The effects of storage on the bacteriological quality of sachet water produced, sold and consumed within Owerri Metropolis, Imo State, Nigeria

Valentine N Unegbu¹, Ndubuisi O Nwachukwu, Emmanuel N Ugbo, and Anthony C Ekennia

¹Department of Microbiology and Brewing, Nnamdi Azikiwe University Awka, Nigeria.
²Department of Microbiology, Abia State University Uturu, Abia State, Nigeria
³Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria
⁴Department of Chemistry/Biochemistry/Molecular Biology, Federal University Ndufu-Alike, Ikwo, Ebonyi State Nigeria

ABSTRACT

The sale and consumption of sachet water is common in Owerri metropolis because the consumers believed it was safer than tap or borehole water. The aim of this study was to assess the effects of storage on the bacteriological quality of sachet water produced, sold and consumed within Owerri metropolis, Imo State, Nigeria. Fifteen sachet water brands were investigated for a period of four months. They were collected within 24 hours of production and stored at ambient temperature for four months. Samples were taken on monthly basis for enumeration of total aerobic heterotrophic bacteria and indicator organisms using APHA and WHO analytical methods. Bacteriological analysis showed that Clets brand had the highest Total heterotrophic bacterial count of $5.7 \times 10^9$ cfu/ml, followed by Elevated and Crystal while Rica no had the least count of $3.2 \times 10^4$ cfu/ml. Total heterotrophic bacteria and total coliform count decreased gradually in all brands throughout this period. Escherichia coli was isolated in three brands. Results from the research indicates that 60% of the brands analyzed met the WHO guideline limit for drinking when stored at ambient temperature within eight weeks period. However, storage beyond this period led to diminished potability of sachet water.

Keywords: Effects, Storage, Bacteriological Quality, Sachet Water, Owerri, Nigeria


INTRODUCTION

Sachet water is any water that is in a sealed plastic and distributed or offered for sale and is intended for human consumption. Sachet water consumption has been steadily growing in Nigeria for the past 10 years. It is one of the most dynamic sectors of all the food and beverage industry. Consumption in Nigeria increases by an average of 12% each year [1]. Consumers may have various reasons for purchasing sachet drinking-water, such as affordability, convenience, or fashion, but for many consumers, safety and potential health benefits are important considerations because they believe that sachet water is safer than borehole or tap water. Some microorganisms, which are normally of little or no public health significance, may grow to higher levels in sachet water [2]. A recent study [3] after randomly sampling sachet water in Ibadan, Oyo State between 2004 to 2005 found them to be bacteriologically very poor, indicating presence of Coliforms and other microbes. In addition, the quality of sachet water can also substantially vary among brands as well as with time and with different production runs depending on its source,
treatment technology, manufacturing operation, packaging material, and shelf-life before use [1]. Although, sachet water should have a shelf life of 3 months unopened, most sachet water companies label fail to indicate this. Moreover, sachet water may be in distribution and storage conditions for several weeks which may adversely affect its quality [4]. Although the microbial quantity levels in processed sachet water are often initially low, they can still decrease to lower levels during storage [5]. This decrease may be due to de-oxygenation of the sachet water or chlorine decay during storage [6]. Growth of microorganisms can occur via carriers such as introduced flakes of human skin, particularly in non-ozonated and non-carbonated water [7]. Tap water in Owerri metropolis is generally inadequate and not considered safe for drinking because of inadequate treatment and post treatment contamination in distribution systems. This situation, linked with the prevalence of waterborne diseases in the city [8], has caused the business of sachet water to flourish, as many people believe that sachet water is safe. There are about 28 brands of sachet drinking water produced and sold in Owerri metropolis. There are no reported studies on the effects of storage on the bacteriological qualities of sachet water produced, sold and consumed in Owerri metropolis of Imo State. The main objective of this study was therefore to examine the effects of storage on the bacteriological qualities of sachet water available in Owerri using total Coliform bacteria as indicators of the possible presence of microbial pathogens.

**MATERIALS AND METHODS**

**Study area**

Owerri, the capital of Imo state is a metropolitan city. It is located in South East Nigeria between longitude 5.485°N and latitude 7.035°E. There are three Local Government Areas making up Owerri Zone; Owerri Municipal, Owerri West and Owerri North. It has a high population density of 401,873 (2006 census report) due to governmental presence and booming business activities. It is approximately 40 square miles (100km²) in area. Owerri was selected for this study because of scarcity of water and extensive use of the ‘sachet water’. This has resulted in residents buying sachet water for drinking purposes and domestic uses. With the high demand for this water, small scale industries have sprung up producing it to meet the demands.

**Sample Collection**

Fifteen brands of sachet water with NAFDAC certification were randomly obtained from different parts of Owerri metropolis, in bags within 24 hours of production and stored in a room at ambient temperature. Sachet samples were collected for bacteriological analysis. These were done immediately after collection and subsequently on a monthly basis. The sachet water samples were also checked for leakages on a monthly interval. Sampling was done between November, 2010 and April, 2011.

