



Original Paper

Microbial Safety of Household Drinking Water Used During Weaning of Children Aged 6-24 Months in Lusaka District, Zambia.

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ABSTRACT

Background: It is estimated that almost 900 million people lack access to improved drinking water worldwide, and over 5 million of those people live in Zambia. Humans can acquire *E. coli* and *salmonella* infections through the consumption of contaminated drinking water. The aim of the study was to investigate the safety of household drinking water provided to children during the weaning period in Lusaka District. **Methods:** We examined the *Salmonella* spp. and *E. coli* levels of contamination in water through a cross-sectional survey conducted over a period of nine months, in 19 townships of Lusaka District. The samples were labelled and transported on ice to the laboratory for analysis, that included bacterial isolation and total bacteria count. Antibiotic susceptibility of the isolated *Salmonella* spp. and *E. coli* was also determined. **Results:** The contaminated water sources with total coliforms amounted to 262 (33.6%). *Salmonella* spp. (150 positive samples 57.2%) contaminated more water tap samples than did *Escherichia coli* (23 positive samples 8.7%). The two bacteria showed varying resistance to the selected antibiotics, with the highest being recorded in metronidazole 130 (95.6%) for *Salmonella* spp. and 53 (89.8%) for *Escherichia coli*, oxacillin 130 (95.6%) for *Salmonella* spp. and 57 (96.6%) for *Escherichia coli*. **Conclusion:** Considerable and extensive contamination of *Salmonella* spp. and *E. coli* in water in households in the Lusaka district has been revealed. Isolated *Salmonella* spp. and *E. coli* isolates unveiled resistance to multiple antimicrobial drugs: mainly to metronidazole, and oxacillin. These results raise serious concerns regarding the safety of drinking water in Lusaka, as well as regarding the prospects for antibiotic treatment of enteric infections in Lusaka.

Key words: *Salmonella* spp., *Escherichia coli*, bacterial contamination, drinking water, post-weaning

1. Introduction

It is estimated that almost 900 million people lack access to improved drinking water worldwide; and over 5 million of those people live in Zambia [1]. *Escherichia coli* (*E. coli*) is a gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the in the lower intestines of endotherm organisms [2].

Escherichia coli is a member of the family Enterobacteriaceae [3,4]. Some pathogenic *Escherichia coli* (*E. coli*) may cause potentially fatal haemolytic uraemic syndrome, bacteraemia and meningitis [5,6]. The presence of *E. coli* in water is a strong indication of recent sewage or faecal contamination from human and animal waste [3,7,8]. *Salmonella* is a genus of rod-shaped (bacillus) gram negative bacterium of the Enterobacteriaceae family [4,9]. *Salmonella* species (spp.) are gram-negative facultative anaerobic bacteria; and they have been isolated from humans, animals, and the environment [3,10,11]. *Salmonella* spp are the leading cause of acute gastroenteritis in several countries and salmonellosis remains an important public health problem worldwide [12].

Humans can acquire salmonella infections through the consumption of contaminated foods, as well as contaminated drinking water [13]. Globally, nearly one in five child deaths – about 1.5 million children under five, die each year due to diarrheal diseases, many of which are caused by food and waterborne pathogens [14,15]. Worldwide, every year, unsafe water, coupled with the lack of basic sanitation kills over 1.6 million children under the age of five years [16].

In Zambia, the Ministry of Health (MoH) promotes the exclusive breast-feeding of infants up to 6 months of age; because fully breast-fed infants have better immunity; thereafter solid food and water can be introduced to the diet [17]. Promoting infant feeding practices, such as exclusive breast-feeding for up to six months is an effective strategy for improving child survival [17]. This practice has shown a decline in infant mortality during the period of exclusive breast-feeding [18]. The weaning of children from breast milk in Zambia usually takes place between 1 year and 1 year and 6 months; although, it may take longer in some cases [18]. The need for safe drinking water is particularly critical – considering that many mothers do not follow the World Health Organisation (WHO) infant-feeding recommendations [19]. Despite clear recommendations to the contrary, most breast-fed infants receive replacement feeds or supplemental water, in addition to breast milk, in the first six (63) months of life [20].

Several studies conducted in the Lusaka District have shown the contamination of water from various sources [19, 21,22]. Under World Health Organisation, water should not contain

any *Salmonella* and any *E. coli* [19]. Nkhuwa and others. [23] in a study conducted in Lusaka found that the aquifer was susceptible to contamination and delicate; because it was shallow; and it was accompanied by an open karst system. They also found that the ground water quality in Lusaka had impairment with regard to most of the selected parameters. In another study by Mulenga and McGranahan [21], they found that the residents of George Township in Lusaka district were heavily dependent on shallow wells and pit latrines as their water source and for the disposal of human excreta, respectively.

