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Original Paper

Contamination of Complementary Weaning Foods for Children with Escherichia coli and Salmonella species in Lusaka District, Zambia

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Abstract

Background: Complementary food is any food other than breast milk given to children in the complementary feeding period. The magnitude of foodborne diseases in Zambia is not fully known. We examined Escherichia coli (E. coli) and Salmonella levels of contamination in complementary foods. These are some of the most harmful potential pathogens that can cause foodborne diseases of human or animal origin. A selected bacterium was analysed for antibiotic susceptibility and their capacity to produce extended spectrum beta-lactamase (ESBL). The aim of the study was to critically explore and investigate the safety of complementary weaning foods in households in Lusaka district.
Methods: A cross-sectional study was conducted over a period of nine (9) months from October 2016 to July 2017. The study sites were in Lusaka district which was clustered into wards then finally into households. At every randomly selected household, the eligible mother/caretaker was asked to submit the complementary foods that she was giving the child, and sampling was done aseptically by taking a portion. A multi-stage sampling type was employed to collect complementary foods which were kept in various types of storage containers: Some (24%) of respondents used refrigerators while others respondents (10.1%) kept complementary foods on vegetable racks. The foods were prepared by boiling maize meal or cassava meal with the addition of pounded ground nuts, sugar, salt and butter. A high confidence limit of 95% (0.95) was calculated.
Results: Out of 244 (100%) complementary food samples contaminated with bacteria, 91 (37.3%) had E. coli and 38 (15.6%) had Salmonella. The rest – 115 (47.13%) – food samples were contaminated with other bacteria such as Staphylococcus 30 (12.30%), Bacillus 68 (27.87) and Streptococcus 17(6.97%). Of the food, 172 (70.49%) were energy foods, 56 (22.95%) protein foods and 16 (6.56%) other food types. Mtendere ward had more E. coli contamination over 6 X 10^4 cfu/g, while Kanyama/Mbasela had around 4 X 10^4. E. coli was found most resistant to metronidazole 72 (94.74%) and Ceftazidime 59 (77.63, while Salmonella isolates were resistant to metronidazole 30 (96.77%), Ceftazidime 25 (80.65%) and penicillin 24 (77.42%).
Conclusion: Complementary foods in this study of Lusaka district were generally contaminated with bacteria. The main one being Escherichia coli which is ubiquitous in nature and the commonest contaminant, followed by Salmonella spp. Contaminated weaning foods are still an unresolved problem in Zambia. Furthermore isolated E. coli and Salmonella exhibited resistance to metronidazole and Ceftazidime. The results raises some serious concerns as the drugs used for first choice for enteric infections. It is recommended that further studies should update the infants/children diarrhoeal cases in the Lusaka district and tackle the problem of antibiotic drug resistance with a view of vaccine development.

Key Words: Escherichia coli, Salmonella, bacterial Contamination, Complementary Foods, Weaning, Children, Suburbs and Lusaka

1.0 Introduction

Complementary food is any food other than breast milk given in the complementary feeding period [1]. Contaminated foods play a major role in the occurrence of infant diarrhoeal diseases. Moreover contamination of weaning foods and water is significant in the transmission of diarrhoeal diseases in developing countries [2]. "Unsafe food" poses global health fears, endangering the population. Infants, young children, pregnant women, the elderly and the terminally ill are particularly vulnerable [3]. Every year 220 million children contract diarrhoeal diseases and 96 000 die as a result [4]. In addition, there is a high incidence of diarrhoeal cases among African countries estimated at 3.3 to 4.1 episodes per child annually. Similarly some 700 000 children and adults die each year from diarrhoeal and dehydration [5]. Shteth and Dwivedi's [3] reports stated that food safety, especially in the weaning group, is one of the main anxieties that had posed a danger to health of infants and children. Millions of children in the world die yearly from diarrhoeal diseases. Likewise, the World Health Organisation (WHO) states that there has been an unprecedented reported number of *E. coli* and *Salmonella* foodborne diseases in the African region [5]. However, the magnitude of foodborne threats in Zambia is not fully known in foods that are used as complementary at weaning.

Numerous cases of foodborne illnesses emanate from inappropriate food handling, preparation and storage in homes [6]. Moreover, most of the child diarrhoeal cases could be due to foods and water that children consume in their homes as most foodborne transmission in developing countries takes place within the home [7]. Worse still is the fact that the role of contaminated weaning food is still unrecognized and more studies are needed to document its impact and develop appropriate interventions for application in developing countries [2].