**Sample inoculation**

The assessment of the various sachet water samples for microbiological quality was done using the culture method applying the spread plate inoculation technique, after ten-fold serial dilution[9]. After serial dilution, three bacterial groups were determined using different culture media. These include total heterotrophic bacteria (THB) using Tryptone Soy Agar, Coliform bacteria (CB) using McConkey Agar and Fecal coliforms using Eosin Methylene Blue (EMB) Agar. The Membrane filter technique was carried out to cross-check the values obtained by the inoculation methods (Most Probable Number). Potential pathogenic bacteria were determined on Blood Agar culture media and coagulase tests performed on the isolates.

**Statistical analysis**

The statistical analysis employed were percentage, range, mean, standard error of mean[10] and Analysis of variance (ANOVA). The parameters were also correlated against the organisms to determine their relationship with each other. This was done using (Ms Excel and SPSS software).

**RESULTS**

The bacterial species isolated from the water samples include *Staphylococcus aureus* (28.6%), *Bacillus spp* (35.7%), *Escherichia coli* (21.4%) and *Staphylococcus epidermidis* (14.3%)(Table 1).
The counts of total aerobic heterotrophic bacteria ranged from 0.0 to $5.4 \times 10^1 \text{cfu/ml}$ while the total coliform ranged from 0.0 to $0.5 \times 10^1 \text{cfu/ml}$. A gradual decrease in total aerobic heterotrophic bacterial counts and total coliform were observed in all the brands tested throughout the investigation period. Total coliform and fecal coliform bacteria were detected in 47% (S2, S4, S8, S10, S11, S12 and S13) of the brands of sachet water analyzed within the first eight weeks. However, they were not detected after this period. The counts of Enterococcus faecalis remained 0.0 cfu/ml in all brands tested throughout the investigation period.

**DISCUSSION**

Results of the investigation indicate that the population of total heterotrophic bacteria and total coliforms decreased with storage time. This may be attributed to the fact that as storage progresses and metabolic wastes accumulates, coliforms and other microbes were eliminated from the environment which was becoming competitive. The modified growth environment (i.e. deoxygenated sachet water) for the microorganisms encouraged a kind of succession as most of the microbes were eliminated. The general effect of these activities is invariably reflected on the physical attributes of the water samples particularly on parameters such as the taste, color and odor. Among the bacteria isolated in this study was *Escherichia coli* which are regarded as the most sensitive indicator of fecal pollution. Its presence in the sachet water samples is of a major health concern and calls for remedial attention. The counts of total aerobic heterotrophic bacteria ranged from 0.0 to $4.8 \times 10^1 \text{cfu/ml}$. This was in agreement with [11] who observed that varying levels of microbial contamination were recorded in samples from different sampling locations in Ibadan. A gradual decrease in total aerobic heterotrophic bacterial counts was observed in all the brands tested up to week 16, a growth pattern typical of microorganisms growing in closed system [12]. The result of total aerobic heterotrophic bacterial count obtained in this study contrasted with previous findings [6]. In a study involving the determination of the microbiological safety of bottled water stored for 30 days, it was reported [6] that the total aerobic heterotrophic bacterial counts increased considerably to a level detrimental to human health when the water was stored at room temperature. Total coliform and fecal coliform bacteria were detected in 47% of the brands of sachet water analyzed within the first eight weeks. However, they were not detected after this period. Indicator organisms loose viability in freshwater environment with time [13]. The counts of Enterococci faecalis remained 0.0 cfu/ml in all brands tested throughout the investigation period. Total coliforms are widely used as indicators of the general sanitary quality of treated drinking water while fecal coliforms give a much closer indication of fecal pollution [14].
**Table 2: Total Heterotrophic Bacteria and Bacterial Count (CFU/mL)**

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*THB = total heterotrophic bacteria, TC = TOTAL COLIFORM, FC = FAECAL COLIFORM, FS = FECAL STREPTOCOCCI, EC = ESCHERICHIA COLI CRYSTAL, 2 RICANO, 3 IROKO, 4 MARLYN, 5 EMMA, 6 AGAD, 7 CLETS, 8 CHIPA, 9 TALENT, 10 IZZY, 11 ANABEBS, 12 ZOPOUE, 13 ENODOZ, 14 COSYRAN, 15 ELEVATED*
WHO limit is that none should be detected. Besides, total and fecal coliform counts also decreased in the sachet water samples up to 16th week of storage. Reduction in the bacterial load of stored water has been attributed to depletion of nutrients due to microbial activities (i.e. mesophilic microbes that grow at moderate temperature) and sedimentation of suspended materials [15]. Among the criteria for indicator organisms, it was stated [16] that indicator bacterium should not reproduce in the contaminated water and produce an inflated value. This justified the choice of coliform bacteria by WHO as indicator organisms [13].

More so, it seems likely that the effects of storage are most directly related to the initial quality of both the finished water and the container [17]. There were significant elimination of coliform bacteria in the sachet waters under storage though the effects of presence of residual chlorine on the survival of fecal coliforms were not statistically different. This proved that the increase in coliform counts was actually due to storage. This was in agreement with the works of [15] who posited that the duration of storage affects the microbiological quality of stored water.

