Child Health and Safety of Complementary Food among Households in Adyel Division, Lira District: A Cross Sectional Study.

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Abstract

Background

Safe complementary food is food that will not cause harm to infants and children when prepared and fed as recommended. Unfortunately, children are frequently suffering from diarrhoea which is not only preventable but is also largely attributed to complementary foods hence this study.

Methodology

The study was carried out in 2015 in Adyel Division, Lira District among caregiver-child pairs. The children were between the ages of 6 – 23 months. Information collected was about the children's diarrhoeal patterns, nutritional status, and complementary food safety.

Results

Analyses of food samples revealed that freshly cooked food was contaminated with fecal coliforms (4.88±1.87 log cfu/g), and the levels of fecal coliforms in stored food increased with prolonged storage period (5.49b±1.86 log cfu/g).

Drinking water too was contaminated with E.coli (2.86 logs cfu/ml). Water in storage containers had total coliform counts of up to 3.14 log cfu/ml. In over half of the households (56.7%), the microbial counts in household drinking water containers (4.48E+03cfu E.coli) were more than those found at the respective water sources (4.46E+02cfu E.coli). Water treatment accounted for 25% of the variation (p=0.005) in E.coli counts in drinking water that was found in water storage containers. About 32.5% of the frequency or recurrence of diarrhea episodes in two weeks among children was explained by the presence of fecal coliforms in freshly cooked complementary food (p=0.001). Overall, diarrheal infections (p=0.030), inappropriate child-feeding practices (p=0.048), and poor hand-washing (p=0.011) played a significant role in influencing child health.

Conclusion

The food safety in this study was compromised by poor complementary food handling practices.

Recommendation

There is a need to study specific food combinations under more controlled conditions to compare the effect of the different handling practices on the microbial load in the various foods.

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1. Background

Children are ideally introduced to complementary foods at 6 months according to the IYCF

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guidelines (Ministry of Health, 2009). These complementary foods must be safe as by the following IYCF policy implementation guideline of Uganda:

"Encourage parents to practice high standards of hygiene when handling the infant's food, and also to maintain sanitation standards and food/water safety among others" (Ministry of Health, 2009).

The safety of complementary foods can be defined as the certainty that they will not cause harm to infants and children when prepared and fed as recommended (WHO, 2009). Some of the most common contaminants that compromise food safety are toxins and microbes, which include bacteria, fungi, viruses, and protozoa. Different food handling practices are associated with microbial hazards that are likely to cause diarrhoea among children. Some of these include: combining different foods to either improve palatability and/or nutritional value, which increases the risk for rapid microbial/bacteria growth during storage (Ehiri, Azubuike, Ubbaonu, Ibe, & Ogbonna, 2001); purchase of foods and/or ingredients from unreliable sources, inadequate cooking and addition of contaminated ingredients, especially after heat treatment, also increases the microbial count (Omemu & Omeike, 2010); storage after preparation, inadequate reheating of food before consumption; unwashed leaves used for wrapping food with inadequate cooking; and intermittent assessment of manual grinding using unwashed hands, dirty utensils, and potentially contaminated wrapping material; as well as market displays all result in food contamination (Ehiri *et al.*, 2001). These findings are in agreement with those of Black and colleagues (1989) who concluded that a particularly important route of transmission of diarrhoea-causing microbes may have been weaning/complimentary foods; which were contaminated due to improper preparation and inadequate cleaning of utensils in Lima, Peru; however, for most food items, the frequency of contamination was related to the amount of time since initial preparation (Black, et al., 1989).

The problem is that children are frequently suffering from diarrhoea which is not only preventable but is also largely attributed to complementary foods (UBOS, 2012); yet the actual causes specifically along the household food chain in Northern Uganda are not documented, hence this study.

This study was limited to identifying the effect of storage and possible reheating of cooked complementary foods on safety. This was done by determining the presence or absence of fecal Coliform bacteria that could have been compromising the complimentary food safety and water quality; thus predisposing the children to diarrhoea and poor nutritional status in Adyel Division, Lira district.

2. Methods

2.1. Study Design

An exploratory cross-sectional study was carried out and quantitative methods were used to collect data.

2.2. Study Area

The area of study was Adyel Division which is located in Lira District. Lira District is bordered by the Districts of Pader and Otuke in the North and North East respectively; Alebtong in the East; Dokolo in the South; and Apac in the West (Ongom, 2011). Lira which is within the Lango Sub-region was classified as food secure by the Uganda Food Security Brief (FAO, 2010). The report also showed that the region had good dietary diversity and food availability, especially after the first season's harvest in July. This was partly attributed to the increase in food access after all the households moved back to their homes and are fully engaged in agriculture. The food security in Lira was also attributed to the use of early maturing varieties and growing security crops such as cassava and sweet potatoes (FAO, 2010).

2.3. Inclusion Criteria

The inclusion criteria were that the household had to have a child of 6-23 months that was reported to have experienced at least one bout of diarrhoea within the 6 months preceding the survey.

The caregiver should have consented to participate in the study.

2.4. *Exclusion Criteria*

Non-consenting participants were excluded from the study. Households that denied participating in the collection of water or food samples were excluded from the study.

2.5. *Sampling*

The 30 caregiver-child pairs were randomly chosen from these households whose children had had at least one bout of diarrhoea within the previous six months of the survey.

2.6. *Study Variables*

The study variables were Nutritional status, diarrhoeal prevalence, and frequency, E. coli in drinking water at the source and the household, as well as Fecal coliforms in freshly cooked, stored, and reheated complementary food.

