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Microbiological Analysis of Sachet Water Vended in Ondo State, Nigeria

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Abstract: Thirty sachet water samples vended to the public were randomly purchased from different vendors in Akure, Ikare, Ondo and Owo in Ondo state, Nigeria. The samples were examined for microbial contamination. The sachet water analysed were coded DC, FT, BF, PT, TC, BB, KW, AD, LL, IT, BS, OP, BW, DT, LA, NI, AN, CL, JS, TD, SW, MS, MH, DS, DL, AT, VA, WT, GL and EF. The counts recovered from PT, BW, BB, LL and TC were low as this conformed with WHO and NAFDAC regulations on drinking water quality. On the other hand, MH water sample had the highest microbial load with marginal coli form count and *Escherichia coli* negative result. This suggests thorough flushing and chemical treatment of the water source. However, samples BF, SW and MS are unsafe for human consumption due to the presence of *Escherichia coli*. The various bacteria encountered in the water samples include; *Alcaligenes faecalis*, *Bacillus subtilis*, *Enterobacter aeogenes*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus* and *Streptococcus faecalis*.

Key words: Sachet water, microbiological analysis, Nigeria

INTRODUCTION

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. Therefore, maintaining a safe drinking water remains essential to human health as transient bacterial contamination may have implication well beyond a period of acute self-limited illness. All living organisms require a wide variety of inorganic compounds for growth, repair, maintenance and reproduction. Water is one of the most important, as well as one of the most abundant of those compounds and it is particularly, vital to organism (Tortora *et al*., 2002). Within the cell, water is the medium for most chemical reactions. It makes up at least 5-95% of every cell and the average being between 65-75%. In addition, water has been traced to be one of the ways by which humans could be infected with various kinds of diseases some water-borne diseases include typhoid fever, cholera, bacillary dysentery and so on. In water borne infections, pathogens are usually spread by water contaminated with untreated or poorly treated sewage.

Water in nature is seldom totally pure. Rainfall is contaminated as it falls to earth, the combustion of fossil fuel put sulphur compound responsible for acid precipitation in the air. Water that moves below the ground's surface undergoes filtration that removes most organisms. For this reason, water from springs and deep wells are generally of good quality. The most dangerous form of water pollution occurs when faecal contaminant like *E. coli* enter the water supply. Also, the fecal-oral routes of transmission, in which pathogens are shed in human or animal's faeces. Contaminants ingested into water supply, perpetuate many diseases. Examples of such pathogens are *Salmonella* sp., *Shigella* sp., *Vibrio cholerae, Escherichia coli* (Tortora *et al*., 2002). Industrial and agricultural chemicals leached from the land enter water in a great amount and uniform that are resistant to biodegradation. Apart from this, rural waters often have excessive amount of nitrite from microbial action on agricultural fertilizers. When ingested, nitrite completes for oxygen in the blood (Lellan *et al*., 2001).

To attain a safe water supply to various communities, an understanding of water that is microbiologically and chemically certified is therefore imperative. Above all, to consider microbial quality of water suitable for human consumption, the Nigeria based NAFDAC in relation to the World Health Organization (WHO), recommended that potable water for human consumption should not contain any microorganism that is known to be pathogen and the coliform number per 100 mL of water must be zero. However, it may contain 3 coli form per 100 mL of water sample in occasional samples (WHO, 1984).

Chlorination is the most common method of ensuring microbiological safety in water supply. It is a

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good reagent for preventing reproduction of microorganism in water (Association of Public Analyst, 2004). Hence, the objective of this study are to isolate, characterize the microorganisms and determine the microbial load of the sachet water samples sold in Ondo state.

MATERIALS AND METHODS

Thirty samples of sachet water of different brands were purchased from different vendors in Akure, Ikare, Ondo and Owo in Ondo State. These includes: DC, FT, BF, PT, TC, BB, KW, AD, LL, IT, BS, OP, BW, DT, LA, NI, AN, CL, JS, TD, SW, MS, MH, DS, DL, ST, VA, WT, GL and EF. Samples were collected twice in a batch of 5 (5 sample per batch) and analyzed within 12 h of collection. Nutrient agar, MacConkey broth and Eosin Methylene Blue (EMB) were prepared according to manufacturer's instruction. The nutrient agar was for the total viable plate count, MacConkey broth was for the presumptive test while the EMB was for the confirmed test and the completed test was done by re inoculating colony from confirmed test into MacConkey broth and acid and gas formation confirmed *E. coli*.

RESULTS AND DISCUSSION

The total viable count of bacterial colonies in water samples is shown in Table 1 from the result sample PT had the lowest plate count while sample MH had the highest count. The results of the Most Probable Number (MPN) is shown in Table 2 sample MS in the second group had the highest coli form number and *E. coli* positive result, followed by sample AD also in the second collection but *E. coli* negative result, then followed by sample BF with *E. coli* positive result, while sample PT had the lowest coli form count and *E. coli* negative result.