Nakaonga and others [24] established that 52.5% of borehole water sources in peri-urban township in the Lusaka district are contaminated with faecal coliforms. These studies did not determine the microbiological safety of drinking water in households. Antimicrobial resistance has been reported in several studies undertaken in Zambia. *Salmonella* spp. and *E. coli* isolated from chickens, dogs and humans have shown resistance to tetracycline, gentamicin, co-trimoxazole and oxacillin [25-27]. The increasing trends of antimicrobial resistance, consequently, pose a public health risk; and they reduce the treatment options available for illnesses caused by the pathogenic bacteria.

The current study was carried out to investigate in households, the safety of drinking water that is provided to children post-weaning in Lusaka District. The safety of drinking water was determined by isolating two waterborne (2) pathogens, *E. coli* and *Salmonella* spp., and thereafter testing their susceptibility to antibiotics.

2. Methodology

The study was a cross-sectional survey aimed at assessing the microbiological quality by determining the presence of *E. coli* and *Salmonella* spp. in drinking water provided to children during weaning period in 19 townships of Lusaka District. The survey correspondingly examined the antibiotic susceptibility of *Salmonella* spp. and *E. coli* isolates from the water samples. Lusaka district total population was estimated to be 1, 747,152 comprising of 886,723 females and 860, 424 males; children 0-2 years were 200,512 and under 5 years children were 391,778 (CSO, 2010).

Sample Size Determination

We selected a confidence level of 95%, margin of error 5%, targeting female population aged 15-49 years residing in the Lusaka urban. This had an assumed response rate (70%), whose sample size was then obtained by using estimated proportions and variance under the assumption of simple random sampling method was given as $s^2=pq$, here p is an

estimate of the proportion of the population that has the characteristic of interest or the probability of success and $q = 1-p$. The safest choice is when $p=0.5$. Fixing the desired precision at $\pm 5\%$ margin and a 95% confidence interval, then the initial simple random sample size, is computed as:

Step 1 $n_1 = P (100-P)*f$

$(1-\alpha)/S^2$

Where, $f (1-\alpha) = 3.84$ (for a 95% confidence interval), $S^2 =$ specified margin of error ± 5 , $z = 1.96$ for a 95 % confidence interval, $p=$ an estimate of the proportion of the eligible population that has a characteristic of interest, this was $q=1-p$. Given the following results: $d =$ the specified margin of error $\pm 5\%$, $p=$ an estimate of the proportion of the population that has a characteristic of interest $q= 1-p$: n_1 is therefore $= 1.96^2 * 50^2/5^2 = 384$ female in the age group 15-49 years.

Step 2

Calculating the modified sample size (n_2) considering the female population aged 15-49 years). In the formula below we use the total number of female aged 15-49 years according to CSO 2010 Census of Population and Housing

$$n_1 = \frac{N}{N + d}$$

data: $n_2 = \frac{N * n_1}{N + n_1}$ where, N is the size of the female population aged 15-49 years = 384
 $* (591,718)/(591,718+384) = 384$ all females in the age group 15-49 years.

Step 3

An adjustment for the design effect is made using: $n_3 = Bn_2$, where $B = 1$ for simple random sampling design, $B < 2$ for stratified sampling design, $B > 1$ for cluster multistage sampling designs.

Adjusting this sample size by the design effect = of say 1.4 the sample size of graduates is calculated as shown below.¹ $n_3 = Bn_2, = 1.4 * 384 = 538$ all females.

Step 4

Adjusting for non-response: $n = n_3/r$, where $r = 70\%$ is the expected response rate, $= 538/0.7 = 769$ all females.

Table 1: Determination of sample size using urban female population

Urban Female population aged 15-49 years	Step 1	Step 2	Step 3	Step 4
	n_1	n_2	n_3	n
591,718	384	384	538	769

This was the target sample of all female population 769 was the target sample size of which 761 all females 15-49 years participated to the study. Showing a response rate of 99 percent.

Statistical Test

The null hypothesis (Analysing dispersion and clustering patterns) which is a Complete Spatial Randomness (CSR), of the values associated with microbiology analysis was done on water and foods given as complementary food to 6 to 24 months during weaning period in Lusaka Urban.

