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Occurrence Of Antibiotic-Resistant Bacteria In Sachet And Bottled Water Brands In Ondo City, Nigeria

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Abstract: Antibiotic resistance is the ability of a microorganism to withstand the effects of an antibiotic. Today, almost all important bacterial infections are becoming resistant to antibiotics. Unavailability of good quality drinking water is widespread and this has serious health implications. The aim of this study was to determine the occurrence of antibiotic-resistant bacteria in some sachet and bottled water brands in Ondo city, Nigeria. Twenty brands of sachet and twelve brands of bottled waters sold in Ondo city, Nigeria were investigated using 3-3-3 regimen of MPN index method. The total coliform count (TCC), faecal coliform count (FCC) and heterotrophic plate count (HPC) in water samples were determined in accordance with standard procedures. The Kirby-Bauer method was used to determine the antimicrobial resistance profiles of the bacterial isolates.Fifty-five percent of sachet water brands belonged to class I (excellent), 25% belonged to class II (satisfactory), 25% belonged to class III (suspicious) while 5% belonged to class IV (unsatisfactory). However, 100% bottled water brands were of class I (excellent). HPC in sachet water brands ranged from 0 - 3.5 x 10^3 cfu/ml while FCC ranged from 0 - 2.7 x 10^2 cfu/ml. HPC in bottled water ranged from 0 - 3.5 x 10^3 cfu/ml while no faecal coliform was present. The bacteria in this study were P. aeruginosa (23.81%), S. aureus (19.05%), Serratia sp (14.29%), Micrococcus sp (14.29%), C. freundii (9.52%), E. aerogenes (9.52%) and K. pneumoniae (9.52%).The overall percentage antibiotic resistance and susceptibility of the bacteria were 43% and 49%, respectively, while 8% of bacteria were intermediately susceptible.This study revealed acceptable bacteriological quality of bottled water brands but questions some sachet water brands from bacteriological standpoints as they fell below WHO drinking water standards. This study also indicted sub-standard packaged waters as a vehicle of spread of antibiotic-resistant potential pathogens, and this poses a high risk to public health.

Keywords: Antibiotic resistance, bacteriological quality, public health, sachet and bottled water.

1. Introduction

Antibiotics have revolutionized medicine in many respects, and countless lives have been saved; their discovery was a turning point in human history. Regrettably, the use of these wonder drugs has been accompanied by the rapid appearance of resistant strains. Medical pundits are now warning of a return to the pre-antibiotic era [1]. Antibiotic resistance is the ability of a microorganism to withstand the effects of an antibiotic. Today, almost all important bacterial infections are becoming resistant to antibiotics. Antibiotic resistance has been called one of the world's most pressing public health concerns [2].The discovery of antimicrobial agents had a major impact on the rate of survival from infections. However, the changing patterns of antimicrobial resistance caused a demand for new antibacterial agents. Water is one of the most essential needs for the continued existence of all living organisms on earth. The day-to-day activities of all living organisms required water in whatever form. Unavailability of good quality drinking water is widespread and this has serious health implications [3]. Water related diseases continue to be one of the major health problems globally. The high prevalence of diarrhea among children and infants can be traced to the use of unsafe water and unhygienic practices. In developing countries, 80% of all diseases and over 30% of deaths are related to drinking water [4], [5].The continuous proliferation of these packaged water products and their indiscriminate consumption are of concern to public health. An understanding of their microbiological quality and safety are therefore pertinent. Microbial contamination by human or animal excreta is the most common reason for water to be considered unsafe for drinking because of the high probability of presence of pathogenic organisms. Bacterial contamination of drinking water is a major public health problem in the world, because this water can be an important vehicle of diarrheal diseases of infectious nature, which makes it necessary to evaluate the microbiological quality [6]. Thereby, monitoring the health conditions of drinking water is done through laboratory testing for the coliform group. The coliforms are widely used as microbiological parameters indicating faecal contamination while total coliforms include coliform and environmental species that