2.7. *Data Sources*

Primary data was obtained from the caregiver-child pairs about diarrhoeal patterns, nutritional status, and food safety. A questionnaire was used to obtain demographic information from the respondents who were primarily the caregivers of children aged 6-23 months of age.

2.8. *Determining Children's Health, Dietary Intake, and Nutritional Status*

Nutritional status is influenced majorly by dietary intake and disease. To obtain associations among these aspects, information was obtained concerning the children's health history, dietary intake, and nutritional status as elaborated below.

2.8.1. *Child's health history*

The child's health history was assessed by asking the caregiver to report on all illnesses the child had experienced within the two weeks preceding the survey. Caregivers were also asked to give an account of the last time the child had diarrhoea, the number of times the child had diarrhoeal episodes in the two weeks preceding the study, and to report on diarrhoeal episodes experienced by the index child within six months preceding the study.

2.8.2. *Child's Dietary intake*

A modification of the Delta NIRI Food Frequency Questionnaire (Tucker, et al., 2004) was used to determine the types and number of times different locally available foods were fed to the index child over seven days. This information was used to determine the commonly used foods. To assess the quality of children's diets, the primary caregivers were also asked to give an account of what the child ate during the past 24 hours preceding the survey using the multiple passes 24-hour food recall questionnaire. The data collected from the 24-hour dietary recalls were used to determine the child-feeding patterns notably: the number of meals, diversity of meals per day, common food preparation methods, and the different sources of food given to the children.

The Child Feeding Index (CFI) and Food Consumption Score (FCS) were used in this study because they are favourable in determining the child feeding patterns in association with child growth (Ma, *et al.*, 2012; Zhang, *et al.*, 2008; Lohia & Udipi, 2014; Srivastava & Sandhu, 2007; Reinbott, *et al.*, 2015).

CFI and FCS computation methods used ***Adapted from Ruel & Menon, (2002) .***

Food Consumption was also assessed using the Food Consumption Score (FCS) which is also known as the "Frequency weighted diet diversity score" is obtained by determining the frequency of consumption of different food groups consumed by a household or individual during the 7 days before the survey. The FCS is calculated by:

· Using the standard 7-day food frequency data, all the food items were grouped into specific food groups, ie, Main Staples, Pulses, Vegetables, Fruit, Meat and Fish, Milk, Sugar, Oil, and Condiments.

· All the consumption frequencies of the food items of the same group were summed up and the value of each group above 7 was recorded as 7.

· The value obtained for each food group was multiplied by its weight (see food group weights in the table below) creating new weighted food group scores.

· The sum of the weighted food group scores was obtained, thus creating the food consumption

Table 1: Variables used in the scoring system to create the Child Feeding Index (CFI) for children 6 to 36 months

Variables	6-9 months	9-12 months	12-36 months
Breastfeeding	No = 0; Yes = +2	No = 0; Yes = +2	No = 0; Yes = +1
Uses bottle	No = 1; Yes = 0	No =1; Yes = 0	No = 1; Yes = 0
Dietary diversity (in past 24 hours)	**Sum of:** (grains + tubers + milk + egg/fish/poultry + meat + other): 0 = 0 1-3 = 1 4+ = 2	**Sum of:** (grains + tubers + milk + eggs/fish/poultry + meat + other): 0 = 0 1-3 = 1 4+ = 2	**Sum of:** (grains + tubers + milk + eggs/fish/poultry + meat + other): 0 = 0 1-3 = 1 4+ = 2
Food group frequency (past 7 days)	**For each of: - egg/fish/poultry - meat** 0 times in past 7 d = 0 1-3 times in past 7 d = 1 4+ times in past 7 d = 2 **For staples (grains or tubers)** 0-2 times = 0; 3+ times = 1 **Food group frequency** = sum of scores for staples + egg/fish/poultry +meat	**For each of: - egg/fish/poultry - meat** 0 times in past 7 d = 0 1-3 times in past 7 d = 1 4+ times in past 7 d = 2 **For staples (grains or tubers)** 0-3 times = 0; 4+ times = 1 **Food group frequency** = sum of scores for staples + egg/fish/poultry + meat	**For each of: - milk - eggs/fish/poultry - meat** 0 times in past 7 d = 0 1-3 times in past 7 d = 1 4+ times in past 7 d = 2 **Food group frequency** = sum of scores for milk + egg/fish/poultry + meat
Meal frequency (past 24 hours)	0 meals/d = 0 1 meal/d= 1 2 meals/d = 2	0 meals/d = 0 1-2 meals/d = 1 3+ meals/d = 2	0-1 meal/d = 0 2-3 meals/d = 1 4+ meals/d = 2
Total score	**12 points**	**12 points**	**12 points**

score (FCS).

· Using the appropriate thresholds (see below), the variable food consumption scores were recorded, from a continuous variable to a categorical variable.

The typical thresholds (FCS Profiles) are: 0-21 for Poor; 21.5-35 for Borderline; and > 35 for Acceptable (WFP, 2008).

2.9. Children's nutritional status

Anthropometrical assessments (mid-upper-arm circumference (MUAC), weight, and height) were conducted to determine the nutritional status of the children aged 6 to 23 months.

