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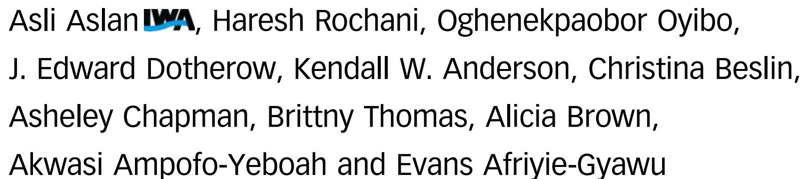
Research Paper

Sources of microbiological contamination in sachet water from Ghana



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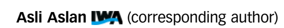
ABSTRACT

Sachet water is one of the primary sources of drinking water in rapidly growing countries. A study to assess the microbiological quality of sachet water in 21 different brands was conducted in Ghana. Culturable total coliform was positive in 87% of the samples collected, where *Escherichia coli* colonies were absent. The analysis of quantitative polymerase chain reaction results indicated the presence of *E. coli* genes in 44.6% of the samples, with the highest concentration up to 3,166 CCE/100 ml. Microbial source tracking analyses showed that the source of *E. coli* genes did not originate from sewage contamination because the human-associated HF183 marker was not detected. Of the 175 samples tested, 71% did not mention any water treatment before filling the packages. These results suggest non-human sources of contamination, such as biofilm formation in the pipelines used to fill these packages due to poor disinfection. Our study shows an urgent need for increased regulation and standardized manufacturing of sachet water to ensure safe drinking water.

Key words | coliform, Ghana, microbial source tracking, sachet water, West Africa



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INTRODUCTION

Ghana has been experiencing rapid urban development and a population increase (World Bank 2019), leading to a significant depletion of safe drinking water supplies. In the capital city of Accra, approximately 75% of the population lacks 24-h water access (Stoler *et al.* 2012a), which is the main driver for the widespread use of packaged water (Fisher *et al.* 2015; Stoler *et al.* 2015a). Sachet water is a form of affordable packaged water, usually sold in 500 ml polythene sealed bags, and is often promoted as 'hygienically produced drinking water' (Stoler *et al.* 2012b). According to a national survey, 27.6% of Ghanaian urban households reported sachet water as their primary source of drinking water (Ghana Statistical Service 2011).

One concern regarding sachet water has been the quality of the product (Stoler 2017). According to the World Health Organization (WHO), safe drinking water should not contain any pathogens (WHO 2017a). Total coliforms (TCs) and *Escherichia coli* are often used as indicators of microbiological contamination (Ashbolt 2004). A recent meta-analysis revealed that about 45% of the studies on packaged water quality detected various levels of microorganisms (Williams *et al.* 2015). Stoler (2017) showed that many studies on sachet water quality reported the presence of TC, fecal coliform, *E. coli*, or in some cases, *Salmonella* or protozoa.

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Studies have shown that indicator bacteria can grow in warm water environments, and some of these strains may not be of human origin (Parveen *et al.* 2011). Microbial source tracking (MST) is a well-established molecular microbiology technique used to identify the sources of fecal contamination in water (Harwood *et al.* 2014). Among many markers, genes belong to the *Bacteroides* genus have been widely studied for MST (Ahmed *et al.* 2009; Aslan & Rose 2013), and *Bacteroides* HF183 is reported as 99% specific for human-associated water contamination (Shanks *et al.* 2010). Because pathogens in water can also originate from non-human or even non-fecal sources, the human health risk associated with exposure may differ significantly. Therefore, identifying the source of microbiological contamination can provide decision makers valuable information when developing water safety policies.

The purpose of this study was to examine the sources of bacteria in sachet water collected from six cities in Ghana. The TC and *E. coli* concentrations were compared among different manufacturers. Samples with elevated levels of indicator bacteria were further tested for a human-associated source tracking marker (HF183) to investigate the origin of contamination. Additionally, the information on package labels was examined for conformity and standards compliance.

MATERIALS AND METHODS

Study design, sample collection and processing

Samples were collected from six locations in Ghana (Tamale, Kintampo, Techiman, Kumasi, Cape Coast, and Accra). A total of 175 sachets from 21 brands (88 samples (12 brands) in 2015 and 87 samples (nine brands) in 2016) were purchased from market vendors or kiosks where sachet water is kept in dark and cold conditions, avoiding direct sunlight. Samples were randomly selected from the piles located at the market vendors and transported in a cooler with ice until arriving at the hotel. These samples were kept in the freezer until the day of departure and transported in ice-packed coolers to the USA. In 2016, samples were also used for enumerating culturable TC and *E. coli* ($n = 87$) and analyzed onsite, on the same day as collection.

