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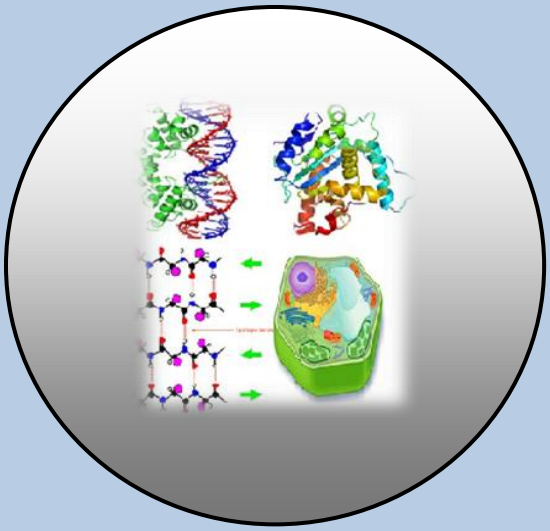

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RESEARCH PAPER

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Microbiological Quality and Safety of Weaning Foods of In-patient Infants in Jimma University Specialized Hospital, Jimma Town, Southwest Ethiopia

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ABSTRACT

Weaning foods which are introduced into the child's diet along with breast milk prepared under unhygienic conditions are shown to be heavily contaminated with pathogenic microorganisms. Thus, this Research was designed to address problems related to the quality of weaning foods preparation practices, storage and handling and their contribution to disease transmission. A total of 90 food samples were collected from mothers feeding their infants with weaning foods in Jimma University Specialized Hospital, Jimma town. Cross sectional study design was employed and microbiological analysis was handled following standard microbiological methods. SPSS statistical software (version 16) was employed for data analysis. Accordingly, 36.7%, 17.8%, 55.6%, 31.1% weaning food samples had aerobic mesophilic bacteria, aerobic spore formers, Lactic Acid Bacteria, and staphylococci of ≥5 log10 CFU/ml respectively. Similarly, 52.2 and 37.8% of samples had Enterobacteriaceae and coliform counts of ≥4 log10 CFU/ml respectively. The aerobic mesophilic flora of the weaning food samples was dominated by Staphylococcus spp. (21.4%) followed by Bacillus spp. (18.9%). Salmonella and Staphylococcus aureus were isolated from 9 (10%) and 57 (63.3%) weaning food samples, respectively. Increasing general hygiene of rural communities and giving more emphasis to infant's food are recommended.

Key words: Cereals, Fruit juices, Gruel, Infants, Milk and Weaning foods.

INTRODUCTION

Nutrition plays crucial role in determining the body defense mechanism against infection. In sub-saharan African countries such as Ethiopia, there is high rate of malnutrition in children which results from the interaction between poor diet and disease (Edward and Parrett, 2003). According to the report of Mamiro *et al*. (2005) good nutrition is essential for adequate growth and cognitive development of children and to resist and fight against infection. During the first 6 months of infant's life, breast milk is a sole and sufficient source of nutrition (WHO, 2000). Thereafter, to meet their evolving nutritional requirements, infants should receive nutritionally adequate and safe weaning (complementary) foods with breastfeeding (WHO and UNICEF, 2003). Weaning foods are the foods which are introduced into the child's diet along with breast milk when breast milk alone is no longer sufficient to meet nutritional requirements of the child (Muhimbula and Issa-Zacharia, 2010). Weaning is the gradual process by which the mother's milk is supplemented and substituted with other foods and an infant is introduced to adult diet (Gubta and Segal, 1991). Weaning period is a very critical period in the life of a child and if not well managed, might lead to malnutrition and other health implications (Ozumba *et al*, 2002). Therefore, adequate nutrition and health care during the first years of infant life is fundamental to prevent malnutrition and child death (Mamiro *et al*., 2005). More than 10 million children die each year from malnutrition and infectious diseases and the majority of children who die are from developing countries. Therefore, adequate nutrition and health care during the first years of infant life is fundamental to prevent malnutrition and child death (Mamiro *et al*., 2005). Timely introduction of appropriate and safe weaning foods promote good nutritional status and growth of infants and young children (Michaelsen *et al*., 2000). The target age range for weaning feeding is generally 6 to 24 months of age although breast feeding may continue beyond the second year (WHO, 2005; Muhimbula and Issa-Zacharia, 2010). In many developing countries, especially those in the low-income class, the introduction of supplementation in terms of weaning foods prepared from easily available and low cost ingredients is of vital importance to meet the requirements of the growing children (Saeeda *et al*., 2009). On the other hand, many weaning food formulations from both animal and plant sources have been reported (Ozumba *et al*, 2002). However, most of these commercially available weaning foods are priced beyond the reach of the majority of the population and may not be feasible in developing countries like Ethiopia due to limited income and inaccessibility (Mamiro *et al*., 2005). The commercial weaning foods which are of excellent quality are generally 10 to 15 times higher than the cost of the common foods due to sophisticate processing, expensive packing, extensive promotion and solid profit margins (Bahlol *et al*., 2007). Therefore, most families frequently use low cost weaning foods which can be prepared easily at home and community kitchens using locally available raw materials and which do not require sophisticated equipment, and can be served quickly and conveniently (Satter *et al*., 2013). The development of weaning foods based on locally available cereals and legumes has been suggested by the Integrated Child Development Scheme (ICDS) and Food and Agriculture Organization (FAO) to combat malnutrition among children of low socio-economic groups (Imtiaz *et al*., 2011). During formulation of any weaning foods made from locally available raw materials; the techniques of food preparation process, handling, storage, sanitation, sensory properties, and food quality and safety issues in general should be taken in to account (Amuna *et al*., 2000; Ifediora, *et al.* 2006).