2.10. Mid-upper Arm Circumference (MUAC)

All measurements were taken from the children's right arms using MUAC measuring tapes. The child's right arm was bent at the elbow at a 90o angle with the upper side parallel to the body. Then using the MUAC tape, the length between the shoulder bone (acrominion) and elbow tip (olecranon) is measured and the midpoint is marked on the child's hand. The child's hand was relaxed and let to hang loosely on the side before the tape was put around the upper arm at the previously marked midpoint- ensuring that the tape was snug, but not very tight. The circumference was recorded to the nearest 0.1 cm and the procedure was repeated once to verify the first result. The measurements were used to get averages. The measurements were entered into the WHO Anthro software (version 3.2.2) and the MUAC – for-Age Z-scores were computed using the WHO reference population. The cut-offs used were: $\leq$ -2 SD for

Table 2: Standard Food Groups and Weights used in calculating the Food Consumption Score (FCS)

	FOOD ITEMS (examples)	Food groups (definitive)	Weight (definitive)
1	Maize, maize porridge, rice, sorghum, Millet, pasta, bread and other cereals Cassava, potatoes and sweet potatoes, other tubers, plantains	Main staples	2
2	Beans. Peas, groundnuts and cashew nuts	Pulses	3
3	Vegetables, leaves	Vegetables	1
4	Fruits	Fruit	1
5	Beef, goat, poultry, pork, eggs and fish	Meat and fish	4
6	Milk yogurt and other diary	Milk	4
7	Sugar and sugar products, honey	Sugar	0.5
8	Oils, fats and butter	Oil	0.5
9	spices, tea, coffee, salt, fish power, small amounts of milk for tea	Condiments	0

Adaptedfrom WFP (2008)

severely wasted; -2 SD to -1.01 SD at risk or moderately wasted; and ≥ -1 SD were considered normal.

2.11. Length

Measurements were taken using a height board (SECA 417 measuring board). The caregiver was asked to remove the child's shoes, socks, hats, and any heavy or tight clothing that made it difficult to take measurements. The child was laid on their back on the measuring mat making sure their legs were straightened with the head held in place against the headboard looking vertically upwards and the toes pointed upwards. Then the footboard was moved against the child's feet and the measurement indicated by the footboard was recorded to the nearest 0.1cm. The procedure was repeated to get an average of the two measurements. The measurements were entered into the WHO Anthro software (version 3.2.2, and the Length – for - Age Z-scores were computed using the WHO reference population. The cut-offs used to characterize children's nutritional status were: ≤ -2 SD severely stunted; -2 SD to -1.01 SD were classified as at risk or moderately stunted; while ≥ -1 SD were considered normal.

2.12. Weight

Children's weight was measured using a bathroom scale (SECA 804 weighing scale). The caregiver was asked to remove excess clothes, accessories, and shoes from the child. Then the caregiver was asked to carry the child and stand on the weighing scale and the measurement on the scale was noted. Then the caregiver was asked to stand on the weighing scale alone and the measurement on the scale was noted. The difference between the two measurements obtained was noted as the weight of the child to the nearest 0.1kg. The procedure was repeated and the average of the two measurements was noted. The measurements were entered into the WHO Anthro software (version 3.2.2) and the Weight for Age Z-scores was computed. Using the WHO reference population, the cut-offs used were: ≤ -2 SD severely underweight; -2 SD to -1.01 SD were classified as at risk or moderately underweight; while ≥ -1 SD were considered normal.

3. Assessment of complementary food quality

Since it was speculated that unsafe food was the major cause of diarrhoea, the existence of disease-causing microbes in cooked food and drinking water was used as a measure of food quality. Hence, microbial analysis was carried out on samples of complementary food or meals and drinking water served to children during the 24-hour survey period. Having noted the different serving times in a household per day, food samples were obtained using sterile containers which were aseptically opened at serving (and other critical periods) while the caretaker put in a sample of the complementary food being served to the child – in the same way as she was serving or feeding the child. The sterile containers were closed and put in ice boxes.

In addition to the food samples, the caregivers' hands and food preparation surfaces such as plates and spoons were swabbed to quantify microbial levels. Before the caregiver put in the food sample, the surface of the hands and utensils onto which food was served were swabbed using sterile swabs that were aseptically removed from their casings. The swabs were immediately aseptically put back in their respective casings and stored in ice boxes.

4. Bacteriological examination of total coliforms and E. coli in water

UNBS (2008), gives the specifications for drinking water that is suitable for human consumption; where the water should contain no E.coli at all. The maximum microbiological limits for drinking water are shown in the table. This study was limited to identifying the Coliform bacterial microbes that could be compromising the complimentary food safety and water quality; thus predisposing the children to diarrhoea.

Water samples were aseptically collected from the household drinking water storage containers and water sources using sterile 200 ml bottles. Water from Jerry's cans was poured into the sterile containers while water from pots was scooped using the cup that the family uses to scoop the water and poured into the sterile container. Water from the water sources was fetched directly from the source into the sterile containers. The samples were then put in ice boxes and transported to Makerere University, School of Food Technology, Nutrition and BioEngineering (FTNB) – Microbiology laboratory for microbial analysis.

About 15ml of the E.coli chromogenic agar was aseptically prepared and poured on sterile Petri dishes and let to set. Then 100 mL of each of the collected water samples were aseptically measured and passed through a membrane filtration unit fitted with a sterile membrane filter (pore size 0.45 μm) placed at the base of the sterile funnel. Aseptically, the membrane filter was then removed from the membrane filtration unit and placed on a well-dried surface of E. coli chromogenic agar in a petri dish. These were then incubated at 37 °C for 24 hours.

5. Bacteriological examination for Total Coliforms and Fecal Coliforms in Food and on Swabbed surfaces

The Horizontal method for the enumeration of total coliforms and fecal coliforms in food and on swabbed surfaces was used.

Preparation of Violet Red Bile Lactose (culture media): The required amount of media powder was weighed and thoroughly mixed with distilled water and heated until boiling with occasional stirring. The mixture was allowed to boil for 2 minutes before it immediately cooled in a water bath at 44°C – 47 °C.