Total coliform and culturable *E. coli* analyses

Samples (100 ml) were pretreated with sodium thiosulfate (0.1 ml of 3% solution) to remove any potential chlorine residuals and processed by membrane filtration technique onsite within 6 h of collection (APHA 2017). Sachet water was filtered under aseptic conditions (i.e., all surfaces were treated with 10% bleach prior to and after analyses, all forceps were wiped with alcohol tissues and flame sterilized). Sterile disposable filtration units (Pall MicroFunnel, NY) with 0.45 μm pore size membrane filters were used for filtration, and all filters were placed on the Endo Nutrient Pad System (Sartorius, Gottingen, Germany). These plates were incubated at 18–24 h at 36 ± 2 °C. Dark to light red colonies are counted as TC, and colonies with a metallic sheen were counted as *E. coli*. Bottled water purchased from the USA was used as the negative control for each batch of filtration. All results were reported as CFU/100 ml. Plates with no growth were reported as below detection limit (<1 CFU/100 ml).

Nucleic acid extraction

Another set of samples (100 ml) was filtered in aseptic conditions onsite as described above. Sterile filtration units (Pall MicroFunnel, NY) with 0.4 μm pore size polycarbonate membrane filters were used for this procedure. Upon filtration, all filters were kept in a freezer until the day of departure and transported on ice to the Jiann-Ping Hsu College of Public Health Core Laboratory in the USA. These filters were then immediately stored at −80 °C until nucleic acid extraction.

DNA was extracted directly from each filter by the crude extraction method (USEPA 2012). The cells were lysed in a bead mill for 60 s at 5,000 rpm, and the debris were removed by centrifugation (Haugland *et al.* 2005). These extracts were immediately used for the quantification of target genes.

Quantitative polymerase chain reaction

Standard curves were prepared by extracting DNA from *E. coli* (ATCC 25922™), and a plasmid is used for the HF183 marker as described previously (Chern *et al.*

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2009; Green et al. 2014). Reagent mixes were prepared by combining 12.5 μl of TaqMan® Environmental Master Mix (Life Technologies, Thermo Scientific, Grand Island, NY), 2.5 μl of 2 mg/ml bovine serum albumin, 1 μM of each primer, and 80 μM of probes for each reaction. Amplification occurred with an initial starting temperature at 50 °C for 2 min followed by 95 °C for 10 min, then 45 cycles of 95 °C for 15 s and 56 °C for 1 min. All amplification reactions were carried out with a StepOnePlus™ thermocycler (Life Technologies, Thermo Scientific, Grand Island, NY), and results were reported as copies/100 ml. For *E. coli*, the inhibition score was detected from the sample processing control cycle threshold values in undiluted samples, as described in Method C (Aw et al. 2019). An internal amplification control was used to observe any potential inhibition in undiluted samples during the human-associated HF183 marker analysis (Green et al. 2014). For each quantitative polymerase chain reaction (qPCR) run, calibrators and a no-template control were run along with the samples for quality assurance and quality control. The detection limit for both qPCR assays was 100 copies/100 ml.

Statistical analysis

The Poisson regression analysis was used to examine the differences between brands, as our dependent variables of interest were discrete data consisting of TC counts and *E. coli* gene copies. Thereafter, two sets of Poisson regression models were fitted (Harper et al. 2011; Hogan et al. 2012).

Model 1 $\log(E(TC)) = \beta_0 + \beta_1(\text{Brand})$

Model 2 $\log(E(E.\,coli)) = \beta_0 + \beta_1(\text{Brand})$

The Proc Genmod procedure in Statistical software SAS 9.4 was used in the analysis of all our models.

RESULTS AND DISCUSSION

This study examined total coliform, *E. coli*, and a human-associated source tracking marker (*Bacteroides* HF183) occurrence in sachet water from Ghana. Overall, TC counts were positive for 76 of the samples out of 87 (87%). The TC counts presented high variability with a maximum count reaching 800 CFU/100 ml (Table 1). When compared among brands, TC counts were significantly different from each other ($p < 0.0001$) (Table 2). Culturable *E. coli* was not detected in any of the samples (Table 1). The absence of *E. coli* is in agreement with Stoler et al. (2014), as they reported low counts of fecal indicator bacteria (FIB) but high total heterotrophic bacteria in many brands regardless of their perceived quality within the communities.