Based on a statistical test on the null hypothesis (H_0):

H_0 : Water and complimentary infant foods given to under 5s during weaning periods are not safe in Lusaka Urban

Based on a calculated chi-square value of 3.84 and the table of critical values with degrees of freedom being 1, and a pvalue of 0.05 was determined. The p-value is a probability. For the pattern analysis tools, it is the probability that the observed spatial pattern was created by some random process. This meant that the null hypothesis could not be rejected, exhibiting statistically significant clustering or dispersion.

¹ The design effect (deff) is the factor by which a simple random sample size is inflated to take into account a design other than a simple random sample. It is the ratio of the variance of the estimate for a particular design to the variance of the estimate for a simple random sample of the same size. This is

usually determined from other surveys of the similar designs. For this kind of a design the deff is usually greater than 1 (usually close to 2 or 3). In this case deff equal to 1.4 and a response rate of 70% are used.

Thus, a random process created the observations associated with microbiology analysis of water and foods given as complimentary to under 5s during weaning period in Lusaka Urban. Which led to obtaining a total of 761 caregivers and women representation from a sample of selected households in 19 communities. From these communities, a total sample of 895 complimentary foods and 771 water samples given to under 5s during weaning period in Lusaka Urban were received whose results have been analysed.

Identification and sampling of the water samples

The study had multiple sources of information, which included at least one woman per household as a primary source of study information, focused group discussions, as well as key informant interviews. In this study, a statistical analytical method was used, based on probability proportion to the sizes of the target population using a circular systematic sampling method without replacement technique to select communities within Lusaka Urban. Recruitment of all eligible respondents were selected using a multistage sampling method with replacement based on the available literature on the high number of under-five mortality cases in urban setup of Lusaka province.

A mathematical sample determined by a formula: $n_2 = n_1 (N/N+n_1)$, where N is the target sample size, $N= 591,718$ (total female 15-49 years in Lusaka urban, based on 2010 Census of Population and Housing (Central Statistical Office)). In this study the target sample size was inflated taking into consideration non-response factors the amount of error margin (5%) due to the design effect adopted for this particular study to come up with a representative sample. Based on the above methodology a representative sample size (n) of 769 households targeting all female respondents of child bearing age (aged 15-49 years), with at least one child aged six (6) months to twenty (24) months, resident within the target communities or standard enumeration areas (SEAs) was envisaged. A total of 761 female respondents participated in the study, giving a response rate of 99%, giving us a sample large enough in which sample estimates could be inferred to give reliable and consistent population estimates of characteristics of all women aged between 15 and 49 years in Lusaka Urban.

At household stage a random inclusion criterion was used based on a KISH Grid method to recruit a caregiver or a woman who had at least an under five-child. Participation of the mothers or caregivers was voluntary and consent was obtained prior to their participation in this study. The Kish Grid method was used to reduce bias of who to interview at household level. From each selected

The identified *E. coli* and *Salmonella* were then subjected to antibiotic susceptibility tests [28]. In order to determine a representative sample of eligible respondents whose perceptions could be generalised from the 19 Lusaka Suburbs, the study adopted a relatively high confidence limit of 95%. In other words, the desired width or precision limit was $\pm 5\%$ margin of error and with a confidence interval of 95%.

Site selection

The research followed a cross-sectional probability sampling design. The townships randomly selected were: Bauleni, Chaisa, Chawama, Chilenje/ Chalala, Chipata, George, Jack, Kabanana, Kamanga, Kamwala, Kanyama, Makeni Villa, Matero, Misisi, Mtendere, Sikanze, Twikatane/Zingalume, Roma and Villa Elizabetha (Figure 1).

