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Full Length Research Paper

Microbiological quality of sachet packaged water vended in three local governments of Oyo State, Nigeria

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Recently in Nigeria, cholera which is a water borne disease has been reported in the major cities of the Southwestern part of which Ibadan is inclusive. The aim of this study was to investigate the microbiological quality of sachet packaged water vended in Oluyole, Ibadan South West and Ibadan South East Local Government Areas in Oyo State, Nigeria. This was to ascertain their compliance to World Health Organisation(WHO) and National Agency for Food and Drug Administration and Control(NAFDAC) standards. Sampling of these water samples was done three times between the months of April and May 2011. Viable bacterial and coliform counts were determined for each sample; obtained isolates were characterized using cultural, morphological and biochemical characteristics of each. Isolates obtained include *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Escherichia coli*. 39.9% of the examined samples contained *Pseudomonas aeruginosa*, 53.3% had *Enterobacter aerogenes* while 13.3% were found to have *Escherichia coli.* Cholera causing pathogen was not isolated from any of the water samples but pathogenic microorganisms and coliform were found in most of the samples.

Keywords: Quality, sachet water, Oyo state, *Pseudomonas aeruginosa, Enterobacter aerogenes, Escherichia coli*

INTRODUCTION

Production and sale of sachet water, popularly called “pure water” in Nigeria, is presently a lucrative business, therefore many people are involved in the production and marketing of the product.

Water is one of the most important as well as one of the most abundant compound on earth, and is vital to the survival of any organism (Tortora et al., 2002).Water in nature is seldom totally pure. Rainfall is contaminated as it falls to earth (Ajewole, 2005). The combustion of fossil fuel put sulphur compounds as being responsible for pollution of rain water by precipitation (Edema et al., 2001). However, water that moves below the ground surface undergoes natural filtration that removes most organisms (Kleiner, 1999.). For this reason, water from springs and deep wells are generally of better quality than flowing water. Water related diseases continue to be one of the major health issue globally. The high prevalence of diarrhoea among children and infants can be traced to the use of unsafe water and unhygienic practices (Omalu et al., 2010).

The most dangerous form of water pollution occur when fecal contaminants like *Escherichia coli* enter the water supply and also through the fecal-oral routes of transmission. Microbial contaminants in water supply are the sources of many diseases such as typhoid fever, cholera, bacillary dysentery and so on. Examples of such microbial contaminants are *Salmonella spp*, *Shigellaspp,Vibrio cholerae*, *Escherichia coli* (Edema *et al.*,2001; Tortora et al., 2002*).*

Various opportunistic pathogens that occur naturally in the environment may cause disease in humans. Those who are at greater risk of infection are infants and young children, people whose immune system is suppressed, the sick and the elderly. In such individuals, drinking water containing large numbers of opportunistic pathogens can occasionally produce infections. Examples of such opportunistic pathogens are *Pseudomonas aeruginosa, Klebsiellaspp, Areomonasspp* and certain slow growing *Mycobacterium* (WHO, 1993).

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In Nigeria, the National Agency for Food and Drugs Administration Control (NAFDAC) in association with the World Health Organization (WHO, 2001) has the responsibility of regulating the standard of drinking water. The agency has published guidelines for the production of sachet- packaged drinking water. Unfortunately most producers do not adhere to these guidelines (Onemano et al., 2003). Thus, this work is aimed at assessing the microbiological quality of sachet packaged water in three Local government areas in Ibadan.

MATERIALS AND METHODS

Study Area

This research work was carried out in three local government areas (LGAs) of Ibadan in Oyo-State, Nigeria. The LGAs are; Ibadan South West, Ibadan South East and Oluyole Local Government Areas.

Study Sample

Different sachet packaged water samples vended in the above named local government areas in Ibadan metropolis, Oyo State were analyzed to know their microbiological quality. The samples were Yomex, Clover, Resogo, Rinot, De Scion, Temidire, Ziddie, Delux, Majok, Aqua Glory, Rioz, Indent, Queenway, Splendid Progress and Zelon. All the samples were bought from vendors in each of the LGAs and taken to the laboratory for analysis within two hours of purchase.

Culture Media

The culture media used include Nutrient agar (Oxoid), Eosin Methylene Blue agar EMB (LAB M), MacConkey agar (Oxoid), Pseudomonas Centrimide agar (PCA, (Oxoid). They were all prepared according to the Manufacturers' specification and sterilized in an autoclave at 121^{o}C at 15 psi for 15 minutes. Nutrient agar was used to determine the total viable plate count, while MacConkey agar was used to enumerate coliforms. EMB and Pseudomonas Centrimide agar were used to enhance identification of specific microorganisms.

