.90 to 23.42°C and is deemed to be at room temperature range by SON standard.

The electrical conductivity (EC) of the packaged bottled water samples ranged from 19 to 21µS/cm which is less than the SON standard of 1000 µS/cm. The total solids of the water samples ranged from 9.60 to 10.20 parts per million (ppm), while the total suspended solids ranged from 0.20 to 0.90 ppm. The total dissolved solid of the water samples ranged from 9.00 to 10.00 ppm which is less than SON standard of 500 ppm. The salinity of the water samples ranged from 0.01 to 0.04 practical salinity unit (PSU) which is in line with SON standard of 200 PSU.

The turbidity level of the water samples ranged from 0.51 to 0.91 Nephelometric Turbidity Units (NTU) which is in line with SON standard of 5 NTU. The level of total hardness in the water samples ranged between 1.60 and 7.00 ppm which is less than 100 to 500 ppm SON standard.

3.2 Physicochemical Profile of Selected Brands of Packaged Sachet Water

The color, taste and odor of collected sachet water samples displayed a colorless, tasteless and odorless profile which are all deemed objectionable according to standard organization of Nigeria (SON) standard. The pH of the packaged bottled water samples ranges from 648 to 7.13 which is less than SON standard. The temperature of the packaged bottled water samples ranges from 23.57 to 23.76°C which is deemed to be at ambient temperature range by SON standard.

The electrical conductivity (EC) of the packaged bottled water samples ranges from 18 to 30 µS/cm which is less than the SON standard of 1000 µS/cm. The total solids of the water samples ranged from 11.00 to 16.20 parts per million (ppm), while the total suspended solids ranged from 1.20 to 1.80 ppm. The total dissolved solid of the water samples ranged from 9.20 to 14.80 ppm which is less than SON standard of 500 ppm. The salinity of the water samples ranged from 0.02 to 0.04 practical salinity unit (PSU) which is in line with SON standard of 200 PSU. The turbidity level of the water samples ranged from 0.07 to 0.38 Nephelometric Turbidity Units (NTU) which is in line with SON standard of 5 NTU. The level of total hardness in the water samples ranged between 6.00 and 13.20 ppm which is less than 100 to 500 ppm SON standard.

Table 1. Physicochemical properties of selected brands of packaged (bottled) water

S/N	PARAMETERS	BS	EV	AQ	STANDARD BY SON (2018)
1	Color	Colorless	Colorless	Colorless	15 TCU
2	Odor	Odorless	Odorless	Odorless	Unobjectionable
3	Taste	Tasteless	Tasteless	Tasteless	Unobjectionable
4	pH	5.68	5.77	6.50	6.5-8.5
5	Temperature (°C)	22.90	23.21	23.42	Ambient
6	Electric conductivity (µS/cm)	19	21	19	1000
7	Total solids (*ppm)	9.90	10.20	9.60	-
8	Total suspended solids (*ppm)	0.90	0.20	0.20	-
9	Total dissolved solids (*ppm)	9.00	10.00	9.40	500
10	Salinity (PSU)	0.01	0.04	0.00	200
11	Turbidity (NTU)	0.51	0.19	0.00	5 NTU
12	Total hardness (*ppm)	1.60	7.00	3.60	100-500

*Keys: *SON- Standard Organization of Nigeria, *ppm – parts per million *PSU- Practical salinity unit *NTU- Nephelometric Turbidity Units *TCU-Total color unit*

Table 2. Physicochemical parameters of selected brands of packaged sachet water

S/N	PARAMETERS	LL	MP	RC	STANDARD BY SON* (2018)
1	Color	0.00	0.00	0.00	15 TCU
2	Odor	Odorless	Odorless	Odorless	Unobjectionable
3	Taste	Tasteless	Tasteless	Tasteless	Unobjectionable
4	pH	7.13	6.48	7.07	6.5-8.5
5	Temperature (◦C)	23.57	23.68	23.76	Ambient
6	Electric conductivity (µS/cm)	18	22	30	1000
7	Total solids (ppm)	11.00	11.40	16.20	-
8	Total suspended solids (ppm)	1.80	1.20	1.40	-
9	Total dissolved solids (ppm)	9.20	10.20	14.80	500
10	Salinity (PSU)	0.00	0.02	0.04	200
11	Turbidity (NTU)	0.38	0.07	0.00	5 NTU
12	Total hardness (ppm)	6.00	7.00	13.20	100-500