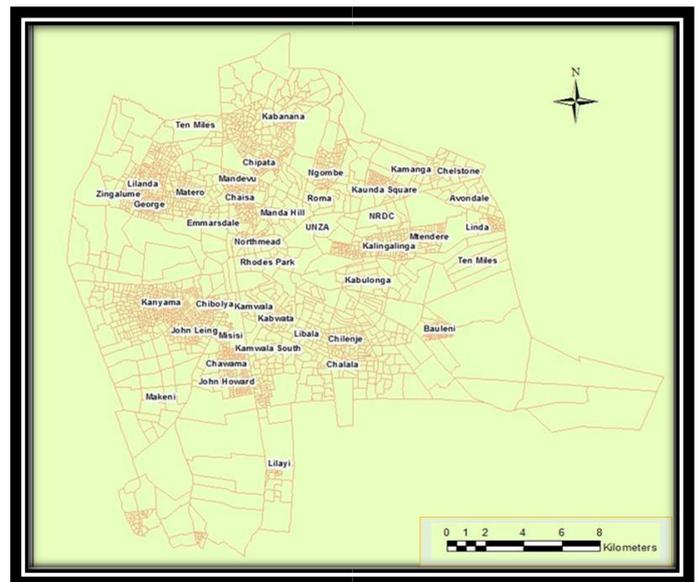


Figure 1: Map of Lusaka district showing location of Townships – CSO – 2018

Bacteriological analysis of the water samples

The Total Bacterial Count was determined by the standard plate count method using standard plate count agar (Himedia™, Mumbai, India), and by employing Serial Dilution Agar Plating Method according to previously described methods by Feng and Weagant, 2014 [29]. For all the samples, three volumes of 100 ml were filtered through a 0.45 μm pore-sized filter (Whatman, Maidstone, England). The membranes were aseptically placed on Blood and MacConkey agar plates (Himedia™, Mumbai, India). Each sample was analysed in triplicate. Furthermore, 0.1 ml of water samples was spread on the nutrient agar plates (Himedia™, Mumbai, India) to identify other fastidious

bacteria. The plates were then incubated at 37°C for 24 hours to attain maximum growth.

Following overnight incubation, the number of colonies on the plates were counted, enumerated and characterized for identification of *Escherichia coli* by the standard methods by Edwards and Ewing, 1972 [30]. For *Salmonella*, 1 ml, of the water sample was thoroughly mixed with 9 ml of selenite broth (enrichment media), and subsequently incubated at 37°C for 24 hours. From the selenite broth, 0.1 ml was streaked on Xylose Lysine Deoxycholate (XLD: Himedia, Mumbai, India) and incubated at 37°C for 24 hours. Suspected *Salmonella* colonies were confirmed on biochemical reactions as previously indicated by Edwards and Ewing, 1972 [30].

The antimicrobial susceptibility testing on *E. coli* and *Salmonella* was done by using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (Becton, Dickinson and Company, MD, USA) based on the Clinical Laboratory Standard Institute (CLSI) [31] guidelines by Steinke, Seaton, Phillips, MacDonald and Davey [32]. The antibiotic discs (Becton, Dickinson and Company, MD, USA) used included Nitrofurantoin (300 µg), Cephoxitin (30 µg), Kanamycin (30 µg), Doxycycline (30 µg), Cotrimoxazole (1.25/23.75 µg), Amoxicillin and Clavulanic acid (AMC) (20 µg/10 µg), Tetracycline (30 µg), Gentamycin (10 µg), Chloramphenicol (30 µg), Oxacillin (1 µg), Cloxacillin (5 µg), Metronidazole (50 µg), Vancomycin (30 µg), Ampicillin (10 µg), Cepodoxime (10 µg), Penicillin G (10 U), Ciprofloxacin (5 µg), Clindamycin (10 µg) and Erythromycin (15 µg).

Interpretation of susceptibility patterns on anti-microbial discs was done using guidelines laid down in the clinical laboratory standards institute (CLSI) [31] which provide break points corresponding to zone of inhibition diameter. *E. coli* ATCC 29922 was used as quality control organisms.

Ethical Considerations

The study/research was conducted after approval from both the Tropical Diseases Ethics review Committee (STC/2016/15), and the National Health Research Authority at the Ministry of Health in Zambia (MH/101/23/10/1).

3. Results

A total of 779 drinking water samples were directly collected from taps, boreholes, kiosks, commercial bottled

water and non-protected wells were analysed from the 19 townships of Lusaka District (Table 1).

The analysis of the water samples from the 19 townships revealed that the most contamination occurred for tap water: 93.3% in Misisi, 77.8% Villa Elizabetha, and 76.3% in Kanyama/Mbasela; for borehole water: 100% for Kanyama/Mbasela, Misisi and Roma; for Kiosk water: 84.6% for Misisi 72.7%; while on total positivity: 90.3% for Misisi, 78.2% for Kanyama/Mbasela and 69.2% for Villa Elizabetha. All non-protected wells had 100% positivity (Table 2).

Contaminated water sources with total coliforms summarised in Table 2 numbered 262 (33.6%). There were five sources of contaminated water, namely: Tap water 204 (32.7%), borehole water 21 (32.3%), kiosk water 29 (41.4%), commercial bottled water 2 (14.3%), and nonprotected wells' water 6 (75%). The microbiological analysis of the drinking water revealed that the most and the least contaminated samples were kiosk and commercial bottled water, at 41.4% and 14.3%, respectively. *Salmonella* spp. 150 (57.25%) was found in more of the contaminated water samples than *Escherichia coli* 23 (8.7%). The most contamination was found in tap water, with high *Salmonella* (79.3%) and *E. coli* (65%) contents (Table 3).

