

MICROBIAL WATER QUALITY ASSESSMENT OF PACKAGED DRINKING WATER OF PRE-SCHOOL CHILDREN IN SOME PARTS OF ACCRA

R. A. BANU, E. D. O. ANSA*, M. O. AKRONG, G. A. ANSA AND M. BELLO,
(*E.D.O.A., R. A. B., M. O. A. & M. B.: CSIR Water Research Institute, P. O. Box AH 38, Accra, Ghana; G. A. A.: University Hospital, Legon, P. O. Box LG 79, Legon-Accra, Ghana.*)

**Corresponding author's email: edoansa@yahoo.com*

Abstract

Episodes of Cholera outbreaks are still common in most developing countries and children usually are at the greatest risk. This study assessed the importance of diarrhoea-related illnesses among children five years of age or less at the University Hospital, Legon and the importance of packaged water handling in the contamination of drinking water of pre-school children. Diarrhea-related diseases (DRD) accounted for 6.3% of cases involving children five years and below reporting at the University Hospital, Legon during the period of the study. Generally, greater number of cases (DRD) were reported among males (6.9%) than among females (5.6%). High counts above recommended levels of either Total Heterotrophic Bacteria, Pseudomonas or Total Coliforms were observed in 88% of the water samples tested. Inadequate cleaning of water bottles and in some instances the use of inappropriate water bottles (reusing PET mineral water containers) contributed to the poor quality of the drinking water of the preschool children. A greater part of the contamination of the drinking water occurred at home and not at the schools. Parents therefore need to be targeted with water, sanitation and hygiene related health education.

Introduction

Diarrhea-related diseases are still prevalent in Africa and children usually are at the greatest risk. In 2013, 6.3 million children died within five years of living and 0.578 million of these deaths (9.2%) was attributed to diarrhea-related diseases. In 2015, of the 5.3 million children who died in their first five years of their lives, 1.2 million deaths were attributed to diarrhea-related diseases, despite the 50% global reduction in diarrhea-related cases (WHO, 2014; UNICEF, 2016). It had been estimated that children below the age of three years gets three episodes of diarrhea each year (WHO, 2005).

In developing countries where diarrhea-related diseases are mostly prevalent, the causes of these risks are not difficult to find. In Ghana, open drainage and lack of waste management systems are major drivers of malaria (the leading cause of death for children under age five) as well as diarrhea-related diseases (UNICEF, 2015), causing thousands of Ghanaian children to die each year from diarrhea-related diseases. Poor handling of food and drinking water of children may result in the spread of diarrhea-related diseases and the role of care givers is significant. Infectious diseases amongst children who have out-of-home child care are known to be two to three times greater than children not in home care (NHS Highland, 2006). Pri-

vate schools in Ghana provide food to pupils during lunch times at least. In public schools, the Government of Ghana through the School Feeding Program, provide meals to about 1.69 million children nationwide, representing a coverage of 37.4% (MOGCSP, 2016). The risk of food contamination among school children is well documented in literature (Osei-Tutu & Anto, 2016; Malm *et al.*, 2015; Monney *et al.*, 2013; Newman, 2005) and 88% of cases of diarrhea-related diseases worldwide are attributable to unsafe water, inadequate sanitation or insufficient hygiene.

Typically, children under five years in crèches go to school with their own packaged drinking water in reusable bottles, refilled polyethylene terephthalate (PET) bottles, commercially packaged bottled water or sachet water from water vendors or from school canteens etc. The average capacity of reusable bottle used by pupil range from 500 ml to 700 ml and this suggests that packaged water sent to school may not be sufficient for the whole day and may need to be refilled. On the other hand, other researchers have suggested that some children may drink less when at school compared to when at home (Rugg-Gunn *et al.*, 1987).

