Microbiological assessment of sachet water “pure water” from five regions in Ghana [version 1; peer review: 2 approved with reservations]

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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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Author roles: Mosi L: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Resources, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; Adadey SM: Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; Sowah SA: Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; Yeboah C: Conceptualization, Funding Acquisition, Investigation, Methodology, Resources, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by the African Academy of Science (AAS) through a DELTAS African grant to GA [DEL-15-007] and by the BCAN Consult. The DELTAS Africa Initiative is an independent funding scheme of the AAS’s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa’s Development Planning and Coordinating Agency (NEPAD Agency), with funding from the Wellcome Trust to GA [107755/Z/15/Z]. Additional support was obtained from The South Africa Medical Research Council; self-initiated research to A.W.; and a National Institute of Health Grant to A.W. [U01 HG009716-01]. S.M.A. is supported by WACCBIP DELTAS PhD fellowship. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Mosi L, Adadey SM, Sowah SA and Yeboah C. Microbiological assessment of sachet water “pure water” from five regions in Ghana [version 1; peer review: 2 approved with reservations] AAS Open Research 2018, 1:12 (https://doi.org/10.12688/aasopenres.12837.1)

First published: 18 Apr 2018, 1:12 (https://doi.org/10.12688/aasopenres.12837.1)
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.

“The 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. Ngmekpele 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 contamination. 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.
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 (https://www.idexx.com/pdf/en_us/water/qt97mpntable.pdf) 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 coliforms 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 tests 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/100ml), Satisfactory (2–3 MPN/100ml), Suspicious (4–10 MPN/100ml) and Unsatisfactory (>10MPN/100ml) (Figure 4) [15]. The majority of the samples (56.09%) were excellent, and the corresponding Most Probable Number (MPN) was obtained from the MPN table (https://www.idexx.com/pdf/en_us/water/qt97mpntable.pdf) 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.

### 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 purchased sachet water was determined after interviewing a number of people the community. The 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 catalog number WP2001-8 from IDEXX Laboratories, Inc., Westbrook, Maine, USA) following the manufacturer’s protocol.

### 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 <em>E. coli</em></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 comparator</td>
<td>Positive for <em>E. coli</em></td>
</tr>
</tbody>
</table>

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**Figure 1.** Map of Ghana showing the sites of sample collection.

**Table 1.** Quanti-Tray result interpretation for presence/absence of total coliform and *Escherichia coli*.
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 E. coli MPN/100mL</th>
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</thead>
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<tr>
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</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.
<table>
<thead>
<tr>
<th>Region</th>
<th>District</th>
<th>Community</th>
<th>Total Coliforms MPN/100mL</th>
<th>Fecal E. coli MPN/100mL</th>
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</table>

**Figure 3. Regional analysis of the samples.** Of the 41 sachet water sampled, 58.5% tested positive for the presence of total coliforms while 17.1% of the total coliform-positive samples also tested positive for fecal *Escherichia coli*.

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 sample based 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\(^\text{10}\) 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 \textit{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\(^\text{16}\) under a CC0 1.0 Universal license

**Competing interests**

No competing interests were disclosed.

**Grant information**

This work was supported by the African Academy of Science (AAS) through a DELTAS African grant to GA [DEL-15-007] and by the BCAN Consult. The DELTAS Africa Initiative is an independent funding scheme of the AAS’s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa’s Development Planning and Coordinating Agency (NEPAD Agency), with funding from the Wellcome Trust to GA [107755/Z/15/Z]. Additional support was obtained from The South Africa Medical Research Council; self-initiated research to A.W.; and a National Institute of Health Grant to A.W. [U01 HG009716-01]. S.M.A. is supported by WACCBIP DELTAS PhD fellowship.

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 \textit{Escherichia coli} positive proportions

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**References**


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


Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 03 May 2018

https://doi.org/10.21956/aasopenres.13903.r26349

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Department of Microbiology and Parasitology, Faculty of Science, Research Foundation for Tropical Diseases and Environment (REFOTDE), Parasites and Vector Biology research unit (PAVBRU), University of Buea, Buea, Cameroon

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Findings presented in this paper are of great public health importance. Mosi et al assessed the microbial content of different brands of sachet water produced, sold and consumed by the majority of the populations in different regions of Ghana with emphasis laid on coliforms and fecal E. coli. They showed that some sachet water brands in Ghana as a whole (since they sampled from 5 geographical distinct regions) are still contaminated by coliforms and fecal E. coli despite awareness-raising from previous reports. However, it would be good to specify the date of water sample collection in the study design to attest that what are referred to as previous reports are really previous reports in term of sample collection period. I will recommend this paper for indexing provided the authors address the comments raised below.

Introduction

The authors present sachet water production and consumption at the beginning of the introduction as something specific to Ghana whereas it is common practice in many countries in Africa (West Africa mainly) (Nigeria, Cameroon). It would be good to move from general to specific.

Methods

Figure 1 and Table 1 are used in the method section to back up the wider geographic coverage of this study. But there is no link between what is found in table 1 and geographical coverage. The authors should clarify that. Was the map drawn by the authors or it is just an adaptation from another map? If that is the case, then the authors should specify that.