A high *Salmonella* contamination of tap water was recorded in Sikanze 20 (17%), followed by Kanyama Bauleni 13 (11%) and Kanyama/Mbasela 10 (8%) townships (Table 4).

Antibiotic sensitivity test of *Salmonella* spp. and *Escherichia coli*

In the water samples, 136 *Salmonella* isolates (40.5%) exhibited antimicrobial resistance; while *E. coli* comprised 59 (17.6%) isolates (Table 5).

Antimicrobial susceptibility testing revealed that the only drugs susceptible to *Salmonella* were Kanamycin 1(0.7%), Chloramphenicol 2 (1.5%), doxycycline 5 (3.7%) and both tetracycline and nitrofurantoin 4 (2.9%).

The two bacteria showed varying resistance to the other antibiotics, with the highest being recorded in metronidazole 130 (95.6%) for *Salmonella* spp. and 53 (89.83%) for *E. coli*, oxacillin 130 (95.6%) for *Salmonella* spp. and 57 (96.6%) for *E. coli*, cepodoxin 121 (89 %) for *Salmonella* spp. and 52 (88.1%) for *E. coli*; and both penicillin G 117 (86. %) and Cephoxitin 115 (84.6 %) for *Salmonella* spp. (Table 6).

Table 1: Number and percentage of drinking water samples from five main water sources per township

Suburbs of Lusaka	Tap		Borehole		Kiosk		Commercial bottled water		Non-protected well		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
Bauleni	24	75.0	8	25.0	0	0.0	0	0.0	0	0.0	32	100
Chaisa	48	94.1	0	0.0	2	3.9	0	0.0	1	2.0	51	100
Chawama	37	100.0	0	0.0	0	0.0	0	0.0	0	0.0	37	100
Chilenje/Chalala	39	88.6	0	0.0	2	4.5	3	6.8	0	0.0	44	100
Chipata	41	100.0	0	0.0	0	0.0	0	0.0	0	0.0	41	100
George	33	76.7	1	2.3	9	20.9	0	0.0	0	0.0	43	100
Jack	36	90.0	2	5.0	2	5.0	0	0.0	0	0.0	40	100
Kabanana	19	61.3	8	25.8	4	12.9	0	0.0	0	0.0	31	100
Kamanga	36	59.0	12	19.7	13	21.3	0	0.0	0	0.0	61	100
Kamwala	46	93.9		0.0		0.0	3	6.1	0	0.0	49	100
Lilanda/Zingalume/ Twikatane	32	82.1	6	15.4	1	2.6	0	0.0	0	0.0	39	100
Makeni Villa	21	58.3	9	25.0	4	11.1	0	0.0	2	5.6	36	100
Matero	52	94.5	0	0.0	0	0.0	3	5.5	0	0.0	55	100
Mbasela Kanyama	38	69.1	2	3.6	11	20.0	0	0.0	4	7.3	55	100
Misisi	15	48.4	3	9.7	13	41.9	0	0.0	0	0.0	31	100
Mtendere	26	65.0	3	7.5	9	22.5	1	2.5	1	2.5	40	100
Roma	7	70.0	1	10.0	0	0.0	2	20.0	0	0.0	10	100
Sikanze	63	88.7	7	9.9	0	0.0	1	1.4	0	0.0	71	100
Villa Elizabetha	9	69.2	3	23.1	0	0.0	1	7.7	0	0.0	13	100
Grand Total	622	79.8	65	8.3	70	9.0	14	1.8	8	1.0	779	100

Table 2: Percentage of contaminated drinking water samples from five main water sources per township

