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COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF BIOLOGY

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

By

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A Thesis Submitted to the Department of Biology, College of Natural Sciences, Jimma University, In Partial Fulfillment of the Requirement for the Degree of Master of Science in Biology (Applied Microbiology)

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ACRONYMS

BPW	Buffered Peptone Water
IPD	In-Patient Department
ICDS	International Child Development Scheme
JUSH	Jimma University Specialized Hospital
MRS	Mann Regosa Sharpe
MSA	Mannitol Salt Agar
OPD	Out-Patient Department
PCA	Plat Count Agar
VRBA	Violet Red Bile Agar
XLD	Xylose Lysine Deoxycholate Agar

ABSTRACT

Food-borne diseases related to unhygienic food handling practices remain a major public health problem across the globe. The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. Weaning foods which are introduced into the child's diet along with breast milk prepared under unhygienic conditions are likely to be contaminated with pathogenic microorganisms. The contribution of unhygienic weaning foods and quality of water used in preparation of foods and drinking are estimated to be high for transmission of diseases. 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 for microbial analysis. Cross sectional study design was employed and microbiological analysis was handled following standard microbiological methods. SPSS statistical soft ware (version 16) was employed for data analysis. Accordingly, 36.7%, 17.8%, 55.6%, and 31.1% of weaning food samples had counts ≥5 log CFU/ml of aerobic mesophilic bacteria, aerobic spore formers, Lactic Acid Bacteria, and Staphylococci respectively. Similarly, 52.2 and 37.8% of samples had Enterobacteriaceae and coliform counts of ≥4 log CFU/ml respectively. However, in most of weaning food samples yeasts (>83%), and moulds (100%) were not detected. 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 28 (31.1%) *weaning food samples, respectively. However, Shigella spp. were not isolated from any of the weaning food samples. Increasing general hygiene of rural communities and giving more emphasis to safety of infant's food are recommended.*

Key words/phrases: *Cereals, fruit juices, gruel, infants, milk, weaning foods*

1. 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) recommends that infants should be exclusively breast-fed for the first 6 months of life to achieve optimal growth, development and health. Breast milk contains all the nutrients and immunological factors that fulfill the requirements to maintain optimal health and growth (Mamiro *et al*., 2005). Thereafter, to meet their evolving nutritional requirements, infants should receive nutritionally adequate and safe weaning foods with breastfeeding (WHO and UNICEF, 2003). The report of Gubta and Segal (1991) indicated that breast milk, even from well-nourished mothers, might be inadequate to meet the nutritional needs of the infant after the first six months of life; hence weaning (supplementary) food is needed. Similarly, Muhimbula and Issa-Zacharia (2010) explained that 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. 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). 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 is generally 6 to 24 months of

age although breast feeding may continue beyond the second year (WHO, 2000; 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 cost of commercial weaning foods which are of excellent quality is 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). The prevalence of enteric bacterial pathogens, such as *Bacillus cereus, Staphylococcus aureus, Salmonella* spp, *Shigella* spp, *Escherichia coli, Vibrio cholerae,* and *Campylobacter jejuni* in weaning foods has been reported in many developing countries (Potgieter *et al*., 2005). 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.

Preliminary survey to Jimma University Specialized Hospital indicated that most of the in-patient infants received three major types of weaning foods namely; milk, cereals, and fruit juices. However, parents and caretakers of infants and young children did not take care of the hygienic conditions of the utensils used to store weaning foods. The parents also practiced improper storage conditions particularly at ambient temperature for several hours. This creates favorable conditions for the growth of food-borne pathogens which accounts for diarrhoeal diseases among infants and young children. Although infants were admitted to the hospital because of health complications additional illness could be resulted due to consumption of contaminated foods. This study was undertaken to ascertain microbial contamination of weaning foods of in-patient infants in Jimma University Specialized Hospital, Jimma town, Southwestern Ethiopia. This study site was selected because of higher rate of infants admitted to the hospital compared to other health institutions found in Jimma town.

2. OBJECTIVES

2.1. General objective

The objective of this study was to evaluate the microbiological quality and safety of weaning foods of in-patient infants in Jimma University Specialized Hospital.

2.2. Specific Objectives

The specific objectives of this study were to:

- Assess the microbial quality (load) of some selected weaning foods collected from Jimma University Specialized Hospital.
- Characterize different microbial groups found in the selected weaning food samples.
- Isolate common food-borne pathogens (*Staphylococcus aureus, Salmonella* and *Shigella* spp.) associated with frequently used weaning foods.

3. LITERATURE REVIEW

3.1. Nutritional Status of weaning foods

Children need a variety of food to ensure that nutritional needs are met. Diet that lacks animal sources (meat, poultry, fish, or eggs, plus milk product) cannot meet the nutritional requirement for children ages 6 to 24 months unless fortified or supplement foods are used. If milk and other animal source foods are not taken in adequate amounts, both grain legumes should be consumed daily, preferably within the same meal, to ensure adequate protein quality (Tempel *et al*., 1996). In developing countries poor feeding practices and shortfall in food intake are the most important direct factors responsible for malnutrition and illness amongst children (Imtiaz *et al*., 2011). Combination of nutritionally inferior diets and improper feeding practices are major contributing factors to the development of childhood malnutrition (Ramakrishna *et al*., 2006). Weaning feeding improvement should be of highest priority for nutrition of infant and young children because of its crucial role in preventing mortality and enhancing child development (Bukusuba *et al*., 2008).

