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Bacteriological safety of plastic-bagged sachet drinking water sold in Amassoma, Nigeria

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ABSTRACT

Objective: To evaluate the bacteriological safety of sachet water sold in Amassoma, a rural community in Bayelsa State, Nigeria. **Methods:** Six samples of each of the different sachet drinking water brands were bought at random from shop shelves, markets and street vendors and were studies for microbial indicators of safety and quality. Bacterial counts were analyzed by one-way Analysis of Variance (ANOVA) and significance of differences was tested at 5% probability. **Results:** Minimum and maximum counts with regard to the sachet water samples investigated were $(4.3\pm1.1)\times10^6$ CFU mL^{-1} and $(8.2\pm1.0)\times10^6$ CFU mL^{-1} for heterotrophic plate counts; $(0.9\pm 0.3)\times10^6$ CFU mL^{-1} and $(1.2\pm 0.4)\times10^6$ CFU mL^{-1} for aerobic spore-former counts; $(1.3\pm0.5)\times10^3$ CFU mL^{-1} and $(2.5\pm 0.8)\times10^3$ CFU mL^{-1} for total coliforms; $(1.6\pm0.9)\times10^3$ CFU mL^{-1} and $(9.5\pm11.2)\times10^3$ CFU mL^{-1} for thermotolerant coliforms. *Klebsiella* spp but not *Escherichia coli* was present in all samples of the brands; non-coliform bacteria detected in some samples were *Staphylococcus, Pseudomonas* and *Bacillus* species. **Conclusions:** The brands of sachet water sold (at the time of this study) in Amassoma did not meet the minimum acceptable standard for microbiologically safe drinking water as recommended by the World Health Organization.

1. Introduction

Microbiological safety and quality of drinking water is of great health concern to all people owing to the potential of drinking water as carrier of microbial pathogens and cause of subsequent illness in both developed and emerging economies of the world[1,2]. Water-related diseases continue to be one of the major health problems globally, with an estimated 3.4 million water-related deaths per year (which represents 4% of all deaths) and 5% of health loss to disability[3,4].

The most common and widespread health risk associated with drinking water is contamination, either directly or indirectly, by human or animal excreta and the microorganisms contained in feces[5]. The health effects of exposure to disease-causing bacteria, viruses, and protozoa in drinking water are varied[6]. The most common manifestation of waterborne illness is gastrointestinal upset (nausea, vomiting, and diarrhoea), and this is usually of short duration. However, in susceptible individuals such as infants, the elderly, and immune-compromised individuals, the effects may be more severe, chronic (e.g., kidney damage), or even fatal. Bacteria (e.g., *Shigella* and *Campylobacter*), viruses (e.g., norovirus and hepatitis A virus), and protozoa (e.g., *Giardia* and *Cryptosporidium*) can be responsible for severe gastrointestinal illness. Other pathogens may infect the lungs, skin, eyes, central nervous system, or liver.

The microbial guidelines seek to ensure that drinking water is free of microorganisms that can cause disease. According to the WHO Guidelines for Drinking Water Quality[7], microbial water quality is to be verified by microbiological testing which in most cases involve the analysis of fecal indicator microorganisms, but in some circumstances it may also include assessment of specific pathogen densities. Approaches to verification include

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testing of source water, water immediately after treatment, water in distribution systems or stored household water. Verification of the microbial quality of drinking water includes testing for *Escherichia coli (E. coli)* as an indicator of fecal pollution. *E. coli* provides conclusive evidence of recent fecal pollution and should not be present in drinking-water. In practice, testing for thermotolerant coliform bacteria can be an acceptable alternative in many circumstances. While *E. coli* is a useful indicator, it has limitations. Enteric viruses and protozoa are more resistant to disinfection; consequently, the absence of *E. coli* will not necessarily indicate freedom from these organisms. Under certain circumstances, it may be desirable to include more resistant microorganisms, such as bacteriophages and/or bacterial spores. Such circumstances could include the use of source water known to be contaminated with enteric viruses and parasites or high levels of viral and parasitic diseases in the community.

Drinking water in most rural communities of developing countries comes from such sources as rivers, streams, lakes, boreholes and wells; and they are likely to be polluted with domestic, agricultural or industrial wastes thereby passing potential health effect to consumer[6,8]. The past decade has seen a dramatic increase in the consumption of sachet water, popularly known as "pure water" in Nigeria. Factory-bagged plastic sachet water was introduced into the Nigerian market as an improvement on the hand-filled hand tied polythene-bagged type of vended waters produced by the poor in Nigeria. Although the standard of hygiene in the various stages in the production of the factory-bagged plastic sachet water may be higher compared to hand-filled hand tied polythene-bagged water, bacteria are also thought to enter as contaminants during filling and sealing. The origin of the water (bore-hole water, treated piped water, or occasionally, well water) may constitute another source of bacterial contamination. The proliferation of such water products raises the question as to whether they are hygienically produced, especially when the poor sanitary environment in Amassoma, like most rural communities in Nigeria, is taken into account.