Procedure for culturing: The serial dilutions were prepared by aseptically adding 10g of each of the food samples to 90ml of diluent (swabs were put directly in 9ml of diluent) and mixed on a vortex; then using a sterile pipette 1ml of the suspension was transferred aseptically to 9ml of diluent; from which 1ml was also transferred to 9ml of diluent till 6 serial dilutions of each food sample were made. Then using a sterile pipette, 1ml of the sample of 3 appropriate dilutions randomly selected were each transferred to the centre of a petri dish in duplicates. Then about 15ml of the molten medium was poured into each petri dish, and the mixture of the medium and inoculum was carefully mixed and allowed to solidify. Also, a control plate with about 20 mL of the medium was prepared for checking its sterility. After complete solidification, the Petri dishes containing the solidified mixture of inoculum and VRBL medium were inverted and incubated at 300C for 24 hours for Total Coliforms; and at 44.50C for Fecal Coliforms.

Enumeration of colonies: The purplish red colonies are considered typical colonies of Coliforms and do not require further confirmation. Counts from those plates with colony numbers ranging from 30 – 300 were taken using colony-counting equipment.

Counting of colonies: Colonies of E. coli appear blue or purple while those of the total coliforms appear pink. These were counted and noted for each of the samples and then the formula for the number of colonies was used as follow

$$\mathbf{C} = \frac{\Sigma \mathbf{X}}{\mathbf{V}[\mathbf{n}_1 + \mathbf{n}_2\ (\mathbf{0.1})]\ \mathbf{d}}$$

Where: C is the number of microbial units

ΣX is the sum of all the counted colonies

n_1 is number of petri dishes at the first counting

n_2 is number of petri dishes at the second counting

d is the dilution factor at which the first counting was done

Expression of obtained observations: Total coliforms or E. coli = x cfu per 100 mL.

In the laboratory, tests were carried out for; the presence of E. coli, and total coliforms in water; feacal coliforms, and total coliforms in food taken at different critical stages of the handling process using standard ISO methods - ISO 4832:2006 of microbe enumeration.

6. Sample size

The number of caregiver-child pairs was 30.

In each household, drinking water was collected from the household and the water source; food samples were also collected (freshly cooked food from every household, as well as stored food and reheated food from households that kept and warmed leftover food respectively). These samples were all collected in replicates (duplicates).

7. Data analysis

The data from interviewer-administered questionnaires were coded and entered into Statistical Package for Social Scientists (SPSS version 20) and analyzed to obtain descriptive statistics such as frequencies and percentages.

Anthropometric data were processed using WHO Anthro to determine the Z-scores. The nutritional status of the children was assessed based on Height-for-Age, MUAC-for-Age, and Weight-for Age Z-scores.

Differences in demographics, food handling practices, feeding practices, and nutritional status between the households were determined using the T-test. Correlations were used to determine the association among complimentary food and water handling practices, feeding patterns, and child health (diarrhoea prevalence or frequency and nutritional status). Simple Linear regression analyses were carried out to test the extent to which: food quality was influenced by handling practices; and the extent to which diarrhoea frequency was influenced by food contamination.

The laboratory analysis data collected were subjected to the Analysis of Variance (ANOVA) test using Statistix Software version 9.0. Mean separation was done using the Least Significant Difference (LSD) test at 1% ($P = 0.01$) to find out if there were any statistically significant differences among the different sets of samples collected.

8. Results

Out of the 30 respondents, the prevalence of diarrhoea at the time of the study (two weeks before survey day) was 50%; and a majority (90% of the households) stored cooked food for later meals. Only 6.9% of the population used soap to wash their hands, with 24.1% of the children's hands not being washed before eating. Meanwhile, 62.1% of the caregivers washed their hands before feeding the children.

The stunting prevalence was 22.2% (Table 1).

Table 3: Nutritional Status of the study population

Indicator	Nutritional Status	Prevalence (%) (n=30)
MUAC Z-score	Severely wasted	0.8
	Moderately wasted	6.3
	Normal	92.9
Height Z-score	Severely stunted	1.6
	Moderately stunted	20.6
	Normal	77.8
Weight Z-score	Moderately underweight	5.6
	Normal	94.4

8.1. ***Microbial Quality of Drinking Water***

The results generally indicated that 90% of the respondents stored their drinking water in locally made ceramic pots while the rest stored their drinking water in Jerry cans. Over 90% of the respondents covered the containers for storing water. The tap was the most commonly used source of water for drinking (see Table 2).

shows the results of the total coliform and E.coli counts from the four main drinking water sources and the two drinking water storage containers used at the household levels. Total coliform count ranged from 3.37 to 5.90 log cfu/ml. Water from unprotected springs recorded the highest levels of the total coliform count of 5.90 log cfu/ml while tap water had a significantly low coliform count (3.37 log cfu/ml). E. coli counts in the drinking water sources ranged from 1.39 log cfu/ml in tap water to 4.35 log cfu/ml in unprotected spring water. Water from unprotected springs also had significantly (p= 0.0000) higher E. coli counts (4.35 log cfu/ml) compared to tap water (1.39 log cfu/ml). Microbial counts in drinking water storage containers were significantly different from the counts at the drinking water sources (p = 0.004).

There were no statistically significant differences in microbial counts concerning drinking water storage containers (2.95 logs cfu/ml in Jerry cans and 2.28 log cfu/ml in pots).