Community	Tap	Borehole	Kiosk	Commercial Bottled Water	Non Protected Well	Total
Bauleni	16 (66.7%)	2 (25.0%)	0	0	0	56.3
Chaisa	12 (25.0%)	0.0	0	0	0	23.5
Chawama	11 (29.7%)	0.0	0	0	0	29.7
Chilenje/Chalala	11 (28.2%)	0.0	0	0	0	25.0
Chipata	12 (29.3%)	0.0	0	0	0	29.3
George	3 (9.1%)	0.0	0	0	0	7.0
Jack	6 (16.7%)	0.0	0	0	0	15.0
Kabanana	6 (31.6%)	0.0	1 (25%)	0	0	22.6
Kamanga	3 (8.3%)	3 (25.0%)	0	0	0	9.8
Kamwala	8 (17.4%)	0.0	0	0	0	16.3
Lilanda/Zingalume/Twikatane	4 (12.5%)	1 (16.7%)	1 (100%)	0	0	15.4
Makeni Villa	13 (61.9%)	3 (33.3%)	2 (50%)	0	2 (100%)	55.6
Matero	12 (23.1%)	0.0	0	1 (33.3%)	0	23.6
Kanyama/Mbasela	29 (76.3%)	2 (100.0%)	8 (72.7%)	0	4 (100%)	78.2
Misisi	14 (93.3%)	3 (100.0%)	11 (84.6%)	0	0	90.3
Mtendere	11 (42.3%)	0.0	6 (66.7%)	0	0	43.6
Roma	3 (42.8%)	1 (100.0%)	0	1 (50.0%)	0	45.5
Sikanze	23 (36.5%)	4 (57.1%)	0	0	0	38.0
Villa Elizabetha	7 (77.8%)	2 (66.7%)	0	0	0	69.2
Grand Total	204 (32.7%)	21 (32.3%)	29 (41.4%)	2 (14.3%)	6 (75%)	262 (33.6)

Table 3: Percentage of drinking water contaminated with total bacteria, *Salmonella* spp. and *Escherichia coli* from five main water sources

Contaminated water Sources	<i>Escherichia coli</i>	<i>Salmonella</i> spp.	<i>Escherichia coli</i> & <i>Salmonella</i> spp	Others	Total Coliform
Tap	15/23 (65%)	119/150 (79.3%)	44/61 (72%)	26/28 (93%)	204 (77.9%)
Borehole	2/23 (9%)	13/150 (8.7%)	6/61 (10%)	-	21 (8%)
Kiosk	5/23 (22%)	15 (10%)	7/61 (11%)	2/28 (7%)	29 (11.1%)
Commercial bottled water	-	2/150 (1.3%)	-	-	2 (0.8%)
Non-protected well water	1/23 (4%)	1/150 (0.7%)	4/61 (7%)		6 (2.3%)
Total	23/262 (8.78%)	150/262 (57.25%)	61/262 (23.28%)	28/262 (10.69%)	262

Table 4: Percentage distribution of water contamination with *Salmonella* spp and *Escherichia coli* from five main water sources in the 19 townships

Water type	Tap		Borehole		Kiosk		Bottled water	Non-protected well	
	<i>E.coli</i>	<i>Salm</i>	<i>E.coli</i>	<i>Salm</i>	<i>E.coli</i>	<i>Salm</i>	<i>Salm</i>	<i>E.coli</i>	<i>Salm</i>
Community									
Bauleni	0 [0%]	13[11%]	0 [0%]	2[15.4%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
Chaisa	0 [0%]	7[6%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
Chawama	0 [0%]	6[5%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
Chilenje/Chalala	1[7%]	8[7%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
Chipata	1[7%]	6[5%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
George	0 [0%]	1[1%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
Jack	0 [0%]	4[3%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
Kabanana	1[7%]	5[4%]	0 [0%]	0 [0%]	0 [0%]	1[6.7%]	0 [0%]	0 [0%]	0 [0%]
Kamanga	0 [0%]	2[2%]	1[50%]	2[15.4%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
Kamwala	0 [0%]	5[4%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
Lilanda/Zingalume/Twikatane	0 [0%]	2[2%]	0 [0%]	1[7.7%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
Makeni Villa	3[20%]	9[8%]	0 [0%]	2[15.4%]	1[20.0%]	1[6.7%]	0 [0%]	0 [0%]	0 [0%]
Matero	3[20%]	4[3%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	1[50.0%]	0 [0%]	0 [0%]
Mbasela Kanyama	1[7%]	10[8%]	0 [0%]	0 [0%]	1[20.0%]	2[13.3%]	0 [0%]	1[100.0%]	1[100.0%]
Misisi	0 [0%]	9[8%]	0 [0%]	1[7.7%]	2[40.0%]	8[53.3%]	0 [0%]	0 [0%]	0 [0%]
Mtendere	2[13%]	1[1%]	0 [0%]	0 [0%]	1[20.0%]	3[20.0%]	0 [0%]	0 [0%]	0 [0%]
Roma	0 [0%]	3[3%]	0 [0%]	1[7.7%]	0 [0%]	0 [0%]	1[50.0%]	0 [0%]	0 [0%]
Sikanze	3[20%]	20[17%]	1[50%]	3[23.1%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
Villa Elizabetha	0 [0%]	4[3%]	0 [0%]	1[7.7%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
Grand Total	15 [100%]	119 [100%]	2 [100%]	13 [100%]	5 [100.0%]	15 [100%]	2 [100%]	1 [100%]	1 [100%]