The objective of this study is to assess the importance of diarrhea-related diseases among children five years of age or less in the West Ayawaso-Wuogon, Madina and Adenta areas as reported by cases in the University Hospital, Legon and to identify the importance of packaged water handling in homes and schools in the contamination of drinking water of pre-school children who are five years old or less. The study therefore assessed the status of packaged drinking water of pre-schoolers in terms of *Escherichia coli*, *Pseudomonas* and *Coliform* contamination both in the morning and in the afternoon.

Experimental

Hospital data acquisition

To assess the importance of diarrhea-related illness in the study area, secondary data was obtained from the records of the University Hospital, Legon, Ghana of patients reporting at the Out Patients Department (OPD) between January-March 2014. Records of 857 patients who are five years of age or below, reporting at the OPD of the hospital was screened for those who were positive for diarrhea-related illness. From the hospital records, the residential location of patients was categorized as Legon, Madina, Achimota, Adenta and Haatso, all of which were within 15 km distance of the University Hospital, Legon.

Cases diagnosed as positive for diarrhea-related illness in the consulting room register are those with three or more loose stools within 24 hours occurring in the previous five days who were with or without clinical symptoms. Stool samples were examined for physical characteristics such as presence of blood and mucus. A portion of the faecal sample emulsified in physiological saline were examined under the microscope to detect the presence of parasites. With the aid of various bacteriological cultural agar, stool samples were cultured for the isolation of enteric bacteria within two hours of sample collection using methods outline in Dutta *et al.* (2013).

Sampling of drinking water from preschools

A list of all pre-schools in the study area was obtained from the Ghana Education Service. 10 pre-schools were selected by simple randomized design using a table of random numbers (Altman, 1991). Five out of the 10 pre-schools randomly selected from these localities agreed to participate in study.

Morning (7:30-8:30am) and afternoon (1:30-2:30pm) drinking water samples as well as swab samples of bottle heads of preschool children (aged 2-5) in the selected schools were obtained for bacteriological analysis. Twenty milliliters of water samples were obtained and for morning samples, care was taken to ensure samples were obtained from only children who have not drunk any of their water. The inner portions of bottle heads were swabbed aseptically into tubes containing 5ml sterile distilled water. Afternoon samples were obtained from the water bottles of the same pupils sampled in the morning. Water samples were immediately kept on ice at 4°C and transported to the laboratory for analysis. All analyses were carried out immediately on reaching the laboratory. The laboratory is within 25 minutes' drive of the sampling locations.

Bacteriological analysis of water samples

Suspension obtained from swabs and water samples were vortexed for 30sec and inoculated for the culture of Total coliform (TC), faecal coliform (FC), *Escherichia coli* (E. coli) and *Pseudomonas aeruginosa* (PA) using the membrane filtration technique. Filtration was done using cellulose nitrate membrane filters of pore size 0.45 µm and diameter 47 mm (Millipore Ltd, UK). Determination of TC and E. coli were done by placing filters on chromogenic HiCrome coliform agar (Fluka) while FC was cultured by inoculating on M-FC Agar (Fluka). Analysis for *Pseudomonas aeruginosa* was done similarly using cetrимide agar (Fluka). Plates were incubated at $37 \pm 0.5^\circ\text{C}$ for 24 hours. The analysis for Total heterotrophic bacteria (THB) was done using the spread plate technique on nutrient agar (Fluka). One (1) milliliter each of water and swabbed samples were pipetted into labeled sterile petri plates

containing nutrient agar. Plates were incubated at $37 \pm 0.5^\circ\text{C}$ for 48 hours. Plating of samples was performed in triplicates and all media were prepared according to manufacturer's instructions. After incubation visible colonies of bacteria were counted with the aid of a colony counter and results expressed in colony forming units (cfu) per 100 ml of analyzed samples. All analysis carried out followed methods outlined in the Standard Methods for the Examination of Water and Wastewater (APHA, 2012) and results obtained were compared to the Ghana Drinking Water Standards GS175-1 (GSA, 2013).