The results obtained from the microbial analysis of sachet water vended in different parts of Ondo state, Nigeria revealed presence of microbes of varying loads. In the total viable plate count, it was observed that, many of the sampled water conform with the WHO, NAFDAC and USEPA (US Environmental Protection Agency), that the Maximum Contaminant Level (MCL) should not be more than 100 colonies and anything above 100 colonies should not be counted as a safe water. While, few samples went against these regulations, which shown that purification process were not properly in use. In the MPN for coli form count an average of 12 samples conformed with the NAFDAC regulation and WHO (2001)

Table 1: Total viable plate count

Sample code	Microbial load cfu mL^{-1}	
	A	B
DC	8×10^{-1}	12×10^{-1}
FT	16×10^{-1}	8×10^{-1}
BF	15×10^{-1}	23×10^{-1}
PT	0×10^{-1}	4×10^{-1}
TC	13×10^{-1}	16×10^{-1}
BB	5×10^{-1}	3×10^{-1}
KW	19×10^{-1}	6×10^{-1}
AD	8×10^{-1}	22×10^{-1}
LL	5×10^{-1}	7×10^{-1}
IT	23×10^{-1}	16×10^{-1}
BS	30×10^{-1}	21×10^{-1}
OP	12×10^{-1}	6×10^{-1}
BW	3×10^{-1}	7×10^{-1}
DT	11×10^{-1}	7×10^{-1}
LA	4×10^{-1}	6×10^{-1}
NI	23×10^{-1}	16×10^{-1}
AN	8×10^{-1}	20×10^{-1}
CL	10×10^{-1}	15×10^{-1}
JS	23×10^{-1}	40×10^{-1}
TD	70×10^{-1}	29×10^{-1}
SW	61×10^{-1}	47×10^{-1}
MS	60×10^{-1}	56×10^{-1}
MH	110×10^{-1}	48×10^{-1}
DS	19×10^{-1}	12×10^{-1}
DL	36×10^{-1}	30×10^{-1}
ST	20×10^{-1}	9×10^{-1}
VA	25×10^{-1}	16×10^{-1}
WT	30×10^{-1}	35×10^{-1}
GL	15×10^{-1}	19×10^{-1}
EF	24×10^{-1}	22×10^{-1}

Sample names have been coded, Key: A (first group of the sample collected], B [second group of the sample collected)

which have maximum contaminant level between 0-10 coli form per 100 mLs, while an average of 18 water samples were noted not to conform with these regulation. From Table 2 sample PT is the best water sample followed by sample LL, sample BB, BW and TS. Samples FT, LA, AN, DS, DL, VA and GL have marginally acceptable coli form count. The WHO (2001) standard is 10/100 which implies that the samples just meet the standard. However, with low plate count and *E. coli* negative result, it is suggested that the water sources should be thoroughly flushed and retested. Also from the results on Table 3 sample DC, KW, AD, IT, BS, OP, DT, NI, CL, JS, TD, MH, ST, WT and EF failed the WHO (2001) standard for potable water, it is suggested that the water sources should undergo chemical treatment, flushed and retested before release for human consumption. Sample MS had the highest coli form count with *E. coli* positive result followed by sample BF with *E.coli* positive result, though sample SW coli form count is not as high as other samples that did not conform with WHO (2001) standard for potable water but there is presence of *E. coli*. Hence, the 3 water samples i.e. MS, BF and SW are

Table 2: Most Probable Number (MPN) of coli form bacteria

	Most probable number		Coli form count per 100 mLs of water		*E. coli*(+) complete test	
Sample code	5-5-5 A	5-5-5 B	A	B	A	B
DC	1-1-2	3-1-3	7	20	-ve	-ve
FT	1-3-1	2-0-2	10	9	-ve	-ve
BF	3-1-2	5-2-1	17	70	-ve	+ve
PT	0-0-1	1-2-0	2	5	-ve	-ve
TC	2-0-2	0-1-1	9	4	-ve	-ve
BB	1-1-2	1-2-0	07	05	-ve	-ve
KW	2-1-1	1-3-1	12	10	-ve	-ve
AD	2-4-0	5-0-4	15	75	-ve	-ve
LL	0-1-2	0-3-0	6	6	-ve	-ve
IT	1-4-0	3-1-3	11	20	-ve	-ve
BS	3-1-3	3-4-1	20	25	-ve	-ve
OP	1-4-0	2-1-1	11	12	-ve	-ve
BW	1-2-0	1-1-2	5	7	-ve	-ve
DT	2-2-2	3-4-1	14	25	-ve	-ve
LA	1-3-1	0-1-1	10	4	-ve	-ve
NI	3-1-2	1-3-1	17	10	-ve	-ve
AN	1-1-2	1-3-1	8	10	-ve	-ve
CL	2-4-0	3-1-3	15	20	-ve	-ve
JS	2-1-1	2-2-2	9	14	-ve	-ve
TD	3-1-3	2-1-1	20	12	-ve	-ve
SW	3-1-2	2-0-2	17	9	-ve	-ve
MS	3-4-0	5-3-1	20	110	-ve	+ve
MH	2-4-0	1-3-1	15	10	-ve	-ve
DS	1-3-1	1-2-2	10	10	-ve	-ve
DL	1-4-0	2-0-2	11	9	-ve	-ve
ST	3-1-2	1-4-0	17	11	-ve	-ve
VA	3-0-2	0-1-2	13	6	-ve	-ve
WT	2-1-1	3-2-1	9	17	-ve	-ve
GL	0-1-2	1-3-1	6	10	-ve	-ve
EF	3-0-1	3-4-0	11	20	-ve	-ve