can serve as a parameter to provide basic information on water quality [7]. High demands of sachet and bottled water for various occasions has led to springing up of small scale entrepreneur who engage in production of package waters, in every nook and cranny of the country, without due regard to hygienic practices in the production steps. The implication of this is lack of guarantee that the products will meet set standard for drinking water quality. Most people living in the major cities of Nigeria like Ondo city under study do not have access to pipe borne water, probably due to unavailability or inadequacy where obtainable. Therefore, the alternative means remain the buying of sachet or bottled water from vendors. This became a major source of drinking water gaining high patronage and to meet the high demand from consumers, proliferation of different brands began and some of which are not even certified by appropriate agency. Generally, packaged water is regulated as a food product in Nigeria by National Agency for Foods Drugs Administration and Control (NAFDAC) in accordance with World Health Organization (WHO) standards for the product regulation, registration and certification. Though, there has been a tremendous improvement in packaged water regulations by NAFDAC as the number of illegal producers has drastically reduced and most brands on sale now have NAFDAC registration, yet some uncertified brands are still in circulation. Sachet and bottled waters are probably being considered for consumption in such high rate because of their reliability in terms of hygiene, purity, tastes, and, most essentially, health safety. Regrettably, however, the problems of its purity and bacteriological status have become questionable in recent years. Packaged water is not completely sterile; it may not be entirely free of all infectious microorganisms. The potential danger associated with sachet water is contamination, which is a factor of the source of the water itself, treatment, packaging materials, dispensing into packaging materials and closure [5], [8]. Under prolonged storage of packaged water at favorable environmental conditions, total aerobic heterotrophic bacteria can grow to levels that may be harmful to humans [9], [10]. These microorganisms can be found in source waters and in treated drinking water. Thus, consumption of water containing large numbers of total aerobic heterotrophic bacteria can lead to diseases such as gastroenteritis and mucous membrane infections particularly in persons whose immune systems are compromised by AIDS, organ transplantation or chemotherapy. The aim of this study was to determine the occurrence of antibiotic-resistant bacteria in some sachet and bottled water brands in Ondo city, Nigeria.

2. Materials and Methods

2.1 Study Area

Ondo City is the second largest city in Ondo State, Nigeria. Ondo City is the trade center for the surrounding region. It has a population close to 360,000 people. Historically, it still remains a key commercial center of the country, with numerous business companies selling tobacco, vegetables, tropic fruits, and other natural products. Individuals from all works of life in this environment patronize packaged (sachet and bottled) water to maintain and sustain everyday activities.

2.2 Collection of Water Samples

Twenty brands of sachet water and twelve brands of bottled water sold in Ondo city, Nigeria, were investigated for their bacteriological qualities. A half bag (10 sachets) of each brand of sachet water and a half pack (6) of bottled water were purchased from different areas in Ondo city, Nigeria and transported to the laboratory same day for the take-off of bacteriological analyses.

2.3 Bacteriological Quality Determination

2.3.1 Total Coliform Count

This was determined by MPN index method using 3-3-3 regimen. MacConkey broth was used and positive result was indicated by acid and gas production on incubation at 37^0C for 48 hours [11].

Most probable number test
This test comprised of three steps:
(a) Presumptive test
(b) Confirmed test
(c) Completed test

2.3.1.1 Presumptive test

The multiple tube fermentation technique was performed as a presumptive test for total coliform using tubes containing MaCconkey broth and inverted Durham tubes. Inoculation was carried out as follows:

i) To each of 3 double-strength MacConkey broth tubes, 0.1 ml of the original sample was added.
ii) To each of 3 single-strength MacConkey broth tubes, 0.01 ml of the original sample was added.
iii) To each of 3 single-strength MacConkey broth tubes, 0.001 ml of the original sample was added. All tubes were incubated at 37°C for 48 hours for the observation of gas production. First reading was taken after 24 hours to record positive tubes, and the negative ones were incubated for another 24 hours [12].

2.3.1.2 Confirmed test

Each gas positive presumptive tube was inoculated into a tube containing 10 ml brilliant green lactose broth medium. All tubes were incubated at 37°C for 48 hours for the observation of gas production [12].

2.3.1.3 Completed test

About 3 loopful of each confirmed positive tube were sub-cultured into EC broth medium and then incubated at 44.5°C for 24 hours. Tubes showing any amount of gas production were considered as positive and the most probable number was recorded (the results were compared with the most probable number table) [12].

2.3.2 Faecal Coliform Count

Faecal coliform count was determined using Eosin Methylene Blue medium employing the pour plate technique. On Eosin Methylene Blue (EMB) agar, E. coli strains appeared as greenish metallic sheen colonies and this was further confirmed by the ability of the organism to ferment lactose at 44.5^0C while Enterobacter aerogenes appeared as large pinkish mucoid colonies.

2.3.3 Determination of Heterotrophic Plate Count (HPC)

Heterotrophic plate count of all water samples were determined using dilution plate technique on standard plate count agar medium. Serial dilutions were prepared to obtain dilution factor of up to 10^{-6} and 1 ml of dilution was transferred to a sterile Petri plate. Plate count agar was prepared according to manufacturer's instructions and then allowed to cool in a water bath to 44 - 46° C. About 16 mls of the agar medium was poured into the Petri plate containing the sample. The sample and agar were mixed thoroughly by rotating the plate several times. The medium was allowed to solidify; the plates were inverted and incubated at 35 °C for 48 to 72 hours. The colonies were counted and expressed as colony forming unit per ml (CFU/ml) of water. The sterility of each batch of test medium was confirmed by incubating one or two uninoculated plates along with the inoculated tests. The uninoculated plates were always examined to show no evidence of bacterial growth.

