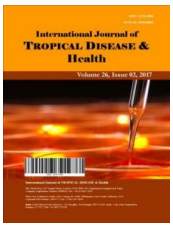

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Assessment of Some Physico-chemical Properties and Bacteriological Status of Sachet Water Consumed in the Hohoe Municipality, Ghana

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Authors' contributions

This work was carried out in collaboration between all authors. Author EA conceived the idea. Author EE performed field data collection. Authors EA and EE performed the laboratory investigations. Author EA wrote the first draft of the manuscript. Authors EA, EE, MK, MC, FZ and FB were involved in the second and final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Sachet water has become an important source of drinking water but little is known about bacteriological quality and quality control improvements with the rapid development of the industry. The study assessed the bacterial load and physical properties of sachet water sold in the Hohoe municipality of Ghana. The study was done in October 2016 and involved nine sachet water manufacturing sites within the municipality. Two (2) samples of each brand of sachet water were randomly selected from the manufacturing sites and the market. Therefore, a total of 36 sachet water samples were processed for Total Coliforms (TC) and *Escherichia coli* (EC) in colony-forming units (cfu) per 100 ml using membrane filtration method and Compact Dry EC media. Physical characteristics of the samples such as temperature, colour, odour, hydrogen ion concentration (pH),

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conductivity, and Total Dissolved Solids (TDS) were also investigated. The mean values for pH, Conductivity and TDS were 6.8, 41.7 µS/cm, 23.0 mg/L respectively. Four (11.1%) samples with blinded labels AV4, EM1, FM1, and IV4 had TC of 5 cfu/100 ml each. Two (5.6%) samples with labels EM1 and FM1 had *E. coli* of 4 cfu/100 ml and 5 cfu/100 ml respectively. Colour was similar (5 HU) for all the samples with unobjectionable odour. Contamination with *E. coli* was statistically dependent on the growth of TC ($P < .05$). The presence of *E. coli* is an indication and presence of faecal elements in the sachet water. This is critical and various stakeholders should sensitize and monitor production of sachet water in the municipality and in the country, Ghana. Further study into causes of contamination is recommended.

Keywords: Sachet water; quality; safety; contamination; bacteria.

1. INTRODUCTION

Water is a vital substance for the survival of all lives and one of the indispensable resources needed for the continued existence of man. It constitutes a sizeable amount of food intake by humans and supply is not reserved in the human body [1,2]. Production, sales and consumption of sachet water is rapidly increasing in most developing countries. However, the quality of production and mode of handling have deteriorated over the years and these pose serious health issues [3,4]. In Ghana, water packaged in 500 ml bags referred to as 'sachets' is now widely consumed and has become the main drinking water for 43.1% urban households [5]. Many public health threats are associated with the consumption of microbial contaminated packaged water. These include diseases such as cholera and diarrhea which sometimes finally results into deaths [2]. In Nigeria, thousands of cholera cases cause deaths each year and studies confirmed water related diarrhea as the most prevalent among the populace after malaria [6]. Consumption of contaminated sachet water was identified as one of the major causes of the 2014 cholera epidemic in the Greater Accra Region of Ghana. The epidemic affected more than 1733 people killing 20 individuals [7].

The sachet water industry is regulated by the Ghana Standards Authority (GSA) and Food and Drugs Authority (FDA) [8]. The FDA is mandated to regulate sachet production through producer inspection and registration [9]. With reference to World Health Organization (WHO) guidelines, GSA and FDA also set standards for quality of water [10,11]. The standards specified that no *E. coli* or Total coliform (TC) should be present in water for human consumption. Hydrogen Ion Concentration (pH) should be 6.5 - 8.5 and colour not exceeding 5 Hazen Units (HU). The water should be tasteless with unobjectionable odour [10,11].