This is because; weaning foods prepared under unhygienic conditions are shown to be heavily contaminated with pathogenic microorganisms (Nkere *et al*., 2011). Contamination of food including drinking water with microbial agents is the major risk factor in the transmission of diarrhoeal diseases in infants and young children. Contaminated hands and cooking utensils contribute greatly to the contamination of weaning foods, especially among mothers who do not observe proper hygienic conditions (Michaelsen *et al*., 2000). Therefore, careful hygienic preparation and storage of weaning foods is crucial to prevent contamination. Personal hygiene plays an important role in feeding infants. If sanitation is not observed, weaning feeding may do more harm than good to the infant by introducing infections to the infant (Satter *et al*., 2013). It is therefore, important that all foods prepared for young infants are handled in a way that they are free from any contamination. Hence, this study was undertaken to ascertain microbial contamination of traditional weaning foods prepared by mothers feeding their infants in Jimma University Specialized Hospital, Jimma town, Southwestern Ethiopia.

MATERIAL AND METHODS

Sample collection

The study was conducted in Jimma town which is located at 353 km southwest of Addis Ababa, the capital city of Ethiopia. Infants admitted to Jimma University Specialized Hospital, pediatrics in- patient department were investigated for microbial contamination of weaning foods. Structured interview was used to obtain preliminary information on the demographic characteristics of the mothers/food makers in JUSH.

A total of90traditional weaning food samples (25ml), comprising 30 samples each of milk, cereals, and fruit juices were collected from mothers feeding their infants in Jimma University Specialized Hospital, in Jimma town from January, 2014 to March, 2014. Weaning food samples were collected using sterilized plastic bag and transported to Jimma University, Biology Department for microbial analysis. Samples were processed within maximum of an hour after its collection and arrival at laboratory.

Microbiological methods

Twenty five (25) ml of each food sample was mixed with 225 ml Buffered Peptone Water (BPW), homogenized in a flask using shaker. The homogenates were serially diluted from 10^{-1} to 10^{-7} and a volume of 0.1 ml aliquot of appropriate dilution was spread-plated on pre-solidified plates of respective media for microbial count: aerobic mesophilic bacteria (AMB) were counted on Plate Count Agar (PCA) after incubation at 32 ^{o}C for 48 hours; Violate Red Bile Agar (VRBA) was used to count coliforms after incubation for 48 hours at 32^{o}C. Purplish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms. Enterobacteriaceae were counted on Mac Conkey agar after incubated at 32^{o}Cfor 48 hours. Pink to red purple colonies were counted as member of Enterobacteriaceae. Staphylococci were counted on Mannitol Salt agar (MSA) after incubation at 32^{o}C for 48 hours. Typical golden yellow color colonies were counted as *Staphylococcus* spp*.* (Acco *et al*., 2003). Lactic acid bacteria were counted on de Mann Rogosa Sharpe (MRS) agar media and incubated at 37 °C for 48 hrs under anaerobic condition using anaerobic jar. All snow white colonies were counted as LAB (Patra, 2011). After enumeration, ten colonies were randomly picked from countable plates of PCA, MSA, VRBA, and Mac Conkey Agar plates and further purified by repeated plating on PCA.