Questionnaire administration

100 questionnaires were administered to parents out of which 83 was retrieved. Responses to structured questionnaires were obtained from parents through the heads of the preschools. Parents were asked to indicate their preference with respect to how they pack water for their children or whether they pay the school to supply potable water for the children. Parents were also asked to indicate if any, their means of packing drinking water for their children, whether they sterilize the drinking water containers used by their children and how it is sterilized.

Ethical clearance

Ethical clearance to conduct this tests on the water of pre-school children was obtained from the institutional committee after consent of the Schools had been obtained. Written permission to access hospital data was also sought and obtained. Researchers did not come into contact with patients nor did they handle any specimen from any patient.

Statistical Analysis

Hospital records of children five years or less were categorized into their various locations or suburbs of residence as well as their sex. These variables were compared using the Chi square test of Epi Info 7.2 software (Center for Disease Control, Atlanta, Georgia). Counts of bacteria in drinking water of morning and afternoon samples from the various schools were compared using paired sample t-test of SPSS 20.0 (Statistical Package for Social Scientists). Responses of parents on their preferences for their wards use of potable water at school, means of packaging potable water and the means of sterilization if any were compared using one-way ANOVA of SPSS 20.0.

Results

Occurrence of diarrhea-related diseases among children Hospital records showed that for the 857 children five years old or less reported at the OPD during the period of the study. Diarrhea-related diseases accounted for 6.3% of cases involving children five years and below reporting at the University Hospital during the period of the study. Generally, greater number of cases were reported among males (6.9%) than among females (5.6%) (Chi square test, $P < 0.05$). Reported cases from Achimota were the highest (7.6%) while Adenta showed the lowest (5.2%) (Table 1). At Madina, Achimota and Haatso males had significantly higher cases than females in the same localities respectively (Chi square test, $P < 0.05$). There were more cases among females than males in the suburbs of Legon and Adenta however (Chi square test, $P < 0.05$).

TABLE 1
Diarrhea-related cases among children 5 years or less in some suburbs of Accra as observed from hospital records.

Localities	Males			Females			Total No. Exam.	Total No. +ve	(%)
	No. Exam.	No. +ve	(%)	No. Exam.	No. +ve	(%)			
Legon	164	9	5.5	123	9	7.3	287	18	6.3
Madina	95	9	9.5	96	4	4.2	191	13	6.8
Achimota	70	8	11.4	61	2	3.3	131	10	7.6
Adenta	99	4	4.0	56	4	7.1	155	8	5.2
Haatso	35	2	5.7	58	3	5.2	93	5	5.4
Total	463	32	6.9	394	22	5.6	857	54	6.3

Bacteriological quality of drinking water of the pre-school children 50 water samples (comprising 25 mornings and 25 afternoon samples) analyzed, showed only six (6) water samples satisfying the criteria for potability (Table 2). Of the six (6) potable samples, only one sample was taken in the afternoon. Drinking water is

deemed potable when all indicator parameters analyzed fall within permissible limits. The Ghana drinking water standards requires that potable water or samples have zero detectable counts of TC, EC and PA per 100ml of water analyzed while THB counts must remain below 500 cfu/100 ml.

TABLE 2
Distribution of both morning and afternoon swabbed and water samples that were potable (-) and those not potable (+) from five preschools in Accra. Potability is based on guidelines of Ghana Standards (GS-175, 2013).

School	Morning		Afternoon	
	Water Sample	Swab Sample	Water Sample	Swab Sample
School 1				
1	-	-	+	+
2	+	+	+	+
3	+	+	+	+
4	-	-	+	+
5	+	+	+	+
School 2				
6	+	+	+	+
7	+	+	+	+
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+
School 3				
11	+	+	+	+
12	+	+	+	+
13	+	+	+	+
14	+	+	+	+
15	+	+	+	+
School 4				
16	+	+	+	+
17	-	-	+	+
18	+	+	+	+
19	+	+	+	+
20	+	+	+	+
School 5				
21	+	+	+	-
22	-	+	-	+
23	+	+	+	+
24	+	-	+	-
25	-	+	+	+
Total Not Potable	20	21	24	23
Potable	5	4	1	2

