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PURE WATER SYNDROME: BACTERIOLOGICAL QUALITY OF SACHET- PACKED DRINKING WATER SOLD IN NIGERIA

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ABSTRACT

Water is one of the indispensable resources for the continued existence of all living things including man. Government has failed to adequately provide safe, pipe-borne water for the increasing population in Nigeria and this has encouraged the sale of drinking water by private enterprises that have little knowledge about good manufacturing practices. This study investigated the bacteriological quality of commercial sachet-packed drinking water at point-of-sale in south-western Nigeria with emphasis on pathogenic bacteria in 108 samples tested, in order to evaluate the contribution of this popular product to the increasing incidence of typhoid fever and related illnesses. Ten-fold serial dilution of water samples and the pour plate technique were used to investigate the presence of *Salmonella* and indicator coliform *Escherichia coli* in sachet-packed water samples. Aerobic and total coliforms were also enumerated. Characterization of isolates was by in-vitro cultural, morphological and biochemical characteristics. Results showed that 87% of the sachet-packed water samples examined contained *Salmonella* and/or *Escherichia coli*, indicative of fecal contamination and inadequate water treatment or no treatment at all. The study also showed that about 65% of the polythene sachets used was not of food-grade quality and imparted polyester taste in the water samples. High aerobic colony counts in the order of 6.0 log CFU/ml was recorded from 93% of water samples examined. *E. coli* counts used as indicator of hygiene criteria were present in the range of 98 and 106 cfu/100ml of water sample, while *Salmonella* counts used as food safety criteria were between $2.12x10^1$ and $2.20x10^1$. These mean values were greater than the international guidelines for drinking water quality. The findings of this study indicate that sachet-packed water samples examined do not meet microbiological standards for drinking water quality. National surveillance agencies need to monitor and enforce compliance with microbiological safety standards of sachet-packed water being sold to the public.

Key words: Sachet, Drinking water, *Salmonella*, *Escherichia coli*

INTRODUCTION

Water is an essential part of human nutrition, both directly as drinking water or indirectly as a constituent of food, in addition to various other applications in daily life. Water is not only essential for life, it also remains a most important vector of illness and infant mortality in many developing countries and even in technologically more advanced countries [1]. It is also a key parameter influencing survival and growth of microorganisms in foods and other microbial environments.

In the order of importance, air, water and food are the three main necessities of life. A person can survive for about a month without food, about a week without water, and less than five minutes without air [2]. The provision of an adequate supply of safe drinking water was one of the eight components of primary health care identified by the International conference on primary health care in 1978. Increase in human population has exerted an enormous pressure on the provision of safe drinking water especially in developing countries.

In the era of colonialism in Nigeria, water was supplied to the public free by the government. But Nigeria has moved from being a mixed economy to capitalist economy. In cities and towns today, water now attracts rates and fees [3]. With insufficient government supply of water, private sector participation has evolved and the idea of packaged drinking water popularly referred to as `pure water' is now a common phenomenon in the country. Drinking water is now commercially packed in easy-to-open 50 – 60 ml polyethylene sacs and is referred to as "sachet or pure water". This packaged water is cheap and convenient and have increasingly become popular. Arising from the popularity of the packaged drinking water is the abuse of its production leading to a situation whereby the pure water is everything but pure. Although there is dearth of documented data on incidence rates of water-borne diseases directly associated with consumption of pure water, it has been widely observed that with its advent, the cases of salmonellosis and typhoid fever have significantly increased in recent years [4]. Between January and August 2010, over 20 deaths and more than 200 hospitalizations were reported in the news in parts of Nigeria arising from cholera outbreaks. Water pollution has continued create negative impacts on health and economic development in Nigeria [5].

There are several rules and regulations for drinking water. In Nigeria, such regulations are monitored by the National Agency for Food and Drug Administration and Control (NAFDAC), which was established as a parastatal of the Federal Ministry of Health by Decree No. 15 of 1993. 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. These malpractices compelled the agency to formulate guidelines for the production of wholesome packaged water. However, despite the standards formulated by NAFDAC to address this problem, the situation has remained bad. In order to

effectively solve the problem, there is a need to fully assess the extent of the problem and its causes. Drinking water regulations require that potable water for human consumption be free from human-disease-causing bacteria and specific indicator bacteria that are indicative of the presence of these pathogens [6]. Some of these bacteria that have greater probability of causing disease in humans are classified as pathogens [7]. Examples of bacterial pathogens and their related diseases are *Salmonella typhi* (typhoid fever), *Shigella dysenteriae* (dysentery), and *Legionella pneumophilia* (Legionnaire's Disease).