At present, there is inadequate information on the microbiological quality of factory-bagged plastic sachet water sold in rural and urban areas of Nigeria; and it is important that the consumers can be assured of its quality and safety. The purpose of this study is to evaluate the bacteriological safety and quality of sachet water sold in Amassoma and its environs by (1) enumerating the heterotrophic bacteria; (2) enumerating spore-former bacteria; (3) detecting and measuring "total" coliforms which are gram-negative bacteria that ferment lactose at 35-37 ℃ within 24-48 h; (4) detecting and measuring "thermotolerant" coliforms which are a subset of total coliform bacteria that ferment lactose at 44-45 ℃; (5) detecting *E. coli*, a specific indicator of fecal contamination; (6) detecting other non-coliform pathogenic bacteria.

2. Materials and methods

2.1. Study area and sampling

The study area (Amassoma), which hosts the state-owned University, is a fast growing community in the Wilberforce Island of Bayelsa State in the South-South region of Nigeria. Drinking water sources in this community include among others, sachet water, bottled water, piped water, bore-hole, wells and river. However, due to the higher demand for plastic-bagged sachet water, it was chosen for our study.

Samples of the different brands of plastic-bagged sachet water were bought during the period October to December 2007 from shops shelves, market and street vendors within Amassoma. The surfaces of the sachet water were disinfected by cleaning with cotton wool soaked in ethyl alcohol (70% v/v) to reduce the risk of contamination from personnel and environment. As a further precaution, the swabbed surface was cut with sterile blade and a sterile syringe and needle was used to draw the required volume of water such that any contaminant observed thereafter was taken to be inherently contained in the packaged sachet water. Microbiological investigations on each of the samples were done within 24 h of collection.

2.2. Heterotrophic plate count (HPC) and aerobic spore-former count (ASC)

Heterotrophs are those microorganisms that use organic compounds for most or all of their carbon requirements. Most bacteria, including many of the bacteria associated with drinking water systems, are heterotrophs[6]. Heterotrophic bacteria were estimated using the spread plate method described by Health Canada[6] with modifications. Briefly, dilutions of 10^{-1} to 10^{-6} of water samples were prepared in peptone water (Fluka Biochemical, Germany) and 0.1 mL of each dilution was aseptically spread on the surface of a sterile nutrient agar (Fluka Biochemical, Germany) in a petri plate. Each dilution was spread in duplicate. The inoculated plates were incubated for 24 h at 37 ℃. Petri dishes from dilutions containing between 20 and 30 discrete colonies were counted and the results expressed as the number of colony forming unit (CFU) per milliliter (mL).

Enumeration of spore-formers was done using the spread plate method adapted from the method for HPC. Briefly, dilutions of water samples (made in the same way as in the HPC) were heated at 80 ℃ for 10 min as described previously[3,9] before spreading on nutrient agar and subsequent incubation.

2.3. Presence/Absence (P-A) test for "total" and "thermotolerant" coliforms (presumptive test)

The P-A test, which is the most probable number method

reduced to a single tube was done by a modification of previously described method[6,10] to detect the presence or absence of coliform bacteria. For each sample, 50 mL double strength MacConkey broth (Fluka Biochemical, Germany) was mixed with 50 mL of undiluted water sample and then incubated at 37 ℃ for 48 h for the "Total" coliform test. Another 50 mL double strength MacConkey broth was mixed with 50 mL of undiluted water sample and then incubated at 44 ℃ for 48 h for "Thermotolerant" coliform test. Each sample was duplicated for the purpose of comparison.

2.4. Estimation of "total" and "thermotolerant" coliform bacteria

"Total" and "Thermotolerant" coliforms were estimated by spread plate method described by Obiri-Danso *et al*[6,11] with modifications. Briefly, 0.1 mL aliquots of 10^{-1} to 10^{-6} dilutions of the water samples in peptone water were spread on MacConkey agar (Fluka Biochemical, Germany) and incubated at 37 ℃ for 48 h (for the total coliform count- TCC) or 44 ℃ for 48 h (for the thermotholerant coliform count- TTCC). Pink to dark red colonies on the agar indicative of total or thermotolerant coliforms were then counted and expressed as CFU mL^{-1}. The experiment was done a total of three times for each sample.