In Ethiopia, the most important nutritional problems in weaning foods consumed by the children in many parts are protein energy malnutrition and deficiency in essential macronutrients and micronutrients (Bukusuba *et al*., 2008). The high cost and inadequacy in production of protein-rich foods have resulted in increased protein energy malnutrition among children and other vulnerable groups in the developing world. Protein energy malnutrition in children is associated with poverty and poor nutrition knowledge resulting in early weaning, delayed introduction of weaning foods, a low-protein diet and severe or frequent infections (Ramakrishna *et al*., 2006). Therefore, vulnerability of infants to problems associated with weaning process is a global concern, but more importantly in economically developing countries (Amuna *et al*., 2000).

3.2. Food safety

A safe food supply is the result of industry and government agencies working together to ensure appropriate handling, processing, packaging, distribution, and storage of foods (Shils *et al*., 2006). Unsafe food causes many acute and life-long diseases, ranging from diarrhoeal diseases to various forms of cancer. It is estimated that food-borne and water-borne diarrhoeal diseases taken together kill about 2.2 million people annually, 1.9 million of them children (WHO, 2000). The scope of food safety problems is broad and diverse. It encompasses problems due to microorganisms and chemical hazards that may either be naturally present in foods or appear as contaminants as a result of pollution (Adams and Mortarjemi, 1999).

3.3. Microbial Contamination of weaning foods

According to the report of Michaelsen *et al*. (2000) contamination of food (including drinking-water) with microbial agents is one of the major causes of diarrhoeal diseases and ill health in infants and young children. Certain pathogens are opportunistic and affect mainly infants and young children. Some have particularly severe, others only mild, health consequences *(*Jay *et al.,* 2005). Contaminated weaning foods account for a substantial proportion of diarrhoeal diseases among infants and young children, especially in developing countries. Worldwide it is estimated that 1400 million episodes of diarrhoea occur annually in children under the age of five years (Motarjemi *et al*., 1993). Infants are exposed to food contaminants through weaning foods and thus subject to the food safety problems experienced by the general population. Because infants and young children are especially susceptible, they are at great risk of acquiring infections. When recurrent or persisting for a long period of time, infectious diseases have an adverse effect on nutritional status (Muhimbula and Issa-Zacharia, 2010).

3.3.1. Sources of contamination of weaning foods

Introduction of weaning foods that are often nutritionally inadequate and microbiologically unsafe increase the risks of multiple nutrient deficiencies (Hotz and Gibson, 2001; Kimmons *et al*., 2005) and gastrointestinal illnesses associated with food-

borne pathogens (Lanata, 2003; Sheth and Dwivedi, 2006). The report of Michaelsen *et al*. (2000) indicated that all types of food may be implicated in food-borne diseases. However, certain food-borne diseases are more frequently associated with specific food. Infants and young children are very susceptible to food-borne diseases and if they consume contaminated foods, they are likely to contract infections or intoxications leading to illness and often death (Gomes, 1991). The report of WHO (2000) indicated that the sources of food contamination are diverse. These include faeces, polluted water, flies, domesticated animals, unclean cooking utensils, baby bottles, food handlers, dust, and dirt. Raw foods themselves are frequently the source of contaminants, since some food-stuffs may naturally harbor pathogenic agents or have been obtained from infected animals and if they are not properly processed, would pose great risk to the infants (Black *et al*., 1989). The major sources of contamination of weaning foods are discussed below.

Preparation

Hotz and Gibson (2001) showed that weaning foods prepared under unhygienic conditions are heavily contaminated with pathogenic agents and are a major risk factor in the transmission of diseases, especially diarrhea. This is because of the lack of facilities, such as electricity and in-house sanitation systems, to support the rapid proliferation of food-borne pathogens, especially weaning foods like porridge, which are usually prepared once on a daily basis for use during the whole day (Sheth and Dwivedi, 2006). On the other hand, Potgieter *et al*. (2005) showed that inadequate cooking and containers/utensils used for preparing food, even when washed, may permit survival of bacteria. According to the report of Sheth and Dwivedi (2006) it is generally recognized that contamination of weaning foods may occur as a result of poor hygiene of food handlers, household equipments and the environment where the preparation of food takes place. Type of water supply and sanitary conditions of personnel preparing weaning foods are the major factors affecting in preparation of safe foods (Mensah *et al.*, 1990; Simango and Rukure, 1992).

Contaminated water can create a public health risk when it is used for drinking, washing of foods, incorporated in the food as an ingredient and used in the processing of food or used for washing equipment, utensils and hands. It is a well known vehicle for enteropathogens such as *E. coli, Salmonella spp*. and other pathogens (Angulo *et al*., 1997).