Key: A [first group of the sample collected], B [second group of the sample collected] –ve = negative, +ve = positive

Table 3: Distribution of bacterial isolates in different water samples

				Isolates						
				Alcaligenes faecalis	*Bacillus subtilis*	*Enterobacter aerogenes*	*Escheichis coli*	*Micrococcus luteus*	*Staphylococcus aureus*	*Streptococcus faecalis*
Water samples	1	DC	A	-	+	-	-	-	-	-
			B	-	-	-	-	-	-	+
	2	FT	A	-	+	-	-	-	-	-
			B	-	+	-	-	-	-	-
	3	BF	A	-	+	-	-	-	-	-
			B	-	-	-	+	-	+	-
	4	PT	A	-	+	-	-	-	-	-
			B	-	-	-	-	-	+	-
	5	TC	A	-	+	-	-	-	-	-
			B	-	-	-	-	-	+	-
	6	BB	A	-	-	-	-	-	+	-
			B	-	+	+	-	-	-	-
	7	KW	A	-	+	-	-	-	-	-
			B	-	+	-	-	-	+	-
	8	AD	A	+	-	-	-	-	-	-
			B	-	+	-	-	-	-	-
	9	LL	A	-	+	-	-	-	-	-
			B	-	-	-	-	-	+	-
	10	IT	A	-	-	+	-	-	-	-
			B	+	-	-	-	-	-	+
	11	BS	A	-	+	-	-	-	-	-
			B	-	+	-	-	+	-	-
	12	OP	A	-	+	-	-	+	-	-
			B	-	-	-	-	-	-	-
	13	BW	A	-	-	+	-	+	-	-
			B	-	-	+	-	-	-	-
	14	DT	A	-	-	-	-	+	+	-
			B	-	-	-	-	+	-	-

Table 3: Continued

			Isolates						
			Alcaligenes faecalis	*Bacillus subtilis*	*Enterobacter aerogenes*	*Escheichis coli*	*Micrococcus luteus*	*Staphylococcus aureus*	*Staphylococcus faecalis*
15	LA	A	-	+	-	-	+	-	-
		B	+	-	-	-	+	-	-
16	NI	A	-	-	+	-	-	-	-
		B	-	-	-	-	+	-	-
17	AN	A	-	+	-	-	-	-	-
		B	+	-	-	-	+	-	-
18	CL	A	-	+	+	-	+	-	-
		B	-	+	-	-	-	-	-
19	JS	A	-	-	-	-	-	-	+
		B	-	-	+	-	-	-	-
20	TD	A	-	+	-	-	-	-	-
		B	-	+	-	-	-	-	-
21	SW	A	+	-	-	+	-	-	-
		B	-	-	+	-	-	-	-
22	MS	A	-	-	+	-	-	+	-
		B	-	+	-	+	-	-	-
23	MH	A	-	+	-	-	-	-	-
		B	-	-	-	-	+	-	-
24	DS	A	-	-	-	-	-	-	+
		B	-	+	+	-	-	-	-
25	DL	A	-	+	-	-	-	-	-
		B	-	+	-	-	-	-	-
26	ST	A	-	-	-	-	+	-	-
		B	-	+	-	-	-	-	-
27	VA	A	-	-	-	-	+	-	-
		B	-	-	-	-	+	-	-
28	WT	A	-	+	-	-	+	-	-
		B	-	+	+	-	-	-	-
29	GL	A	-	-	+	-	-	-	-
		B	-	+	+	-	-	-	-
30	EF	A	-	+	-	-	-	-	-
		B	-	+	+	-	-	-	-

Sample names have been coded, Key: - = Absent, + = Present

not safe for human consumption hence the water sources should be re-examined by the NAFDAC. The isolation of *Escherichia coli* is these water samples is indicative of feacal contamination as *E. coli* is used as an indicator of water borne pathogen (Tortora *et al*., 2002; Chao, 2004) from the results samples BF, AD and MS agree with Morgan (1991) statement that supplied fund with 1-10 coliform 100 mL^{-1} that such water is satisfactory, supplies found with 11-50 coliform mL^{-1}water needs further testing and supplies found with 751 coliform mL^{-1} water unsafe and needs treatment. Therefore, all these water samples that failed these regulations should be retreated and flushed before they are release to the public for human consumption. Also NAFDAC should intensify effort on batch number, manufacture date and expiry date of all these sachet water vended to the public.

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