Microbiological assessment of sachet water “pure water” from five regions in Ghana [version 1; referees: awaiting peer review]

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Abstract

Background: Sachet water, popularly known as “pure water” has become an invaluable entity in most Ghanaian households. Despite its importance, there is no extensive nationwide investigations on its wholesomeness for consumption. The aim of this study was to determine the microbiological quality of 41 brands of sachet water sampled in 16 districts across 5 regions in Ghana.

Methods: The samples were analyzed for the presence of total and fecal coliform (Escherichia coli) using the Colilert*-18 Test Kit.

Results: Majority of the samples (56.09%) were excellent, 4.87% satisfactory and 14.63% suspicious. Ten samples (24.4%) were unsatisfactory. For the degree of fecal contamination, (85.56%) were satisfactory, four (9.76%) were suspicious, and two others (4.88%) were unsatisfactory. The contaminations observed could be attributed to poor sanitary conditions (during and/or after production) and failure of some production facilities to adhere to standard manufacturing practices.

Conclusion: Our data suggest that microbiological quality sachet water from some sources have not yet attained levels that make it absolutely pure and wholesome for consumption in many areas.

Keywords

microbiology, sachet water, coliforms, E. coli, most probable number (MPN)
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Abbreviations
Ghana Water Company Limited (GWCL)
Most Probable Number (MPN)
Hazard Analysis Critical Control Point (HACCP)

Introduction
The occurrence of packaging water into sachets popularly referred to as “pure water” is one of the most lucrative business ventures across Ghana. This business has gained much popularity and acceptance among the Ghanaian populace particularly because in the past drinking water was sold in cups and plastic bags hand-tied at one end; a practice which was faced with a lot of sanitary issues. Currently, the exact numbers of sachet water companies is unknown, as new ones spring up almost daily. There are more unregistered producers than registered ones, with the current estimate of registered producers reaching 3,000.

“Pure water” contains 500ml of water in a clear plastic bag that is electrically heated and sealed at opposite ends. Water used for “pure water” is mostly obtained from ground water, springs and potable pipe-borne water. Prior to packaging, the water goes through a number of treatment processes, mainly filtration, in an attempt to make it purer and safer for consumption. Most households and families depended greatly on tap water from the Ghana Water Company Limited (GWCL) for drinking and household activities including cooking. However, with the frequent shortages associated with the supply of potable water across the country, and the questionable quality of the water supplied, many households and families in Ghana have resorted to “pure water” mostly for drinking and cooking purposes.

According to the WHO Guidelines, water for drinking must not present any significant risk to the health of the consumer over a lifetime of consumption. Neither should the consumption of such water present different sensitivities that may arise between life stages. Invariably, safe drinking water should be colorless and tasteless, free from harmful chemicals as well as other suspended materials and most importantly should be devoid of disease-causing organisms. Among many other concerns, the possibility of drinking water being the source of disease-causing organisms and related illnesses has been a huge hurdle to overcome, especially in parts of developing countries where availability, accessibility and affordability of potable and safe drinking water continues to be a challenge.

Although the introduction of sachet water was intended to provide affordable and readily available safe drinking water for Ghanaians, investigations on its quality and wholesomeness for consumption have revealed considerable gaps especially with regards to microbial quality. Ngemepele and Hawkins (2015) analyzed the microbial and physicochemical properties of sachet water sold in Obuasi in the Ashanti region and found total coliform levels exceeding the WHO and the Ghana Standards Authority’s accepted levels for drinking water. In addition, fecal coliform was also detected in one of the samples.

In a study to investigate the bacteriological quality of sachet water produced and sold in Teshie-Nungua, a suburb known for perennial water shortages, Addo et al. (2009) reported sachet water sampled with suspicious microbial contaminations based on the most probable number (MPN) values. Fecal coliforms were detected in several samples while some of the samples were also contaminated with Escherichia coli. Given the vast number of people that rely on sachet water for their drinking needs, it is imperative that its quality is of the highest standard to avert any future waterborne outbreaks related to its consumption.

The quality of packaged water assessed in Nigeria showed some levels of microbial contaminations. The results of the study indicated that bottled water has lower microbial load than sachet water. E. coli, Clostridium perfringens spore and fecal Streptococcus were the most common isolated microbe from the packaged water. A similar study on the quality of potable water in Benin showed that some of the drinking water tested had microbiological pollution exceeding the approved levels, hence making the drinking water not wholesome for consumption. Although packaged water is an improved source of drinking water, it is not totally free from microbial contaminations; hence the need for enhanced monitoring strategies to ensure that packaged water is always safe for human consumption.