2.3.4 Identification of isolates

The pure cultures of the bacterial isolates were subjected to various morphological and biochemical characterization tests to determine the identity of the bacteria isolates with reference to Bergey's Manual of systematic Bacteriology. MacConkey agar was used to isolate lactose fermenting gram negative bacilli, blood agar was used to isolate fastidious organisms and Mannitol salt agar for the isolation of salt resistant bacteria. Pure isolates were Gram differentiated and then biochemically identified with adequate number of tests.

2.4 Preparation of 0.5 McFarland standards and standardization of bacterial concentrations (Inocula)

In this study, 0.5 mL of 0.048 M $BaCl_2$ (1.175% W/V $BaCl_2 \cdot 2H_2O$) was added to 99.5 ml of 0.18 M H_2SO_4 (1% V/V) with constant stirring to make 0.5 McFarland Standards. The standard was distributed into a screw capped test tube for color comparison of the test inoculums. Hundred microliter (100 μl) bacteria sample from nutrient broth culture media was added into 5 mL saline and the concentration was adjusted to 1×10^8 colony forming unit per milliliter (CFU/ml) by comparing with McFarland 0.5 standardized.

2.5 Antibiotic sensitivity testing

The Kirby-Bauer method was used to determine the antimicrobial resistance profiles of the bacterial isolates. The antibiotics investigated were azithromycin (15µg), pefloxacin (5 µg), ciprofloxacin (5 µg), kanamycin (30 µg), ceftriazone (30 µg), gentamicin (30 µg), ofloxacin (5 µg), nitrofuratoin (300 µg), tetracycline (30 µg), vancomycin (30 µg), teicoplanin (30 µg) and minocycline (30µg). The medium used was Mueller Hinton (MH) agar. Pure cultures of organisms were enriched in nutrient broth and incubated at 37^0C to a turbidity of 0.5 Macfarland standards. The MH agar was inoculated by streaking using sterile cotton swab of each of the cultures. The antibiotic disks were applied using sterile forceps and sufficiently separated from each other in order to prevent overlapping of the zones of inhibition. The agar plates were left on the bench for 30minutes to allow diffusion of the antibiotics and the plates were incubated inverted at 37^0C for 24 hours. Results were recorded by measuring the zone of inhibition and comparing with the Clinical and Laboratory Standards Institute (CLSI) interpretive performance standard for antimicrobial disk susceptibility testing [13].

3 Results

Table 1 showed the bacteriological quality of sachet and bottled sold in Ondo city, Nigeria. This table revealed the quality of water based on 3-3-3 index of most probable number (MPN), heterotrophic plate count (HPC) and faecal coliform count (FCC) in analyzed water samples while Table 2 showed WHO criteria for acceptability of drinking water samples using MPN. There were no coliform present in eleven of the twenty (55%) sachet water brands investigated in this study and, according to the WHO criteria for acceptability of drinking water samples, the samples were regarded excellent from bacteriological point of view. The brands in this category were SW1, SW3, SW5, SW6, SW9, SW11, SW12, SW13, SW15, SW18 and SW20. However, four of the twenty (25%) brands gave satisfactory bacteriological quality and these include SW2, SW10, SW16 and SW19. Also, twenty-five percent (four of twenty) brands which included SW4, SW7, SW8 and SW14 showed suspicious bacteriological quality while five percent (one of twenty) of brands was of unsatisfactory bacteriological quality. Further assessments of the water samples showed that the HPC in sachet water brands ranged from 0 - 3.5×10^3 cfu/ml while FCC ranged from 0 - 2.7×10^2 cfu/ml. It was discovered that sachet water brands SW1, SW6, SW9, SW11, SW12, SW13, SW15, SW18 and SW20 were not contaminated with heterotrophic bacteria. However, brands SW2, SW4, SW7, SW8, SW10, SW14, SW16, SW17 and SW19 had FCC of 1.2×10^2, 1.9×10^2, 1.6×10^2, 1.4×10^2, 1.7×10^2, 2.1×10^2, 2.0×10^2, 2.7×10^2 and 4.0×10^1 cfu/ml, respectively. It was noted that six of the brands investigated in this study were not NAFDAC certified and these brands which included SW4, SW7, SW8, SW14, SW16 and SW17 were found to be contaminated with faecal coiliforms in amount higher than SW2, SW10 and SW19 which were NAFDAC certified. In the case of bottled water brands sampled and investigated in this study, it was found that all brands were NAFDAC certified and showed no presence of coliforms. They were, thus, categorized excellent based on WHO criteria for acceptability of potable water (Tables 1 and 2).. However, brands BW3, BW6 and BW10 had HPC of 1.4×10^1, 1.2×10^1 and 2.3×10^1 cfu/ml, respectively. Invariably, 55% of sachet water brands belonged to class I (excellent), 25% belonged to class II (satisfactory), 25% belonged to class III (suspicious) while 5% belonged to class IV (unsatisfactory) while 100% bottled water brands were of class I (excellent) (Table 2). Table 3 showed morphological and biochemical characteristics of bacteria isolated from sachet and bottled water brands. Twenty-one bacterial isolates were encountered and these were characterized into seven genera. These organisms were Serratia sp, Citrobacter freundii, Enterobacter aerogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Micrococcus sp and Klebsiella pneumoniae. The percentage occurrence of bacteria isolated from sachet and bottled water in Ondo city, Nigeria was shown in Figure 1. P. aeruginosa had the highest percentage occurrence of 23.81%, followed by S. aureus (19.05%), Serratia sp