Kajogbola [8] revealed the prominence of dysentery as one of the leading causes of morbidity within the Ibadan region in Nigeria. The National Health Policy report of the Federal Ministry of Health, Nigeria gave the morbidity pattern in Nigeria in 2006 with infectious and parasitic diseases being responsible for over 38% of deaths in the country [9]. It is therefore important to determine the likely sources of food/water-borne infections in order to plan sustainable drinking water quality improvement. This study aimed to investigate the presence of pathogenic and disease indicator organisms in sachet- packed drinking water sold in south-western Nigeria.

MATERIALS AND METHODS

Study Sites

Nigeria is located in the tropical zone of West Africa between latitudes 4°N and 14°N and longitudes 2°2'E and 14°30'E and has a total area of 923,768 km^2. The country is bounded by Cameroon to the east, Chad to the northeast, Niger to the north, Benin to the west, and the Gulf of Guinea on the Atlantic Ocean forms the southern limits of Nigerian territory. Land cover ranges from thick mangrove forests and dense rain forests in the south to a near-desert condition in the northeastern corner of the country. Nigeria is by far the most populous country in Africa, with its 140 million people accounting for about one-seventh of the total population of Africa's 53 countries. In 2002, 60 percent of the total population was using improved drinking water sources, with 72 percent in urban areas and 49 percent in rural areas [10]. The study site was South-western Nigeria, comprising six states Lagos, Ogun, Oyo, Osun, Ondo and Ekiti states.

Sampling of sachet water

A total of 108 samples of sachet water were collected between 2006 and 2009. Six brands of sachet water were randomly selected from the numerous brands available in the capital cities of each of the states. Three replicate samples from different lots were collected per brand. The samples were purchased and taken to the laboratory in insulated containers with ice packs. Analyses were usually carried out within 8 hrs after sampling. Where immediate microbiological evaluation was going to be delayed, the samples were refrigerated at 4^0C and analyzed within 24 hours of collection.

Physico-Chemical Examination of Water Samples

The water samples were examined for turbidity, odor, taste, pH and sliminess. Odor, sliminess and taste were evaluated by perception with the sense organs by the

investigators upon opening each sample while pH was determined by a combined glass electrode and a pH meter (Mettler-Toledo, Essex M3509 Type 340).

Microbiological Analyses of Water Samples

Ten milliliters of each water sample for bacteriological evaluation were aseptically transferred into 90 ml of 0.1% sterile peptone water, mixed thoroughly and appropriate dilutions (up to 10^4) prepared for microbiological studies. Bacteriological evaluation of water samples was made on selective media for groups of bacteria investigated as follows:

Total viable counts: Total viable counts of aerobic mesophilic bacteria were made on Plate Count Agar (PCA, Oxoid, Hampshire, UK) by using the pour plate technique [11]. Incubation was at 37^0C for up to 48 h. Representative colonies were identified by morphological and biochemical characteristics according to Sneath *et al*. [12].
Coliforms: Total coliforms were isolated and enumerated by the pour plate technique on MacConkey Agar incubated at 37^0C. Bright pink colonies on incubated MacConkey plates were recognized as *Escherichia*, and confirmed by indole test and mannitol fermentation as recommended by Harrigan and McCance [11]. Other members of the family Enterobacteriacae, arising as a heterogeneous micro-flora on MacConkey Agar were characterized and differentiated by biochemical properties as *Citrobacter* and *Enterobacter* - Gram negative, oxidase negative, glucose fermenting rods and *Aeromonas* which is oxidase positive [13]. *Enterococcus fecalis* was identified by its ability to hydrolyze esculin in the presence of bile using the bile esculin medium [14].

Salmonellae: A 10^1 dilution of each water sample was enriched in tetrathionate broth (Difco), incubated at 37°C for 6 h before inoculation on *Salmonella-Shigella* agar (Oxoid) for isolation of salmonellae. Incubation was at 37^0C for 48 h. Colonies with black spots were recognized as *Salmonella* and confirmed by the production of H_2S, urease test and citrate utilization, differentiating it from *Shigella* which also grew on the same medium as normal colonies.