Water from the protected and unprotected springs had more coliform counts (TC=4.24 log cfu/ml and 5.90 logs cfu/ml respectively; E.coli=3.11 log cfu/ml and 4.35 log cfu/ml respectively) at the source compared to the corresponding water in the storage containers (TC =2.03 log cfu/ml and 4.37 log cfu/ml respectively; E.coli=1.85 log cfu/ml and 2.04 log cfu/ml respectively). This was probably because the water in the containers was obtained days earlier when the contamination levels at the source could have been different or less.

To test the hypothesis that different handling practices of complementary food and water in the household do not have any effect on microbial growth in the food hence no effect on the health of the children, correlations and regression analyses were carried out. Of the possible direct associations (correlations), water purification was found to be associated with microbial growth in drinking water (r= -0.500, p = 0.005) at the point of use (in the drinking water storage container). In other words, water that was treated in one way or another at the household had lesser microbial growth compared to water that was not treated. Untreated drinking water at the point of use favoured microbial growth in the drinking water storage containers. It was found that treatment of drinking water at the household accounted for 25% of the variation in E.coli counts in drinking water in the storage container (p = 0.005) at α-level 1%.

8.2. Safety of Complementary Food

The food samples collected were those of freshly cooked food (58 samples), stored cooked food for 4 hours that had not been warmed or reheated (22 samples), cooked food kept overnight (6 samples),

Table 4: Water Sources, Treatment and Hygiene Practices

Characteristic	**Variables**	**Percentage of households (%)**
Source of drinking water	Tap	66.7
	Borehole	20.0
	Protected Spring	3.3
	Unprotected Spring	6.7
	Rain water	3.3
Drinking water treatment method used	Boiling	6.7
	Using Chemicals	3.3
	No treatment	90.0
Method used to wash child's hands	Wash hands in a container that has water communally*	77.1
	Wash hands using water pouring from a container	22.9

* Everybody uses the same container one after another without changing the water.

Table 5: Microbial quality of drinking water by source and storage containers

Drinking water	**Total Coliform ± Sd (log cfu/ml)**		**E. coli ± Sd (log cfu/ml)**		
	Water source	**Storage container**	**Water source**	**Storage container**	
Tap water		$3.37^b \pm 1.37$	4.13 ± 0.90	$1.39^b \pm 1.27$	2.18 ± 1.40
$3.37^b \pm 1.37$					
Borehole	$4.24^{ab} \pm 1.56$	3.87 ± 1.20	$2.59^{ab} \pm 1.95$	2.91 ± 1.20	
Protected Spring	$4.24^{ab} \pm 0.12$	2.03 ± 0.02	$3.11^{ab} \pm 0.05$	1.85 ± 0.04	
Unprotected Spring	$5.90^a \pm 0.01$	4.37 ± 0.52	$4.35^a \pm 0.03$	2.04 ± 1.49	

Means with the different superscript are significantly different($p \leq 0.05$).

and food that had been reheated after storage for 4 hours (24 samples).

A variety of food combinations were collected, they comprised: posho and beans or pigeon peas (30.9%), cassava and beans or pigeon peas (23.6%), followed by simsim or groundnut-pasted foods including vegetables (23.6%), rice and beans (3.6%), plain maize porridge (3.6%), plain beans (1.8%), and other food combinations (12.7%).

The highest counts of both Total coliforms (TC) and fecal coliforms (FC) were found in food kept overnight while the lowest was observed in freshly cooked food (see Tables 4 and 5). There were statistically significant differences in the microbe counts for both Total Coliforms and Fecal Coliforms in the different sets of food samples ($p < 0.05$); for instance, food warmed after storage had counts that were not significantly different from those of food stored. However, food stored for longer had significantly different counts compared to freshly cooked food, and food stored for shorter periods.

Table 6: Means of the microbial safety of the complementary food samples collected

Variable description	Total Coliform ±Sd (log cfu/g)	Fecal Coliform ±Sd (log cfu/g)	Percentage of samples that were contaminated
Freshly cooked food (n=58)	$5.15^{c}\pm1.66$	$4.88^{c}\pm1.87$	93.1
Food stored for over 4 hours (n=22)	$6.16^{b}\pm1.26$	$5.49^{bc}\pm1.86$	100.0
Food kept overnight (n=6)	$8.92^{a}\pm0.26$	$8.16^{a}\pm0.31$	100.0
Food warmed after storage for over 4 hours (n=24)	$6.76^{ab}\pm1.09$	$6.17^{b}\pm1.97$	100.0

Means with the same letters (superscript) are not significantly different from one another.

Table 7: Means of the microbial quality of complementary food samples given to children 6-23 months inAdyel Division, Lira District

	Mean Fecal Coliform(log cfu/g)	Mean Total Coliform (log cfu/g)
Freshly cooked food		
Posho and beans, or pigeon peas (n =20)	6.50	6.55
Cassava and beans, or pigeon peas (n = 12)	6.75	6.76
Pasted foodsα (n = 14)	6.66	6.80
Rice and beans (n =2)	2.06	2.29
Other food combinations (n = 10)	6.81	6.81
Food stored for over 4 hours (not warmed)		
Posho and beans or peas (n= 8)	6.68	6.49
Cassava and beans (n = 2)	4.86	3.12
Maize Porridge (n = 4)	6.30	5.40
Pasted foods (n = 6)	7.20	7.16
Other food combinations (n = 2)	3.10	5.31
Food kept overnight (not warmed)		
Rice and beans (n= 2)	6.89	6.96
Cassava and pigeon peas (n =2)	7.60	7.65
Beans (n = 2)	7.19	7.31
Food warmed after storage for over 4 hours		
Posho and beans or peas or ants (n=6)	6.08	6.14
Cassava and beans (n = 10)	6.90	7.02
Pasted foods (n = 6)	6.97	7.26
Other food combinations (n = 2)	4.85	5.48

Pasted foods$^{\alpha}$ means that the food was cooked and apaste made from grinding roasted simsimand/or roasted groundnuts was added and mixed in the food or sauce.

