

MICROBIAL CONTAMINATION OF PACKAGED DRINKING WATER IN ADO-EKITI METROPOLIS, SOUTH WESTERN NIGERIA

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Abstract

Water is an important resource for the sustenance of human life. It is a basic human need and essential constituent of life. A cross sectional study was conducted on sachet and bottled water samples to determine their microbial quality. A total of 19 samples were collected from different vendors in Ado metropolis, the samples were analysed for the physicochemical qualities, total bacterial count, total coliform count and antibiotic susceptibility test. The mean total bacterial count for bottled water was $\log_{10} 4.69$ cfu/ml, ranging from $\log_{10} 4.43$ cfu/ml to $\log_{10} 4.85$ cfu/ml and the mean total coliform count for bottled water was $\log_{10} 4.38$ cfu/ml ranging from $\log_{10} 4.23$ cfu/ml to $\log_{10} 4.68$ cfu/ml. The mean bacteria count for sachet water $\log_{10} 5.67$ cfu/ml, ranging from $\log_{10} 4.10$ cfu/ml to $\log_{10} 6.00$ cfu/ml, while the mean total coliform count was $\log_{10} 5.18$ cfu/ml, ranging from $\log_{10} 5.00$ cfu/ml to $\log_{10} 5.48$ cfu/ml. The organisms isolated were screened for susceptibility to various commonly used antibiotics. These organisms include: *Escherichia coli*, *Pseudomonas* sp., *Serratia* sp., *Micrococcus* sp., *Enterococcus* sp., *Bacillus* sp., *Streptococcus* sp., *Klebsiella* sp., *Proteus* sp., *Citrobacter* sp. *Staphylococcus aureus* and *Salmonella* sp. Most of the organisms were resistant to antibiotics and this pose a serious public health risk. Most of the water brands fell below the WHO drinking water standard hence have a doubtful quality and should be treated to make it save for drinking.

Keywords: Antibiotic resistance, public health risk, packaged water

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Introduction

Bottled water is defined as water that is intended for human consumption and this is sealed in bottles or other container with no added ingredients except that it may contain safe and suitable fluorides. Small scale entrepreneurs introduced small nylon sachets which are electrically heated and sealed at both ends to the market and is popularly called pure water. It finds patronage from members of low socio-economic class (Narasimhan and Himabindu, 2010). The sale of sachet water is one of the local interventions in Nigeria that is unreliable (Egwari and Aboaba, 2002). Most sachet water manufacturers in Nigeria obtain their raw water mostly from local, municipal piped water or well water, hence adherence to production and analytical standards are doubtful as most of the factories are observed to lack appropriate technology for achieving these (Oyedele *et al.*, 2010). Surveillance carried out by NAFDAC between 2004 and 2005 revealed that some producers of packaged water indulge in sharp practices such as packaging of untreated water, production under unhygienic conditions, illegal production of unregistered water in unapproved premises, use of non-food grade sachets and release of packaged water for distribution and sale without date marking (Edema and Atayese, 2010).

The demand for bottled water has increased over years due to the fact that non-availability of reliable safe municipal water has left the impression that most bottled water offers a healthy, safer and water with better quality (Gardner, 2004). Continuous increase in the sale and indiscriminate consumption of packaged water in Nigeria is of public health significance (Ogundipe, 2008). High demand for packaged water for various occasions has led to springing up of small scale entrepreneurs who engage in production of packaged water without due regard to hygienic practices in the production processes. The implication of this is lack of guarantee that the product will meet set standards for drinking water.

Generally, drinking water contamination can arise from chemical (industries and farms) and other sources (sewage material). Microbial faecal contaminant indicators are *Escherichia coli*, *Clostridia*, *Enterococci* that could be of human and non-human origin and *Streptococci* (Binnie *et al.*, 2002; Simpson *et al.*, 2002; Scott *et al.*, 2002).

Inadequate sanitation and unhygienic practices account for the major sources of microbial contamination of any potable water (Sahota, 2005).

Water borne diseases continue to be a major problem in developing countries. The high occurrence of typhoid, cholera, dysentery and diarrhea has been traced to the consumption of

unsafe water and unhygienic drinking water production processes (Mead *et al.*, 1999). Several studies have documented the detection of coliform and heterotrophic bacteria counts in bottled water which has exceeded national and international standards of portable water (Bhareth *et al.*, 2003). It has been widely observed that the advent of pure water has significantly increased the cases of Salmonellosis and typhoid fever in recent years (Adelegan, 2004). In addition to natural contamination, the product can also deteriorate before it reaches the consumer (Da Silva, 2007) However, drinking water can be carefully evaluated for microbial contamination to ensure informative updates on the quality of water and provide authorities with on-time records for national control programs before any microbe could pose any health and economic problems.