Different laboratory investigations were used by many scientists to carry out research on the physical and microbial analyses of packed water in various communities. These studies reported various results of microbial contamination in specific geographical locations [3,12,13,14]. No such studies have been carried out in the Hohoe municipality. Sachet water consumption in this population was assumed to be safe and hygienic. It is obligatory that the quality of water being offered for sale is determined. Hence this study assessed the bacteriological status and physical properties of sachet water consumed in the Hohoe municipality of Ghana. The study targeted the manufacturers as well as vendors of sachet water.

2. STUDY OBJECTIVES

1. To assess bacteriological contamination levels of sachet water in the Hohoe municipality of Ghana.
2. To observe and characterize physical properties of sachet water during prolonged storage Scenarios.
3. To create awareness amongst potential and target communities of sachet water users.

3. MATERIALS AND METHODS

3.1 Study Area

The study area Hohoe municipality is one of the twenty-five administrative districts of the Volta Region. The municipality with Hohoe as its capital is located in the Volta Region of Ghana with a population of 167,000. It is located about 78 kilometers away from Ho, the regional capital and 220 kilometers from Accra, the nation's capital. The municipal has a total land surface area of 1172 km^2 and is located within longitude 0 degrees 15' E and 0 degrees 45' E and latitude 6 degrees 45' N and 7 degrees 15' N and lies

almost in the heart of the Volta Region. The major sources of water are pipe borne but there are few bore holes and a Dayi river which takes its source from the Akpafu ranges before entering the Volta Lake in the Kpando District. There were nine sachet water manufacturing companies in the municipality using either pipe-borne water or bore-hole for production. Water related diseases such diarrhoea and dysentery were recorded in the municipality. Furthermore, the 2014 Ghana Demographic Health Survey recorded 97.0% improved drinking water sources including bottled and sachet water [5].

3.2 Study Design

The study was a cross-sectional survey. From 10th to 29th October 2016, sachet water samples were randomly selected from the manufacturing sites and the vendors (market). Two (2) samples of the same brand were collected from the sites and the market. The names of the manufacturers and the vendors were blinded and coded. Codes with 'M' and 'V' in the middle denote a sample from the manufacturing site and vendor respectively. The codes for manufacturing sites were: AM1, AM2, BM1, BM2, CM1, CM2, DM1, DM2, EM1, EM2, FM1, FM2, GM1, GM2, HM1, HM2, IM1, and IM2. Vendors were coded as: AV3, AV4, BV3, BV4, CV3, CV4, DV3, DV4, EV3, EV4, FV3, FV4, GV3, GV4, HV3, HV4, IV3, and IV4.

3.3 Laboratory Investigations

3.3.1 Sample preparation

After taking the temperature at the point of sampling, the rest of the sachet water samples were transported to the laboratory within two (2) hours in an iced chest and stored in a refrigerator on reaching the testing site, Ghana Water and Sewerage Cooperation (GWSC) Volta Regional Laboratory. The edge of the sachet water was cleansed with 70% ethanol, cut with a pair of sterile scissors and aseptically introduced into sterile measuring cylinders for the laboratory investigations.

3.3.2 Physical examinations

The samples were tested for physical qualities such as temperature, colour, odour, pH, conductivity, and Total Dissolved Solids (TDS) and compared with international standards.

Conductivity meter (ExStik Multimeter, Accepta, UK) was used to measure three parameters (temperature, conductivity and TDS) by dipping a probe into the sachet water in a sterile test tube and values of the respective parameters recorded.

The pH was also measured using electric pH meter (Edutech Limited, India, Automated pH meter) and manufacturer's protocol observed. Each water sample was poured and measured into 100 cm^3 beaker, the probe was then inserted into the beaker, stirred for at least 1 to 2 minutes and the reading was observed and recorded.

Measurement of colour was by visual comparator. This was done by comparing the sample with standard colour solution. The standard colour solution was prepared using 1.2 g potassium chloroplatinate (K_2PtCl_6) and 1.0 g cobalt chloride ($CoCl_2.6H_2O$). The test samples and the prepared standard were dispensed into sterile test tubes. The tubes were placed on a white surface to be able to match the colours. The colour of the test sample closed to the standard was chosen and the matching unit recorded.