Table 5: Percentage of Antimicrobial Resistant *Escherichia coli* and *Salmonella* spp. in isolates from drinking water samples

	<i>Escherichia coli</i>	<i>Salmonella</i>
Isolates	59/336	136/336
Per cent	17.6	40.5

Table 6: Percentage of Antimicrobial Resistant *Escherichia coli* and *Salmonella* spp. isolated from the drinking-water samples

Antibiotics	<i>Salmonella</i> Resistant Isolates		<i>E. coli</i> Resistant Isolates	
	Number	Percentage (%)	Number	Percentage (%)
Ciprofloxacin	7	5.1%	3	5.1%
Cepodoxime	121	89 %	52	88.1%
Metronidazole	130	95.6%	53	89.8%
Clindamycin	100	73.5%	38	64.4%
Cotrimoxazole	107	78.7%	43	72.9%
Tetracycline	4	2.9%	3	5.1%
Cephoxitin	115	84.6%	46	78 %
Penicillin G	117	86.0%	40	67.8%
Gentamycin	10	7.3%	5	8.5%
Oxacillin	130	95.6%	57	96.6%
Doxycycline	5	3.7%	1	1.7%
Kanamycin	1	0.7%	0	0.00%
Cloxacillin	52	38.2%	33	55.9%
Nitrofurantoin	4	2.9%	3	5.1%
Chloramphenicol	2	1.5%	1	1.7%
Vancomycin	6	4.4%	5	8.5%
Ampicillin	6	4.4%	5	8.5%
Erythromycin	35	25.7%	12	20.3%
Total Number of Isolates	136		59	

4. Discussion

To the best of our knowledge, this is the first study in Zambia to examine drinking water given to infants and children post-weaning in households, as well as establishing the antibiotic susceptibility of *Salmonella* and *E. coli* of the water provided to children during the weaning period. The current study has demonstrated that the water used in households in Lusaka suburbs during the weaning period of children is generally contaminated with bacteria, mostly *Salmonella spp.* and *E. coli*.

Lusaka households in the surveyed communities/wards obtained their water supply for drinking and various household activities from five main sources, namely: tap water 622 (79.8%), borehole water 65 (8.3%), kiosk water 70 (9%), commercial bottled water 14 (1.8%) and nonprotected well water 8 (1%), (Table 1). In the present study, the contamination of different sources of household water was summarised in Table 2. Although these sources of water never met the guidelines of the WHO for quality of drinking water [33], the availability of safe water is vital to the satisfactory practice of food hygiene [34, 35].

Amongst the 19 townships, high total positivity was found in Misisi community 90.3%, followed by Kanyama/Mbasela 78.2%, Sikanze 69.2% and Makeni Villa 55.6%. These settlements were characterised by high population density, overcrowding, haphazardly laid out housing infrastructure and the poor provision of basic social services, including water supply and sanitation [36]. Many of these high-density settlements relied mainly on pit latrines or open defaecation to dispose their excreta; and were heavily dependent on shallow wells for water supply as described by other workers [22,37]. As a result of contaminated water and poor hygiene, resulting from the observed factors, basic hygiene-linked infections and diseases, such as diarrhoea, acute gastro-enteritis and salmonellosis, were a severe problem [13]. This was worse still if infants and children who were not on extended breast-feeding, as full breast-feeding provides a stronger protective effect among infants living in such crowded and highly contaminated settings [38].

Salmonella spp. 150 (57.3%) were found in most water samples that tested positive to bacterial contamination, (Table 3). Contaminated-water samples that tested positive to *E. coli* amounted to 23 (8.78%), and mixed contamination water samples of both *E. coli* and *Salmonella spp.* were found in 61(23.3%) and the other bacteria samples were 28 (10.7%). Furthermore, tap water had the highest contamination level of all the sources; *Salmonella spp.* had water samples that tested positive 119 (79.3%).

Escherichia coli water tap contamination was 15 (65%). Correspondingly, in their study on the prevalence of *E. coli* in drinking water sources in Nyakapala, Ghana, Adzitey and others [39] found that 29% of the water samples examined were positive for *E. coli*. All the well water samples (100%) were positive for *E. coli*; while 80% of the water samples collected from drinking sources, and tap water (12%), were likewise positive for *E. coli*.