(14.29%), Micrococcus sp (14.29%) while C. freundii, E. aerogenes and K. pneumoniae had same percentage of 9.52% each. Table 4 showed the antibiotic resistance profiles of bacteria isolated from sachet and bottled water while Table 5 showed the CLSI interpretive performance standard for antimicrobial disk susceptibility testing. Serratia sp exhibited resistance to ceftriaxone, nitrofurantoin, vancomycin and minocycline. The bacterium showed intermediate susceptibility to tetracycline but sensitive to azithromycin, pefloxacin, ciprofloxacin, kanamycin, gentamicin and teicoplanin. C. freundii exhibited resistance to pefloxacin, ceftriaxone and vancomycin but susceptible to azithromycin, ciprofloxacin, gentamicin, ofloxacin, nitrofurantoin, tetracycline and teicoplanin; the organism was intermediately susceptible to kanamycin and minocycline. Enterobacter aerogenes was resistant to azithromycin, pefloxacin, ceftriaxone, nitrofurantoin, tetracycline, vancomycin and minocycline. It is, however, sensitive to ciprofloxacin, kanamycin, gentamicin and ofloxacin, but intermediately susceptible to teicoplanin. Staphylococcus aureus showed resistance to tetracycline and minocycline but susceptible to azithromycin, pefloxacin, ciprofloxacin, kanamycin, ceftriaxone, gentamicin, nitrofurantoin, vancomycin and teicoplanin. S. aureus showed intermediate susceptibility to ofloxacin. P. aeruginosa exhibited resistance to azithromycin, pefloxacin, ceftriaxone, tetracycline, vancomycin, teicoplanin and minocycline; it was sensitive to ciprofloxacin, gentamicin, ofloxacin and nitrofurantoin, but intermediately susceptible to kanamycin. Micrococcus sp was resistant to pefloxacin, nitrofurantoin, tetracycline, vancomycin, teicoplanin and minocycline; it is, however, susceptible to azithromycin, ciprofloxacin, kanamycin, ceftriaxone, gentamicin and ofloxacin. K. pneumoniae exhibited resistance to pefloxacin, ofloxacin, nitrofurantoin, tetracycline, vancomycin, minocycline, teicoplanin and minocycline. The organisms encountered in this study showed relatively high level resistance to the investigated antibiotics. Each of E. aerogenes, P. aeruginosa and K. pneumoniae exhibited 58.33% resistance, Micrococcus sp (50% resistance), Serratia sp (33.33% resistance), C. freundii (25% resistance) while S. aureus showed 16.67% resistance to the antibiotics under study. The overall percentage antibiotic resistance/susceptibility of bacteria isolated from sachet and bottled water in Ondo city, Nigeria was shown in Figure 2. The overall percentage antibiotic resistance and susceptibility of the bacteria were 43% and 49%, respectively, while 8% of bacteria were intermediately susceptible.

Table 1: *Bacteriological quality of sachet and bottled water sold in Ondo city, Nigeria*

Category	Sample Code (Brand)	NAFDAC Registration	3-3-3 Index			MPN/ 100ml	95% Confidence Range	HPC (cfu/ml)	FCC (cfu/ml)
			0.1	0.01	0.001				
Sachet Water	SW1	√	0	0	0	0	-	-	-
	SW2	√	0	0	1	3	0.15-9.6	2.3×10^2	1.2×10^2
	SW3	√	0	0	0	0	-	1.1×10^2	-
	SW4	x	0	1	1	6.1	1.2-18	1.5×10^3	1.9×10^2
	SW5	√	0	0	0	0	-	2.7×10^2	-
	SW6	√	0	0	0	0	-	-	-
	SW7	x	1	2	1	15	4.5-42	2.1×10^3	1.6×10^2
	SW8	x	1	0	1	7.2	1.3-18	3.5×10^3	1.4×10^2
	SW9	√	0	0	0	0	-	-	-
	SW10	√	1	0	0	3.6	0.17-18	1.8×10^2	1.7×10^2
	SW11	√	0	0	0	0	-	-	-
	SW12	√	0	0	0	0	-	-	-
	SW13	√	0	0	0	0	-	-	-
	SW14	x	0	2	0	6.2	1.2-18	3.2×10^3	2.1×10^2
	SW15	√	0	0	0	0	-	-	-
	SW16	x	1	0	0	3.6	0.17-18	3.8×10^2	2.0×10^2
	SW17	x	2	0	0	9.2	1.4-38	2.5×10^3	2.7×10^2
	SW18	√	0	0	0	0	-	-	-
	SW19	√	0	0	1	3	0.15-9.6	1.0×10^2	4.0×10^1
	SW20	√	0	0	0	0	-	-	-
Bottled Water	BW1	√	0	0	0	0	-	-	-
	BW2	√	0	0	0	0	-	-	-
	BW3	√	0	0	0	0	-	1.4×10^1	-
	BW4	√	0	0	0	0	-	-	-
	BW5	√	0	0	0	0	-	-	-
	BW6	√	0	0	0	0	-	1.2×10^1	-
	BW7	√	0	0	0	0	-	-	-
	BW8	√	0	0	0	0	-	-	-
	BW9	√	0	0	0	0	-	-	-
	BW10	√	0	0	0	0	-	2.3×10^1	-
	BW11	√	0	0	0	0	-	-	-
	BW12	√	0	0	0	0	-	-	-