MATERIALS AND METHODS

Media Used for Isolation

The media used were Nutrient agar (NA), MacConkey agar (MA), Eosin methylene blue (EMB), Salmonella-Shigella agar (SSA). They were prepared in line with the manufacturers' specification and were sterilized at 121⁰C for 15minutes in an autoclave.

Collection of Samples

A total of 19 samples of table water, comprising 6 samples for bottled water and 13 samples of sachet water from different brands were randomly collected from commercial vendors at different parts of Ado-Ekiti metropolis. The samples were sealed and unopened until microbiological analysis was carried out on them.

Physico-chemical analysis of water samples

The water sample temperature was taken at the site of collection using a simple thermometer calibrated in °C, electrical conductivity was measured with a CDM 83 conductivity meter (Radio Meter A/S Copenhagen, Denmark). The pH of each water sample was determined by using a pH meter and turbidity of each water sample was determined using Spectrophotometer at a wavelength of 520nm. Other physicochemical characteristics determined were hardness

determined by titrimetry; total dissolved solid and total suspended solid were determined by gravimetric method.

Microbiological Analyses

Enumeration of bacterial population

Determination of total bacterial count and Coliform count in water samples were done in triplicates. Serial dilutions of water samples (1 ml fresh volume) were made with peptone water. 1ml of dilutions 10^{-2} and 10^{-3} was plated on Nutrient agar to determine Total Bacteria Count (TBC) and MacConkey agar to determine Total Coliform Count (TCC). The plates were incubated at 37°C for 24 h.

Identification of Organisms

The bacteria isolated were identified based on the biochemical tests outlined in the Bergey's Manual of determinative bacteriology (Holt, 1994).

Antibiotic susceptibility test

The antibiotic resistance patterns of the isolates were determined by inoculating on Oxoid-Mueller-Hinton agar (Difco Laboratories, Detroit, Mich USA) plates, using the disc diffusion method. The inocula were prepared directly from an overnight agar plate. Commercially prepared antibiotics impregnated discs, containing the following antibiotics: Augmentin (30 μ g), Cloxacillin, Chloraphenicol (30 μ g), Erythromycin, Ofloxacin (5 μ g), Gentamycin (10 μ g), Cotrimoxazole (25 μ g), Nitrofurantoin (300 μ g), Tetracycline (30 μ g), Nalcillin (30 μ g) were aseptically placed on the inoculated plates and incubated overnight. The zones of inhibition were measured and interpreted according to NCCLS (NCCLS, 2000) after incubation at 37°C for 24 h. The diameter of the zone of clearance (including the diameter of the disk) was measured to the nearest whole millimeter and interpreted on the basis of CLSI guideline (CLSI, 2005).

Results

The results of the total bacteria and coliform count of sachet and bottled water samples are shown in table 1. The mean of the total bacteria count and total coliform count of sachet water samples was observed to be $\log_{10} 4.69$ cfu/ml and $\log_{10} 4.51$ cfu/ml respectively, while the

mean of the total bacteria count and total coliform count of bottled water samples was observed to be $\log_{10} 5.67$ cfu/ml and $\log_{10} 5.18$ cfu/ml respectively.

Table 1: Bacterial density and physicochemical properties of Bottled and sachet water

Parameters	Bottled water (n=10)		Sachet water (n-13)		W.H.O standard for Drinking water
	Mean values	Ranges	Mean value	Ranges	
Total Bacterial count (\log_{10} cfu/ml)	4.69	4.43 – 4.85	5.67	4.10 – 6.00	0.0
Total Coliform count (\log_{10} cfu/ml)	4.38	4.23 – 4.68	5.18	5.00 – 5.48	0.0
pH	7.2	6.8-7.5	7.17	6.6 – 7.5	7.00-8.9
Turbidity (NTU)	0.16	0.1-0.8	0.79	0.04 – 1.5	5.0
Electrical conductivity (mS/cm)	0.07	0.01-0.15	0.10	0.01 – 0.16	1.20
Total dissolved solid (mg/l)	56.08	25-92	75	12 – 117	500.0
Total hardness (ppm)	29.2	18-50	31.6	10 – 66	100.0