Isolation of Microorganisms

For each brand of sachet water from each LGA, a bag of twenty sachets was purchased and two sachets were randomly picked from each bag for sampling. Sampling was done 3 times and analyzed within 12hours of collection. 1ml of each sachet water sample was serially diluted and 1ml of an appropriate dilution was inoculated on sterile, Nutrient agar, Eosin methylene blue agar and Pseudomonas centrimide agar using spread plate technique, while Macconkey agar was used to enumerate coliform by membrane filter technique . The plates were incubated at 37^{o}C$\pm$ 2^{o}C for 24 hours, after which visible colonies were counted and results were expressed in cfu/ml. The sterility of each batch of culture media was established by incubating uninoculated plates along with inoculated plates.

Characterization and Identification of Isolates

This was carried out according to standard techniques described by Olutiola et al. (1991). Pure culture of each isolate was obtained by aseptically picking distinct colony and culturing them on newly prepared nutrient agar which was subsequently incubated at 37^{o}C$\pm$ 2^{o}C for 24 hours . The pure cultures of the bacterial isolates obtained were subjected to morphological identification using the Gram-Staining procedure, microscopy of the isolates was done under X100 objective of the light microscope and other biochemical tests were carried out. Characterization of the isolates and identification of the isolates was carried out using the method Cruickshank et al. (1975) and Bergey's Manual of Determinative Bacteriology as reference (Buchanan and Gibbon, 1974).

RESULTS

Fifteen brands of sachet water were analyzed and a total of six bacterial isolates were obtained from the sachet water vended from these LGAS in Ibadan metropolis. The isolates were initially differentiated on the basis of their cultural and morphological characteristics after which they were subjected to various biochemical tests. The isolates were identified to be *Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Enterobacter aerogenes* as shown on Table 4.

Table 1 shows the bacterial load of the water samples obtained from Ibadan South West local government.

Water sample T10 had a bacterial load of 8.5x10^{6}cfu/ml, T3 4.5x10^{6}cfu/100ml, T8 3x10^{6}cfu/100ml, T12, 8.5x10^{6}cfu/100ml and T7 23x10^{6}cfu/100ml. Out of the water samples analyzed as shown on Table 3, T8 has the lowest bacterial load while T7 had the highest bacterial load.

Table 1 shows bacterial load of sachet water sample obtained from Ibadan South West LGAs.

Water sample T10 had a coliform count of 4x10^{6}cfu/100ml, T3,6x10^{6}cfu/100ml, T8, 1.5x10^{6}cfu/100ml, T12, 5.5x10^{6}cfu/100ml and T7, 9x10^{6}cfu/100ml. Table 1 shows that sample T8 had the lowest coliform count while T7 had the highest coliform count.

Table 2 shows bacterial load of sachet water sample obtained from Ibadan South West LGAs.

Table 1. Bacterial Load of Sachet Water Sample Obtained From Ibadan South West LGAs.

Sample Names	Sample Codes	Batch no	Sampling date	NAFDAC No.	Average Bacterial Load (cfu/ml)	Average Coliform Count (cfu/100ml)
Yomex	T 10	Nil	21/04/11	4985L	$8.5x10^6$	4.0×10^6
Clover	T 3	Nil	21/04/11	1787L	$4.5x10^6$	6.0×10^6
Resogo	T 8	Nil	21/04/11	0331L	$3x10^6$	1.5×10^6
Rinot	T 12	Nil	21/04/11	0176L	$8.5x10^6$	5.5×10^6
De Scion	T 7	Nil	21/04/11	3426	$23x10^6$	$9.0x10^6$

Table 2. Bacterial Load and Coliform count of Sachet Water Sample Obtained From Ibadan South East LGAs.

Sample Name	Sample Code	Batch no	Sampling date	NAFDAC NO	Average Bacterial Load (cfu/ml)	Average Coliform count (cfu/100ml)
Temidire	T 1	Nil	27/04/11	0040L	$50x10^6$	14.0×10^6
Ziddie	T 2	Nil	27/04/11	5008L	$10x10^6$	3.0×10^6
Delux	T15	Nil	27/04/11	0792L	$15x10^6$	8.0×10^6
Majok	T 5	Nil	27/04/11	0936L	$32x10^6$	10.0×10^6
Aqua Glory	T 6	Nil	27/04/11	1151L	$17.5x10^6$	$4.5x10^6$

Table 3. Bacterial Load of Sachet Water Sample Obtained From Oluyole LGA.