Keys: TVC - Total viable count; TCC – Total coliform count; FC - Faecal coliform; √ = NAFDAC registration number; x = No NAFDAC registration number; - = Zero count

Table 2: *WHO criteria for acceptability of drinking water samples using MPN*

Class	Grade	Count	Satchet water		Bottled water	
			n=20	% positive	n=12	% positive
I	Excellent	0	11	55	12	100
II	Satisfactory	1-3	4	25	-	-
III	Suspicious	4-9	4	25	-	-
IV	Unsatisfactory	>10	1	5	-	-

Source: WHO [14].

Table 3: *Morphological and biochemical characteristics of bacteria isolated from sachet and bottled water sold in Ondo city, Nigeria*

Isolate	Pigment	Gram Reaction	Cellular morphology	Catalase	Oxidase	Indole	Motility	Methyl-Red	VogesProskauer	Urease activity	Citrate Utilization	Starch Hydrolysis	Gelatin Hydrolysis	Casein Hydrolysis	Spore test	NO_3 Reduction	Glucose	Sucrose	Arabinose	Maltose	Mannitol	Xylose	Galactose	Sorbitol	Invositol	Raffinose	Frauction	Most Probable Identity
1	Red	-ve	R	+	+	-	+	-	+	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	Serratia sp
2	Pink	+ve	R	+	+	+	+	+	-	-	+	-	-	-	-	-	+	+	-	+	+	-	-	+	-	+	+	Citrobacter freundii
3	Orange	+ve	C	+	-	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	ND	ND	ND	+	S. aureus
4	Pink	-ve	R	+	+	-	+	-	+		+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	P. aeruginosa
5	Red	-ve	R	+	+		+		+		+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	Serratia sp
6	Pink	-ve	R	+	-	-	+	-	+	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	ND	+	Enterobacter aerogenes
7	Yellow	+ve	C	+	-	-	-	-	-	+	-	-	+	-	-	-	+	+	-	+	+	-	-	-	-	ND	-	Micrococcus sp
8	Pink	-ve	R	+	+	-	+	-	+		+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	P. aeruginosa
9	Pink	-ve	R	+	-	-	+	-	+	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	ND	+	Enterobacter aerogenes
10	Pink	-ve	R	+	+	-	+	-	+		+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	P. aeruginosa
11	Orange	+ve	C	+	-	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	ND	ND	ND	+	S. aureus
12	Pink	-ve	R	+	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	K. pneumoniae
13	Green	-ve	R	+	+	-	+	-	+		+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	P. aeruginosa
14	Pink	-ve	R	+	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	K. pneumoniae
15	Red	-ve	R	+	+	-	+	-	+	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	Serratia sp
16	Green	-ve	R	+	+	-	+	-	+		+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	P. aeruginosa
17	Orange	+ve	C	+	-	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	ND	ND	ND	+	S. aureus
18	Pink	-ve	R	+	-	-	+	-	+	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	ND	+	Micrococcus sp
19	Pink	+ve	R	+	+	+	+	+	-	-	+	-	-	-	-	-	+	+	-	+	+	-	-	+	-	+	+	Citrobacter freundii
20	Orange	+ve	C	+	-	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	ND	ND	ND	+	S. aureus
21	Yellow	+ve	C	+	-	-	-	-	-	+	-	-	+	-	-	-	+	+	-	+	+	-	-	-	-	ND	-	Micrococcus sp

Keys: R = Rods; + = Positive reaction; - = Negative reaction; ND = Not determined