Coliforms, defined as aerobic and facultative anaerobes, gram negative, non-spore forming, rod-shaped bacteria that ferments lactose with gas formation within 48hrs at 35°C are generally used as indicators of water portability [18]. They include Escherichia coli, Klebsiella pneumoniae and Enterobacter aerogenes. Coliform bacteria may not cause disease, but can be indicators of pathogenic organisms that cause diseases [18]. The latter could cause intestinal infections, dysentery, hepatitis, typhoid fever, cholera and other illnesses. The presence of indicator organisms indicated that the water was contaminated by potentially dangerous fecal matter and hence their absence denotes in general that water is safe. Although coliform organisms may not always be directly related to the presence of fecal contamination or pathogens in drinking water, the coliform test is still useful for monitoring the microbial quality of drinking water [19]. Quist [20] confirmed that only E. coli is considered as a specific and reliable indicator of fecal pollution of water; since the more general test for Fecal Coliforms (FC) also detects thermotolerant non-fecal coliform bacteria.

Other important pathogens identified in the samples were Staphylococcus epidermidis, Staphylococcus aureus and Bacillus spp. These organisms have been variously implicated in gastro-intestinal disorders [21]. This confirms the result of an earlier study [4], indicating that most sachet water sold in Ibadan, Nigeria, are heavily contaminated with microbial pathogens. The sources of these contaminations could be attributed to careless handling by workers during sachet water production and also to the deliberate and indiscriminate littering of human and animal waste in adjoining bushes to the sites used for sachet water production.

Only six sachet water brands, S2, S6, S8, S11, S13 and S14 were found to be free from all pathogens indicating a very high microbial quality. Following biochemical tests, Staphylococcus aureus was identified in the sachet water brands, S1, S5, S9 and S10. E. coli were identified in S1, S9 and S15. Staphylococcus epidermidis was discovered in S4 and S7. Lastly, Bacillus spp were discovered in S5, S4, S5, S9 and S12. These bacteria have been implicated in water related diseases [22]. The presence of Bacillus species, Staphylococcus aureus and Staphylococcus epidermidis in the sachet water samples could be as a result of contamination from poor staff handling during processing of the water samples. Bacillus species produces enterotoxin which could be toxic when ingested into the body. They are also spore formers and are able to resist chemical and physical agent used during sachet water production. That explained why they were more prevalent, 35.7% in the sachet waters samples.

The presence of Escherichia coli in this study, a fecal contaminant of human origin, is a cause for concern as these sachet water brands are not safe for human consumption [23]. Staphylococcus epidermidis has been implicated in urinary tract infection. Presence of these bacteria in water may be unnoticed, even in transparent packaged water and the presence of these microorganisms may pose a potential risk to consumers. The consumption of such contaminated water may facilitate the widespread of infections and can lead to outbreak of epidemic [24].

CONCLUSION

All sachet water samples analyzed showed 100 cfu/ml for total aerobic heterotrophic bacteria which is below the WHO limit. Total and fecal coliform appeared in 47% of sachet water samples analyzed within the first eight weeks and died off.

Staphylococcus aureus and Bacillus spp. These organisms have been variously implicated in gastro-intestinal disorders [21]. This confirms the result of an earlier study [4], indicating that most sachet water sold in Ibadan, Nigeria, are heavily contaminated with microbial pathogens. The sources of these contaminations could be attributed to careless handling by workers during sachet water production and also to the deliberate and indiscriminate littering of human and animal waste in adjoining bushes to the sites used for sachet water production.

Only six sachet water brands, S2, S6, S8, S11, S13 and S14 were found to be free from all pathogens indicating a very high microbial quality. Following biochemical tests, Staphylococcus aureus was identified in the sachet water brands, S1, S5, S9 and S10. E. coli were identified in S1, S9 and S15. Staphylococcus epidermidis was discovered in S4 and S7. Lastly, Bacillus spp were discovered in S5, S4, S5, S9 and S12. These bacteria have been implicated in water related diseases [22]. The presence of Bacillus species, Staphylococcus aureus and Staphylococcus epidermidis in the sachet water samples could be as a result of contamination from poor staff handling during processing of the water samples. Bacillus species produces enterotoxin which could be toxic when ingested into the body. They are also spore formers and are able to resist chemical and physical agent used during sachet water production. That explained why they were more prevalent, 35.7% in the sachet waters samples.

The presence of Escherichia coli in this study, a fecal contaminant of human origin, is a cause for concern as these sachet water brands are not safe for human consumption [23]. Staphylococcus epidermidis has been implicated in urinary tract infection. Presence of these bacteria in water may be unnoticed, even in transparent packaged water and the presence of these microorganisms may pose a potential risk to consumers. The consumption of such contaminated water may facilitate the widespread of infections and can lead to outbreak of epidemic [24].

CONCLUSION

All sachet water samples analyzed showed 100 cfu/ml for total aerobic heterotrophic bacteria which is below the WHO limit. Total and fecal coliform appeared in 47% of sachet water samples analyzed within the first eight weeks and died off.
No fecal *Enterococcus* was detected throughout the investigation period while *Escherichia coli* were detected in only three brands.

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**Conflict of interest:**
We have no conflict of interest.

**REFERENCES**