After incubation, all colonies from a representative sector of incubated plates were picked, purified by repeated sub-culturing before being examined microscopically for Gram reaction [15], cell morphology (using 24 h old cultures), motility, pigmentation and sporulation [11]. Biochemical analysis included catalase and oxidase activities, nitrate reduction, patterns of sugar utilization as well as urea and starch hydrolysis [11, 16]. The isolates were identified on the basis of the results obtained from conventional biochemical characterization supplemented with the corresponding API identification kits (API Systems, Biomerieux, France). API 20E strips for enteric bacteria and other gram negative rods was used for bacterial identification, primarily to distinguish between *Klebsiella* and *Enterobacter* and between *Enterobacter* and *Escherichia coli*. The results were analyzed using Bergey's manual of systematic bacteriology [12].

Statistical Analyses of Data: The data obtained were subjected to statistical analysis (percentages, means and ANOVA) using SPSS 11.0 for Windows software.

RESULTS

The physico-chemical and bacteriological properties of the samples of sachet-packed drinking water from south-western Nigeria, examined in this study are presented in Table 1. Mean pH values for all samples were between 6.9 and 7.7. Total viable counts were in the order of 10^5 and 10^6 colony forming units per ml of samples, while counts of salmonellae were between 20 and 23 per ml of sample enriched in tetrathionate broth. Mean coliform counts per ml of sample ranged from $1.51x10^2$ to $1.54x10^2$ cfu/100ml while fecal coliform represented by *E. coli* were between 98 and 106 cfu/100ml sample. Mean counts of salmonellae on S-S agar plates ranged from $2.12x10^1$ to $2.20x10^1$. Values obtained in this study for the counts made on bacteriological plates, were significantly different by Duncan's Multiple Range Test at 5% confidence level. These values are relatively high for point-of-sale samples.

Pathogenic organisms: *Escherichia coli, Enterococcus fecalis, Shigella* sp, *Enterobacter aerogenes, Citrobacter,* and *Salmonella* sp were isolated from the samples (Table 2). This indicates that pure water presented under a wide variety of brand names, is potentially unsafe for human consumption by the very high counts of potential pathogens enumerated and identified. The presence of these organisms could have contributed to off odours, tastes, cloudiness and sliminess that were observed in the samples (Table 3). The results also showed that about 65% of the sachets used were not of food-grade quality thereby leading to polyester taste in many of the samples examined.

DISCUSSION

The pH values of water samples examined in this study were within the recommended WHO values; however, the numbers of indicator bacteria were comparatively higher than permissible values [17]. Previous studies in other parts of the country reported similar bacterial load indicative of poor water quality [18, 19]. Relatively high aerobic colony counts are indicative of poor, unhygienic handling and processing, while indicator organisms like *Escherichia coli* are likely to result from sewage contamination [20].

In a previous study [21], different sources of drinking water were examined in Abeokuta, South western Nigeria, which was one of the locations in the present study, and all the samples including dug well, stream and tap water samples were contaminated with bacteria above the WHO stipulated guidelines. Though the values obtained for sachet-packed water in this study were higher than those obtained from the drinking water sources examined by Edema *et al.* [21], it is likely that the contamination of samples in this study can be traced to the sources of the water packed. 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.

Some of the isolates such as *Aeromonas* sp have been implicated in diarrhea [22, 23]. Old cells of *Azotobacter* species tend to form thick-walled cysts, which have capsules consisting largely of alginates and other polysaccharides that enhance resistance to heat, desiccation and adverse environmental conditions. However, unlike bacterial endospores these cysts cannot withstand extreme temperatures. Under favorable environmental conditions, the cysts germinate and grow as vegetative cells. Their presence in samples examined in this work could have contributed to the sliminess observed in the plastic sachet containers.

The presence of potentially pathogenic organisms is indicative of the possibility that about 90% of packed pure water sold in the part of the country examined are not fit for human consumption and are potentially hazardous to health [24].

From the foregoing, it has become imperative for government and concerned regulatory bodies to address the issue of safety in sachet-packed water in Nigeria. A large number of the population currently stands at risk because many people depend on this sachet-packed water for convenience and assumed safety, believing that it is better than pipe-borne water since it is believed to have undergone further treatment (hence the acronym “pure water”) and is relatively cheap [25]. Unfortunately, the incidence of water-borne infections continues to rise [17].