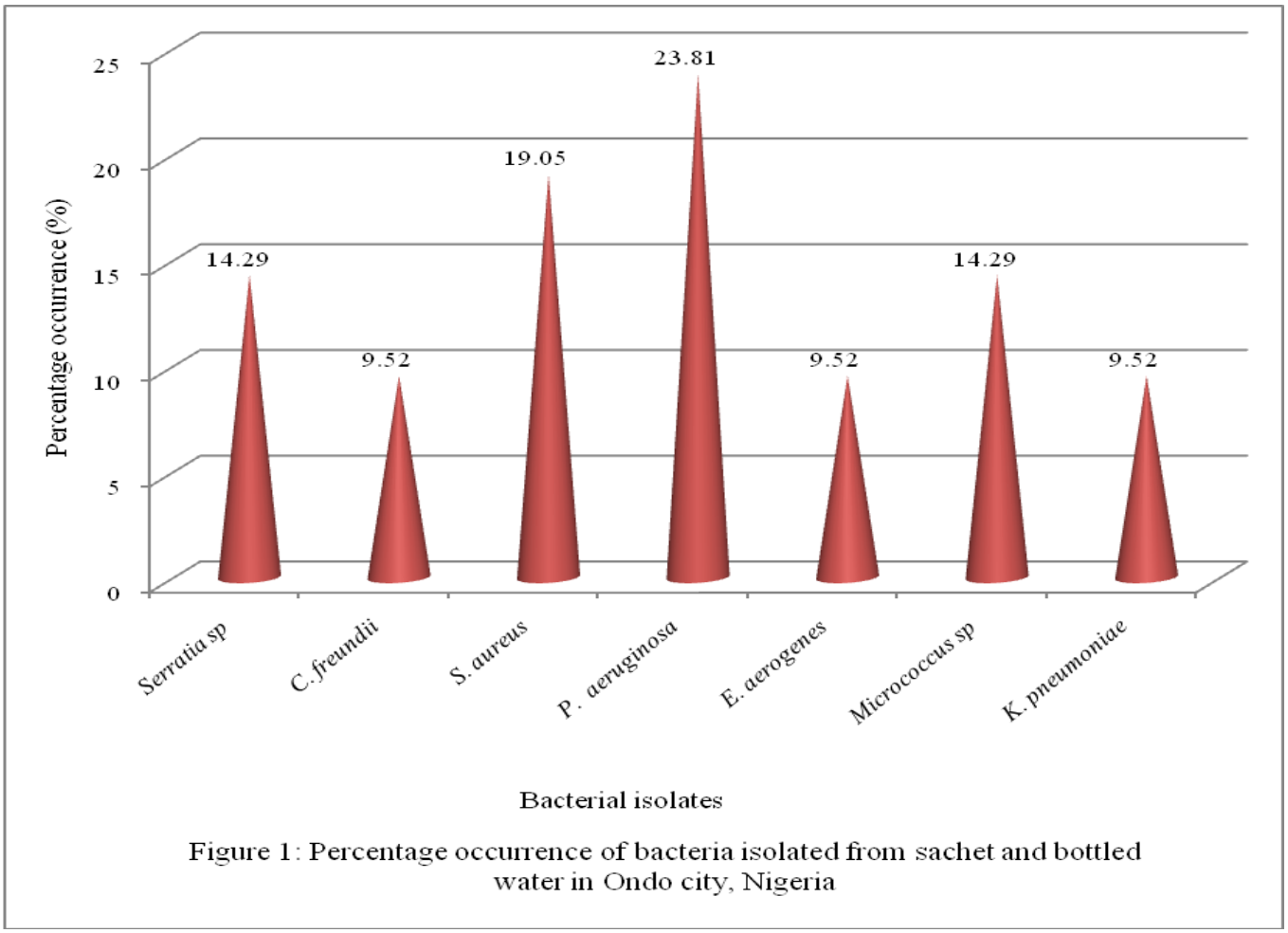

Figure 1: Percentage occurrence of bacteria isolated from sachet and bottled water in Ondo city, Nigeria

Table 4: *Antibiotic resistance profiles of bacteria isolated from sachet and bottled water in Ondo city, Nigeria*

	Conc. (µg)	Serratia sp	C. freundii	E. aerogenes	S. aureus	P. aeruginosa	Micrococcus sp	K. pneumoniae
Azithromycin	15	15S	21S	11R	21S	4R	17S	14S
Pefloxacin	5	25S	19R	16R	25S	17R	13R	16R
Ciprofloxacin	5	21S	22S	23S	26S	20S	22S	24S
Kanamycin	30	22S	17I	19S	22S	16I	23S	22S
Ceftriaxone	30	11R	10R	5R	23S	12R	23S	23S
Gentamicin	10	15S	15S	17S	16S	21S	21S	19S
Ofloxacin	5	13I	21S	19S	15I	18S	17S	10R
Nitrofurantoin	300	6R	19S	13R	19S	17S	14R	12R
Tetracycline	30	12I	17S	6R	8R	10R	5R	0R
Vancomycin	30	8R	9R	12R	18S	0R	11R	0R
Teicoplanin	30	21S	17S	12I	15S	0R	0R	0R
Minocycline	30	14R	18I	7R	8R	0R	7R	13R
Percentage resistance (n=12)		33.33%	25%	58.33%	16.67%	58.33%	50%	58.33%