Study site and design
Sachet water samples were collected from 41 selected communities. How many samples were collected per community? The reader may have the impression that only one sample was collected per community. If that is the case, why? If many samples were collected then, your sample size should be 41 times the number of sample per brand. The authors should have collected more samples per community and even evaluate the storage conditions in each store, which information would have helped them discuss better their results. Did the authors collect each sachet water brands from the source (packaging company) to rule out the fact that water leaves the company already contaminated? If not, they should put that as a point in the discussion.

Giving the brand’s names as ObiriDanso et al (2003) did in their paper “The microbiological quality of drinking water sold on the streets in Kumasi, Ghana” could be of great importance to the consumers in making the choice of which brands to take. Maybe ethically this is not correct but the authors should think about it.

“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 “ the authors mentioned this in their study design, the reader would expect them to present the results and discuss them with respect to level of urbanisation which is not the case in the current version of the paper.

Sample collection

-There is a word missing in the following sentence “The most commonly patronized sachet water was determined after interviewing a number of people the community [...] to central point laboratories within each region where analysis were carried out.” There seems to be a grammatical error in this sentence “where analysis was carried out or where analyses were”...

Again, in the methods, the authors did not describe clearly the measures taken to avoid contamination during sample transport and manipulation in the lab which could have contributed to the number of micro-organisms found.

Results

……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)

There is no link between the sentence and table1. The authors should make sure tables and figures are properly reference in the text.

The title of figure 3:”Regional analysis of the samples” does not really describe the figure. The reader may think from the title that the figure summarizes the methods used to analyse the sample in different regions and not the analysis outcomes which the authors are referring to.

Discussion

The authors could consider adding a small paragraph on the impact of used sachets on the environment, e.g. they constitute breeding sites for mosquitoes such as anopheles which transmit other diseases.

References

1. Obiri-Danso K, Paul N, Jones K: The effects of UVB and temperature on the survival of natural populations and pure cultures of Campylobacter jejuni, Camp. coli, Camp. lari and urease-positive
thermophilic campylobacters (UPTC) in surface waters. *Journal of Applied Microbiology*. 2001; *90* (2): 256-267 Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

*Competing Interests*: No competing interests were disclosed.

*Reviewer Expertise*: Microbiology, parasitology and biochemistry

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 07 Jan 2019

**Samuel Mawuli Adadey**, University of Ghana, Accra, Ghana

Comment:
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Response:
Comments addressed in manuscript

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Response:
The study was designed to examine the wholesomeness of sachet water in Ghana. Therefore, the target was focused on microbial contaminants at the time it gets to the end user. It would have been interesting to sample water at the source (manufacturing treatment plant), and at the market but it was difficult to incorporate it in the study due to some technical challenges.

Comment:
Giving the brand’s names as ObiriDanso et al (2003)1 did in their paper “The microbiological quality of drinking water sold on the streets in Kumasi, Ghana” could be of great importance to the consumers in making the choice of which brands to take. Maybe ethically this is not correct but the authors should think about it.
Response:
As rightly said, we considered the ethics of publishing the brand names and decided to leave them out.

Comment:
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Again, in the methods, the authors did not describe clearly the measures taken to avoid contamination during sample transport and manipulation in the lab which could have contributed to the number of micro-organisms found.
Response:
Comments addressed in manuscript. In addition, transportation, as described by Johnson et al., was stated in the manuscript which gives reference to the aseptic measures used during the sample collection.

Results

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Discussion

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The authors could consider adding a small paragraph on the impact of used sachets on the environment, e.g. they constitute breeding sites for mosquitoes such as anopheles which transmit other diseases.
Response:
Since the publication was focused on the microbial contaminants and there was no result or evidence from our study on the environmental impact of sachet water, it would be difficult to discuss the effect of sachet water on the environment and it serving as breeding grounds for mosquitoes. This is however well noted for future studies.

Competing Interests: No competing interests were disclosed.
Olusegun A. Olaoye
Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, Nigeria

The study presents microbiological analysis of sachet water samples across Ghana, with a view to ascertain their fitness for consumption. The research is an interesting one and of public health significance to the people that consume sachet water in Ghana.

The study reported occurrence of coliforms and useful microbial indicator of water quality, *Escherichia coli* in some of the water samples analyzed, signaling public health alarm to consumers.

Authors need to be consistent in their citations/references; with the use of numbers (1,2 etc) or Surname with years, eg Nmekpele and Hawkins (2015). Authors should choose a particular style of citation and use such throughout the manuscript.

Why did authors only take samples for analysis from 5 out of 10 regions? Why were the other regions not considered? Authors should be able to justify this.

Authors need to discuss briefly the efforts of the water regulatory body in Ghana in ensuring good quality drinking water, especially the regulations to companies involved. Are there laid down regulations by required body, are companies not adhering to such regulations? What possible sanctions can the regulatory body take on erring companies to serve as deterrent to others?

I suggest that the authors include a Conclusion section.

I recommend acceptance of the manuscript after the authors address the above points raised and other areas as highlighted in the reviewed PDF article, which can be downloaded here.

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