Table 2 displays the frequency of isolation in sachet water samples. *Klebsiella pneumonia* (25%), showed the highest frequency followed by *Pseudomonas sp* (20.31%) while *Micrococcus sp* (1.56%) and *Salmonella typhi* (1.56%) showed the lowest frequency. Other isolates showed the following frequency: *Escherichia coli* (9.37%), *Bacillus sp* (6.25%), *Streptococcus sp* (7.81%), *Staphylococcus aureus* (6.25%), *Serriatia sp* (7.81%), *Enterococcus sp* (6.25%) and *Citrobacter sp* (7.81%).

However, in bottled water samples the frequency of isolation is as follows: *Staphylococcus aureus* (22.22%) showed the highest frequency, closely followed by *Proteus sp* (19.44%) while *Micrococcus sp* (2.78%) showed the lowest frequency. Other isolates showed the following frequency: *Klebsiella pneumonia* (11.11%), *Bacillus sp* (16.69%), *Pseudomonas sp* (11.11%) and *Citrobacter sp* (16.67%).

Table 2: Distribution of isolates from Sachet and Bottled water samples

ISOLATES	SACHET WATER		BOTTLED WATER	
	No. of isolate	Frequency (%)	No. of isolate	Frequency (%)
<i>Escherichia coli</i>	6	9.37	-	-
<i>Klebsiella pneumonia</i>	16	25	4	11.11
<i>Pseudomonas sp</i>	13	20.31	4	11.11
<i>Proteus sp</i>	-	-	7	19.44
<i>Serriatia sp</i>	5	7.81	-	-
<i>Citrobacter sp</i>	5	7.81	6	16.67
<i>Enterococcus sp</i>	4	6.25	-	-
<i>Bacillus sp</i>	4	6.25	6	16.67
<i>Staphylococcus aureus</i>	4	6.25	8	22.22
<i>Micrococcus sp</i>	1	1.56	1	2.78
<i>Streptococcus sp</i>	5	7.81	-	-
<i>Salmonella spp.</i>	1	1.56	-	-

The antibiotic resistance patterns of bacteria isolated from sachet water are shown in table 3. The *Escherichia coli* isolates showed 100% resistant to Amoxycillin, Augmentin, Ofloxacin and Tetracycline, while the resistance to Gentamycin, Cotrimoxazole, Nitrofurantoin and Nalcillin was observed to be 6.67%, 83.33%, 50%, 16.67% respectively. It was also observed that *Klebsiella pneumoniae* isolate showed 100% resistance to Amoxycillin while resistance to Augmentin, Ofloxacin, Tetracycline, Gentamycin, Contrimoxazole, Nitrofurantoin and Nalcillin respectively. *Micrococcus sp* was observed to have 100% resistance to Augmentin, Cloxacilin, Chloraphenicol, Gentamycin and Cotrimoxazole. *Bacillus sp* also showed 100% resistance to Cloxacilin, Ofloxacin, Gentamycin, while resistant to Augmentin, Chloraphenicol, Erythromycin, Cotrimoxazole and Nitrofuratoxin was observed to be 50%, 25%, 75%, 25% and 75% respectively. *Enterococcus sp* showed 100% resistance to Augmentin, Cloxacilin, Erythromycin, Ofloxacin, Gentamycin and Cotrimoxazole while it showed 50%, 75% to Chloraphenicol, Nitrofuratoxin. It was observed that *Staphylococcus aureus* showed 100% resistance to Cloxacilin, Erythromycin, Ofloxacin and 50% to Augmentin, Chloraphenicol, Gentamycin., while Augmentin showed 40% resistant, Chloraphenicol and Cotrimoxazole showed 60% resistant. It was observed that *Serriatia sp* showed 100% resistance to Amoxycillin

and Nitrofurantoin, 40% to Gentamycin, Nalcillin while Augmentin and Cotrimoxazole showed 20% resistant and Ofloxacin and Tetracycline showed 80% and 60% respectively. It was also observed that *Citrobacter sp* showed 80% to Amoxycillin, Nitrofurantoin and Nalcillin. Augmentin and Tetracycline showed 100% while ofloxacin and Gentamycin showed 20% and Cotrimoxazole showed 60%.