2.5. Confirmatory test for E. coli

Pink to dark red colonies were picked from both the 37 ℃ (total coliform) and 44 ℃ (thermotolerant coliform) MacConkey agar plates, and streaked on eosin methylene blue (EMB) agar (International Diagnostics Group, UK). The presence/absence of a metallic sheen indicates the presence/absence of *E. coli*.

2.6. Identification of organisms

Non-pink colonies from 37 ℃ MacConkey agar plates (non-coliforms); pink non-metallic sheen colonies from EMB agar plates (non-*E. coli* coliforms); and pink (from 44 ℃ MacConkey agar) non-metallic sheen following sub-culture on EMB agar (non-*E. coli* coliforms) were selected and maintained on nutrient agar at 4 ℃ for microscopic (Gram stain), cultural, motility and biochemical characterization. Minimal biochemical tests namely: indole, citrate, urease and methylred-Voges Praskauer test were done as adapted from Cheesebrough[12].

2.7. Statistical analysis

Bacterial counts were analyzed by one-way Analysis of Variance (ANOVA) using Smith Statistical Package, Version 2.80; designed by David Smith, Pomona College, Claremont, California 9171. Significance of differences was tested at 5% probability (P), that is $P = 0.05$.

3. Results

3.1. Heterotrophic plate counts

Heterotrophic plate count varied from a minimum (4.3 ±1.1) $\times 10^6$ CFU mL^{-1} (Brand E) to a maximum (8.2±1.0) $\times 10^6$ CFU mL^{-1} (Brand D). For all the brands examined (A-E), intra-brand differences in the HPCs of samples were significant ($P<0.05$). While the differences between HPCs of Brand A [Mean±SD =(5.3±1.8)$\times 10^6$ CFU mL^{-1}, (3.2-8.2) $\times 10^6$ CFU mL^{-1}] and those of Brand B [Mean±SD =(6.0± 1.2) $\times 10^6$ CFU mL^{-1} , (3.8-7.1)$\times 10^6$ CFU mL^{-1}] or Brand E [Mean ± SD =(4.3±1.1)$\times 10^6$ CFU mL^{-1} ,(3.5-6.6) $\times 10^6$ CFU mL^{-1}] were insignificant ($P>0.05$), HPCs of Brand A differ significantly ($P<0.05$) from those of Brands C [Mean±SD =(7.5±1.6) $\times 10^6$ CFU mL^{-1} , (5.0-9.2) $\times 10^6$ CFU mL^{-1}] and D [Mean±SD =(8.2 ±1.0)$\times 10^6$ CFU mL^{-1}, (6.1-9.1) $\times 10^6$ CFU mL^{-1}]. HPCs of Brand B differ significantly ($P<0.05$) from those of other brands examined. Although HPCs of Brand C were insignificantly ($P>0.05$; $P=0.24$) different from those of Brand D, they were different significantly ($P<0.05$; $P=0.00$) from those of Brand E. The HPCs of samples of Brand D differ significantly ($P<0.05$; $P = 0.00$) from those of Brand E. Overall, the inter-brand differences in HPCs were significant ($P<0.05$; $P = 0.00$).

3.2. Aerobic spore-former counts

Aerobic spore-former count ranged from a minimum (0.9 ± 0.3)$\times 10^6$ CFU mL^{-1} (Brand E) to a maximum (1.2 ± 0.4) $\times 10^6$ CFU mL^{-1} (Brand D). Intra-brand differences in ASCs were significant ($P<0.05$) for samples of Brand A [Mean± SD =(0.9±0.8)$\times 10^6$ CFU mL^{-1}, (0.4-2.4)$\times 10^6$ CFU mL^{-1}] ($P = 0.00$), Brand B[Mean ± SD =(0.9±0.3) $\times 10^6$ CFU mL^{-1}, (0.4 -2.4)$\times 10^6$ CFU mL^{-1}] ($P = 0.00$) and Brand E [Mean±SD =(1.2 ± 0.4)$\times 10^6$ CFU mL^{-1}, (0.5-1.6)$\times 10^6$ CFU mL^{-1}] ($P = 0.01$); but insignificant ($P>0.05$) for samples of Brand C [Mean±SD =(1.2 ±0.2) $\times 10^6$ CFU mL^{-1}, (1.1-1.5)$\times 10^6$ CFU mL^{-1}] ($P = 0.58$) and Brand D[Mean±SD =(1.2 ± 0.2) $\times 10^6$ CFU mL^{-1}, (0.9 -1.4)$\times 10^6$ CFU mL^{-1}] ($P = 0.28$). ASCs of Brand A are insignificantly ($P>0.05$) different from those of Brand B ($P = 0.87$), Brand C ($P = 0.17$), Brand D ($P = 0.25$) and Brand E ($P = 0.28$). ASCs of Brand B differ significantly ($P<0.05$) from those of Brand C ($P=0.00$), Brand D ($P = 0.01$) and Brand E ($P = 0.04$). ASCs of Brand C differ insignificantly ($P>0.05$) from those of Brand D ($P = 0.54$) and Brand E ($P = 0.72$). ASCs of Brand D were also insignificantly ($P>0.05$) different from those of Brand E ($P = 0.95$). Overall, inter-brand differences in the ASCs were insignificant ($P>0.05$; $P = 0.13$).