Cell morphology, Gram reaction, colony characterization were determined following standard microbiological methods: KOH test, a test for lipopolysaccharide, was made to distinguish between gram-positive and gram- negative bacteria. In addition, the isolates were further identified based on their biochemical tests. Catalase test was performed by adding few drops of 3% H_2O_2 on plates of an overnight culture of the pure isolates. Coagulase test was done to identify *Staphylococcus aureus*. Triple sugar iron agar, lysine iron agar, simmons citrate agar, urea agar, and Sulfide in dole motility (SIM) medium were used for detection of *Salmonella* spp. and *Shigella* spp.

Statistical methods

Microbiological data were expressed as average of the total samples for each weaning food analyzed. The significance of differences ($P<0.05$) of the mean microbial count among the weaning food was evaluated with one-way ANOVA using SPSS for windows version 16.0.

RESULTS

Of the 90 parents of infants' interviewed in this study, a significant number (85.6%) of the respondents were mothers and (56.7%) of the respondents were within an age group of 30 to 39 years. Most (83%) of the in-patient infants in Jimma University Specialized Hospital fed on milk obtained either from the hospital or arranged by the parents (Figure 2). Others (65%) fed on fruit juices of commercial origin, and locally prepared cereal-based weaning foods (50%) including gruel (heat treated) and "besso" (non-heat treated).All infants age fall within the age range of 6-24 months. Breast feeding infants were 80% and most (63.3%) of the in-patient infants began feeding weaning foods after six months. Most of the infants (68.9%) fed with spoon and/or cup and the rests (31.1%) were bottle-feeding (Table 1).

Table 1. Feeding practices of in-patient infants in Jimma University Specialized Hospital, Jimma Town, Southwestern Ethiopia, 2014.

Characteristics		Number of respondents (N=90)	
		Frequency	Percent (%)
Breast feeding	Yes No	72 18	80.0 20.0
Age when feeding on weaning foods started	<6 month ≥6 month	33 57	36.7 63.3
Methods of feeding infants	-Bottle-feeding -Feeding with spoon/cup	28 62	31.1 68.9
Bottle washing practices	-Cold water -Cold water and soap -Warm water and soap	7 13 8	25.0 46.4 28.6
No. and frequency of use of bottles	-Using more than one bottle exchanging one after the other -A single bottle all the time	2 26	7.0 93.0

The mean microbial counts for selected weaning foods of in-patient infants in Jimma University Specialized Hospital were shown below (Table 2). The overall mean microbial counts of AMB, EB, coliforms, ASF, *Staphlococcus* spp., LAB, and yeasts were 5.21, 3.65, 2.90, 3.67, 3.26, 4.65, and 0.65 log CFU/ml, respectively.

Table 2. Mean microbial counts (log CFU/ml) of some weaning foods of in-patient infants, Jimma University Specialized Hospital, Jimma Town, Southwest Ethiopia, 2014.

Variables	Log CFU/ml (mean± S.D)						
Weaning food types	**AMB**	**EB**	**COLI**	**ASF**	**STAPH**	**LAB**	**YEAST**
Milk (N=30)	6.60±0.7	5.12±2.4	4.45±2.3	4.84±0.7	3.41±2.2	6.17±1.3	0.76±1.7
Cereals (N=30)	5.42±1.5	3.78±1.6	2.25±2.3	3.88±1.8	4.60±2.5	4.94±2.2	0.85±1.6
Fruits (N=30)	3.62±0.5	2.07±2.7	2.02±2.7	2.29±1.1	1.78±2.5	2.84±2.3	0.35±1.1
Over all mean of the three (N=90)	**5.21±1.5**	**3.65±1.5**	**2.90±1.3**	**3.67±1.3**	**3.26±1.4**	**4.65±1.7**	**0.65±0.3**