Furthermore, according to the Ministry of Health reports in Zambia in 2015, non-bloody-diarrhoea has been consecutively ranked number three since 2006 and is among the top ten [10] diseases, causing visitations to health facilities for all ages combined in Zambia. There were at Out Patients department (OPD) records on first (1st) attendance of diarrhoea non-bloody for one (1) year to under five years children: 478,598 patients in 2013, 520, 380 patients in 2014 and 458,987 patients in 2015 [8]. Seemingly, most children being given complementary foods in Lusaka Suburbs generally suffer from diarrhoeal diseases. The safety of complementary foods and quality of water in Lusaka suburbs households is therefore questionable or compromised. Moreover, here in Zambia, according to the Ministry of Health [9], reports of diarrhoea (non-bloody) case fatality rate (hospitals only), which is defined as the number of deaths due to diarrhoea (non-bloody) per 1000 admissions of diagnosed diarrhoea (non-bloody) for the age group 5 years and below, has been higher in each of the years 2011, 2012 and 2013.

Studies that are investigating problems linked to safety of complementary food and water in households in Zambia are non-existing, especially during the weaning period. This is particularly important as it has been postulated that food, not water, may be the most important route of transmission of diarrhoea in less-developed countries [2]. To our knowledge this is the first study to analyse the safety of complementary foods given to children during weaning time in the Lusaka suburbs of Zambia. Food handling is an important factor in food safety, and includes the safety practices among those preparing and/or serving food as well as mode of storage [10]. Factors of food hygiene that include handling, preparation and storage practices are generally measured on the level of bacterial contamination [1]. Foods which are nutritious should also be wholesome and safe to prevent infection. Food should therefore be prepared hygienically and safely [12]. It is also acknowledged that bacterial contamination of complementary foods may occur as a result of poor hygiene of those who prepare food, the household, equipment, and the environment where the preparation of food takes place [13].

This study was initiated to fill up the data gap on the safety of complementary foods used in Zambian households at the time of weaning. The aim of the study, therefore, was to investigate and analyse the levels of contamination of complementary foods that children below or at two years of age are being provided in Lusaka suburbs during the weaning period. Furthermore, a selected bacterium was analysed for antibiotic susceptibility and the capacity to produce extended spectrum beta-lactamase (ESBL) [14].

2.0 Methodology

Study Design

This study was a mixed method study design combining both quantitative and qualitative approaches.

Identification and Sampling of complementary foods

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 and key informant interviews. The 2010 Census of Population and Housing Area sampling frame was used to select a mathematically determined representative sample of 769 respondents obtained from Central Statistical Office. A total of 761 women aged 15 years and older with at least a child were selected using probability sampling giving a 98.9 percent response rate. All eligible respondents at each selected household gave at least one or more food and water samples used for weaning the child during enumeration. These constituted the various samples of food and water types that were submitted to the School of Veterinary Medicine, Microbiology laboratory for Bacteriological analysis and possible identification of contaminants. The identified bacterial isolates were then subjected to antibiotic susceptibility tests. Complementary foods were kept in various types of storage containers: (24%)

of respondents used refrigerators while some respondents (10.1%) kept complementary foods on vegetable racks, (10.6%) respondents kept them in bucket and bottles, (11.8%) respondents kept them in plastic bags or sacks and (16.2%) respondents kept them in cupboard or kitchen units and (17.1%) respondents kept complementary foods in storage bins. When handling and before preparing complementary foods, 19.2% percent of respondents were not washing hands with soap, 8.8% of respondents never washed hands and 72% ensured their hands were clean. Complementary foods were prepared by boiling maize meal or cassava meal and they added pounded ground nuts, sugar and butter.

At every randomly selected household, the eligible mother/caretaker was asked to show us the complementary foods that she was giving the child, and sampling was done by aseptically taking a portion of the complementary foods. The samples were aseptically collected to avoid any contamination. All samples were clearly labelled to ensure identification of their source. This included: date and time collected, place or location and type of food sample.

The sterilized plastic bags were used as complementary food sample containers. The same equipment, such as spoons used to feed the child, was used to collect the complementary food samples. Every research assistant had cotton swabs soaked in 70% alcohol to wash their hands before and after collecting each sample. Sample containers were immediately put in cooler boxes that contained frozen ice packs and were transported to the laboratory for analysis.

Bacterial culture and identification

Samples were processed according to recommended techniques by International Organizations for Standardization (ISO-6579, 2000), and those of the Global *Salmonella* Surveillance (GSS) and National Health Services for Wales (NHS) [15, 16] at the School of Veterinary Medicine laboratories, University of Zambia.

Isolation of *Salmonella* involved three stages: firstly, 1 gram or equivalent of food samples was pre-enriched into 9 ml of buffered peptone water (BPW) (HIMEDIA Laboratory Pvt, Ltd, M614, Mumbai, India) and incubated for 24 hours at 37°C. A portion (1.0 ml) of the pre-enriched aliquot was transferred to 10 ml selective enrichment Rappaport Vassiliadis Soya bean Meal Broth (RVSM) (Himedia M1448) and incubated at 42°C. A loopful of the inoculum was transferred from the RVSM broth and streaked onto Xylose Lysine Deoxycholate (XLD) (HIMEDIA, M031) agar then incubated at 37°C for 24 hrs. The suspected colonies from the culture were cloned onto non-selective agar for pure culture. For a definitive *Salmonella* confirmation, pure colonies were serologically screened using polyvalent O antisera and phenotypically tested using Analytical Profile Index (API) 20 E systems (BioMérieux SA, Marcy-1 Etoile France). Further confirmation was achieved using PCR targeting the *invA Salmonella* gene.