3.3. Presumptive "total" and "thermotolerant" coliform tests

"Total" and "Thermotolerant" coliforms were present in

the samples of Brands A to D tested. This is evident by the color change to pink and/or production of gas in MacConkey broth incubated for 48 h at both 37 ℃ (for total) and 44 ℃ (for thermotolerant).

3.3.1. "Total" coliforms counts

"Total" coliforms (observed as pink colonies on the 37 ℃ MacConkey agar) were counted and the statistical comparison of the counts within and between brands were listed as following. The counts ranged from a minimum (1.3 ±0.5)×10^3 CFU mL^{-1} (Brand C) to a maximum (2.5 ± 0.8)× 10^3 CFU mL^{-1} (Brand B). Within-brand differences in TCCs were insignificant (P>0.05) for Brand A [Mean±SD =(2.2±0.9) ×10^3 CFU mL^{-1}, (1.5-3.5)×10^3 CFU mL^{-1}] (P = 0.13), Brand B [Mean±SD =(2.5±0.8)×10^3 CFU mL^{-1}, (2.0-3.5)×10^3 CFU mL^{-1}] (P = 0.53), Brand C [Mean± SD =(1.3±0.5)×10^3 CFU mL^{-1}, (1.0 -2.0)×10^3 CFU mL^{-1}] (P = 0.21) and Brand D [Mean ±SD =(2.2±0.7)×10^3 CFU mL^{-1}, (1.5-2.5)×10^3 CFU mL^{-1}] (P = 0.77). TCCs of Brand A differ from those of Brand C significantly (P<0.05; P = 0.01), but insignificantly (P>0.05) from those of Brand B (P = 0.36) and Brand D (P = 1.00). TCCs of Brand B also differ from those of Brand C significantly (P<0.05; P = 0.00), but insignificantly (P>0.05; P=0.29) from Brand D. TCCs of Brand C differ significantly (P<0.00; P = 0.00) from those of Brand D. Overall, inter-brand differences in TCCs were significant (P<0.05; P = 0.00).

3.3.2. "Thermotolerant" coliform counts

"Thermotolerant" coliforms (pink to dark red colonies on the MacConkey agar incubated at 44℃ for 48 h) were counted and the statistical comparison of the counts within and between the different brands was given as following. The counts ranged from (1.58±0.90) ×10^3 CFU mL^{-1} (Brand B) to (9.50±11.23)×10^3 CFU mL^{-1} (Brand D). Within-brand differences in TTCCs of samples were insignificant (P>0.05) for Brand A [Mean±SD =(4.3±2.7)×10^3 CFU mL^{-1}, (1.0 -7.0) x10^3 CFU mL^{-1}] (P = 0.11) and Brand B [Mean±SD =(1.6± 0.9)×10^3 CFU mL^{-1}, (1.0-3.0)×10^3 CFU mL^{-1}] (P=0.24), but significant (P<0.05) for Brand C[Mean±SD =(7.9± 5.0)×10^3 CFU mL^{-1}, (3.5~16.0)×10^3 CFU mL^{-1}] (P = 0.01) and Brand D[Mean±SD =(9.5±11.2) ×10^3 CFU mL^{-1}, (2.0-33.0)×10^3 CFU mL^{-1}] (P = 0.00). TTCCs of Brand A differ significantly (P<0.05) from those of Brand B (P = 0.00) and Brand C (P = 0.04), but insignificantly (P>0.05; P = 0.13) from Brand D. TTCCs of Brand B differ significantly (P<0.05) from those of Brand C (P = 0.00) and Brand D (P = 0.02); but differences in TTCCs of Brand C were insignificant (P>0.05) when compared with those of Brand D (P = 0.66). Overall, inter-brand differences in TTCCs were significant (P<0.05; P = 0.02).