*Keys: *SON- Standard Organization of Nigeria, *ppm – parts per million *PSU- Practical salinity unit *NTU- Nephelometric Turbidity Units *TCU- Total color unit*

Table 3. Total viable count of enumerated bacteria in packaged sachet water samples

Packaged sachet water samples (brands)	CFU/ml*
RCC	0.7×10^2
RCS	1.5×10^2
LLS	0.9×10^2
LLC	0.6×10^2
FEC	No growth
FES	1.4×10^2
FVS	2.3×10^2
FVC	0.8×10^2
FLS	2.0×10^2
FLC	1.0×10^2
BNS	4.2×10^2
BNC	0.2×10^2
PMS	2.3×10^2
PMC	0.4×10^2
FTC	0.3×10^2
FTS	2.1×10^2
JDS	3.2×10^2
JDC	0.2×10^2
MPS	0.3×10^2
MPC	2.6×10^2
DCS	$4.5\times10^{\backslash 2}$
DCC	0.9×10^2

**CFU/ml – Colony forming unit per ml*

3.3 Total Bacterial Count Profile of Packaged Sachet Water Samples

The total bacterial count of packaged sachet water brands ranged from 0.2×10^2 to 4.5×10^2 CFU/ml.

3.4 Total Bacterial Count Profile of Packaged Bottled Water Samples

The total bacterial count of packaged bottled water brands ranged from 0.1×10^2 to 4.2×10^2 CFU/ml.

Table 4. Total Viable count of enumerated bacteria in packaged bottled water samples

Packaged bottled water samples	CFU/ml
MVS	0.1×10^2
MVC	0.1×10^2
BSC	0.2×10^2
BSS	0.4×10^2
ELC	3.0×10^2
ELS	4.0×10^2
EVC	0.2×10^2
EVS	2.4×10^2
AQS	4.2×10^2
AQC	0.5×10^2
BGS	0.3×10^2
BGC	No growth
FTTC	0.7×10^2
FTTS	No growth

**CFU/ml – Colony forming unit per ml*

Table 5. Percentage occurrence of bacterial isolates enumerated from packaged water samples

Suspected bacteria isolates	Isolate occurrence	% Occurrence
Staphylococcus aureus	11	32.35
Enterobacter aerogenes	4	11.76
Staphylococcus epidermidis	4	11.76
Corynebacterium diphtheriae	3	8.82
Enterococcus faecalis	3	8.82
Escherichia coli	1	2.94
Salmonella typhi	1	2.94
Bacillus cereus	1	2.94
Shigella dysenteriae	1	2.94
Klebsiella pneumoniae	1	2.94
Total	34	100

3.5 Percentage Occurrence Profile of Bacterial Isolates Enumerated from Packaged Bottled and Sachet Water Samples

Staphylococcus aureus had the highest percentage occurrence of 32.35 % among bacterial organisms associated with the packaged bottled and sachet water samples while *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae*, *Salmonella typhi* and *Shigella dysenteriae* had the least bacterial occurrence of 2.94 % as illustrated in Table 5.

3.6 Biochemistry of Bacterial Organisms Isolated from Packaged Water Samples (Sachet and Bottled Water)

The bacterial enumerated and identified after the biochemical characterization of the bacterial organisms include;*Bacillus subtilis, Corynebacterium diphtheriae, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus, S. epidermidis and Shigella dysentariae. Streptococcus pneumonia.*

3.7 Antibiotic Susceptibility Pattern of Bacterial Isolates from Packaged Water Samples

Streptococcus pneumoniae had the highest zone of inhibition (ZOI) on ofloxacin at 29.67±00.33 mm while the same bacteria also had he least ZOI on cefuroxime at 7.00±10.10 mm.