Sample Name	Sample Code	Batch no	Sampling date	NAFDAC No.	Average Bacteria Load (cfu/ml)	Average Coliform count (cfu/100ml)
Rioz	T 4	Nil	07/05/11	9790L	$4.5x10^6$	-
Indent	T 9	Nil	07/05/11	2404L	$56.5x10^6$	$7.5x10^6$
Queenway	T13	Nil	07/05/11	9691L	$6.5x10^6$	$0.5x10^6$
Splendid Progress	T 14	Nil	07/05/11	0963L	$21x10^6$	$6.5x10^6$
Zelon	T 11	Nil	07/05/11	9311L	$20.5x10^6$	8.0×10^6

Water samples T1 had a bacterial load of $50x10^6$cfu/100ml, T2, $10x10^6$cfu/100ml, T15, $15x10^6$cfu/100ml, T5, $32x10^6$cfu/100ml and T6, $17.5x10^6$cfu/100ml. Out of the water samples analyzed Table 5 shows that sample T2 had the lowest bacterial load while T1 had the highest bacterial load.

Table 2 shows the coliform count of the water samples obtained from Ibadan South East local government. T1 water sample had a coliform count of 14x106cfu/100ml, T2 water sample 3x106cfu/100ml, T3, 8x106cfu/100ml, T5 water sample 10x106cfu/100ml and T6 water sample 4.5x106cfu/100ml. Out of the water sample analyzed in table 6, T2 water sample has the lowest coliform count while T1 water sample have the highest coliform count.

Bacterial load of the water samples obtained from Oluyole local government area revealed that T4 water sample had a bacterial load of $4.5x10^6$cfu/100ml, T9 water sample $56.5x10^6$cfu/100ml, T13 water sample $6.5x10^6$cfu/100ml, T14 water sample $21x10^6$cfu/100ml and T11 water sample $20.5x10^6$cfu/100ml. Of these water samples, T4 water sample has the lowest bacterial load while T9 water sample have the highest bacterial load (Table 3). Table 8 shows the coliform count of the water samples obtained from Oluyole local government area. There was no coliform in the sample of T4 water that was analyzed, T9 water sample had a coliform count of 7.5x106cfu/100ml, T13 water sample had 0.5x106cfu/100ml coliform count, Splendid progreT14 water sample had $6.5x10^6$cfu/100ml while T14 water sample's coliform count was $8x10^6$cfu/100ml. Out of the water sample analyzed in Table 3, T4 water sample had no coliform present while T14 water sample had the highest coliform count of $8x10^6$cfu/100ml.

Table 4 shows the biochemical characteristics of the bacterial isolates gotten from the water samples. The isolates were identified to be *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Enterobacter erogenes.*

Table 5 shows the occurrence of the identified bacterial in each of the water samples with samples T5, T8 and T12 having the highest occurrence of these isolates.

Table 4. shows the biochemical characteristics of the bacterial isolates

Isolates code	Lactose	Glucose	Sucrose	Mannitol	Maltose	Starch hydrolysis	Motility	M.R	V.P	Indole	Acid	Gas	Citrate	Oxidase	Catalase	Coagulase	Probable Organism
SA		+		+	NA			+	+	+	+	+	+	-	+	+	*Staphylococcus aureus*
ML	-	-	NA	-	NA		-	NA	NA	NA	-	-		+	+	-	*Micrococcus luteus*
BS	+	+	NA	+	+	+	+	NA	+	NA	+	-	+	-	+	-	*Bacillus subtilis*
PA	-	+	NA	+	NA	+	+	-	-	-	+	-	-	+	+	-	*Pseudomonas aeruginosa*
EA	+	+	+	+	NA	+	+	-	+	-	+	+	-	-	-	-	*Enterobacter aerogenes*
EC	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	*Escherichia coli*

NA- Not applicable

Table 5. Occurrence of Bacterial isolates in the analyzed sachet water samples in the Three LGAs in Ibadan

S/N	Samples Names	Nafdac no	*E. coli*	*P. aeruginosa*	*E. raerogenes*	*S. aureus*	*B. subtilis*	*M. luteus*
1.	Resogo	0331L	-	-	-	+	-	-
2.	Ziddie	5008L	-	-	+	+	-	-
3.	De Scion	342L	-	-	+	+	+	-
4.	Majok	0936L	-	+	-	+	-	+
5.	Clover	1789L	-	+	+	+	+	+
6.	Aqua Glory	1151L	-	+	-	-	-	-
7.	Qway	9691L	-	-	-	+	-	+
8.	Temidire	0040L	+	+	+	+	-	+
9.	Rioz	9790L	-	-	-	+	-	-
10.	Delux	0792L	-	+	+	+	-	+
11.	Rinot	0176L	-	+	-	+	+	+
12.	Indent	2404L	+	+	+	+	-	+
13.	Yomex	4985L	-	-	+	-	+	-
14.	Progress	0963L	-	+	+	+	-	-
15	Zelon	9311L	-	+	-	+	-	-