E. coli 23S rDNA genes were positive in 44.6% of the samples ($n = 175$). The highest concentration was 3,166 copies/100 ml. *E. coli* gene copies in all 21 brands were also significantly different from each other ($p < 0.0001$; Table 3), indicating that the microbiological quality of sachet water varied among brands and even among different batches of the same brand. Detection of culturable TC and *E. coli* genes, but the absence of culturable *E. coli*, showed that, although during the time of analysis no live *E. coli* cells were detected, there were *E. coli* in these samples.

One possible explanation of these results is that bacteria can enter a viable but nonculturable (VBNC) stage under stress conditions (Roszak & Colwell 1987), where bacteria lose their ability to form colonies on solid media (Rozen & Belkin 2001). Recent studies showed that VBNC can be triggered by prolonged solar radiation exposure (Jozić & Šolić 2017; Sagarduy et al. 2019) or chlorination (Chen et al. 2018), where they can still display pathogenicity (Zhang et al. 2015). During our study, we often observed that sachet water was transported to the market vendors from other

Table 1 | Descriptive statistics of microbiological parameters tested in sachet water

	Number of samples (*N*)[a]	Minimum	Maximum	Mean	Standard deviation
Total coliform (CFU/100 ml)	87	<1	800	35.6	96.00
E. coli (CFU/100 ml)	87	<1	<1	<1	–
E. coli 23S rDNA (copies/100 ml)	175	<100	1,503	172.5	284.35
HF183 (copies/100 ml)	175	<100	<100	–	–

[a]Molecular data collected from 21 brands over the course of 2 years (2015 and 2016). Culture data (total coliform and *E. coli* CFU/100 ml) only collected in 2016 with a total of nine brands.

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Table 2 | Comparison of total coliform counts by brand ($n = 87$)

Variable	Model 1: response total coliform					
	Levels	***B***	**Standard error**	**Wald 95% confidence limits**		***P*-value**
	Intercept	4.7122	0.0316	4.6503	4.7742	<.0001
Brand (*n*)	Angel (10)	−0.5502	0.0506	−0.6493	−0.4511	<.0001
	Cool Pac (5)	−2.3599	0.1025	−2.5609	−2.1589	<.0001
	Century (14)	−3.8121	0.2869	−4.3743	−3.2498	<.0001
	Darkofresh (9)	−0.5516	0.0523	−0.6540	−0.4492	<.0001
	Deep (15)	−1.5189	0.0714	−1.6589	−1.3789	<.0001
	Ever Pac (12)	−1.4319	0.0643	−1.5579	−1.3059	<.0001
	Kama (12)	−2.2889	0.0916	−2.4684	−2.1094	<.0001
	Mobile (10)	−2.9596	0.1354	−3.2249	−2.6942	<.0001
	Reference = Special Ice (14)	0.0000	0.0000	0.0000	0.0000	.

Table 3 | Comparison of *E. coli* 23S rDNA gene copy numbers by brand ($n = 175$)

Variable	Model 2: response *E. coli* 23S rDNA genes					
	Levels	***B***	**Standard error**	**Wald 95% confidence limits**		***P*-value**
	Intercept	5.5084	0.0318	5.4460	5.5708	<.0001
Brand (*n*)	Angel (10)	−2.2237	0.0690	−2.3589	−2.0885	<.0001
	Aquafill (5)	−0.1962	0.0447	−0.2838	−0.1085	<.0001
	Aspet (5)	−0.6135	0.0501	−0.7117	−0.5153	<.0001
	Blue Sea (5)	−0.3820	0.0469	−0.4740	−0.2901	<.0001
	Century (14)	−1.0063	0.0568	−1.1177	−0.8949	<.0001
	Cool Pac (5)	0.1716	0.0355	0.1021	0.2410	<.0001
	Cool Pack (17)	−0.5195	0.0376	−0.5932	−0.4458	<.0001
	Darkofresh (9)	−2.4377	0.0785	−2.5917	−2.2838	<.0001
	Deep (15)	−0.5826	0.0387	−0.6584	−0.5067	<.0001
	Ever Pac (12)	−2.3657	0.0679	−2.4987	−2.2326	<.0001
	First Klass (5)	−0.4183	0.0474	−0.5112	−0.3255	<.0001
	Glory (5)	−0.3904	0.0470	−0.4825	−0.2982	<.0001
	Ice Pak (4)	−0.9183	0.0596	−1.0351	−0.8015	<.0001
	JAF Lover (5)	−0.2021	0.0448	−0.2899	−0.1143	<.0001
	Kama (12)	−2.1268	0.0620	−2.2484	−2.0053	<.0001
	Life Natural (5)	−0.6135	0.0501	−0.7117	−0.5153	<.0001
	Mobile (10)	−2.3729	0.0732	−2.5164	−2.2294	<.0001
	NSUPA (5)	0.9054	0.0366	0.8336	0.9772	<.0001
	Special Ice (14)	−1.5822	0.0492	−1.6786	−1.4857	<.0001
	Standard (9)	−0.1042	0.0389	−0.1804	−0.0280	0.0074
	Reference = Tepco (4)	0.0000	0.0000	0.0000	0.0000	