3.8 Multiple Antibiotic Resistant Index (MAR) Profile of Bacteria Organisms Enumerated from Packaged Water Samples

The multiple antibiotics resistance index (MAR) of bacterial organisms isolated from the packaged water samples ranged from 0.63 to 0.88 as shown in Table 8.

Table 6. Biochemical tests and identification of enumerated bacterial isolates

Isolates	Gram stain	Cell morphology	Ct	Co	Sf	Sh	H_2s	Mo	In	Ur	Ctr	Suspected Organism
RCC	+	COCCI	+	+	NS	-	-	-	-	+	+	*Staphylococcus aureus*
BNS	+	COCCI	+	-	NS	-	-	+	-	+	-	*Staphylococcus epidermidis*
BNC	-	ROD	+	-	NS	-	-	-	-	+	+	*Klebsiella pneumoniae*
DCS	+	COCCI	-	-	NS	+	-	-	-	-	-	*Streptococcus pneumoniae*
MPS	-	ROD	+	-	N S	-	-	+	-	-	+	*Enterobacter aerogenes*
ELS	-	ROD	+	-	NS	+	-	+	+	-	-	*Escherichia coli*
JDS	+	COCCI	-	-	NS	-	-	-	-	-	-	*Enterococcus faecalis*
FVC	+	ROD	+	-	S	+		+	-	-	+	*Bacillus subtilis*
FLC	-	ROD	+	-	NS	-	-	-	+	-	-	*Shigella dysenteriae*
PMC	-	ROD	+	-	NS	+	+	+	-	-	-	*Salmonella typhi*
BSS	+	ROD	+	-	NS	+	+	-	-	-	-	*Corynebacterium diptheriae*

Key: CT-Catalase; CO-Coagulase; SP-Spore formation; SH-Starch hydrolysis; H_2S-Hydrogen sulphide production; MO-Motility; IN-Indole; UR-Urease; CTR-Citrate