For the isolation of *Escherichia coli*, the food samples were processed by transferring one gram of the food sample into 9 ml peptone water and homogenized by vortexing for five minutes at 120 xg. The aliquot was incubated at 37ºC for four hours. After incubation, a loopful of the mixture was inoculated onto MacConkey agar (Oxoid, Basingstoke, UK) then incubated at 37°C for 24 hours. After incubation, the colonies were identified as lactose fermenters or non-lactose fermenters. Identification of *E. coli* lactose-fermenting positive colonies was done using the Analytical Profile Index (API) 20 E systems.

Total bacteria counts in complimentary foods

From each food sample, 25g was weighed, suspended and homogenised in 225ml sterile buffered saline (10^{-1} dilution). After homogenisation, 10 ml of the homogenate was transferred into 90 ml of buffered saline (10^{-2} dilution), mixed thoroughly then 1ml transferred asceptically serially over a set of tubes containing 9 mls buffered saline (10^{-3} , 10^{-4} , 10^{-5} , 10^{-6}……10^{-9}). For standard plate count agar technique (Oxoid, Basingstoke, UK) by the pour plate method with a first choice from (10^{-3} dilution), dilutions were used for each sample up to no growth. One (1) ml from each of these chosen dilutions were asceptically transferred onto petri dishes in duplicates. Molten (PCA) agar at the right temperature was added to the aliquot in the petri dishes, mixed thoroughly, let to set at room temperature and incubated at 37 ºC to the maximum of 72 hours. Controls with diluents (buffered saline) and agar (PCA) plates were included then incubated at 37 ºC to the maximum of 72 hours.

Antibiotic susceptibility testing

The Kirby-Bauer diffusion method was used in the susceptibility testing of bacterial isolates using 17 antimicrobials agents belonging to eight antibiotic classes (Beta-lactams, macrolides, fluoroquinolones, tetracyclines, aminoglycosides, sulphanomides, nitrofurantoin and phenicols). The method used Mueller-Hinton agar with single concentration antibiotic disc potency by following the manufacturer's recommendations (Himedia Ltd). Pure colonies were emulsified in 5ml sterile physiological saline (0.85% NaCl) to make a bacterial suspension compared with a barium chloride standard (0.5 McFarland). Prior to bacterial inoculation, the surfaces of Muller-Hinton agar plates were dried at 37ºC. This was followed by dipping a sterile swab into the bacterial suspension, removed excess fluid by pressing the swab against the wall of the test tube and applied the swab contents evenly on to the surface of the agar. Sensitivity drug discs used included ampicillin (10µg), azithromycin (15µg), ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg), chloramphenicol (30µg), ciprofloxacin (5 µg), cotri-moxazole (25µg), erythromycin (15µg), gentamycin (10 µg), levofloxacin (5µg), nalidixic acid (30µg), nitrofurantoin (300µg), norfloxacin (10µg), penicillin G (10 units), streptomycin (300µg) and tetracycline (30µg). Culture plates were incubated at 37ºC for 24 hour and isolates were

determined as susceptible or resistant according to the breakpoints as described in the guidelines of the Clinical and Laboratory Standards Institute [17]. *Escherichia coli* (ATCC 25922) was used as a control organism.

Laboratory procedures for detecting ESBL production

For genetic detection, all *E. coli* isolates that showed resistance to Ceftazidime were analysed for extended spectrum beta-lactamase-producing capacity using PCR to detect the chromosome-encoded genes of intrinsic cefotaximases (bla_{CTX-M}). The *E. coli* was cultured in BHI (Brain Heart Infusion, HiMedia laboratories, India) at 37ºC for 18 hours. After incubation, 1ml of bacterial suspension was centrifuged at 5800 *xg* for five minutes. After centrifuging, the supernatant was discarded. The remaining cell pellet was washed with 500µl of normal saline, centrifuged at 13000 *x g* for 5 minutes and the supernatant was discarded. After washing, 500µl of TE buffer (pH 8.0) was added to the cell pellet and then heat treated until boiling, then immediately transferred to ice for 10 minutes. The cell debris was removed after centrifuging at 13000 *x g* for five minutes, while the supernatant was transferred into a new microfuge tube and maintained at -20ºC until use [18]. The master mix per reaction tube was made with 5µl of Phusion Flash, 2µl sterile water, 1µl of reverse primer, 1µl of forward primer and 1µl of DNA template, giving total volume of 10µl per reaction tube which was mixed using a vortex mixer. The PCR primers used for the detection of bla_{CTX-M} gene responsible for beta-lactamase-production was:

***bla*CTX-M -F 5'-CGATGTGCAGTACCAGTAA-3'** and
***bla*CTX-M –R 5'-TAAGTGACCAGAATCAGCGG-3'.**

The PCR conditions were set as 98℃ for 30 seconds, 98℃ for 0 seconds, 60℃ for 5 seconds (35 cycles), 72℃ for 15 seconds, 72℃ for 2 minutes, and holding at 4℃ Infinitely. After PCR, the products were visualized on a trans-illuminator machine following staining with ethidium bromide.