Table 5: *CLSI interpretive performance standard for antimicrobial disk susceptibility testing*

	Conc. (µg)	S	I	R
Azithromycin	15	≥ 13	-	≤ 12
Pefloxacin	5	≥ 24	-	≤ 23
Ciprofloxacin	5	≥ 21	16–20	≤ 15
Kanamycin	30	≥ 18	14–17	≤ 13
Ceftriaxone	30	≥ 23	20–22	≤ 19
Gentamicin	10	≥ 15	13–14	≤ 12
Ofloxacin	5	≥ 16	13–15	≤ 12
Nitrofurantoin	300	≥ 17	15–16	≤ 14
Tetracycline	30	≥ 15	12–14	≤ 11
Vancomycin	30	≥ 17	15–16	≤ 14
Teicoplanin	30	≥ 14	11–13	≤ 10
Minocycline	30	≥ 19	15–18	≤14

Source: CLSI [13].

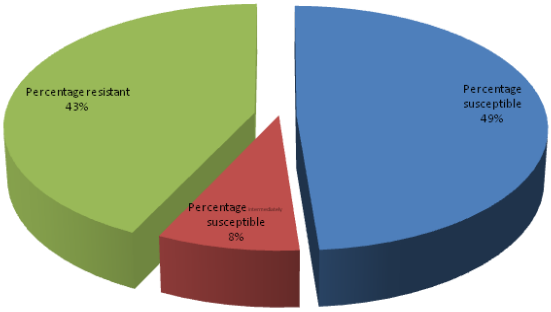

Figure 2: Overall percentage antibiotic resistance/susceptibility of bacteria isolated from sachet and bottled water in Ondo city, Nigeria

4 Discussion

It has become a common knowledge the major health problems globally are associated with water related diseases. Unhygienic practices and use of unsafe water had been indicted in prevalence of diarrhea among children and infants. Thus, ensure that water meant for drinking, cooking and domestic purposes (including washing of plates and utensils) are safe as transient bacterial contamination may have implication well beyond a period of acute-self limited illness. An estimated 4.1% of the total daily global burden has been associated with diarrhoeal diseases, and this has been reported to be responsible for deaths of 1.8 million people every year [15]. There were no coliform present in eleven of the twenty (55%) sachet water brands investigated in this study and, according to the WHO criteria for acceptability of drinking water, the samples were regarded excellent from bacteriological point of view. However, four of the twenty (25%) brands gave satisfactory bacteriological quality; twenty-five percent (four of twenty) brands showed suspicious bacteriological quality while five percent (one of twenty) of brands was of unsatisfactory bacteriological quality. This study revealed that 55% of sachet water brands belonged to class I (excellent), 25% belonged to class II (satisfactory), 25% belonged to class III (suspicious) while 5% belonged to class IV (unsatisfactory) while 100% bottled water brands were of class I (excellent) (Table 2). Although, total coliforms were isolated from some brands of sachet waters, E. coli was not isolated from any of the brands of sachet and bottled water samples in this study. In a study in Ghana, none of the microbial indicators were present in bottled water whereas 4.5% of sachets contained total coliforms and 2.3% had faecal coliforms [16]. In another study on packaged drinking waters in Ibadan, Nigeria, Ajayi et al. [17] reported that larger proportions of sachet water showed positive counts than bottled water. The absence of coliform bacteria in all brands of bottled drinking water could be attributed to the better hygienic practices observed in the industry compared to the sachet water producing industry. These include use of protective sealed caps on bottles, improved hygienic filling system and use