locations, during which they may have been exposed to direct sunlight resulting in the inactivation of the existing live *E. coli* cells in these packages. qPCR allows for quantifying all *E. coli* DNA from live and dead cells as well as free-floating DNA. It is known that the time to decay in 90% of the initial concentrations of *E. coli* cells (T90) in freshwater stored at ambient temperature can be short and up to 5 days (Noble *et al.* 2004), whereas *E. coli* genes T90 vary from 6 to 12 days (Klein *et al.* 2011; Brooks *et al.* 2015). The absence of *E. coli* cells but frequent detection of their genes in our study may be due to differences in the persistence of cells versus genes in the environment.

Another advantage of using qPCR in this study was the ability to test for the presence of the human-associated fecal contamination. Our results showed that all samples were negative for the HF183 marker; a universal indicator of

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human fecal contamination in water (Mayer *et al.* 2018). The absence of this marker in the sachet samples indicated that the source for TC cells and *E. coli* genes could have originated from non-human sources.

Based on these findings, one potential source of contamination can be biofilms cross-contaminating these packages during the filling process. Biofilms produced by a homogenous group of bacterial communities can occur in distribution systems when disinfection practices are poorly executed (Bertelli *et al.* 2018). According to the WHO (2017a) guidelines for drinking water, TC should be used to assess the bacteriological and biofilm contamination in distribution systems. Because there would have been no TC growth in a recently disinfected system, detecting high concentrations of this group of bacteria in many of these brands indicates the poor treatment, which might have led to biofilm growth that sustained TC survival within the pipelines. Our findings were supported by Stoler *et al.* (2015b) who reported that 41% of the sachet samples collected from Accra were positive for *Pseudomonas aeruginosa*, a microorganism that thrives in distribution system biofilms.

The information on the sachet bags were also examined (Table 4). The majority (78%) of the labels had no information on treatment. The sources of the water used for manufacturing were not mentioned in the majority (89%) of the samples. Almost 30% of the brands tested had no batch number nor date of manufacture. The quality of sachet water and how it is classified can change a nation's investment on water infrastructure and associated health policies. Vedachalam *et al.* (2017) analyzed the positive and negative outcomes of classifying sachet water as an improved or unimproved source of drinking water and concluded that, no matter how sachet water is classified, it should serve to advance public health. WHO already recognizes the intensive use of sachet water in Ghana and classifies as an improved water source in their recent report based on extensive household use (WHO 2017b). Our study shows that even though there has been a significant improvement in sachet water quality, such as not detecting any live *E. coli* cells, there still seem to be problems related to sachet production. Further studies are needed to address challenges related to the sachet manufacturing process, in particular, a comprehensive assessment of industrial hygiene practices across all brands.

Table 4 | Summary of information presented on sachet labels

Characteristics	(*N* = 21) *n* (%)
Definition	
Drinking water	15 (71.4)
Mineral water	6 (28.6)
Source of water	
Ground water	4 (19.0)
Not available	17 (81.0)
Company name and address	
Yes	21 (100)
Country of origin	
Ghana	21 (100)
Mark of conformity	
GSB	16 (76.2)
No	5 (23.8)
Other[a]	0 (0)
Registration number	
Yes	20 (95.2)
No	1 (4.8)
Batch number	
Yes	17 (81.0)
No	4 (19.0)
Date of manufacture	
Yes	17 (81.0)
No	4 (19.0)
Best before	
Yes	21 (100)
No	0 (0)
Storage instructions	
Yes	13 (61.9)
No	8 (38.1)
Treatment	
Yes	6 (28.6)
No	15 (71.4)

GSB: Ghana Standards Board.
[a]Other: Association of Sachet Water Producers.

CONCLUSIONS

To our knowledge, this is the first study that applies a rapid molecular method (MST) to investigate human-associated markers in sachet water. Based on our findings, more stringent treatment regulations at the manufacturing stage and

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standards compliance of the packages are needed to ensure the provision of safe drinking water.

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DISCLOSURE STATEMENT

There are no competing interests for the present study, financial or otherwise.

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