In this study we sought to examine the microbiological quality of sachet water sampled across Ghana, with primary focus on fecal contaminations. Although there have been several similar studies in the country, our study has a wider geographic coverage (with samples from five regions out of the ten regions in Ghana) including mostly peri-urban and rural settings compared to previous studies where mostly urban settings were considered.

Methods
Study sites and design
Sachet water samples were collected from 41 selected communities within 16 districts in 5 out of the 10 regions in Ghana. The communities were selected from the southern/coastal belt (comprising communities in Central and Volta Regions), the middle belt (comprising communities in the Ashanti and Eastern Regions) and the northern belt (comprising communities in the Northern Region) of Ghana. These communities gave a fair representation of rural, urban as well as peri-urban communities in Ghana thus giving the study a wider geographic coverage (Figure 1 and Table 1). The selected communities comprised of towns and villages where sachet water is sold by retail shops and hawkers. The study was designed to evaluate the microbial quality at the point of consumption of the most commonly sold sachet water.
catalog number WP2001-8 from IDEXX Laboratories, Inc., Westbrook, Maine, USA) following the manufacturer’s protocol. A set of quality controls were run for the lot of sachet water sampled within each region (Figure 2). Briefly, ATCC strains of Escherichia coli (ATCC 25922), Klebsiella pneumonia (ATCC 31488) and Pseudomonas aeruginosa (ATCC 10145) were each transferred with sterile loops into three sterile containers coated with sodium thiosulfate and each filled with 100 ml of sterile water. A similar set up with deionized water and Colilert*-18 substrate only served as negative control. After 18 hours of incubation at 35°C, each Quanti-Tray was compared with a Quanti-Tray comparator for the presence or absence of total coliform. E. coli enumeration was performed by observing wells under a 6-watt, 365-nm UV light in the dark. The results were read following the interpretations in Table 2.

The number of positive wells in each Quanti-Tray was counted and the corresponding Most Probable Number (MPN) was obtained from the MPN table provided by the manufacturer. With reference to the work done by Addo et al. (2009), the WHO [4] and U.S. FDA standards, the MPN values were used to categorize the samples.

### Statistical analysis
Proportions of sachet water samples that were positive for E. coli and total coliform were compared across the five regions using Chi-square test with Yates correction for continuity using Microsoft excel (Microsoft Office 2013). The Marascuilo’s test of equality of several proportions was then used to compare pairs of proportions where the Chi-square test rejected the null hypothesis.

### Results
#### Sachet water microbiological quality
One branded sachet water with the most patronage was collected in triplicate from each of the 41 communities. Twenty-four (58.5%) samples tested positive for the presence of total coliform with 7 (17.1%) of the positive samples positive for fecal E. coli. No E. coli was detected in the Central Region samples. The highest MPN for total coliform and fecal E. coli was estimated at 1299.7 and 27.5 respectively with samples from the Volta region having more positive total coliforms and E. coli compared to other regions (Table 1). However, results from Chi-square and Marascuilo test showed that, the proportion of sampled sachet water that were positive for E. coli was significantly different across the five regions while the proportion that were positive for total coliform did not differ significantly across the five regions (Figure 3 and Supplementary File 1; Supplementary Table 1 and Supplementary Table 2).

The results indicated that 11 out of the 41 (26.83%) sachet water brands sampled had coliforms exceeding the World Health Organization (WHO) and U.S. Food and Drugs Administration (U.S. FDA) approved limits of 9.2 MPN. The coliform MPN of the samples was used to grade the samples as Excellent (<2MPN/100), Satisfactory (2–3 MPN/100ml), Suspicious (4–10 MPN/100ml) and Unsatisfactory (>10MPN/100ml) (Figure 4). The majority of the samples (56.09%) were excellent.