Odour was investigated by simply using human nose (olfactory nerve) as receptor. An odourless bottle with stopper was used. The bottle was vigorously shaken for 2-3 seconds to determine any odour. The odour was interpreted as objectionable or unobjectionable.

3.3.3 Bacteriological examination

Bacteriological parameters of Total coliform (TC) and *E. coli* (EC) were investigated. The sachet water samples were first filtered using membrane filter and Millipore (filtering device). Compact Dry EC medium (NISSUI Pharmaceutical Co. Ltd., Tokyo, Japan, Lot # = 218509) was used for the culturing. Compact Dry EC is a ready to use chromogenic plate for detection of *E. coli* and coliforms. Manufacturer's instruction in the package insert was followed. After opening the cap, one milliliter specimen was dropped onto the middle of each Compact Dry plate. The specimen diffused automatically and evenly into the sheet and transformed the dried sheet into a gel within seconds. Using forceps the membrane filter from the Millipore was gently placed on the Compact Dry Plate. The plate was recapped, turned over and incubated. After 24 hour incubation period, the number of coloured colonies underneath the plate were counted.

E. coli forms blue to blue-purple colonies. Coliforms show a red to pink colouration. Red/pink and blue/blue-purple colonies together are the total coliform group count. One Compact Dry plate was used for each sachet water sample.

3.4 Data Analysis Plan

Data was analyzed using Microsoft Office Excel 2013 and STATA software (Stata 12.0, Statacorp, Texas, USA). Data was presented in frequency tables, means and percentages. Regression analysis was done to determine the association between growth of TC and EC. Association between growth of microbes (TC and EC) with other confounding variables was also determined. P-value less than 0.05 was considered significant.

4. RESULTS

Findings were categorized into physical and microbiological properties. Predictors of sachet water contamination were also examined.

The value for colour was similar (5 Hazen Units) for all the brands of sachet water analyzed with unobjectionable Odour. Few samples (16.67%) has pH values below the international standard and most of them were in the range (6.8-7.1). The bulk of the water samples were in the temperature range 23.5 – 24.0℃. However, the conductivity and TDS of 9.5 – 39.5 µS/cm, 28.8 – 40.8 mg/L respectively were recorded for the greater portion of the sachet water analyzed (Table 1).

Table 1. The physical properties of the sachet water brands

Physical parameters	Frequency N = 36; n (%)	Sample label
pH **(6.5 – 8.5) Average = 6.8**		
< 6.5	6 (16.67)	AM2,FV4,GM2,GV3,GV4,HM1.
6.5 – 6.7	7 (19.44)	AM1,BM1,BM2,BV3,GM1,HM2,HV3.
6.8 – 7.1	23 (63.89)	AV3,AV4,BV4,CM1,CM2,CV3,CV4,DM1,DM2,DV3,DV4,EM1, EM2,EV3,EV4,FM1,FM2,FV3,HV4,IM1,IM2,IV3,IV4.
> 8.5	0 (00.00)	
Temperature **(°C) Average = 23.7**		
22.9 – 23.4	6 (16.67)	BV3,CM2,EM2,EV4,HM2,HV4.
23.5 – 24.0	26 (72.22)	AM1,AV3,AV4,BM1,BM2,CM1,CV3,CV4,DM1,DM2,DV3,EM 1, EV3,FM1,FM2,FV3,FV4,GM1,GV3,GV4,HM1,HV3,IM1,IM2,I V3, IV4.
24.1 – 24.6	4 (11.11)	AM2,BV4,DV4,GM2.
Conductivity **(µS/cm) Average = 41.7**		
9.5 – 39.5	17 (47.22)	AM1,AM2,AV3,AV4,BM1,BM2,BV3,BV4,CM1,CM2,CV3,CV4, EM1,EM2,EV3,EV4,IM1.
39.6 – 69.6	11 (30.56)	DM1,DV3,FM1,FV3,HM1,HM2,HV3,HV4,IM2,IV3,IV4.
69.7 – 99.7	8 (22.22)	DM2,DV4,FM2,FV4,GM1,GM2,GV3,GV4.
TDS **(mg/L) Average =23.0**		
4.6 – 16.6	13 (36.11)	AM1,AM2,AV3,AV4,BM1,BM2,BV3,BV4,CM1,EM1,EM2 EV3,EV4.
16.7 – 28.7	9 (25.00)	CM2,CV3,CV4,HM1,HM2,HV3,HV4,IV3,IV4.
28.8 – 40.8	14 (38.89)	DM1,DM2,DV3,DV4,FM1,FM2,FV3,FV4,GM1,GM2,GV3,GV 4, IM1,IM2.