of non-returnable plastic containers [18]. HPC of sachet water brands ranged from 0 - 3.5 x 10^3 cfu/ml while FCC ranged from 0 - 2.7 x 10^2 cfu/ml. Six of the brands were not NAFDAC certified and these brands were found to be contaminated with faecal coiliforms in amount higher than certified, but contaminated samples (SW2, SW10 and SW19). All bottled water brands were NAFDAC certified and showed no presence of coliforms. They were, thus, categorized excellent based on WHO criteria for acceptability of potable water (Tables 1 and 2). However, brands BW3, BW6 and BW10 had HPC of 1.4 x 10^1, 1.2 x 10^1 and 2.3 x 10^1 cfu/ml, respectively. Ten of the twenty sachet water brands had HPCs above 100 ml^{-1}; one (SW19) had exactly 100 ml^{-1} HPC while no heterotrophic bacterium was encountered in the remaining nine brands. However, heterotrophic bacteria were encountered in only three of twelve bottled water brands analysed and with HPCs less than 100 ml $^{-1}$. The high level of the heterotrophs in the sachet water questions the quality of sachet water when compared to that of the bottled water. According to WHO [19] report, a high HPC concentration does not itself present a risk to human health. Nevertheless HPCs are used as good indicators of the overall quality of production [5], [20]. However, based on the recommended standard limits of 100 HPCs per ml of drinking water by WHO, 50% of the sachet water brands were considered unfit for human consumption while 100% of bottled water brands are safe. Omalu et al. [8] reported that bacteriological quality of sachet water deteriorates considerably as products moved farther down the distribution chain. The authors further reported that less than 7% of sachet water contamination took place after production while between 40 and 45% of the products observed between distribution sheds and the street hawkers. The bacteria isolated in this study were P. aeruginosa (23.81%), S. aureus (19.05%), Serratia sp (14.29%), Micrococcus sp (14.29%) while C. freundii (9.52%), E. aerogenes (9.52%) and K. pneumoniae (9.52%). This result was supported by reports of studies carried out elsewhere in and outside Nigeria by some authors [21], [22], [23], [24]. The presence of indicator organisms in some of the sachet water brands indicated that the water was contaminated with matter of faecal origin, and, hence, their absence denotes safety of the water. Although coliform organisms may not always be directly related to the presence of fecal contamination or pathogen in drinking water, the coliform test is still useful for monitoring the microbial quality of drinking water [25]. Ineffectiveness or malfunctioning of the treatment process employed could also result in the presence of coliform bacteria in the water samples. Appropriate treatment processes should therefore be utilized for production of quality and safe packaged drinking waters. In a study carried out in Canada, screening of bottled water for indicator bacteria revealed that 3.7 % of the samples had total coliforms and 23.3 % had more than 100 colonies of heterotrophic bacteria per ml of sample (Warburton et al., 1998). A similar study of brands of bottled water in Trinidad revealed the presence of total coliforms, E. coli and colonies of E. faecalis in the samples [20]. The organisms isolated in this study were similar to those commonly encountered in water and aquatic environments. In Cameroun, Kuitcha et al. [23] reported Klebsiella pneumoniae and Pseudomonas sp in their study. These bacteria might have contaminated the water from source [26], [27]. The presence of these bacteria isolated in the presumed treated sachet water used in this study may be as a result of improper handling, processing and purification procedures, unhygienic handling after production. However, the occurrence of these isolates in sachet water used in this study is in contrast with the findings of some other studies in Nigeria which found no enteric pathogens [28], [29]. Presence of these bacteria in water may be unnoticed even in transparent packaged water and the presence of these microorganisms may pose a potential risk to consumers. Even the consumption of such contaminated water facilitates the widespread infections and can ultimately lead to outbreak of epidemic [5]. The presence of these bacteria in the presumed treated sachet water used in this study may be as a result of improper handling, processing and purification procedures, unhygienic handling after production. The presence of E. aerogenes, P. aeruginosa and K. pneumoniae, potential pathogens, renowned for their high resistance to antibiotics is a point of concern. The organisms encountered in this study showed relatively high level resistance to the investigated antibiotics. Percentage resistance of individual organism ranged from 16.67% to 58.33%. However, the overall percentage antibiotic resistance and susceptibility of the bacteria were 43% and 49%, respectively, while 8% of bacteria were intermediately susceptible.

5 Conclusion

This study revealed acceptable bacteriological quality of bottled water brands but questions some sachet water brands from bacteriological standpoints as they fell below WHO drinking water standards. This study also indicted sub-standard packaged waters as a vehicle of spread of antibiotic resistant potential pathogens, and this poses a high risk to public health. There is, therefore, need for NAFDAC to intensify efforts in the routine monitoring of activities in the packaged drinking water industry. The safety of bottled and sachet drinking water should be ensured through comprehensive regulatory programs at both the federal and state levels. NAFDAC regulations for packaged waters should be protective of public health and there should be continuous adoption of packaged water quality standards. Testing of market samples will be a good way of detecting if the water is actually pure as claimed by these producing companies. High premium should also be placed on ascertaining compliance with Good Manufacturing Practice (GMP) with emphasis on management of raw water source to the consumer product point as recommended by the International Bottled Water Association. Application of good manufacturing practices (GMP), strict process control and personal hygiene should be maintained at processing facilities. Knowledge of the susceptibility of an organism to a specific agent in a hospital or community setting is important in the selection of empiric therapy. Pharmacokinetic differences among agents with similar antimicrobial spectrums may be exploited to reduce the frequency of dosing. Finally, increasing consideration is being given to the cost of antimicrobial therapy, especially when multiple agents with comparable efficacy and toxicity are available for a specific infection. Changing from intravenous to oral

antibiotics for prolonged administration can be particularly cost-effective.

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