When correlations to explain the presence of fecal coliforms in freshly cooked food was run (see Table 6) the water source used for cooking was weakly associated with fecal coliforms in freshly cooked food. Where using water from taps, boreholes, and protected springs was safer than using unprotected springs. The duration the eating utensils had been shelved was associated with the fecal coliform counts in freshly cooked complementary food. The longer the time the utensils are spent on the shelves before cooking, the greater the risk for increased fecal coliform counts in freshly cooked food.

8.3. Microbial quality of hands and eating utensils

Significant counts of both total coliforms and fecal coliforms were found on the caregivers' hands while the lowest counts (4.01 log cfu/ml total coliforms; 1.81 log cfu/ml fecal coliforms) were found on spoons (see Table 7). The results indicate that utensils, caregivers' hands, and children's hands were contaminated with coliforms. Caregivers' hands recorded the highest total coliform counts (4.51 cfu/ml) and fecal coliform counts (4.34 cfu/ml).

8.4. Diarrhoea Reoccurrence among children

The mean number of diarrhoea bouts or incidences among the children that were reported to have experienced diarrhoea (50% of the children in the study) within the two weeks preceding the survey was 1.68. Of the children that had diarrhoea within the two weeks preceding the survey, 9 (30%) of the mothers/caregivers reported that their children had had one diarrhoea episode, 1 (3.3%) reported 2 diarrhoea episodes, while 5 (16.7%) reported a minimum of 3 diarrhoea episodes.

8.5. Factors associated with diarrhoea prevalence and frequency

As shown in Table 8, children that finished all the food served ($r = 0.216$, $p = 0.015$) and children whose caretakers washed their hands after cleaning the child following toilet use ($r = 0.224$, $p = 0.011$) were less likely to have had a diarrhoea episode within the two weeks preceding the survey.

The frequency of diarrhoea episodes within the study period (two weeks before the survey) was found to be associated with the storage of utensils for long before use ($p=0.007$); the source of drinking water –where using taps and boreholes was safer than using unprotected springs for drinking water; and not treating drinking water ($r = 0.188$, $p = 0.035$). Those who did not treat their drinking water were more prone to frequent diarrhoea episodes as opposed to those who treated their drinking water in some sort of way, by either boiling or adding chemicals.

At the α-level of 5%, the Child Feeding Index (CFI) was also found to be negatively associated with the frequency of diarrhoea in the two weeks preceding the study ($r = 0.176$, $p=0.048$) implying that: the better the child-feeding practices, the lesser the risk of diarrhoea.

From the correlations to determine factors associated with diarrhoea frequency and prevalence among children, one of the factors found to be significantly associated with diarrhoea episode recurrence in this study was Fecal Coliform counts in freshly cooked food. To determine the extent to which this was so, a regression model was run.

At an α-level of 5%, ($p=0.001$), it was found that 32.5% of the frequency or recurrence of diarrhoea episodes among children in two weeks in this study could be explained by the fecal coliforms in freshly cooked complementary food.

8.6. Factors associated with nutritional status

The nutritional status of the participants was associated with diarrhoea prevalence, and pneumonia – see Table 9. The presence of infections was associated with being underweight as observed by the low MUAC and Weight Z-scores. There were no significant bivariate associations between wasting and stunting.

To further ascertain the degree to which dietary intake and diarrhoea were associated with nutritional status, odds ratios were determined and the following positive results were obtained (see Table

Table 8: Factors associated with safety of freshly cooked complementary food

Factor associated with food safety	**Correlation coefficient, r**	**p-value**
Water source used for cooking	0.380	0.042
Duration eating utensils had been shelved	0.563	0.001

Table 9: Microbial safety of utensils and hands used to serve and feed children

Variable description	**Total Coliform ±Sd(log cfu/ml)**	**Fecal Coliform ±Sd (log cfu/ml)**
Plate (before serving)	$4.08^{a}\pm$ 1.50	$3.68^{b}\pm$ 1.41
Spoon (before eating)	$4.01^{a}\pm$ 0.08	$1.81^{c}\pm$ 0.07
Caregivers' hands (before feeding)	$4.51^{a}\pm$ 1.17	$4.34^{a}\pm$ 1.70
Child's hands (before eating)	$4.15^{a}\pm$ 1.84	$3.87^{ab}\pm$ 1.51

Means with the same letters (superscript) are not significantly different from one another

Table 10: Bivariate analysis showing factors associated withdiarrhoea

Factor	Prevalence of diarrhoea (r)n = 30	Frequency of diarrhoeal episodes (r) n = 30
Not finishing all the food served	r=0.216, p=0.015	
Caretakers not washing hands after cleaning child following toilet use	r=0.224, p=0.011	
Not treating drinking water at point of use		r=0.188, p=0.035
Storage of utensils for long before use		r=0.481, p=0.007
Drinking water source		r=0.381, p=0.038
Low CFI		r=0.176, p=0.048
Fecal Coliforms in freshly cooked food		r=0.570, p=0.001

r - Correlation coefficient

Table 11: Bivariate analysis of factors associated with nutritional status of children

Factor		**Correlation coefficient, r**	**p-value**
Diarrhoea prevalence	MUACZ	0.234	0.008
	WAZ	0.194	0.030
Pneumonia prevalence	MUACZ	0.177	0.047
	WAZ	0.204	0.022
FCS	HAZ	0.045	0.179

10). Diet and diarrhoea were less likely to influence the wasting of the children compared to the effect on stunting.