mg/L = Milligram per Liter, µS/cm = Micro-Siemens per centimeter

Table 2. Sachet water brands with positive microbial population

Labels	Microbial load	
	Total coliform (cfu/100 ml)	*E. coli* (cfu/100 ml)
AV4	5	0
EM1	5	4
FM1	5	5
IV4	5	0

Adjusted R-squared= 0.4488, P-value=0.000, 95% CI (0.646-1.419)

Four (11.1%) water samples with labels AV4, EM1, FM1, and IV4 had TC of 5 cfu/100 ml each. However, 2 (5.6%) samples EM1 and FM1 had *E. coli* of 4 cfu/100 ml and 5 cfu/100 ml respectively (Table 2). Using TC as independent variable and EC as dependent variable, the probability that the presence of TC could lead to the development or growth of EC is significant {P-value = 0.000, 95% CI (0.282-0.618)}. From the binary regression, there was 44.9% chance of *E. coli* contamination in the presence of TC (Adjusted R-squared = 0.4488) (Table 2).

The predictors in Table 3 showed pH as positive predictor for bacterial contamination of sachet water. This was determined using TC as outcome variable with an adjusted R-square of 0.0340. This represents 3.4% chance of contamination using the four predictor variables. The pH as the only predictor could be due to low values for other statistical parameters aside the p-value (Table 3).

5. DISCUSSION

In Ghana, 46.5% of households use pipe-borne water as their main source of drinking water, 29.1% use borehole or protected well. In addition, 9.4% depend on sachet or bottled water [15]. The question we posed was how safe is this water for consumption in the Hohoe municipality? Are quality standards observed by the manufacturing companies?

The physical analysis of sachet water in previous studies showed that all sample brands were colourless, odourless and tasteless [16,17]. The present study confirmed these physical characteristics. Similar values of colour reported by the current study contradicts variability of colour of water samples by another study in Nigeria [18]. However, the unobjectionable odour reported by the current study conforms to the same study in Nigeria [18]. Previous study showed that chemical and bacterial parameters tested were above recommended levels and so could pose health treats to consumers [4]. The recent study showed pH as the only physical predictor of contamination and making sachet water unsafe for consumption. This is possible because microorganisms can alter the pH. Water with pH value below 6.5 is acidic and could cause health issues due to acidosis. Furthermore, pH above 8.5 could also lead to in-balance in homeostasis due to alkalosis [19]. These acid-base defects could lead to complications such as kidney stones, heart and kidney failures. Few sachet water sampled recorded pH below the acceptable standard of 6.5 (acidic). This is contrary to a study in Ghana [3] and Nigeria [20]. However similar acidic pH was reported in Nigeria [21]. Conductivity which is also an indication of TDS varies and this conforms to a study by Ackah and his colleagues [22]. The standard is silent on conductivity, however the recommended value for TDS is based on taste effects than health [3,22].