Ethical Considerations

This study was conducted after approval from both Tropical Diseases Ethics review Committee – STC/2016/15. Additional approval was obtained from National Health Research Authority – MH/101/23/10/1 for recruitment through health facilities.

3.0 Results

Sample Characteristics

1. ***Identification of Complementary Foods in this study***

The study was conducted in 19 randomly selected wards (communities) among the Lusaka suburbs. The wards (communities) included Villa Elizabetha, Roma, Chilenje/Chalala, Sikanze, Misisi, Kamanga, Bauleni, Mtendere, Lilanda/Twikatane Zingalume, Kamwala South, Kabanana/ Chazanga, Chipata, Jack, Chaisa, George, Matero, Chawama, Makeni Villa, and Kanyama/Mbasela. A total of 244 (100%) food samples (*table 1*) belonging to different types were collected in 19 community target areas (CTA) and were found with bacteria contamination. Out of 244 (100%) food samples contaminated with bacteria, 91 (37.30%) food samples were found with *Escherichia coli* and 38 (15.57%) food samples with *salmonella.* The rest 115 (47.13%) food samples were contaminated with other bacteria such as *Bacillus 68 (27.87%)*, *Staphylococcus30 (12.3%)* and *Streptococcus 17 (6.97%)*. In this article we considered only at *E. coli* and *Salmonella* because *Escherichia coli* is the commonest bacteria contaminant, followed by *Salmonella*. There were amongst the bacteria-contaminated complementary foods 172 (70.49%) energy foods, 56 (22.95%) protein foods and 16 (6.56%) other food types.

There were 30 energy food types. In *table 2* the most commonly found contaminated energy complementary food was maize meal 76 (31.15%), followed by nshima 19 (7.79%); boiled rice and bread were each 12 (4.92%), and porridge mixed with ground nuts 19 (7.79%).

There were 29 protein food types, as shown in *table 3,* where the most commonly found contaminated protein complementary food was pounded ground nuts 12 (4.92%), followed by cooked beans 8 (3.28%), chicken soup and fresh milk each 3 (1.23%) and chicken meat, fried egg, peanut butter and sour milk each 2 (0.82%).

There were 13 other food types. *Table 4* shows other complementary food types with bacterial contamination. A as shown in Table 4 these are cooked pumpkin leaves 3 (1.23%) cooked vegetables and cabbage each 2 (0.82%).

Table 1: Identified foods with bacterial contamination in various communities of study

S/N	Study Wards (Communities)	Energy Foods		Protein Foods		Other Foods		Total Foods
		n	**%**	**N**	**%**	**n**	**%**	
1&2	Villa Elizabetha/Roma	5	2.05%	-	-	2	0.82%	7
3	Chilenje/Chalala	3	1.23%	5	2.05%	-	-	8
4	Sikanze	12	4.92%	3	1.23%	2	0.82%	17
5	Misisi	6	2.46%	-	-	2	0.82%	8
6	Kamanga	10	4.10%	4	1.64%	-	-	14
7	Bauleni	5	2.05%	1	0.41%	-	-	6
8	Mtendere	26	10.66%	10	4.10%	2	0.82%	38
9	Lilanda/Twikatane	14	5.74%	3	1.23%	1	0.41%	18
10	Kamwala South	11	4.51%	3	1.23%	1	0.41%	15
11	Kabanana/Chazanga	11	4.51%	8	3.28%	1	0.41%	20
12	Chipata	19	7.79%	-	-	-	-	19
13	Jack	8	3.28%	3	1.23%	-	-	11
14	Chaisa	11	4.51%	3	1.23%	1	0.41%	15
15	George	11	4.51%	5	2.05%	3	1.23%	19
16	Matero	5	2.05%	3	1.23%	1	0.41%	9
17	Chawama	7	2.87%	1	0.41%	-	-	8
18	Makeni Villa	3	1.23%	4	1.64%	-	-	7
19	Mbasela Kanyama	5	2.05%	-	-	-	-	5
	Total	**172**	**70.49%**	**56**	**22.95%**	**16**	**6.56%**	**244 (100%)**