Producing water of appropriate quality is becoming increasingly difficult because of the ever-increasing demand. Coupled with this fact is the difficulty in establishing an universal standard for drinking water due to different sociological conditions, varying climates, and other specific circumstances found all over the world. However, water quality can be improved if treatments applied are adapted to the initial quality of the raw water. This often requires the use of combinations of different treatments [26] such as storage in open reservoirs or ponds, coagulation, filtration, and treatment with activated carbon or disinfectants. In tropical areas, the microbial flora may be dominated by mesophilic and thermo-tolerant bacteria and the diversity is usually greater [27]. A wide range of potentially infectious agents including bacteria, viruses, protozoa, and helminths may also be introduced to drinking water sources. Viable bacteria that were identified in drinking water include *Pseudomonas* spp., *Flavobacterium spp.*, *Aeromonas* spp., *Micrococcus* spp., and *Bacillus* spp. Pathogenic bacteria linked to waterborne outbreaks include *Campylobacter jejuni*, *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Escherichia coli* O157 [28]. Some of these pathogenic organisms were isolated from water samples in this study, indicative of contamination. It is the responsibility of water regulatory authorities or suppliers (in case of private sources such as sachet water producers) to ensure the microbiological safety of drinking water supplies. Regular monitoring of the water for pathogens and indicators of recontamination, including faecal contamination performed by water authorities and/or water companies provides important information to users as to

possible microbiological deviations. Small to medium scale private enterprises can analyse their samples regularly through certified analytical laboratories at reasonable costs. Guidelines on microbiological criteria for drinking water provided by WHO or by local legislations are normally used.

The regulatory agency NAFDAC is doing a lot to address the problem. In the last 5 years, several workshops have been organized by the agency for producers of packaged water to enable their production staff acquire relevant information and experience on the production of packed water. No doubt, more effort is required to reduce the ever-increasing cases of typhoid fever among other water borne diseases which are also on the increase, like enteric hepatitis and zoonosis. By the FAO/WHO guidelines for drinking water quality which NAFDAC adopts, *E. coli* or thermotolerant coliform bacteria must not be detectable in any 100-ml sample of drinking water unlike what was observed in this study. Monitoring and inspection will therefore be required to enforce existing regulations and if need be, promulgate new ones to ensure that the health of the populace is guaranteed. For example, there could be a law concerning the source of water for producing sachet-packed drinking water like deep wells or boreholes, which usually provide water of excellent bacteriological quality. These ground water sources are often used without any treatment, except physicochemical ones to reduce hardness or eliminate off-flavours and odours. Active inspection, surveillance and preventive maintenance will all be required for sustainable drinking water management and safety assurance [29].

Microbial spoilage of potable drinking water is presently not a concern, although, organoleptic deterioration of water quality may occur due to the release of compounds with earthy, musty, or chemical odours. In this study, the most important organoleptic factor was probably the impartation of polyester taste in the water samples arising probably from leaching of chemicals into the water. PolyEthylene TerEphthalate (PETE / Polyester) type of plastic material used is considered non-food grade, although it is not certain that there are possible risks associated with the use of such packaging material for drinking water, other than the change in organoleptic perception. In fact, food grade plastics such as polystyrene used for burger wrapping and beverage cups may leach styrene into the food it comes into contact with. Styrene compounds leaching from food containers are estrogenic (meaning they can disrupt normal hormonal functioning). Styrene is also considered a possible human carcinogen by the World Health Organization [30]. Polycarbonate, another food grade material used for water containers, some baby bottles, and metal can linings, could release Bisphenol A, another suspected hormone disruptor, into liquids and foods. The FDA generally considers an estimated daily intake (EDI) that does not exceed 1.5 micrograms/person/day (0.5 ppb dietary concentration, DC) to be of negligible risk for a contaminant migrating from recycled plastic. While the present study did not determine the EDI or DC levels of the polyester packaging material used, it is believed that the material does not constitute significant health risk.

CONCLUSIONS AND RECOMMENDATIONS

The incidence of bacterial water-borne infections particularly typhoid fever caused by *Salmonella typhi*, is increasing in Nigeria. The findings of this investigation showed that a large percentage of the popular sachet-packed water is microbiologically unsafe for human consumption, constituting potential health risk; and this can be linked to the increasing incidence of typhoid fever among other food and water-borne infections. Commercialization of drinking water is inevitable considering the increasing population of the country. Therefore, the production and distribution of commercial sachet-packed water in Nigeria requires increased attention and heavy monitoring by the regulatory agencies. The aim is not to point accusing fingers at any location, but to call the attention of government to a need for more effective monitoring and control. Some of these products are even produced at certain locations and then distributed to other parts of the country for sale. There is also a need for public enlightenment and awareness with respect to consumers' responsibility towards assuring their own safety by avoiding this source of drinking water until corrective actions have been put in place to assure safety of these products. However, there is an urgent need to effectively communicate the risks associated with consumption of sachet packed water of unacceptable microbial quality to the populace. Government must also be determined to increase resource allocations to relevant regulatory agencies to ensure adequate monitoring and enforcement of drinking water quality in public and private distribution systems.