The results of the antibiotic resistance patterns of bacteria isolated from bottled water are shown in table 4. It was observed that *Klebsiella pneumoniae* isolate showed 100% resistance to Amoxycillin and Augmentin, 75% to tetracycline and Gentamycin, 50% resistant to Cotrimoxazole, Nitrofurantoin and 25% resistant to Ofloxacin and Nalcillin. It was observed that *Pseudomonas sp* showed 100% resistance to Amoxycillin and Nitrofurantoin, 75% resistance to Augmentin and Cotrimoxazole, while Oflaxacin, Tetracycline, Gentamycin and Nalcillin showed 25% resistance. *Micrococcus sp* was observed to have 100% resistance to Augmentin, Cloxacilin, Chloraphenicol, Gentamycin and Cotrimoxazole. *Bacillus sp* also showed 100% resistance to Cloaxacilin, 50% resistant to Erythromycin, Gentamycin, Cotrimoxazole and Nitrofuratoxin while Augmentin and Ofloxacin showed 66.67% and Chloraphenicol showed 33.33% resistant. *Proteus sp* showed 85.71% resistant to Amoxycillin, Nitrofurantoin and Tetracycline, 71.43% resistance to Ofloxacin and Cotrimoxazole while Gentamycin showed 28.57% resistant. It was observed that *Staphylococcus aureus* showed 100% resistance to Cloxacilin, 75% resistance to Erythromycin and Nitrofurantoin, 12.25% resistance to Gentamycin and Cotrimoxazole while Augmentin, Chloraphenicol and Ofloxacin showed 50%, 37.5% and 62.5% respectively. It was also observed that *Citrobacter sp* showed 100% to Amoxycillin, Augmentin and Tetracycline, 83.33% resistance to Contrimoxazole and Nitrofurantoin, 33.33% resistance to Ofloxacin and Gentamycin, while Nalcillin showed 66.67% resistance.

The results of the multiple antibiotic resistance patterns of bacteria isolated from sachet water samples are shown in table 5. *Escherichia coli* isolated was observed to show 2 resistance pattern to antibiotics while *Klebsiella pneumoniae* showed 6 resistance patterns and *Salmonela sp* showed 1 pattern of resistance to antibiotic. It was also observed that *Pseudomonas sp* showed 4 different antibiotic resistance patterns while *Micrococcus sp* and *Bacillus sp* showed 1,3 antibiotic resistance pattern respectively, while *Enterococcus sp*, *Serriatia sp* and *Citrobacter sp* showed 2 antibiotic resistance pattern each and *Staphylococcus aureus*, *Streptococcus sp* showed 3 antibiotic resistance patterns each.

Table 3: Antibiotic resistance patterns in bacteria isolated from sachet water samples

ANTIBIOTICS (μg)	Antibiotic resistance patterns of the bacterial isolates (%)										
	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Salmonella typhi</i>	<i>Pseudomonas sp</i>	<i>Micrococcus sp</i>	<i>Bacillus sp</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus sp</i>	<i>Streptococcus sp</i>	<i>Serratia sp</i>	<i>Citrobacter sp</i>
NO. OF ISOLATES	6	16	1	13	1	4	4	4	5	5	5
AMOXYCILIN (25 μg)	100	100	100	100	-	-	-	-	-	100	80
AUGMENTIN (30 μg)	100	50	100	69.23	100	50	50	100	40	20	100
CLOXACILIN	-	-	-	-	100	100	100	100	100	-	-
CHLORAPHENICOL (30 μg)	-	-	-	-	100	25	50	100	60	-	-
ERYTHROMYCIN	-	-	-	-	0	75	100	100	100	-	-
OFLOXACIN (5 μg)	100	12.5	0	23.07	0	100	100	100	100	80	20
GENTAMYCIN (10 μg)	16.67	43.75	0	61.54	100	100	50	100	100	40	20
COTRIMOXAZOLE (25 μg)	83.33	93.75	100	84.61	100	25	25	100	60	20	60
NITROFURANTOIN (300 μg)	50	68.75	100	92.31	0	100	100	75	100	100	80
TETRACYCLINE (30 μg)	100	93.75	100	38.45	-	-	-	-	-	60	100
NALCILLIN (30 μg)	16.67	12.5	100	46.15	-	-	-	-	-	40	80

The result of the multiple antibiotic resistance patterns of bacteria isolated from bottled water samples are shown in table 6. *Klebsiella pneumoniae*, *Pseudomonas sp*, *Bacillus sp*, *Staphylococcus aureus* and *Citrobacter sp* showed 3 antibiotic resistance patterns each, while *Micrococcus sp* showed only 1 antibiotic resistance pattern. It was also observed that *Proteus sp* showed 4 antibiotic resistance patterns.