Table 2: Energy-complementary food types with bacterial contamination

Energy Complementary Foods with Bacterial Contamination	Number	Type	Percentage
Banana	2	Energy	0.82%
Boiled Rice	12	Energy	4.92%
Bread	15	Energy	6.15%
Cerelac	1	Energy	0.41%
Cerelac Porridge	1	Energy	0.41%
Cooked Nshima	1	Energy	0.41%
Cooked Potatoes	2	Energy	0.82%
Cooked Rice & Butter & Milk	1	Energy	0.41%
D'lite	4	Energy	1.64%
Dlite Porridge	2	Energy	0.82%
Infant Formula	1	Energy	0.41%
Maize Meal Mixed With Ground Nuts	6	Energy	2.46%
Macaroni	1	Energy	0.41%
Maize Meal	76	Energy	31.15%
Nakonde Rice	1	Energy	0.41%
Nshima	19	Energy	7.79%
Nshima & Eggs	1	Energy	0.41%
Nshima With Sausage	1	Energy	0.41%
Pombe Nshima	1	Energy	0.41%
Pork Soup	2	Energy	0.82%
Porridge Mixed with Maize Meal	11	Energy	4.51%
Porridge Mixed With Ground Nuts	3	Energy	1.23%
Potatoes	2	Energy	0.82%
Rice & Ground Nuts	2	Energy	0.82%
Rice With Peanut	1	Energy	0.41%
Rice/Millet/ Ground Nuts	1	Energy	0.41%
Sample Maize	2	Energy	0.82%
Total	172		70.49%

Table 3: Protein-complementary food types with bacterial contamination

Protein Complementary Foods with Bacterial Contamination	Number	Type	Percentage
Cooked Beans	8	Protein	3.28%
Beans Leaves/ Ground Nuts	1	Protein	0.41%
Chicken	8	Protein	3.28%
Cooked Vegetables With Kapenta	1	Protein	0.41%
Cooked Kapenta	1	Protein	0.41%
Cooked Meat (Goat)	1	Protein	0.41%
Eggs	1	Protein	0.41%
Fried Eggs	2	Protein	0.82%
Ground Nuts With Mealie Meal	1	Protein	0.41%
Groundnuts Porridge	1	Protein	0.41%
Kapenta/Beans	1	Protein	0.41%
Katapilla	1	Protein	0.41%
Mabisi Lacto	1	Protein	0.41%
Meat Soup (Beef)	1	Protein	0.41%
Milk Formula 2	1	Protein	0.41%
Fresh Milk	3	Protein	1.23%
Peanut Butter	2	Protein	0.82%
Peanut Porridge	1	Protein	0.41%
Pounded groundnuts	12	Protein	4.92%
Sausage Roll	1	Protein	0.41%
Sour Milk	2	Protein	0.82%
Sour Milk & Sugar (Porridge)	1	Protein	0.41%
Soya Meal (Instant)	1	Protein	0.41%
Tea Milk	2	Protein	0.82%
Uncooked Kapenta	1	Protein	0.41%
Total	56		22.95%

Table 4: Other complementary food types with bacterial contamination

Protein Complementary Foods with Bacterial Contamination	Number	Type	Percentage
Apple Max	1	Other	0.41%
Cabbage	2	Other	0.82%
Chikanda	1	Other	0.41%
Cooked Rape	1	Other	0.41%
Cooked Vegetables	2	Other	0.82%
Cooking Oil	1	Other	0.41%
Impwa	1	Other	0.41%
Pumpkin Leaves	3	Other	1.23%
Rape With Groundnuts	1	Other	0.41%
Raw Sausage	1	Other	0.41%
Relish	1	Other	0.41%
Shake And Sip	1	Other	0.41%
Total	**16**		**6.56%**

2. *Contamination Levels of E. coli and Salmonella*

Figure 1 above shows that Mtendere Ward had more *E. coli* bacterial contamination over 6 X 10^4 cfu/g, while Kanyama/Mbasela had around 4 X 10^4, and Chipata Ward had around 0.5 X 10^4 cfu/g.

3. *Antibiotic susceptibility testing*

Antimicrobial Susceptibility Testing

Antibiotic susceptibility tests conducted on selected *E. coli* and *Salmonella* isolates revealed resistance (*Table 5*). *E. coli* was found resistant to metronidazole 72 (94.74%), oxacillin 67 (88.16%), penicillin 60 (78.95%), ceftazidime 59 (77.63%) cloxacillin 41 (43.42%), clindamycin 34 (44.74%) and cotrimoxazole 33 (43.42%) while *Salmonella* isolates were resistant to metronidazole 30 (96.77%), oxacillin 28 (90.32%), Ceftazidime 25 (80.65%), penicillin 24 (77.42%) cotrimoxazole 22 (70.97%) and cloxacillin 19 (61.29%). Of the 59 *E. coli* isolates that were resistant to Ceftazidime, subjected to PCR for the detection of the $bla_{\text{CTX-M}}$ genes, the majority 53 (90%) were found to harbour the CTX gene.