Table 1: Physico-Chemical and bacteriological properties of sachet-packed water samples

Location	pH	Total viable counts (cfu/ml)	Total coliform counts (cfu/ml)	*E. coli* (cfu/100ml)	*Salmonellae (cfu/ml of sample in enrichment broth)
A	Range 6.80-7.50 Mean 7.10	Range 5×10^5-$1x10^6$ Mean $9.54x10^5$	Range $1.50x10^2$-$1.56x10^2$ Mean $1.53x10^2$	Range 0.94-1.20 Mean 1.06	Range $2.10x10^1$-$2.36x10^1$ Mean $2.20x10^1$
B	Range 6.83-7.80 Mean 7.41	Range $9x10^5$-$1x10^6$ Mean $1.26x10^6$	Range $1.49x10^2$-$1.56x10^2$ Mean $1.53x10^2$	Range 0.92-1.13 Mean 0.98	Range $2.03x10^1$-$2.20x10^1$ Mean $2.12x10^1$
C	Range 7.50-8.03 Mean 7.70	Range $1x10^6$-$2x10^6$ Mean $1.47x10^6$	Range $1.48x10^2$-$1.57x10^2$ Mean $1.53x10^2$	Range 0.84-1.23 Mean 1.02	Range $2.07x10^1$-$2.23x10^1$ Mean $2.17x10^1$
D	Range 7.07-7.82 Mean 7.58	Range $1x10^6$-$2x10^6$ Mean $1.39x10^6$	Range $1.47x10^2$-$1.57x10^2$ Mean $1.51x10^2$	Range 0.97-1.20 Mean 1.06	Range $2.00x10^1$-$2.30x10^1$ Mean $2.17x10^1$
E	Range 6.89-7.87 Mean 7.50	Range $1x10^6$-$2x10^6$ Mean $1.47x10^6$	Range $1.49x10^2$-$1.57x10^2$ Mean $1.54x10^2$	Range 0.87-1.23 Mean 1.01	Range $2.10x10^1$-$2.23x10^1$ Mean $2.15x10^1$
F	Range 6.10-7.43 Mean 6.91	Range $1x10^6$-$2x10^6$ Mean $1.47x10^6$	Range $1.48x10^2$-$1.59x10^2$ Mean $1.54x10^2$	Range 0.87-1.23 Mean 1.02	Range $2.03x10^1$-$2.37x10^1$ Mean $2.16x10^1$
*WHO Guideline Value	No guideline but other sources suggest 6 – 8.5	Not a health concern to ma but other authors suggest less than 100 cfu/ml	Not detectable in any 100 ml sample	Not detectable in any 100 ml sample	Not detectable in any 100 ml sample

*** WHO's Guidelines for Drinking-water Quality (2002)**

Key: Locations A-F represent Lagos, Ogun, Oyo, Osun, Ondo and Ekiti states, respectively

Table 2: List of isolated microorganisms from sachet-packed water samples

Isolate	Description
Aeromonas	Gram negative, oxidase positive, non-spore forming rods
Azotobacter	Gram-negative, aerobic soil-dwelling bacteria, typically polymorphic, form thick-walled cysts
Bacillus	Gram positive spore-forming rods, catalase positive
Citrobacter	Gram negative rod, lactose fermenting, utilize citrate, does not produce acetoin
Enterobacter aerogenes	Gram negative rod, lactose fermenting, utilize citrate, produces acetoin
Enterococcus fecalis	Gram positive cocci in chains, catalase negative, grows in bile and 6.5% NaCl
Escherichia coli	Gram negative rod, heavy lactose fememnter, does not utilize citrate, positive for indole test, ferments mannitol
Salmonella	Non-lactose fermenting, urease negative, utilizes citrate, produces H_2S
Shigella	Non-lactose fermenting, does not utilize citrate, produces H_2S

Key: Locations A-F represent Lagos, Ogun, Oyo, Osun, Ondo and Ekiti states, respectively

Table 3: Percent physical quality of sachet-packed water samples (n=108)

Parameter	Number of positive samples	Percent of total sample size
Cloudiness (Turbidity)	16	14.8
Off odor	12	11.1
Metallic taste	09	8.3
Sharp taste	15	13.9
Polyester taste	70	64.8
Sliminess	42	38.9

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