Table 4: Antibiotic resistance patterns in bacteria isolated from bottled water samples

ANTIBIOTICS	Antibiotic resistance patterns of the bacterial isolates (%)						
	<i>Klebsiella pneumonia</i>	<i>Pseudomonas sp</i>	<i>Micrococcus sp</i>	<i>Bacillus sp</i>	<i>Staphylococcus aureus</i>	<i>Proteus sp</i>	<i>Citrobacter sp</i>
NO. OF ISOLATES	4	4	1	6	8	7	6
AMOXYCILIN (25µg)	100	100	-	-	-	85.71	100
AUGMENTIN (30µg)	100	75	0	66.67	50	42.86	100
CLOXAICILIN	-	-	100	100	100	-	-
CHLORAPHENICOL (30µg)	-	-	100	33.33	37.5	-	-
ERYTHROMYCIN	-	-	100	50	75	-	-
OFLOXACIN (5µg)	25	25	100	66.67	62.5	71.43	33.33
GENTAMYCIN (10µg)	75	25	100	50	12.5	28.57	33.33
COTRIMOXAZOLE (25µg)	50	75	100	50	12.5	71.43	83.33
NITROFURANTOIN (300µg)	50	100	100	50	75	85.71	83.33
TETRACYCLINE (30µg)	75	25	-	-	-	85.71	100
NALCILLIN (30µg)	25	25	-	-	-	0	66.67

Table 5: Multiple antibiotic resistance patterns of bacteria isolates in sachet water samples

BACTERIA ISOLATES	MAR TYPE	% RESISTANCE PATTERN	RESISTOTYPE
<i>Escherichia coli</i>	Multiple R- type 5	33.33	AMX-AUG-OFL-TET-NAL-COT
	Multiple R- type 6	66.66	AMX-AUG-COT-NIT-TET-OFL-GEN
<i>Klebsiella pneumoniae</i>	Multiple R- type 3	12.5	AMX-AUG-TET-NIT-COT
	Multiple R- type 4	37.5	AMX-AUG-COT-NIT-TET
	Multiple R- type 5	31.25	AMX-AUG-GEN-TET-NIT-COT
	Multiple R- type 6	6.25	AMX-GEN-TET-NIT-COT-NAL
	Multiple R- type 7	6.25	AMX-AUG-TET-NIT-COT-OFL-GEN
	Multiple R- type 8	6.25	AMX-AUG-TET-NIT-COT-OFL-GEN-NAL
<i>Salmonella typhi</i>	Multiple R- type 6	100	AMX-AUG-TET-NIT-COT-OFL-GEN-NAL
<i>Pseudomonas sp</i>	Multiple R- type 3	7.69	AMX-COT-NAL
	Multiple R- type 4	61.50	AMX-GEN-COT-NIT-AUG-OFL
	Multiple R- type 6	15.38	AMX-AUG-GEN-NIT-TET-NAL

The results of the physiochemical characteristics of sachet and bottled water samples are shown in table 1. The turbidity of the bottled water samples ranged from 0.04 – 1.5NTU, with the mean of 0.79NTU. The pH ranged from 6.6 – 7.5, with the mean of 7.17. The range of electrical conductivity was observed to be 0.01 – 0.16mS/cm, with the mean of 0.10mS/cm. The mean total dissolved solid of the sachet water samples was 75mg/I and ranged from 12 to 117mg/I, while the mean total hardness was observed to be 31.6ppm and ranged from 10 to 66ppm.

Likewise, the turbidity of the sachet water samples ranged from 0.1 – 0.8 NTU, with the mean of 0.16NTU. The pH ranged from 6.8-7.5, with the mean of 7.2. The range of electrical conductivity was observed to be 0.01-0.15mS/cm, with the mean of 0.07mS/cm. The mean total dissolved solid of the sachet water samples was 56.08mg/I and ranged from 25-92mg/I, while the mean total hardness was observed to be 29.2ppm and ranged from 18-50ppm.