Table 7. Zones of inhibition of bacterial isolates from packaged water samples

Suspected isolates	CAZ S = ≥21 I =18-20 R = ≤17	CRX S = ≥23 I =20-22 R = ≤19	GEN S = ≥15 I =13-14 R = ≤12	CTR S = ≥23 I =20-22 R = ≤19	ERY S = ≥23 I =14-22 R = ≤13	CXC S = ≥15 I = 12-13 R = ≤14	OFL S = ≥16 I =13-15 R = ≤12	AUG S = ≥18 I =14-17 R = ≤13
FLS (*Staphylococcus aureus*)	00.00±00.00^{a}	16.67±00.88^{cd}	15.00±00.58^{c}	13.00±00.58^{b}	20.67±00.88^{e}	20.00±00.58^{e}	17.33±00.88^{d}	00.00±00.00^{a}
FLS (*Staphylococcus epidermidis*)	00.00±00.00^{a}	16.67±00.88^{cd}	15.00±00.58^{c}	13.00±00.58^{b}	20.67±00.88^{e}	20.00±00.58^{e}	17.33±00.88^{d}	00.00±00.00^{a}
BNC (*Klebsiella pneumoniae*)	00.00±00.00^{a}	00.00±00.00^{a}	00.00±00.00^{a}	00.00±00.00^{a}	00.00±00.00^{a}	00.00±00.00^{a}	27.67±00.88^{b}	00.00±00.00^{a}
DCS *(Streptococcus pneumoniae)*	00.00±00.00^{a}	7.00±10.10^{a}	00.00±00.00^{a}	00.00±00.00^{a}	00.00±00.00^{a}	00.00±00.00^{a}	29.67±00.33^{b}	00.00±00.00^{a}
FTTC (*Enterobacter aerogenes*)	00.00±00.00^{a}	00.00±00.00^{a}	17.33±00.88^{b}	00.00±00.00^{a}	00.00±00.00^{a}	00.00±00.00^{a}	18.67±00.33^{c}	00.00±00.00^{a}
ELS (*Escherichia coli*)	00.00±00.00^{a}	00.00±00.00^{a}	19.00±00.58^{b}	00.00±00.00^{a}	00.00±00.00^{a}	00.00±00.00^{a}	23.00±00.58^{c}	00.00±00.00^{a}
JDS (*Enterococcus faecalis*)	00.00±00.00^{a}	00.00±00.00^{a}	17.33±00.88^{b}	00.00±00.00^{a}	00.00±00.00^{a}	00.00±00.00^{a}	19.33±00.88^{c}	00.00±00.00^{a}
FVC (*Bacillus subtilis*)	00.00±00.00^{a}	00.00±00.00^{a}	14.33±00.33^{b}	00.00±00.00^{a}	00.00±00.00^{a}	00.00±00.00^{a}	18.00±00.58^{c}	00.00±00.00^{a}
FLC (*Shigella dysenteriae*)	00.00±00.00^{a}	00.00±00.00^{a}	13.00±00.58^{b}	00.00±00.00^{a}	00.00±00.00^{a}	00.00±00.00^{a}	17.00±00.58^{c}	00.00±00.00^{a}
PMC (*Salmonella typhi*)	00.00±00.00^{a}	00.00±00.00^{a}	18.33±00.88^{c}	00.00±00.00^{a}	00.00±00.00^{a}	00.00±00.00^{a}	20.00±00.58^{d}	16.00±00.58^{b}
ELC (*Corynebacterium diptheriae*)	00.00±00.00^{a}	00.00±00.00^{a}	18.33±00.88^{c}	00.00±00.00^{a}	15.33±00.88^{b}	00.00±00.00^{a}	20.33±00.88^{d}	00.00±00.00^{a}

KEYS: CAZ= Ceftazidime, CRX= Cefuroxime, GEN= Gentamycin, CTR= Ceftriaxone, ERY=Erythromycin, CXC=Cloxacillin, OFL= Ofloxacin, AUG=Augmentin. Data are presented as mean ± standard deviation (Where n=3). Values exhibiting the same superscript in the same column are not significantly different (p≤0.05)

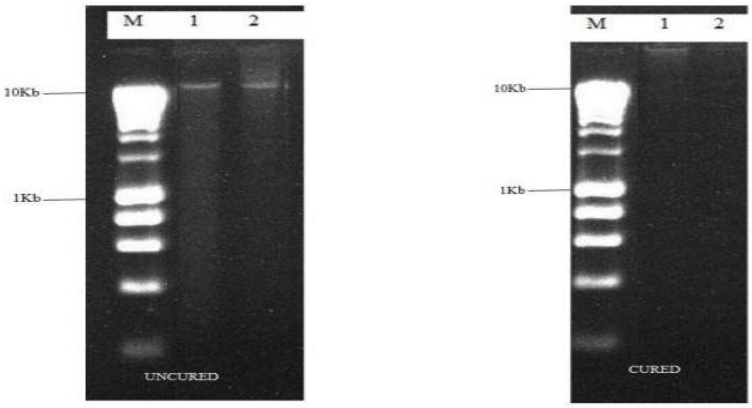
Table 8. Multiple antibiotic resistant index (MAR) of bacteria organisms enumerated from packaged water samples

Bacterial isolates	Resistant	Tested	MAR index
Staphylococcus aureus	6	8	0.75
Staphylococcus epidermidis	6	8	0.75
Klebsiella pneumoniae	7	8	0.88
Streptococcus pneumoniae	7	8	0.88
Enterobacter aerogenes	6	8	0.75
Escherichia coli	5	8	0.63
Enterococcus faecalis	6	8	0.75
Salmonella typhi	6	8	0.75
Shigella dysentariae	7	8	0.88
Corynebacterium diptheriae	6	8	0.75
Bacillus subtilis	7	8	0.88