DISCUSSION

All the sachet water samples analyzed were contaminated with bacterial isolates identified as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Micrococcus luteus* and *Escherichia coli*. These bacteria have been implicated in water related diseases (APHA, 1998)

Poor personal hygiene of handlers and poor environmental hygiene have been reported to contribute significantly to the level of contamination in packaged water in developing countries (Ashbolt, 2004). The poor microbiological quality of the drinking water samples recorded in this study has been observed in other developing countries by some researchers (Dada, 2009; Olaoye and Onilude, 2009). In a study carried out by (Obiri et al., 2003) on the microbiological evaluation of drinking water in Ghana, the author reported that the water samples were of poor quality. According to them, the occurrence of the indicator organisms in the water constitutes a serious threat to the community and they called for strict observance of good manufacturing practices by the processors and handlers. In a related study, Yassin et al. (2006) reported poor microbiological quality of water in Gaza, which deviated from the recommended limits of the World Health Organization (WHO).

The detection of *E.coli* and *E. aerogenes* which are indicator organisms in some samples implies that such water samples may have been contaminated from faecal sources and as such were not safe for cons-umption (WHO). This is also in accordance with the result

obtained by Olaoye and Onilude (2009) on their assessment of sachet drinking water from Western Nigeria. WHO has reported that the occurrence of pathogens or indicator organisms in water sources mainly depends on the intrinsic physical and chemical characteristics of the catchment area, the magnitude and range of the human activities and animal sources that release pathogens to the environment (WHO). Hence the bacteria found in the drinking water samples in the three LGAs in Ibadan metropolis could be due to the sources of water used as the raw material by the processors.

NAFDAC has published guidelines for the small-scale production of the sachet-packaged drinking water (NAFDAC) with which processors are expected to comply. Unfortunately, many of the processors fail to do so, as the study has shown. Moreover, poor practices of the water handlers, curing processing as well as distribution from factory to consumer, may contribute to the contamination with unwanted organisms. It has been observed that sanitation and hygiene practices by many food handlers in Nigeria are poor (Omemu et al., 2008). It is suggested that public enlightenment through reinforced and consistent health education on hygiene could contribute significantly towards improving microbiological safety in water processing plants in the country.

The coliform count recorded in this study falls within the slightly polluted limits as published by the (WHO) on the pollution of drinking water. Detection of coliform shows the danger of faecal pollution and the consequent hazard of contracting disease through pathogenic organisms. Disease causing organisms are transmitted via drinking water predominantly of faecal origin, but the coliform count would not constitute much concern without the detection of *E. coli* in the water samples. It has been reported that typical enteropathogenic *Escherichia coli* is a leading cause of the infantile diarrhoea in developing countries, whereas this is rare in industrialized countries (Trabulsi et al., 2002).

The presence of *Bacillus spp* in the sachet water could be as a result of contamination from poor staff handling during processing of the water samples (Okonko et al., 2008).Among the microbial isolates identified from the sachet water samples and which are of public health concern is *Staphylococcus aureus*. The micro-organism is a pathogenic bacterium responsible for severe health problems such as food spoilage, chronic infections and vomiting in humans (Jay, 2006). *Staphylococcus aureus* generally occurs in water that contains organic pollutants that is mineral ions and organic matter (Tortora *et al*, 1988).

The presence of enteric bacteria, *E.aerogenes* as reported in this study are indication of faecal contamination as a result of possible burst along pipe lines or unhygienic handling of the water right from the treatment plant for tap water and borehole water. The presence of *Micrococcus sp*. and *Enterobacter aerogenes* reported in this study has also been reported by (Umeh et al., 2005) in a study on the bacteriological quality and safety of pure water sold in Awka, Nigeria. Bacterial growth in water may be unnoticed even in transparent packaged water and the presence of some of these microorganisms may pose a potential risk to consumers (Oladipo et al., 2009).

The non-compliance of the water processors with NAFDAC regulation, as noted in this study could pose serious concerns to public health. Many of the defaulting processors may not have been licensed for their operations, and this could be the reason their plant location details were not printed on the water samples. They also do this to avoid their unlicensed plants from been sealed off by the regulatory agency. Such practices may have led to outbreaks of waterborne diseases in many parts of Nigeria (Hutin et al., 2003).

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