Table 6: Multiple antibiotic resistance patterns of bacteria isolated from bottled water sample

BACTERIA ISOLATES	MAR TYPE	% RESISTANCE PATTERN	RESISTOTYPE
<i>Klebsiella pneumoniae</i>	Multiple R – type 3	25	AMX-AUG-TET
	Multiple R – type 4	50	AMX-AUG-COT-NIT-TET-GEN
	Multiple R – type 8	25	AMX-AUG-TET-NIT-COT-OFL-GEN-NAL
<i>Pseudomonas sp</i>	Multiple R – type 4	25	AMX-COT-AUG-NIT
	Multiple R – type 5	50	AMX-AUG-GEN-NIT-TET-NAL
	Multiple R – type 8	25	AMX-AUG-COT-NIT-TET-NAL-OFL
<i>Micrococcus sp</i>	Multiple R – type 5	100	AUG-CXC-CHL-GEN-COT-ERY-OFL-NIT
<i>Bacillus sp</i>	Multiple R – type 1	16.6	CXC
	Multiple R – type 5	50	AUG-CXC-ERY-OFL-NIT-COT-CHL
	Multiple R – type 6	33.33	AUG-CXC-COT-ERY-OFL-GEN-NIT
<i>Staphylococcus aureus</i>	Multiple R – type 3	12.5	CXC-ERY-AUG
	Multiple R – type 4	62.5	AUG-CXC-CHL-ERY-OFL-NIT
	Multiple R – type 8	25	AUG-CXC-CHL-ERY-OFL-GEN-NIT-COT
<i>Proteus sp</i>	Multiple R – type 3	14.28	AUG-AMX-TET
	Multiple R – type 4	14.28	AMX-COT-NIT-TET
	Multiple R – type 5	57.14	AMX-AUG-OFL-COT-NIT-TET-NAL-GEN
	Multiple R – type 8	14.28	AMX-AUG-OFL-COT-NIT-TET-NAL-GEN
<i>Citrobacter sp</i>	Multiple R – type 5	33.3	AMX-AUG-OFL-TET-NAL-NIT-COT
	Multiple R – type 6	50	AMX-AUG-COT-NIT-NAL-NIT-COT
	Multiple R – type 7	16.7	AMX-AUG-OFL-COT-NIT-TET-NAL

Discussion

The bacteriological quality of sachet and bottled water commercially sold in Ado – Ekiti metropolis was examined in this study. The results obtained show that the bottled and sachet drinking waters sold in various parts of Ado metropolis in South Western Nigeria exhibited variable characteristics in terms of their microbiological quality. Ten out of the thirteen brands of sachet water studied were contaminated with at least one type of coliform bacteria, making them unsuitable for human consumption. However, there are non – coliform bacteria present in the brands of sachet water. Bacteria such as *Escherichia coli*, *Streptococcus sp*, *Bacillus sp*, *Enterococcus sp*, *Klebsiella sp*, *Staphylococcus aureus*, *Serriatia sp*, *Proteus sp*, *Pseudomonas sp*, *Citrobacter sp* and *Salmonella sp* were isolated from the samples. 77% of the sachet water brands failed to meet the WHO drinking water standard of zero coliform per 100 ml water (WHO, 1993). This is similar to the result of the study carried out on package drinking water sold in Dar es Salaam, Tanzania (Kassenga, 2007).

The bottled water brands had relatively better microbiologocal quality, with mean total coliform count of $\log_{10} 4.38$ cfu/ml compared to $\log_{10} 5.18$ cfu/ml mean total coliform count of sachet water. *Escherichia coli*, *Salmonella sp*, *Enterococcus sp*, *Streptococcus sp* and *Serriatia sp* are however absent from all brands of bottled water. Similar to Oyedeleji *et al.* (2009), the absence of coliform bacteria in most brands of bottled drinking water was attributed to better hygienic practices observed in the industry compared to the sachet water producing industry. Ajayi *et al.* (2008) had reported an earlier study of packaged drinking waters in Ibadan, Nigeria in which larger proportions of sachet water were found to show positive coliform counts compared to bottled waters.

The microbial contaminations of packaged drinking water, especially sachet water could be influenced by factors such as their raw water source, treatment process employed and hygienic practices observed in production (Geldreich, 1996). Most sachet water manufacturers are observed to utilize well water or at best shallow, contaminated boreholes and municipal tap water as raw water source. Most of these sources have been implicated with microbial contamination. Study of the quality of tap drinking water in Quebec City of Canada showed that 36 and 28% of water samples were contaminated by at least one coliform or indicator bacterium and or at least one pathogenic bacterium (Levesque *et al.*, 1994). Inadequate sanitation and

unhygienic practices account for the major source of microbial contamination of any potable water (Sahota, 2005).