Generally, no significant differences were observed in bacterial counts of morning and afternoon water samples for Total Coliforms (Table 3), *E. coli* (Table 4), *Pseudomonas aeruginosa* (Table 5) and Total Heterotrophic bacteria (Table 6). A similar observation was made for swab samples taken in the morning and afternoon. School 1 showed significantly higher counts in Total Coliforms in the morning than in the afternoon but Total Coliform counts in swab samples from that same school were significantly higher in the afternoon than in

the morning ($P < 0.05$). *E. coli* counts were observed only at schools 5 and 2, with school 2 showing contamination only in water samples in the afternoon. All the water and swab samples of all the five schools showed high counts in *Pseudomonas aeruginosa*, with very high counts ($26,340 \pm 6,914$ cfu/100 ml) in afternoon water sample of School 1, suggesting significant contamination. At school 2, significantly higher counts of total heterotrophic bacteria were observed in the afternoon water samples compared to the morning samples ($P < 0.05$).

TABLE 3
Mean Total Coliform (TC) concentrations \pm standard deviation in water samples and cover of water bottles (swab sample) in cfu/100 ml.

Location	Number of samples	Water Samples		Swab samples	
		Morning	Afternoon	Morning	Afternoon
School 1	5	3162 \pm 1194	874 \pm 224	46 \pm 25	388 \pm 58
School 2	5	2139 \pm 1581	7298 \pm 2256	804 \pm 91	681 \pm 161
School 3	5	935 \pm 311	1223 \pm 715	476 \pm 267	103 \pm 37
School 4	5	1680 \pm 821	1034 \pm 477	277 \pm 91	809 \pm 109
School 5	5	2418 \pm 1357	1782 \pm 734	472 \pm 252	195 \pm 132

TABLE 4
Escherichia coli (EC) concentrations in water samples and cover of water bottles (swab sample) in cfu/100 ml.

Location	Number of samples	Water Samples		Swab samples	
		Morning	Afternoon	Morning	Afternoon
School 1	5	0	0	0	0
School 2	5	0	63 \pm 47	0	0
School 3	5	0	0	0	0
School 4	5	0	0	0	0
School 5	5	243 \pm 77	266 \pm 68	34 \pm 22	57 \pm 33

TABLE 5
Pseudomonas aeruginosa (PA) concentrations in water samples and cover of water bottles (swab sample) in cfu/100 ml.

Location	Number of samples	Water Samples		Swab samples	
		Morning	Afternoon	Morning	Afternoon
School 1	5	5208 \pm 2628	26340 \pm 6914	3943 \pm 1641	1668 \pm 1280
School 2	5	11514 \pm 1127	16114 \pm 8367	509 \pm 104	353 \pm 58
School 3	5	3000 \pm 2053	1152 \pm 476	1574 \pm 383	153 \pm 76
School 4	5	143 \pm 23	188 \pm 94	70 \pm 29	103 \pm 54
School 5	5	693 \pm 271	1024 \pm 353	167 \pm 128	192 \pm 69

TABLE 6
Total Heterotrophic Bacteria (THB) concentrations in water samples and cover of water bottles (swab sample) in cfu/100 ml.

location	Number of samples	Water Samples		Swab samples	
		Morning	Afternoon	Morning	Afternoon
School 1	5	2353 \pm 1276	3233 \pm 1828	168 \pm 122	180 \pm 106
School 2	5	1698 \pm 1449	3427 \pm 1632	100 \pm 56	112 \pm 82
School 3	5	1022 \pm 353	1899 \pm 1437	110 \pm 58	137 \pm 85
School 4	5	1777 \pm 480	1375 \pm 603	77 \pm 67	84 \pm 31
School 5	5	1779 \pm 763	3140 \pm 455	98 \pm 21	68 \pm 31