This current study reported four (11.1%) samples of the sachet water contained TC and two (5.6%) *E. coli* contaminants. Previous studies informed relative contaminations using different laboratory investigations [3,12,13,14]. A study in India revealed that 50% of 20 packaged water samples were unsatisfactory for human consumption. This was due to the presence of coliforms and *E. coli* [1]. Presence of *Escherichia coli, Enterococcus faecalis, Moraxella catarrhalis, Listeria monocytogenes, Enterobacter spp., Klebsiella spp*., *Pseudomonas spp., Bacillus spp., Proteus spp*. And *Staphylococcus spp.* were detected in sachet water sold in various communities in Ghana and Nigeria [2,13,23,24]. The current study could not identify these specific organisms because of the method

Table 3. Predictors of microbial contamination (*E. coli* and Total coliform)

Predictor variables	Coefficient	Standard error	t - value	p - value	95% CI
Temperature	-0.389	0.848	-0.46	0.650	-2.119 – 1.341
Conductivity	0.035	0.030	1.15	0.261	-0.027 – 0.096
TDS	-0.075	0.063	-1.20	0.241	-0.204 – 0.053
pH	2.178	0.999	2.18	0.037	0.142 – 4.215

used with a corresponding type of media. The media used was selective for only coliforms and *E. coli*. Furthermore, biochemical testing was not done to isolate specific organisms. In a similar study, 73% reported TC between 3-43 MPN/100 ml and 35% had *E. coli* between 15 and 78 cfu/ml [23]. The contaminations recorded in this study is higher compared to the current study. The current study and other studies proved that most sachet water sold in the Ghanaian market does not conform to international standards for drinking water. The presence of *E. coli* in water indicates faecal contamination. This raises the possibility that other pathogens may be present in the water [25]. *E. coli* 0157:H7 is the main serotype in water and causes hemorrhagic diarrhoea and haemolytic uremic syndrome [26]. Therefore, TC and *E. coli* are used as gauges to ration the degree of contamination and wholesome quality of water [4].

The cause of contamination of the investigated sachet water samples could be due to treatment processes, raw water sources, and hygienic practices during production [1,4]. Another study suggested packaging materials, dispensing into packaging materials and closure as potential dangers associated with sachet water contamination [27]. However, the current study did not probe the cause of contamination. The absence of TC and faecal elements in some of the brands could be due to good hygienic practices and observing standard operating procedures in the treatment process. The use of Compact Dry EC medium and conductivity meter could be an improved approach in this current study.

6. CONCLUSION

The mean values for pH, Conductivity and TDS were 6.8, 41.7 µS/cm, 23.0 mg/L respectively. Four (11.1%) samples with blinded labels AV4, EM1, FM1, and IV4 had TC of 5 cfu/100 ml each. Two (5.6%) samples with labels EM1 and FM1 had *E. coli* of 4 cfu/100 ml and 5 cfu/100 ml respectively. Colour was similar (5 HU) for all the samples with unobjectionable odour. Contamination with *E. coli* was statistically dependent on the growth of TC ($P < .05$). The presence of *E. coli* implies that the quality of water consumed is compromised with human excreta and this is crucial.

7. RECOMMENDATIONS

The study recommends involvement of all manufacturing companies in good hygiene practice. We also recommend that, the region must organize continual professional development programme in good personal and manufacturing hygiene. It is important that the Government of Ghana, Food and Drugs Authority, Hohoe Municipal Health Directorate, Environmental Health Unit of the Hohoe Municipal Assembly, and other responsible agencies focus on education and training of sachet water vendors and manufacturers. We also recommend further studies into causes of sachet water contamination and measurement of other trace elements in the municipality.

CONSENT

A written informed consent was obtained from managers of the sachet water companies and vendors.

ETHICAL APPROVAL

Ethical clearance was obtained from the Ghana Health Service Ethical Review Board (GHS/ERC) before the commencement of the study with the approval number GHS/ERC/90/10/2016. A written permission was obtained from management of the Ho Municipal Health Directorate. Respondents were assured of full confidentiality in every aspect of the study including all information collected from them. The sachet water brands sampled in the municipality were also coded.

COMPETING INTERESTS

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

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