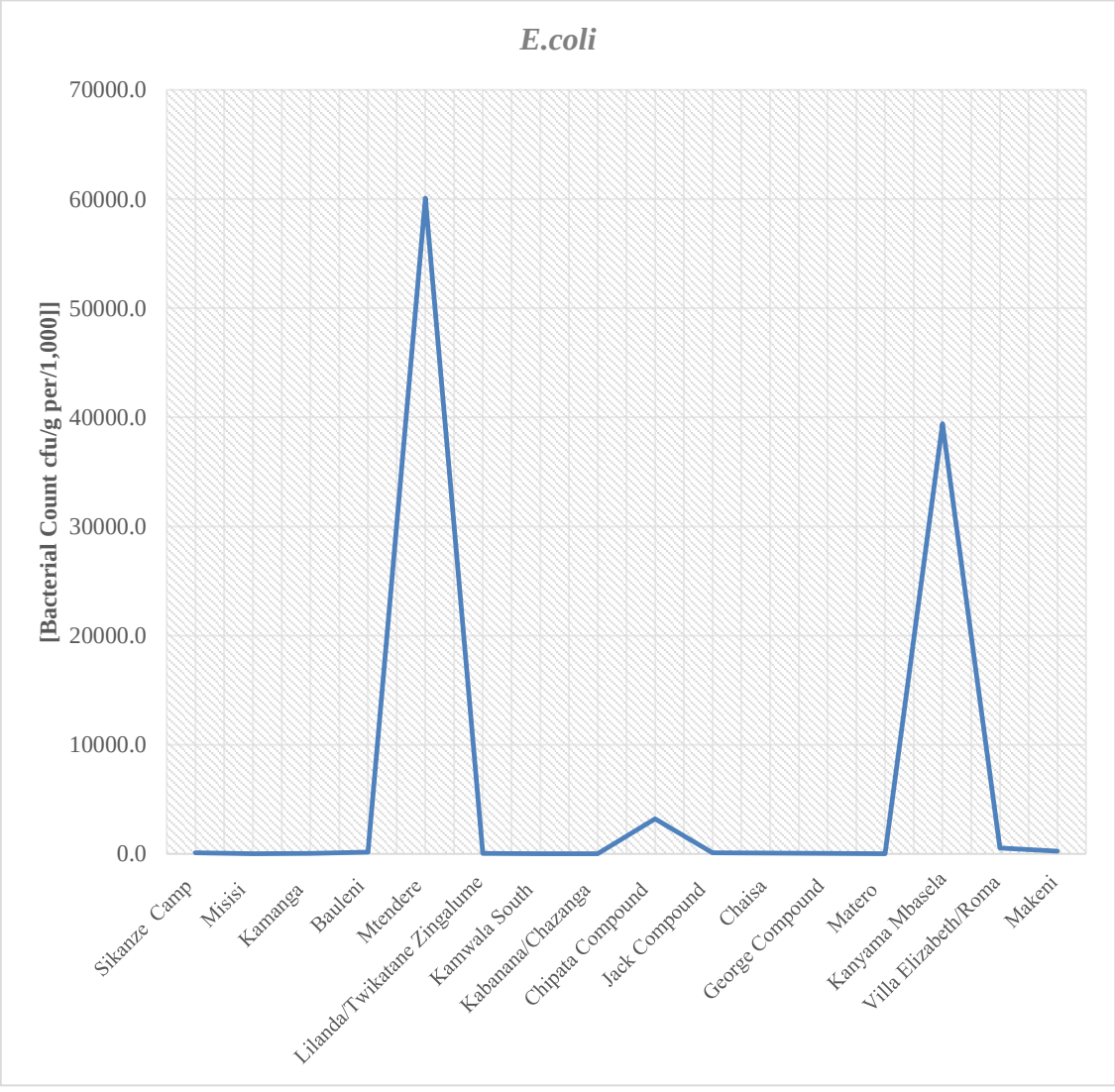

Figure 1: *E. coli* Distribution per Ward/Community as Bacterial count cfu/g per/1,000

Table: 5 Antibiotics Susceptibility of *E. coli* (n=76) and *Salmonella* (n=31) Isolates