The presence of *Bacillus sp* and *Pseudomonas sp* in commercial bottled water is similar to result obtained by (Kawther and Suaad, 2007). The isolation of *Escherichia coli*, *Streptococcus sp*, *Enterobacter sp*, *Citrobacter sp*, and *Salmonella sp* from water are similar to isolates obtained by (Edema and Atayese, 2010).

In the present study, antibiotic resistant bacteria were wide spread in sachet and bottled water commercially sold in Ado metropolis. This is not surprising since the intrinsic resistance of many organisms to antibiotics is well documented (Ajayi and Akonai, 2003). The *Escherichia coli* isolates of sachet water showed 100% resistant to Amoxycillin, Augmentin, Ofloxacin and Tetracycline, while the resistance to Gentamycin, Cotrimoxazole, Nitrofurantoin and Nalcillin was observed to be 6.67%, 83.33%, 50%, 16.67% respectively. The isolates of *E. coli* showed MAR of 33.33% to Amoxycillin, Augmentin, Ofloxacin, Tetracycline, Cotrimoxazole and Nalcillin and 66.67% MAR to Amoxycillin, Augmentin, Ofloxacin, Tetracycline, Gentamycin, Cotrimoxazole, Nitrofurantoin.

Generally, most of the isolates from sachet and bottled water commercially sold in Ado metropolis showed multiple antibiotic resistance (MAR, table 5 & 6). This may be related result obtained by (Ajayi and Akonai, 2003) only 17% of the isolates were resistant to single antibiotics while other 83% were multiple antibiotics resistant. It was observed that *Bacillus sp*, *Staphylococcus aureus*, *Micrococcus sp*, *Streptococcus sp* and *Enterococcus sp* have 100% resistance to Cloxacillin. The result of antibiotic resistance pattern of this study showed that all the bacteria isolated from both sachet and bottled water have 100% resistance to Amoxycillin except *Citrobacter sp* and *Proteus sp* which showed 80% and 85.71% respectively.

The pH range of 6.8 to 7.5 recorded for sachet water samples and 6.6 to 7.5 recorded for bottled water samples obtained from commercial vendors in Ado metropolis could be considered as being within acceptable range for natural waters. According to (Medera *et al.*, 1982), the pH of most natural waters ranged from 6.5 to 8.5 while deviations from neutral 7.0 are as a result of CO₂/bicarbonate/carbonate equilibrium. While the turbidity range of 0.1 to 0.8 recorded for sachet water samples and 0.4 to 1.5 recorded for bottled water samples could be considered

within acceptable range of water turbidity in accordance with the NSDWQ (SON, 2007). The total hardness range of 10 to 66 recorded for bottled water and 18 to 50 recorded for sachet water samples are also within acceptable range in accordance to (SON, 2007) Nigeria Standard for Drinking Water Quality.

One of the main requirements for drinking water is it must be free of coliform. Generally the presence of coliforms including *E. coli*, *Citrobacter sp* and *Klebsiella pneumoniae* are usually determined by presumptive coliform test. This results in the production of gas (following the lactose fermentation) and acid at 37°C. Turbidity and gas production at 44°C imply the presence of faecal coliforms i.e. *E. coli* is considered as foreign enteric indicator bacteria when they are present in water thus, the water cannot be appropriate for human consumption.

An adequate, safe and accessible supply must be available to all improving access to safe drinking water can result in significant benefits to health. Every effort should be made to achieve a drinking water quality as safe as possible (WHO, 2008).

Conclusion

The presence of microorganisms especially coliform bacteria in packaged water which serve as major sources of water for consumption purposes by the inhabitants of Ado-Ekiti poses great health risk. Since, coliform bacteria have been detected in packaged water especially sachet water, they are therefore not good enough for human consumption according to World Health Organisation (WHO) and National Agency for Food and Drug Administration and Control (NAFDAC). 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, compliance with Good Manufacturing practices (GMP). Also random testing of water samples should be employed to know if water is actually pure as claimed by manufacturers.

The results of multiple antibiotic resistances obtained from this study challenges the scientist on the need for more or developments of new antibiotics to fight against the infections caused by these resistant strains.

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