The Child Feeding Index and increased diarrhoea frequency played a significant role in influencing stunting among children in the Adyel division. Children who were stunted were either 2.29 more times likely to have been exposed to a low CFI; or 3.8 more times likely to have been exposed to an increase in episodes of diarrhoeal infections.

Stunting is an indicator of chronic undernutrition while wasting usually indicates acute undernutrition. The odds ratios in Table 18 above show that the CFI and diarrhoeal frequency were associated with chronic undernutrition which is indicative of traits that have happened over time as opposed to being short-term in the study population.

9. Discussion:

9.1. Microbial quality of the complimentary food and water

The majority of the food and drinking water served to children in this study area was not safe as it contained fecal coliforms - an indication of fecal contamination - hence predisposing children to infections like diarrhoea (Ehiri *et al.,* 2001; Schmitt, *et al.*, 1997; Michanie *et al.*, 1988). Studies have reported that hand hygiene, food storage, and inadequate reheating of food compromised the safety of complementary food and drinking water at the point of use to bacterial contamination (Imong, *et al.*, 1989; Ehiri, *et al.*, 2001; Anigo, *et al.*, 2007; Wright, *et al.*, 2004). This study showed that freshly cooked complementary food given to children was contaminated and this was associated with handling practices at the households like not treating drinking water at the point of use, storage duration of utensils before use, and the source of water used for cooking. Some sources of water were more contaminated (unprotected springs) than others (taps, boreholes, and protected springs) as they had significantly more *E.coli* counts.

Since most of the water sources were already contaminated, the children's drinking water at the point of use needed to be treated as recommended by the IYCF guidelines (Ministry of Health, 2009). Storage of already contaminated water without boiling or treating it predisposes the water to increased microbial proliferation (Jay, 1996). It is important to note that bacterial load plays an important role in causing infections thus compromising the children's health status. As already discussed above (section 5.1.2), storage of cooked food compromises the safety of the food by increasing the microbial load thus predisposing children to diarrhoeal infections (Gupta, *et al.*, 2016).

Storage of utensils for long before use predisposes the utensils to accumulation of dust particles from the air. Over time, they increase to levels where the dust becomes host to microbes. If used without washing, can predispose the children to infection caused by the microbes now thriving on the utensils; as evidenced by the presence of microbial contaminants on the utensils in this study.

9.2. Factors associated with diarrhoea and nutritional status

In the second phase of this study, the prevalence of diarrhoea at 50% among children between the ages of 6 to 23 months was more than double the national average of 23% (UBOS, 2012) most likely due to the rainy season. This diarrhoea prevalence was also higher than the prevalence in the first phase due to the difference in the seasons when the different phases of the study were carried out. This was comparable with findings from Burkina Faso, where diarrhoeal infection rates varied depending on the season due to the proliferation of different causal pathogens in the different seasons (Bonkoungou, *et al.*, 2013).

The Infant and Young Child Feeding guidelines recommend that children's complementary food be prepared separately from family meals (Ministry of Health, 2009). Children (13.4%) in this study whose food was separately prepared from the rest of the family meals were less likely to have had diarrhoea episodes within the two weeks preceding the survey. When preparing separate meals for children, special care is usually taken

Table 12: Odds ratio of factors influencing nutritional status

Exposure (Factor)	Event (Poor nutritional status)	Odds Ratio
Low CFI	WHZ	0.46
Diarrhoea prevalence	WHZ	0.46
Increased Diarrhoea frequency	WHZ	0.06
Low CFI	HAZ	2.29
Diarrhoea prevalence	HAZ	∞
Increased Diarrhoea frequency	HAZ	3.8

CFI – Child Feeding Index; ∞ - Positive infinity

to prepare just what is enough for the child, thus eliminating the need to store the food for later. It also ensures that the food is of the right consistency and well-balanced nutritional composition (Berhanu, *et al.*, 2015; Nabugoomu, *et al.*, 2015). Implying that preparing special meals for children not only ensures children's access to safe foods but is likely to have significant effects in reducing the prevalence of diarrhoea among children because special care is taken to give the right foods in terms of age appropriateness, consistency, composition, and safety among others.

This study also found that children who finished all the food they were served and thus had no leftovers left on the plate, were less likely to have diarrhoea episodes in the two weeks and six months preceding the study. Those who did not finish the food served to them were more likely to get frequent diarrhoea episodes. This can be explained by the fact that the leftovers were left for the children to pick up later on increasing the chances of eating contaminated food that had been poorly stored and not reheated and/or with unwashed hands. IYCF guidelines recommend that food not finished by young children be given to older siblings to finish at meals and not be stored for later meals (Ministry of Health, 2009). This is meant to avoid unhygienic storage of cooked complementary food for later to decrease the possibility of eating contaminated food and the frequency of diarrhoea infections.

Drinking water treatment at the point of use by boiling is a key recommendation for the prevention of water-borne diarrhoeal diseases among children (Ministry of Health, 2009). The frequency of diarrhoea episodes within this study population was found to be associated with the treatment of drinking water at the point of use. Children in households whose drinking water was not treated were more prone to frequent diarrhoea episodes compared to those who treated their drinking water specifically by boiling. Unhygienic handling of drinking water is one of the factors that influenced the frequency of diarrhoea among children in Bangladesh (Afroza, Sk. Shahinur, Hafizur, & Sabir, 2013). Therefore, improving water handling practices have the likelihood of improving child health by decreasing the prevalence and incidence of diarrhoea among children (Wright, *et al.*, 2004).