Antibiotics	*E. coli* Resistant Isolates		*Salmonella* Resistant isolates	
	Number	Percentage	Number	Percentage
Ceftazidime	59	77.63%	25	80.65%
MT – Metronidazole	72	94.74%	30	96.77%
CO – Cotrimoxazole	33	43.42%	22	70.97%
COX - Cloxacillin	41	53.95%	19	61.29%
TE – Tetracyline	6	7.89%	3	9.68%
GEN – Gentamycin	0	0.00%	0	0.00%
DO – Doxycycline	16	21.05%	12	38.71%
CX – Cephoxitin	38	50.00%	17	54.84%
OX – Oxacillin	67	88.16%	28	90.32%
CD – Clindamycin	34	44.74%	19	61.29%
K – Kanamycin	18	23.68%	9	29.03%
P - Penicillin	60	78.95%	24	77.42%
CIP – Ciproflaxacin	0	0.00%	0	0.00%
CID -	3	3.95%	0	0.00%
VA: Vancomycin	5	6.58%	2	6.45%
Amp: Ampicillin	5	6.58%	2	6.45%
Nit - Nitrofurantoin	3	3.95%	0	0.00%
C: Chloramphenicol	2	2.63%	1	3.23%
E – Erythromycin	0	0.00%	2	6.45%
Number of Isolates	**76**	**100.00%**	**31**	**100.00%**

4. Discussion

Our study has established general bacteria contamination of complementary foods in Lusaka suburbs. *Escherichia coli* is the commonest bacterial contaminant, followed by Salmonella. This is as expected, as *E. coli* is ubiquitous in nature. Motarjemi et al.'s [19] study also identified infections due to pathogenic *E. coli* as being the commonest illness in developing countries that produce up to 25% of all diarrhoeal deaths. They also indicated that transmission of *E. coli* has been specifically associated with weaning foods.

Contaminated weaning food is still an unsolved problem in developing countries [20]. Studies have indicated that a great portion of diarrhoea and other foodborne diseases are due to unhygienic preparation of foods in households [7, 21, 22]. Identified foods with bacterial contamination in nineteen [19] randomly sampled communities, in *table 1*, were 244 (100%) sampled complementary foods. They were grouped into categories and described as energy foods, 172 (70.49%); protein foods, 56 (22.95%) and other foods 16 (6.56%). The study objective of determining the quality and safety of complementary foods was met. In this study most of energy-contaminated foods as well as protein foods have come from and overpopulated and high density suburbs characterized by poor hygienic environments and practices: Mtendere (10.66% energy foods and 4.10% protein foods; Chipata (7.79%) Kabanana/Chazanga, and George (4.51%) energy foods). In a similar study by Black and others [23] in Bangladesh, results showed that 41% of samples of food items fed to children of weaning age contained *E. coli*. Also, in a study conducted in Myanmar, food consumed by children aged 6-29 months was examined for four enteric bacterial pathogens. Of 775 samples of food tested, 505 were positive for *E. coli*, 28 for *V. cholera*, and six for *Salmonella* spp. [24]. These results have generated data on household complementary foods in Zambia and they have filled the gap on the lack of households' food safety data. The study data have also answered the research question on why feeding practices, food preparation methods and temperatures are important with weaning foods. These results are similar to other studies referred to, where hygiene is not maintained during food preparation and weaning foods are exposed to contamination at all steps of their preparation and handling and feeding.

In the current study, in *table 2*, the most common food type that is given to most children in the 19 community targeted areas, is the starchy energy food made from maize mealie-meal (staple food in Zambia), which maize meal porridge and nshima. These were the most contaminated: maize meal 76 (31.15%), nshima 19 (7.79%), porridge mealie meal 11 (4.51%). The other energy foods that are generally consumed are boiled rice 12 (4.92%) and bread 12 (4.92%). The study objective to identify common ingredients added or used as complementary foods and their safety has been met as well. In a similar study in peri-urban Mali, Toure and others [7] found that "Moni", a weaning food made by cooking flour from up to six local cereals, was contaminated by coliforms during storage, producing counts up to 550TTC/g. Also, a study conducted in Zanzibar, Tanzania, demonstrated higher bacterial numbers in infant porridges held for four hours after preparation in the households. In Zanzibar, Tanzania, and

many other low-income countries, foods made from local grains predominate in the diet of children other [25].

Commercially available fortified complementary foods are considered to be convenient and nutritious in developing countries [25]. In this study, D'lite 4 (1.64%) and D'lite porridge (0.82) were contaminated with bacteria. Our results are in agreement with other studies in Zanzibar, Tanzania that observed that a commercially fortified instant soy-porridge (SRP) and cooked porridge (Lishe bora LB) had high bacteria counts [25]. This study has produced data to fill up the gap on complementary food studies in households in Zambia. Similarly to other studies in African countries referred to contamination of such energy foods happen during storage of weaning foods held at warm temperatures.

The most contaminated protein foods (*table 3*) were pounded ground nuts 12 (4.92%), cooked beans (3.28%), chicken soup and tea with milk, both at 2 (0.82%). The study objective also, to identify common ingredients added or used as complementary foods and their safety has been met accordingly. These results are consistent with results in Mali, where the levels of contamination found in fish soup, a protein food, at baseline and continuing in the foods of the control group, were shockingly high as home-produced foods consumed by children [7, 26]. Comparing to other studies in Africa and Asia, results are not different probably due to unhygienic conditions that prevail in households. The same arguments above will be applicable to table 4 for other food types.