AMB: aerobic mesophilic bacteria, EB: Entrobacteriacae, COLI: coliforms, ASF: aerobic spore formers, STAPH: *Staphylococcus* spp., LAB: lactic acid bacteria, S.D: standard deviation

Analysis of variance of the mean counts (log CFU/ml) revealed that there was statistically significant difference ($P<0.05$) among the mean counts of all the microbial groups in the weaning food types except yeasts ($P>0.05$). Weaning milk samples obtained from home of the parents and/or bought from cafes had higher counts of all the microbial groups than milk prepared in the hospital (Figure 1).

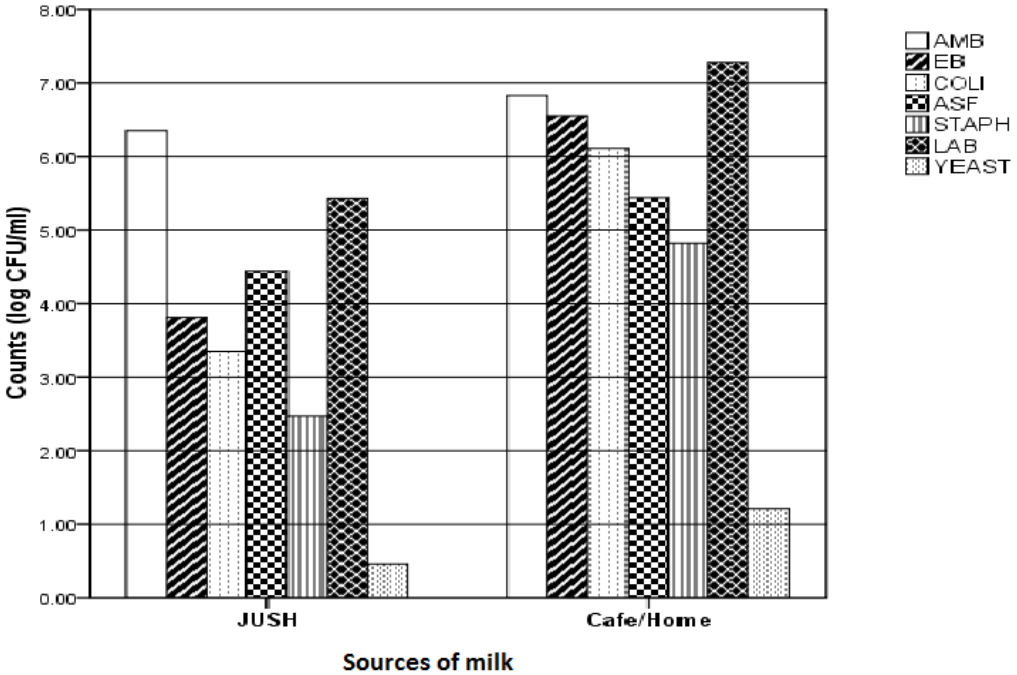

Figure 1. Mean microbial load of weaning milk of in-patient infants in Jimma University Specialized Hospital, 2014.

Where; AMB: Aerobic mesophilic bacteria, COLI: Coliforms, EB: Enterobacteriaceae, STAPH: *Staphylococcus* spp., ASF: Aerobic spore formers, LAB: Lactic acid bacteria, JUSH: Jimma University Specialized Hospital.

Microbial analysis

Based on cultural, morphological, and biochemical characteristics of the isolates, a total of 697 bacteria were isolated from 90 weaning food samples. A total of seven bacterial genera were identified (Table 3).

Table 3.Frequency distribution (%) of dominant bacterial isolates in some weaning foods of in-patient infants in Jimma University Specialized Hospital, 2014.