Hand-washing after cleaning the children following defecation is one of the recommended practices especially before handling food (WHO, 2009; Ministry of Health, 2009). This practice is known to minimize the transfer of microbial contaminants on the caregiver's hands and thereby reduce the exposure of children to diarrhoea. A study in Tanzania found that drinking water safety was influenced by the presence of microbial contaminants on the hands of caregivers (Mattioli, *et al.*, 2014). However, several studies have shown that improved hand-washing practices significantly reduce the rates of diarrhoeal infections among children (Cousens *et al.*, 1996; Aiello & Larson, 2002; Laskar *et al.*, 2005; Luby, *et al.*, 2005; Curtis & Cairncross, 2003).

The IYCF guidelines specify that the hands be washed under clean running water with soap (WHO, 2009; Ministry of Health, 2009) which was not observed in the study population. Over threequarters of the children's hands were washed in a container with water one after another without replacing the water between individuals. Those who did not use communal containers to dip in and wash one after another used a cup or container to pour water over the child's hands as the child washed their hands. In most cases the water was not clean but had been used to previously wash utensils or clothes, a practice contrary to the IYCF guidelines (WHO, 2009; Ministry of Health, 2009). The inability to use running water to wash hands in this population was due to the absence of water sources in most homesteads, besides, the tap water is paid for and it is economical to rather ration the water used for handwashing. This results in less expenditure on tap water. Studies have shown that food-hygiene practices such as inappropriate child and caregiver handwashing practices increase episodes of diarrhoea among children below the age of 2 years within low socioeconomic households (Agustina, et al., 2013); Oloruntoba, Folarin, & Ayede, 2014; Mohammed & Tamiru, 2014). Therefore, this inappropriate hand-washing practice is predisposing the children to increased diarrhoeal infections.

Overall, this study shows that children's nutritional status is influenced by diarrhoea frequency, as well as dietary intake. The positive odds ratios greater than 1 equally showed both diet and diarrhoeal infections as contributors to stunting among children in the Adyel division. While studies have shown associations between diarrhoeal frequency and stunting (Neumann, *et al.*, 2004; Martorell, *et al.*, 1980), this study too agreed with these findings. The composite Child Feeding Index (CFI) is an indicator that is used to assess child-feeding practices that can be related to nutritional status. According to the UNICEF conceptual framework for malnutrition, dietary intake, and disease were cited as the two immediate causes of malnutrition (UNICEF, 1990) just as reflected in this study. None of the handling practices were directly associated with the nutritional status of the children- the association was indirect by predisposing the food to diarrhoea causing microbes (fecal coliforms). This in turn was associated with diarrhoea which in turn influenced the nutritional status of the children.

The high prevalence of diarrhoea morbidity associated with contaminated food and water is an indication that strategies for the prevention of childhood undernutrition ought to extend beyond dietary patterns and requirements. Prevention strategies should focus on ensuring that children consume food and water that is safe and free from pathogens (Marino, 2007).

10. Conclusion

The handling practices are likely to have compromised child health by predisposing the children's complementary food to contamination and growth of diarrhoea causing microbes like E.coli. Some of the significant handling practices that were found to be associated with the presence of microbial contaminants in drinking water were the drinking water treatment method used. Freshly cooked contaminated complementary food on the other hand was associated with the water source used for cooking and the duration utensils had been shelved before use.

Diarrhoea in this study population was significantly influenced by the presence of fecal contaminants in freshly cooked complementary food, shelving utensils for long before use, poor handwashing practices, low CFI (dietary intake), and not treating drinking water at the point of use. While the nutritional status was associated with both diarrhoeal disease burden as well as dietary intake. The strong associations between stunting (HAZ) and diarrhoeal suggest that dietary intake alone is inadequate in ensuring a good nutritional status if infections are not prevented or controlled.

The children aged 6 -23 months in the Adyel division had chronic undernutrition which was related to frequent diarrhoeal episodes which were associated with the consumption of contaminated food. Food safety was influenced by poor complementary food handling practices such as not treating drinking water at the point of use and storage of washed utensils for a long before using them.

11. Recommendations

The findings of this study showed that infections like diarrhoea which were partly associated with food handling played a role in influencing the nutritional status of children in the Adyel division. Therefore, nutrition and health interventions for children should always target both dietary practices as well as infection control practices as both can independently affect the nutritional status of children. Aspects of hand hygiene and safety should be emphasized especially during the preparation and handling of the complementary food- some of which include: washing hands even after cleaning children following toilet use; opting to use tap water or water from a protected spring for cooking; regularly wash and dry utensils especially if they have been shelved for long, boiling water for drinking before it is stored and used. Dietary interventions to consider are ways of increasing the use of animal-source foods as part of the complementary feeding regimen without imposing an extra financial burden on the caregivers and/or the breadwinners of the household.

This study found that the complementary food was generally contaminated. However, there is a need to study specific food combinations under more controlled conditions to compare the effect of the different handling practices on the microbial load in the various foods; and the rate of increase of the microbial load in those foods.

The effect of using pots as drinking water storage containers needs to be further investigated, for instance, the effect of storing water in pots on the microbial, physical, and chemical qualities of water under controlled conditions.

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13. List of Abbreviations

ANOVA Analysis of variance
CFI Child feeding index
FAO United Nations Food and Agriculture Organisation
HIV Human immunodeficiency virus
IYCF Infant and young child feeding practices
MOH Ministry of Health, Uganda
UBOS Uganda Bureau of Statistics
WHO World Health Organisation

14. Conflict of interest

The authors have no conflicts of interest in this study.

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