Responses to questionnaires

Eighty-three percent (83%) of the 100 questionnaires administered were retrieved from the five (5) preschools. The survey of parents showed that only 12% of parents preferred the school providing drinking water for the pre-school children during school hours (Fig. 1). Majority of the parents (77%), preferred packing their wards drinking water from home (Fig. 1a). Additionally, of the 77% parents who

provided for their wards drinking water from home, 68% sterilized their water while 11% did not (Figure 1c). Forty-three percent of parents (43%) used water bottles, 27% purchased factory packaged bottled water while 15% used PET mineral water bottles (Fig. 1b). Means of cleaning water containers included washing with soap and brush (48%), washing in warm water (23%), while others use other methods (5%).

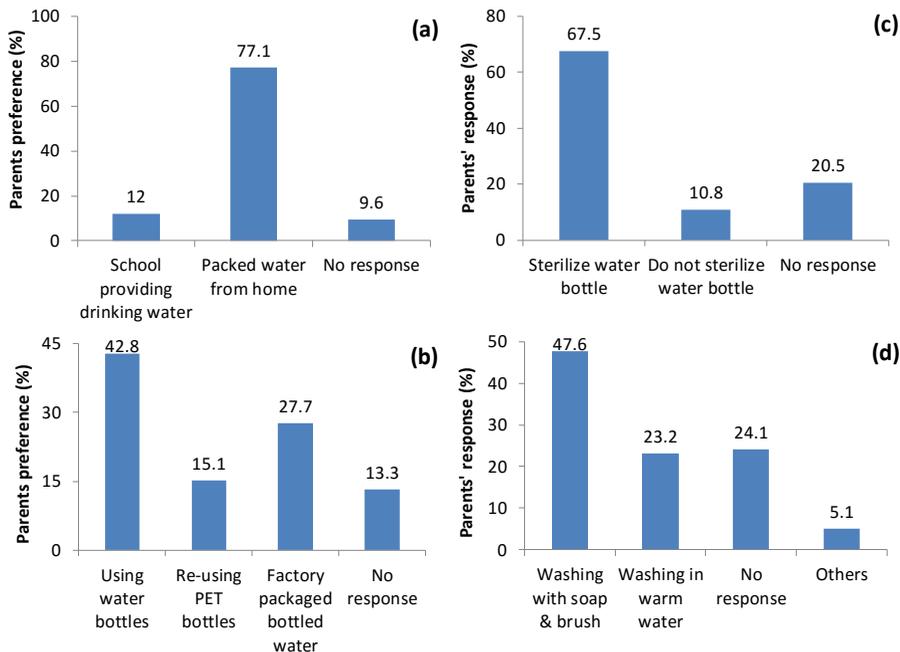


Fig. 1: Drinking water preferences of parents for their wards (a), types of containers used by parents for storing their children's drinking water (b), attitude of parents to sterilization of water bottles (c) and parents current water bottle sterilization practices (d).

Discussion

Occurrence of diarrhea-related diseases among children. The occurrence of 54 positive cases (6.3%) of diarrhea-related diseases from January to March of children five years and below as indicated by hospital records shows that diarrhea-related diseases is still a public

health challenge in Ghana. The highest number of positive cases (7.6%) came from Achimota while the lowest number of positive cases were recorded for residents coming from Adenta. The socio-economic status of a locality may influence the occurrence of the disease, with affluent areas expected to have less cases

of the disease and vice versa. Understandably, poorer areas may have little sanitary infrastructure and less hygienic conditions for drinking water or food preparation (Motarjemi *et al.*, 1993). Personal hygiene of individuals is also likely to improve with better standard of living. This may explain the lower number of positive cases coming from Adenta as opposed to Achimota. Adenta is a more affluent locality than Achimota. Hospital records showed that males (463 patients) had significantly more positive cases (6.9%) than females (5.6%) although Legon and Adenta showed a contrary trend (Table 3). Studies by Tawiah (2004) at the Princess Marie Louis Children's Hospital in Ghana observed more female children having cases of diarrhea-related illness than male children. This they attributed to the presence of more female children in the community. Hospital visits among male children is known to be higher than among female children (Parashar, 1999; Boccolini, 2012) and this observation is consistent with the findings of this study (Table 3). This may explain the higher overall percentage of positive cases of diarrhea-related illness among male children observed in this study.