Perhaps the water was stored in dirty utensils, acquired cross-contamination via handling; while flies could have been the mechanism of stored household water pollution [40]. Cross-contamination can introduce microbial contaminants via contact with hands, dippers and other faecally contaminated sources or the intrusion of vectors [41]. Several other studies investigating salmonellosis outbreaks have linked those outbreaks to the use of water contaminated with human faeces or animal manure [8,42-44]. Moreover, Kinkese and others. [28] reported that there were, in Zambia at "Out Patients Department (OPD)" records on first (1st) attendance of diarrhoea non-bloody for children of one (1) year to children under five years: 478,598 patients in 2013, and 520,380 patients in 2014 and 458,987 patients in 2015, according to the Ministry of Health reports. The emphasis of health education to mothers, on the importance of extended breast-feeding during the period when the immune system of the infants and young children is still maturing is essential, in order to reduce diarrhoeal diseases.

On the other hand, the findings in this study that water samples from commercial bottled water tested positive 2 (14.3%) was a matter of serious concern. Such water sources were not expected to be contaminated with any bacteria by the Zambia Bureau of Standards [45] and the World Health Organisation Standards guidelines [46]. The WHO [45] on drinking water standards, states that the level of *E. coli* or thermotolerant bacteria should be zero (0) in a 100 ml sample of water directly intended for drinking. Furthermore, the Zambian bureau of Standards (ZABS) for Safety and Quality Assurance states that: Drinking water should contain no *E. coli* per MPN/100ml; while drinking water should contain no *Salmonella spp.* per coliform forming unit (cfu)/100ml and 2 Coliforms per cfu/100ml [45]. Therefore, all the household water samples that were analysed and tested positive did not comply with the WHO water safety standards and with the ZABS water-quality standards. As such, the drinking water provided to children post-weaning in Lusaka Suburbs is not safe. The findings from this study have filled up the gap on households' drinking water safety investigation in Zambia.

Antimicrobials are essential for the treatment of infections caused by *Salmonella spp.* and *E. coli* [47]. However, antimicrobial resistance has been recognised as an emerging worldwide problem in human and veterinary medicine: both in developing and developed nations [48]. In this study, we had *Salmonella spp.* isolates 136 (40.5%) and *E. coli 59* (17.6%) from water samples that were tested for antibiotic sensitivity tests (Table 5).

Our study has revealed that *Salmonella spp.* and *E. coli* exhibited resistance to a number of antibiotics, such as metronidazole (*Salmonella spp.* 95%, *E. coli* 89%), oxacillin (*Salmonella spp.* 95.59%, *E. coli* 96.61%) and cepodoxime (*Salmonella spp.* 88.97%, *E. coli* 88.14%) (Table 6). These results raise serious concerns; as these are some of the drugs used for enteric infections [13], such as metronidazole and co-trimoxazole. Furthermore *Salmonella spp.* and *E. coli* have been found resistant to penicillin and oxacillin

Correspondingly, Adzitey and others [36], in their study found that 34 *Salmonella spp.* isolated from drinking water samples sources in Tamale Metropolis in Ghana were highly resistant to erythromycin (100%), vancomycin (94.1%) and amoxicillin (23.53%). In Malaysia, although few data were published and were available on the prevalence of *E. coli* and its resistance to antibiotics, *E. coli* strains were resistant to multiple antibiotics [49]. Similarly, Lim and others, [50] found that *E. coli* were resistant to ceftazidime (11%) in Malaysia and 28% in China; amoxicillin-clavulanic acid (17%) in Malaysia and in China 84%; and forty *E. coli* isolates were classified as Extendedspectrum beta-lactamases (ESBL) producers, based on the phenotypic or genotypic detection of ESBL. Likewise, Papadopoulos et al [14], found in Greece, antibiotic resistance of *Salmonella* strains to nine antimicrobials: Streptomycin (59.1%), tetracycline (47.7%), nalidixic acid (46.6%), ampicillin (37.5% and oxolinic acid (35.2%).

5. Conclusion

This study has revealed that there is considerable and extensive contamination with *Salmonella spp.* and *E. coli* in water in households in the Lusaka district. This is likely to be conducive to water-related diarrhoeal diseases in infants post-weaning, who are provided with this water. This study has also determined that *Salmonella spp.* and *E. coli* isolates revealed resistance to multiple antimicrobial drugs, mainly: metronidazole, Oxacillin and Cepodoxime. These results raised serious concerns regarding the safety of drinking water given to weaning children in Lusaka, as well as regarding the prospects for antibiotic treatment of enteric infections in Lusaka. [13,47]. The limitation of this

study was that there was no available literature to compare our findings with in Zambia. Further investigation should therefore study the impact of drinking-water storage quality in households.

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