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**Table 1. Quanti-Tray result interpretation for presence/absence of total coliform and Escherichia coli.**

<table>
<thead>
<tr>
<th>Appearance</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow less than comparator</td>
<td>Negative for total coliform and E. coli</td>
</tr>
<tr>
<td>Yellow equal to or greater than comparator</td>
<td>Positive for total coliform</td>
</tr>
<tr>
<td>Yellow and fluorescence equal to or greater than</td>
<td>Positive for E. coli</td>
</tr>
</tbody>
</table>

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**Sample collection**
A total of 41 branded sachet water samples; one from each community (from different sachet water manufacturing companies), were randomly purchased from vendors on the streets. For each brand of sachet water, we tested 3 independent samples to correct for bias in sampling. The choice of purchase was based on the most commonly patronized sachet water by the inhabitants of a given community. The most commonly patronized sachet water was determined after interviewing a number of people in the community. Samples were stored on ice and transported (as described by Johnson et al., [7]) to central point laboratories within each region where analysis were carried out.

**Sample inoculation and incubation**
We analyzed samples for the presence of total as well as fecal coliform (E. coli) using the Colilert*-18 Test Kit (with...
Table 2. Total coliform and *Escherichia coli* contamination of sachet water from 41 communities in Ghana.

<table>
<thead>
<tr>
<th>Region</th>
<th>District</th>
<th>Community</th>
<th>Total Coliforms MPN/100mL</th>
<th>Fecal <em>E. coli</em> MPN/100mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashanti</td>
<td>SEKYERE WEST</td>
<td>WORASO</td>
<td>325.5</td>
<td>1</td>
</tr>
<tr>
<td>Ashanti</td>
<td>SEKYERE WEST</td>
<td>APAAH</td>
<td>2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ashanti</td>
<td>SEKYERE WEST</td>
<td>ADIDWAN</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ashanti</td>
<td>SEKYERE WEST</td>
<td>BUNUSU</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ashanti</td>
<td>SEKYERE WEST</td>
<td>NINTING</td>
<td>2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ashanti</td>
<td>SEKYERE WEST</td>
<td>MPRIM</td>
<td>24.3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ashanti</td>
<td>SEKYERE EAST</td>
<td>NAAMA</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ashanti</td>
<td>EJURA SEKYEDUMASE</td>
<td>KOBRTI</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ashanti</td>
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<td>AFRAAMSO</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ashanti</td>
<td>EJURA SEKYEDUMASE</td>
<td>BABASO</td>
<td>325.5</td>
<td>27.5</td>
</tr>
<tr>
<td>Ashanti</td>
<td>EJURA SEKYEDUMASE</td>
<td>MBANAA</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ashanti</td>
<td>SEKYERE EAST</td>
<td>OGUAA</td>
<td>1299.7</td>
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</tr>
<tr>
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<td>EJURA SEKYEDUMASE</td>
<td>KASEI</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ashanti</td>
<td>EJURA SEKYEDUMASE</td>
<td>HIWAOANWU</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ashanti</td>
<td>SEKYERE EAST</td>
<td>ABOTANSO</td>
<td>155.3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Central</td>
<td>AWUTU-EFFUTU-SENYA</td>
<td>OSIMO 1</td>
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<td>&lt;1</td>
</tr>
<tr>
<td>Central</td>
<td>GOMOA</td>
<td>GOMOA LOME</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Eastern</td>
<td>AKWAPIM SOUTH</td>
<td>FOTOBIX</td>
<td>3.1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Eastern</td>
<td>AKWAPIM SOUTH</td>
<td>OTU KWADJO</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Eastern</td>
<td>YILO KROBO</td>
<td>TROM</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Figure 2. Total coliform and *Escherichia coli* enumeration: Quality control test for (A) *Pseudomonas aeruginosa* (ATCC 10145), (B) *Klebsiella pneumonia* (ATCC 31488) and (C) *Escherichia coli* (ATCC 25922) to confirm negative result for both total coliform and fecal *E. coli*, positive results for total coliforms and positive results for fecal *E. coli* respectively. The positive wells for *E. coli* were observed under a 6-watt, 365-nm UV light in the dark.
with 4.87% and 14.63% as satisfactory and suspicious respectively. 10 samples (24.4%) however, were unsatisfactory (Figure 4).

To determine the degree of fecal contamination, WHO and U.S. FDA standards were used to sort the samples on the total fecal E. coli. The samples were graded Excellent (0 MPN/100ml), Suspicious (1–2 MPN/100ml) and Unsatisfactory (>2 MPN/100ml). The majority of the samples (85.56%) were satisfactory, however, 4 samples (9.76%) were suspicious and 2 others (4.88%) were unsatisfactory (Figure 5).
Figure 4. Grading of sampled water based on total coliforms. Classification of sampled water for total coliform contamination was based on the total coliform MPN according to Addo et al. (2009), WHO (2011) and U.S. FDA standard for water purity. Approximately fifty six percent (56.09%) were excellent, 4.87% and 14.63% were satisfactory and suspicious respectively. The remaining samples (24.41%) were unsatisfactory.