Bacterial isolates	Weaning food types			
	Milk (N=30)	Cereals (N=30)	Fruits (N=30)	**Total isolates (%)**
	No. of isolates (%)	No. of isolates (%)	No. of isolates (%)	
Staphylococcus spp.	46 (18.2)	65 (27.7)	38 (18.1)	149 (21.4)
Bacillus spp.	74 (29.4)	58 (24.7)	-	132 (18.9)
Micrococcus spp.	35 (13.9)	52 (22.1)	24 (11.5)	111 (15.9)
Enterobacteriacae	28 (11.1)	24 (10.2)	32 (15.2)	84 (12.1)
Pseudomonas spp.	9 (3.6)	8 (3.4)	29 (13.8)	46 (6.6)
*Aeromonas*spp.	3 (1.2)	-	11 (5.2)	14 (2.0)
Lactobacillus spp.	42 (16.7)	15 (6.4)	58 (27.6)	115 (16.5)
Other Gram positive bacteria	15 (5.9)	13 (5.5)	18 (8.6)	46 (6.6)
Total	**252 (36)**	**235 (34)**	**210 (30)**	**697 (100)**

Frequency of *Salmonella*, *S. aureus*, and *Shigella* isolates

Among 90 weaning food samples 9 (10%) samples were positive for *Salmonella* spp. With regard to frequency distribution of *Salmonella* among the weaning food types,it was more prevalent in milk (16.7 %) than all others. Higher prevalence of *S. aureus* (46.7%) was observed in cereal-based weaning foods, followed by milk (36.7%)(Table 4).

Table 4. Prevalence of *Salmonella* spp, *S. aureus*, and *Shigella*spp in weaning foods of in-patient infants, Jimma University Specialized Hospital, Jimma town, Southwest Ethiopia, 2014.

Sample types	Sample size	No. of *Salmonella* positive samples (%)	No. of *S. aureus* positive samples (%)	No. of *Shigella* positive samples (%)
Milk	30	5 (16.7)	11 (36.7)	0 (0.0)
Cereals	30	3 (10.0)	14 (46.7)	0 (0.0)
Fruit juices	30	1 (3.3)	3 (10.0)	0 (0.0)
Total	**90**	**9 (10.0)**	**28 (31.1)**	**0 (0.0)**

DISCUSSION

The present study revealed that most of parents had no training on basic hygienic practices to be followed during food preparation and had no awareness to give special attention to the microbial safety of diet of the children. This calls for designing of strategies for community level training as suggested by Cuprasitrut *et al*. (2011).

In this study about 45% of the parents prepared weaning foods in their home in a form of thin porridge (gruel) and brought to the hospital to feed their infants. According to the report of WHO (2000) microbial contamination of weaning foods can be prevented by using safe water for preparation, frequent washing of hands and proper storage. However, in low income settings this can be constrained by lack of economic resources and absence of facilities for storage of food (Obi and Nwozor, 2012). In the current study, 89.7% of the parents stored food for more than six hours. Similar to this study, Omemu and Omeike (2010) from Nigeria reported that 90.3% mothers stored weaning foods for more than six hours. However, foods should be prepared hygienically and eaten at one sitting or stored safely until consumption (Potgieter *et al*., 2005). This study showed that the counts of aerobic mesophilic bacteria ranged between 3.51 log CFU/ml (fruit juice) to 8.93 log CFU/ml (non-heat treated cereals). A similar study carried out in Lagos by Uzeh*et al*. (2009) showed that total aerobic mesophilic bacteria count ranged from 3.5 l to 6.8 log CFU/ml which reflects the existence of favorable conditions for multiplication of microorganisms. The overall mean of aerobic mesophilic bacteria count (5.21 log CFU/ml) of weaning foods in this study was lower than Nwogwugwu *et al*. (2012) who reported the counts between 7.0 to 7.3 log CFU/g in Nigerian novel weaning food (DUPAP). Acceptable level (<5.0 log CFU/ml) was observed in fruit juices. This is possibly due to quality control measures employed by manufacturers using automated machine directing aseptic processing as well as for the application of some preservatives (Obi and Nwozor, 2012; Rashad *et al*., 2013).Low pH and low water activity of fruits also restrict spoilage and pathogenic microorganisms (Ejechi *et al*., 1998). The mean counts of Enterobactericae in the present study was 3.65 log CFU/ml which is higher compared to Kungu *et a*l. (2009) who reported the mean count 2.54 log CFU/g in weaning porridge samples from Zanzibar. Enterobacteriacae and the high counts clearly prove that poor hygiene meals that could be a source of food-borne illness (Motarjemi *et al*., 1993). The mean count of coliforms in the present study (2.90 log CFU/ml) is higher compared to the report by Omemu and Omeke (2010) where the mean count was 1.22 log CFU/ml from household ogi used as a weaning food in Nigeria. In this study, the maximum mean count (4.45 log CFU/ml) were detected in milk samples. Hence, the high count of coliforms in milk in this study could be attributed to insufficient boiling, unclean utensils such as feeding bottles and teats, or probably due to initial contamination of the milk samples either from the cows, milk containers or the milking environment. General lack of hygienic practices and cleanness observed during visits to the hospital indicated a strong likelihood of cross-contamination between unclean utensils, and the weaning foods. The mean aerobic spore count of the present study was higher in milk (4.84 log CFU/ml) and lower in fruit juices (2.29 log CFU/ml). A study conducted by Pathak *et al*. (2012) in India indicated that the spore formers were found dominants (38%) in boiled milk samples. The higher count of spore formers in milk in this study is probably due to the spore formers require a temperature above 135°c to be completely eliminated from the milk and this temperature range could hardly be achieved in non-industrial boiling procedures (Pathak *et al*., 2012).