3.4. Identification of organisms

The non-pink colonies from 37 ℃ MacConkey agar plates (non-coliforms) were identified as species of *Staphylococcus, Pseudomonas, Bacillus*, using cultural, microscopic and biochemical characterization. There was an absence of a metallic sheen on EMB agar following sub-culture of pink colonies from 37 ℃ MacConkey agar plates and pink colonies from 44 ℃ MacConkey agar plates. This signifies the possible absence of both thermotolerant and non-thermotolerant *E. coli* in the entire water samples of the various brands. The pink (from 37 ℃ MacConkey agar plates), non-metallic sheen colonies from EMB agar plates (that is, non-*E. coli* coliforms) and pink (from 44 ℃ MacConkey agar plates), non-metallic sheen following sub-culture on EMB agar (that is, thermotolerant non-*E. coli* coliforms) were identified by cultural, morphological, microscopic and the minimal biochemical tests as *Klebsiella* spp.

4. Discussion

Safe drinking water, as defined by the Guidelines for Drinking Water Quality[7], is one which does not represent any significant risk to health, over a lifetime of consumption, including different sensitivities that may occur between life stages. Thus, water is considered safe when there is no indication of pathogenic organism, although this water may have undesirable taste or odor due to the presence of organic or inorganic substances or chemicals as contaminants.

The very high content of heteroptrophic bacteria in all the brands is indication that the source water is of a low quality. Since HPC test assesses drinking water quality rather than drinking water safety[6,13], low and consistent levels of HPC bacteria in any finished drinking-water add assurance that the treatment or disinfection process was effective. As guidelines for HPC bacteria in drinking water vary slightly between different nations, no numerical limits are set by the WHO[13]. However, it is suggested that they be maintained at the lowest level possible for aesthetic reasons and as a demonstration of treatment sufficiency. There is no clear-cut evidence that heterotrophic bacteria as such pose a public health risk, particularly when they are ingested by healthy people via drinking-water[6].

Counts of aerobic spore-forming bacteria are useful indicators for effective treatment or disinfection of the source water[3]. The rather high spore-former counts obtained are clear signs that the disinfection or treatment of source water from which the sachet water were produced was not effective.

According to the WHO Guidelines for Drinking Water Quality[7], safe drinking water should not contain "total" coliforms, *E. coli* or any "thermotolerant" coliforms in a 100 mL water sample. However, because total coliforms of non-fecal origin can exist in natural waters, their presence can occasionally be tolerated in unpiped and untreated water, in the absence of more specific index parameters[3]. The presence of both total and thermotolerant coliforms in all the water samples investigated is indication that they are unsafe for human consumption. The presence of coliforms is usually taken as clear evidence of an inadequate treatment

of the source water[3]. The demonstrated absence of *E. coli* in the sachet water samples indicates that the observed coliform contamination was of non-fecal origin. This is because *E. coli* is always present in the feces of humans, other mammals, and birds in large numbers, and rarely, if ever, in water or soil in temperate climates that have not been subject to fecal pollution, even though re-growth can occur in hot environments[14].

The presence of *Klebsiella*, a thermotolerant coliform, in the water samples is evidence that subtropical or tropical waters may contain thermotolerant coliforms other than *E. coli*, and this does not necessarily suggest fecal contamination by humans. Thermotolerant coliforms other than *E. coli* may originate from organically-enriched water such as industrial effluents or from decaying plant materials and soil[3].

The detection of non-coliform bacteria in the water samples was not unexpected since many organisms of environmental origin, that are not normally associated with the gastrointestinal system, such as the protozoan *Naegleria fowleri*, *Pseudomonas*, *Klebsiella*, *Legionella*, *Acinetobacter*, *Aeromonas*, and *Aspergillus* and non-tuberculous Mycobacterium, can be found in drinking water [7]. Although there is no evidence that these microorganisms represent a health concern through water consumption by the general population, including most patients in health care facilities, additional processing may be required to ensure safety for consumption by severely immune-suppressed persons, such as those with neutrophil counts below 500 per mL[13].

This study has observed that all the brands of sachet water sold at the time of collection for the study did not meet the acceptable criteria for a safe drinking water set out by the World Health Organization. Although the source water type used to package as sachet is not indicated by the producers, the quality of the source water is by no means poor due to the demonstrated contamination by total and non-fecal thermotolerant coliforms, an indication of inadequate treatment. Caution should be taken by boiling the water and allowing it to cool before consumption.

Conflict of interest statement

The authors declare no competing interest with the present work.

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