Table 9. Pre and post susceptibility curing of selected multidrug resistant bacteria isolate

	ZONE OF INHIBITION (DIAMETER IN MM)	
Antibiotics	**DCS (*Streptococcus pneumoniae)***	**MPC *(Staphyloococcus epidermis)***
CAZ (BF)	0.00	0.00
CAZ (AF)	16.33	0.00
CRX(BF)	0.00	0.00
CRX(AF)	18.67	0.00
GEN(BF)	0.00	0.00
GEN(AF)	20.67	0.00
CTR(BF)	0.00	0.00
CTR(AF)	21.33	0.00
ERY(BF)	0.00	0.00
ERY(AF)	15	0.00
CXC(BF)	0.00	0.00
CXC(AF)	20.33	0.00
OFL(BF)	22.33	19.00
OFL(AF)	29.67	21.67
AUG(BF)	0.00	0.00
AUG(AF)	17.67	0.00

KEYS: CAZ= Ceftazidime, CRX=Cefuroxime, GEN= Gentamycin, CTR= Ceftriaxime, ERY= Erythromycin, CXC=Cloxacillin, OFL= Oflaxacin, AUG= Augmentin, BF= Before curing, AF= After curing



Lane M = 1kb ladder plus molecular weight marker; lane 1 = 17A;
Lane 2 = 5A

Plate 1. Plasmid Profiling and curing of selected isolates LANE 1 DCS (*Streptococcus pneumonia*), LANE 2 MPC (*Staphylococccus epidermidis*)

4. DISCUSSION

This study revealed that from the physiochemical analysis of selected brands of packaged water 50% of the brand samples worked on was outside the benchmark for pH specified by the regulatory body in Nigeria (SON) as shown in Tables 1 and 2. This was also a deviation from the benchmark of safe drinking water [1] and were consistent with that of Onifade and Ilori, [31].

The bacteria count (Tables 3 and 4) showed that there were more microorganisms on the surface of the of the packaged water sample than the content of the packaged water sample with the exception of *Staphylococcus aureus* that had a low number of bacteria count on the surface as compared to other swabbed surface of packaged water and also a higher count in sachet water than in bottled water. The total viable bacteria count showed that *Streptococcus pneumoniae* had the highest count while *S. aureus* and *Enterobacter aerogenes* had the lowest count.

The results obtained from this total bacteria count (Tables 3 and 4) and microbial analysis of packaged water vended in Akure metropolis showed that some of the water samples conformed with world health organization (WHO) and USEPA (United states environmental protection agency) specifying the maximum contaminant level must not be more than 100 colonies forming unit per milliliter (CFU/ml) and anything asides that renders the water unsafe while others that didn't abide by this standard showed improper purification process for the packaged water content and poor handling [31].

Bacteriological analysis carried out on the packaged water collected from vendors around Akure metropolis indicates that most of the packaged water did not meet the requirement of WHO standard for potable water as there were presence of some enterobacteria (Table 5), the completed result does show positive result for *E. coli* which signifies contamination in the water sample as reported by Ogueche et al. [32]. There was visible resistance to multiple antibiotics (Table 8) including; *S. aureus*, *S. epidermidis, Klebsiella pneumoniae* and *Streptococcus pneumonia*e as previously reported by Onilude et al. [2]; Daniel and Daodu, [33].

Table 7 showed that Ofloxacin had a considerable inhibitory effect on almost all the suspected organisms, a similar trend observed by Daniel and Daodu, [33]. This could be attributed to the bacterial contaminants which could have been introduced during the processing, packaging, distribution stages, handling during purchase from the vendors and even the water used in cooling this water samples [33] and even long period of storage [34]. Various organisms such *Staphylococcus aureus, Klebsiella pneumoniae, Corynebacteria diphtheriae, Salmonella typhi, Shigella dysenteriae, Enterobacter aerogenes* and so on were synonymous with literature and previous works were also isolated from the water samples.