Figure 5. Grading of water samples based on total fecal Escherichia coli. Classification of sampled water for Escherichia coli contamination was based on the fecal E. coli MPN according to Addo et al. (2009), WHO (2011) and U.S. FDA standard for water purity. Majority of the samples (85.56%) were satisfactory, 9.76% were suspicious and 4.88% were unsatisfactory.

Discussion

The study of the microbiological quality of sachet water has been a topic of interest to researchers since mid-1990s. Nonetheless, publications on the microbial content of sachet water are still scarce, with only a few studies having a large sample size and are not representative of a nationwide study. Our study collected branded sachet water samples from 41 communities within 5 regions in Ghana to provide a better representation of the microbial water quality across the nation.

Based on the WHO and U.S. FDA standards, analytical samples should not have total coliform more than 9.2 MPN/100ml of water and must be free from fecal E. coli (thus 0 MPN/100ml). Our study revealed that 26.83% of our samples tested did not meet the above requirement as far as total coliform is concerned while 14.63% of the samples tested positive for fecal E. coli. The poor sanitary conditions could be a probable contributor to the above observations as well as failure of some production facilities to adhere to good sanitation practices. However, it is very important to further investigate the source of these contaminations.

Ideally, treated water should not have any coliform, however, several studies have indicated the presence of microbial contamination in sachet water from different parts of the country. Although the sachet water samples collected for this study were presumptively treated by the manufacturer, 58.5% of the samples tested positive for total coliforms which is indicative of the risk associated with their consumption. The presence of fecal E. coli in these samples point to fecal contamination. The trend observed in the contamination across the regions suggests more of a generalized rather than a centralized contamination. These microbial contaminants could have been either introduced during the manufacturing or post-manufacturing processes. Should these contaminants be as a result of gaps in the manufacturing processes, then it raises a lot of concern about the efficiency of the treatment processes involved in making the water wholesome for consumption.

We recommend that further studies be carried to investigate the efficiency of the treatment processes. Ngmekpele & Hawkins (2015) attributed the failure of most sachet water companies to adhere to the Hazard Analysis Critical Control Point (HACCP) system as another cause of contamination. The HACCP seeks to help check and eliminate the various levels of contamination that may occur in the sachet water production processes. Therefore, it is very essential that the manufacturing of sachet water be closely monitored by the regulatory bodies in charge to ensure strict adherence to the standard manufacturing procedures. Addo et al. (2009) randomly collected 30 sachets of 10 different brands of “pure water” from Teshie and Nungua, in the Greater Accra Region of Ghana. From their analysis none of the brands sampled met the WHO standards.
for drinking water based on their microbial contamination. Also, other related studies implicated both vendors and poor production practices as the source of the microbial contamination. To effectively identify the sources of contamination of sachet water, a progressive study should be done with different brands of sachet water following them from production to consumers.

The strengths of this study include (1) obtaining random samples from each of the five regions, (2) accounting for overestimation of statistical significance for small samples using Yates correction for continuity in the Chi-squared test, and (3) the use of fisher’s exact test where cell counts are less than five.

The study identified microbial contamination; most alarming being contamination with *E. coli* in sachet water sampled in five regions across Ghana. As far as microbiological quality is concerned, sachet water has not yet attained levels that make it absolutely pure and wholesome for consumption.

**Data availability**

Underlying data for this study is available from Open Science Framework: Dataset 1. Microbiological assessment of sachet water “pure water” from five regions in Ghana. http://doi.org/10.17605/OSF.IO/J968K under a CC0 1.0 Universal license

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**Competing interests**

No competing interests were disclosed.

**Grant information**

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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**Supplementary material**

**Supplementary File 1** – file containing the following Supplementary Tables:

Click here to access the data.

Supplementary Table 1: Chi-Square Test

Supplementary Table 2: Marasculo test for *Escherichia coli* positive proportions

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**References**

10. Dodoo DK, Quagrainie EK, Okai-Sam F, et al.: Quality of “sachet” waters in the


Reference Source