Bacteriological quality of swabbed and drinking water samples

Generally, no significant differences ($P < 0.05$) were observed in bacterial counts of morning and afternoon water samples for Total Coliforms (Table 3), *E. coli* (Table 4), *Pseudomonas aeruginosa* (Table 5) and Total Heterotrophic bacteria (Table 6). A similar observation was made for swab samples taken in the morning and afternoon (Tables 3, 4, 5 and 6). Bacteria counts of swab samples (Total Coliforms, *Pseudomonas aeruginosa* and Total Heterotrophic bacteria Table 3, 4, 5 and 6) were also high and well above the recommended GSA (2013) levels. This was unexpected as

swabbed samples tend to be lower in count due to low recovery of bacteria by this technique (Eissa & Mahmoud, 2012).

The Ghana drinking water standards requires that potable water must have zero detectable counts of TC, EC and PA per 100 ml of water analyzed while THB counts must remain below 500 cfu/100 ml. Thus samples contaminated by only one bacteriological parameter would still not be adjudged as potable. Fifty (50) water samples were analyzed (for both morning and afternoon samples), and out of this, only six (6) satisfied the criteria for potability (Table 2). The presence of coliforms and THB above recommended levels is indicative of inadequate treatment of the water or contamination. The presence of PA can result in illness in persons with compromised immune system while presence of EC is indicative of faecal contamination of drinking water. Drinking water or food contaminated with faecal matter can result in the spread of diarrhea-related diseases. School 5 (Table 4) showed contamination with faecal matter for both swab and water samples, both in the morning and afternoon based on EC count observed.

Contamination of drinking water bottles of the pre-school children is obviously due to poor handling of drinking water package as well as poor personal hygiene. School 2 (Table 4) showed contamination of drinking water with faecal matter in the afternoon but not in the morning suggesting that contamination occurred at school as a result of inadequate hygienic practices.

Sixty-eight percent (68%) of parents who use water bottle or used PET bottles to package their wards drinking water claimed they sterilize the water (Fig. 1c). However, 82% of the drinking water and swabbed samples were contaminated (Table 2), suggesting that their method of sterilization is not effective. Methods of

sterilization used by parents included washing with soap and brush (48% of parents), washing in warm water (23% of parents) and others (5%). It was observed during the sampling period that some of the water bottles had sophisticated bottle heads with several levels of crevices that can make cleaning difficult. This may contribute to the ineffectiveness of the cleaning methods. WHO (2011) and (2015a) recommends the use of water heated to rolling boil point for rinsing after washing with brush and detergent. Stainless steel water bottles should also be preferred to plastic reusable ones as it is easier to clean.

Conclusion

Diarrhea-related diseases accounted for 6.3% of cases involving children five years and below reporting at the University Hospital during the period of the study. Generally, greater number of cases were reported among males (6.9%) than among females (5.6%). Reported cases from Achimota were the highest (7.6%) while Adenta showed the lowest (5.2%). This figure suggests that diarrhea-related diseases is still important in the West Ayawaso, Wuogon, Madina, Adenta and nearby municipalities. High counts above recommended levels of either Total Heterotrophic Bacteria, *Pseudomonas* or Total Coliforms were observed in 88% of the water samples tested. Inadequate cleaning of water bottles and in some instances the use of inappropriate water bottles (reusing PET mineral water containers) contributed in the poor quality of the drinking water of the preschool children. A greater part of the contamination of the drinking water occur at home and not at the schools. One school however showed contamination of *E. coli* only in the afternoon suggesting that contamination of the drinking water occurred at the school.

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