Nonetheless, our findings in figure 1, of *Escherichia coli* up to a bacterial count of more than10 X 10^6 cfu/g in Mtendere locality and of more than 10 X 10^6 cfu/g in Kanyama/Mbasela in various foods do meet the study objective of determining the quality and safety of complementary foods. These findings, they support other studies by Motarjemi [27] that indicated that transmission of E. *coli* has been specifically associated with weaning foods; Enterotoxigenic E. *coli* was the commonest organism isolated in infants aged 6-12 months. Also *E. coli*-contaminated foods were considered to be responsible, in part, for the diarrhoea-induced weight faltering [27]. Similarly, studies by Onyangore and others [12] stated that patients, especially children, who are affected by Enterotoxigenic *E. coli* can develop haemolytic uraemic syndrome (HUS), which is characterized by acute renal failure. Furthermore, Gama and others [28] established in their study that *E. coli* 10^6 is the minimum ID50 infectious dose to cause disease in humans. Lanata [2] also observed that many other studies that have been carried out on weaning foods given to children, just at the point before consumption were found with faecal coliform in high proportion. Data of this study have rightly answered research questions why are feeding practices, food preparation, storage methods and temperatures are important with weaning foods in households. The similarity to other studies are that communities lives in overcrowded environment that also lack good hygiene and sanitation.

Antimicrobial resistance has been recognised as an emerging worldwide problem in human and veterinary medicine both in developing and developed nations Rasheed et al, [29]. In this study, as shown in table 5 the isolated *E. coli* and *Salmonella* exhibited resistance to a number of antibiotics such as Ceftazidime (*E. coli 77.63%, Salmonella 80.65%*) and metronidazole (*E. coli 94.74%, Salmonella 96.77%*). Of the 59 E. coli isolates that were resistant to Ceftazidime, subjected to PCR for the detection of the blaCTX-M genes, the majority 53 (90%) were found to harbour the CTX gene. Though the study objectives were met; this result raises serious concerns as these are some of the drugs used for enteric infections. As expected in the case of penicillin, resistance may be due to intrinsic mechanisms as Gram-negative bacteria do not have a target site for penicillin. Ciprofloxacin remains a drug of choice in case of any infections. The ESBL *E. coli* detected are beta lactamases capable of conferring bacterial resistance to the penicillins, first, second, and third generation cephalosporins, and aztreonam (but not cephamycins or carbapenems) by hydrolysis of these antibiotics, and are inhibited by β-lactamase inhibitors such as clavulanic acid [30, 31]. Just as in the Korea study by Ryu and others [32] they found that 13 E. coli isolates were multidrug resistant to three or more antibiotics and twelve isolates carried at least one antimicrobial resistant gene. Likewise Rasheed and others, [29] study in Hyderabad, India; their investigation showed that organisms harbouring Extended Spectrum B-Lactamase enzymes were multi-drug resistant showing resistance to 12 or more drugs tested and thus, could pose serious challenge to the public health. This study, similarly to the foregoing studies in India and Korea, has provided comprehensive data to fill the gap of information in food safety in Zambia. Further investigation may be required to ascertain the extent of diarrhoea cases in these communities.

Conclusion

Our findings have revealed that complementary foods in Lusaka district are generally contaminated with bacteria. *Escherichia coli* is the commonest bacteria contaminant, followed by *Salmonella*. Contaminated weaning foods are still an unsolved problem in Zambia. Furthermore, findings of *Escherichia coli* up to a bacterial count of more than10 X 10^6 cfu/g in the Mtendere locality and of more than 10 X 10^6 cfu/g in Kanyama/Mbasela in various foods, indicate that transmission of *E. coli* has been specifically associated with weaning foods. There were, in Zambia at "Out Patients Department (OPD)" records on first (1^{st}) attendance of diarrhoea non-bloody for one (1) year to under five years children: 478,598 patients in 2013, 520, 380 patients in 2014 and 458,987 patients in 2015 (8). This study also has concluded that the isolated *E. coli* and *Salmonella* exhibited resistance to a number of antibiotics such as Ceftazidime and metronidazole. This result raises serious concerns as these are some of the drugs used for enteric infections.

Further investigation should update the infants/children diarrhoeal cases in the Lusaka district. Also further studies should tackle the problem of antibiotic drug resistance with a view of vaccine development. Finally any further study could review the processes of complementary food preparation, storage and feeding practices and study on the implementation of HACCP (hazard analysis critical control point) in households.

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Declarations

The Corresponding Author, on behalf of all co-authors, has read and agreed to the terms of the *Journal of Preventive and Rehabilitative Medicine* protection notice. Informed consent was obtained from all respondents that information that they provided could be published. All Authors have seen and approved the final manuscript. We also do hereby declare that all the work in this study is original and has not been submitted elsewhere.

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