The percentage occurrence of bacteria represented in Table 5 shows *Staphylococus aureus,* had the larger percentage of occurrence with 32% followed by *Enterobacter aerogenes* and *Streptococcus pneumoniae* 12%, *Corynebacterium diphtheriae* and *Enterococcus faecalis* 9% and so on, occurring organisms such as (*Klebsiella*, *Streptococcus*, *Enterobacter*) are synonymous with previous similar works [35], high presence of *Staphylococcus aureus* and presence of *Corynebacterium diphtheria, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli.,* was also recorded in previous works [33].

The presence of Enterobacter may be from the contamination of bottled water due to poor storage because it exists at high temperature, presence of *Bacillus* species may be from industrial machines and chemical usage and the presence of *Staphylococcus aureus* is because of its opportunistic and ubiquitous nature [36].

The resistance pattern of multidrug resistant bacteria isolates shows that each of the multidrug resistant isolates resist at least not less than five (5) antibiotics out of the eight antibiotics they were subjected to except the *S. epidermidis*. This revealed that the level of resistance exhibited by the bacteria with multidrug resistance is disturbing because these resistant genes can be transfer to susceptible microbial populations, and this will render commonly available antibiotics useless [37].

The multiple antibiotics resistance (MAR) index of multidrug resistant isolates was calculated on the ratio of the number of antibiotics to which an isolate is resistant to, divided by the total number of antibiotics to which the isolates was exposed, ($^{a}/_{b}$), where "a" represents the number of antibiotic to which the isolates were resistant and "b" represents the total number of antibiotics to which the isolates was exposed. From the result

obtained (Table 8) the MAR index ranges from 0.63 to 0.88 which is high and MAR index of 0.2 or higher indicates elevated contamination sources in concomitance with antibiotic prescription [37].

Plate 1 shows the agarose electrophoresis plate showing the plasmid profile of the selected multidrug resistant isolates, plasmid analysis result and post susceptibility test as shown in Table 9 indicates resistance displayed by *Streptococcus pneumoniae* was plasmid mediated while that of staphylococcus epidermidis was chromosomally mediated.

Major health problems such as cholera, gastrointestinal infections etc. are associated with water related diseases, prevalence of diarrhea and typhoid in adults, children and infants have also been linked with unsafe water [38]. Antibiotic resistance of isolated bacteria from packaged water is of great concern, poses a potential risk to consumers health, and if not checked reduces the effect of other strong antibiotics and this is a draw back in the fight against Antimicrobial Resistance.

5. CONCLUSION

Water is an essential commodity needed for human metabolism and most especially vended is one of the most taken forms of packaged water and it is also a major source of transmission of pathogenic waterborne diseases; a major menace in the public health sector of the Nigerian economy and it does not abide by seasonal changes.

This study revealed that adherence to physicochemical and bacteriological standard of some packaged water (bottled and sachet water) fell below SON and WHO drinking water standards, it also indicted improper packaging, handling and storing of packaged waters when consumed can facilitate the spread of antibiotic resistant potential pathogens, which poses a threat to public health.

The physicochemical and microbiological safety of this vended water is therefore of utmost importance and it is one of the major ways of curbing water borne diseases that are transmitted through packaged water and because there is a certain level of choice less trust from the consumer to the producer and there should be an utmost avoidance of contamination by all means because the contamination of a batch of water sample either from producer or the vendor is deadly and can affect a whole number of the community.

It is therefore pertinent that NAFDAC and other regulatory bodies intensify surveillance efforts by making sure roadside vendors are checked regularly and randomly tested and the water they use for cooling and storing the water samples should be taken to the laboratory and checked randomly, and also mandate sachet water producers to follow bottled water specification and they should specify the expiry date, batch no, manufacturing date etc. Public Health agencies should sensitize the general public on the hazards of buying packaged water from roadside vendors and the proper storage for packaged water.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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