The mean counts of staphylococci in the present study were3.26 log CFU/ml, and the highest counts were detected in cereals (4.60 log CFU/ml) and lowest in fruit juices (1.78 log CFU/ml). The presence of *S. aureus* in food is indication that such food is potentially hazardous (Amissah and Owusu, 2012). The mean count of LAB was 4.65 log CFU/ml. In agreement with this study, Omemu and Omeke (2010) reported the higher count ranging between 4.5 to 9.2 log CFU/ml in cooked ogi used as weaning food in Nigeria. The predominant microflora of weaning foods in the present study was generally *Staphylococcus* spp. (21.4%) followed by *Bacillus* spp. (18.9%), and *Lactobacillus* spp. (16.5%). Nwogwugwu *et al*. (2012) showed that *Staphylococcus* and *Bacillus* occur in 100% weaning food samples. The prevalence of *S. aureus* in the present study was 31.1%. The presence of these pathogens in foods is dangerous to consumers, and the problems are severe in infants and young children. Higher number of *S. aureus* was isolated from weaning cereals (46.7%). This is probably due to in the preparation of gruels for prolonged cooking is often avoided, since sustained cooking produces a food that is too viscous for young infants to consume. In the present study, the prevalence of *Salmonella* spp. were 10% in which more prevalence (16.7%) was observed in milk. The report by Erku and Ashenafi (1998) indicated that three *Salmonella* isolates were encountered from bottle contents made of cow's milk and gruel made from cereal blend. The presence of these organisms indicates poor food preparation such as inadequate cooking and unclean utensils which contribute to cross contamination (Tunung *et al*., 2007). The report of Potgieter *et al*. (2005) showed that in South Africa 3.2% of Vhuswa (local weaning food) samples were contaminated with *Shigella*. However, in the present study, *Shigella* spp. was not isolated from any of the weaning food samples. This is probably due to *Shigella* are not as persistent in the environment as *Salmonella* (Cetinkaya *et al*., 2008) or most likely killed during cooking process (Muleta and Ashenafi, 2001).

CONCLUSION

The results obtained in this study indicate that most weaning foods available to the infants have high level of microbial contamination that does not meet the international standards. This could be due to unhygienic preparation and improper storage. Milk was the most contaminated food observed in this study. However, milk provided by the hospital showed less contamination. On the other hand the lowest microbial load was observed in fruit juices. The most predominant microbial groups isolated from weaning food samples in this study were *Staphylococcus* spp., *Bacillus* spp., *Lactobacillus* spp., and *Micrococcus* spp. S*almonella* isolates were found more prevalence in milk and *Staphylococcus aureus* in weaning cereals. Therefore, foods should be prepared hygienically, not stored for longer time or stored safely until consumption by giving greater emphasis to infants' foods.

Competing interest

The authors declared that they have no competing interest.

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