Personal Hygiene

According to WHO (1989) food handling personnel play an important role in ensuring food safety throughout the chain of food production, processing, storage and preparation. Poor personal hygiene causes more than 90% of the food safety problems. Murat *et al.* (2006) highlighted that unsafe and inefficient practices followed by food makers and manufacturers were due to unhygienic practices and lack of personnel hygiene knowledge. The report of Cairncross and Curtis (2003) showed that improper hand washing alone accounts for more than 25% of all food-borne illnesses. Therefore, hand washing should always be needed after using the restroom, touching raw foods, touching the hair, face or body, sneezing, coughing, or using a tissue, smoking, eating, or chewing gum or tobacco, handling chemicals, taking out or handling trash, cleaning a table, touching clothing and touching anything else that may contaminate hands (Murat *et al.*, 2006).

Storage

Improper storage and handling of cooked food are responsible for food-borne illnesses. During storage especially at ambient temperature (28 to 35°C) there is the risk of multiplication of pathogenic organisms (Motarjem *et al*., 1993). Holding foods at high ambient temperatures for long periods of time have been reported to be major contributor to the occurrence of food poisoning out-break (El-Sherbeeny *et al*., 1985). Under favorable conditions, a single bacterium can multiply to 500 million bacteria in 10 hr. Considering that the minimum infective dose of pathogens varies from a few (10 or less) to as many as 10^4 or 10^5, the survival of even a small number of pathogens in freshly prepared food can become health threatening, particularly if the food is stored at ambient temperature for several hours or overnight (Muhimbula and Issa-Zacharia, 2010).

Besides, some of the foods are held in the pans in which they are cooked, until reheated, which results in longer holding time, hence creating favorable conditions for the growth of food borne pathogens. In such foods, the counts of *Staphylococcus aureus, Bacillus cereus, salmonella, and Escherichia coli* are reported to be high (Bryan, 1998). The presence of *Bacillus* coupled with the storage of these foods at ambient temperatures for several hours under high temperature and high relative humidity showed that the product could be hazardous (El-Sherbeeny *et al*., 1985). Moreover, during food preparation and storage, there is an added risk of cross contamination as well as an opportunity for pathogenic bacteria to multiply. The report of Muhimbula and Issa-Zacharia (2010) indicated that a careful analysis of food-borne diseases has shown that there are two particular errors in food preparation that increase this risk, as they permit the survival and growth of pathogens to disease causing level.

These are:

- The storage of food several hours before consumption, combined with its storage temperatures favoring the growth of pathogens and/or formation of toxins

- Insuffient heating or reheating of food to reduce or eliminate pathogens

3.4. Microbiology of Weaning Foods

Several food-borne pathogens have been associated with weaning foods which are prepared under unhygienic conditions and some of these are discussed below (Becker *et al.,* 1994).

Staphylococcus aureus

S. *aureus* is ubiquitous organism occurring in mucus membranes and skin of most homiothermic animals. It is an opportunistic pathogen that causes illness due to changes in host physiology. This organism has previously been implicated in food poisoning incidences involving the consumption of contaminated food such as dairy products and unpasteurized milk. S. *aureus* strains reportedly produce up to seven different enterotoxins which result in diarrhea (Ewald, 1987). They compete poorly with other

bacteria in foods; however, the toxins produced are very heat stable, often surviving cooking and sterilization processes of low acid canned foods (Dinges *et al*., 2000).

***Salmonella* spp.**

Salmonella reside in the intestinal tracts of infected animals and have been isolated from contaminated foods and those subject to sewage pollution. Contamination is believed to be spread to humans during animal slaughter and processing and symptoms of infection include diarrhoea (Beyene, 2008). This pathogen is primarily a contaminant of foods of animal origin such as meat, poultry, eggs and milk, however, it has also been found to contaminate foods of non-animal origin such as cereals and spices (Dargatz *et al*., 2000). Infection by *Salmonella typhimurium* strain results in salmonellosis which is a gastrointestinal infection characterized by; fever, diarrhoea, and intestinal cramps. Although the rates of mortality as a result of infection are low, the disease has been found to be severe in infants, children and the elderly (Barnhart *et al*., 1991). The infective dose in health person varies depending on its ability to survive during transit through the stomach. Growth of *Salmonella* has been found to be inhibited at temperatures below 5 ºC and at pH below 4 (Stock and Stolle, 2001).

***Shigella* spp.**

Shigella spp. are primarily transmitted through contaminated foods and water and from person to person. They have the ability to cause bacillary dysentery or acute enteritis in man and have been found to produce endotoxins which are responsible for diarrhoeal infections Jay *et al*., 2005). *Shigella* strains are also invasive pathogens which penetrate the epithelial tissue and cause ulcerative lesions in the mucosa. The infective dose has been found to be very small (≤100 cells) and as such food containing low numbers of *Shigella* may cause disease. Their growth is inhibited at temperatures below 5 ºC and pH below 4 (Klein *et al*., 2006).

Escherichia coli

There are six types of pathogenic *E. coli* that have been associated with food-bome diarrhoeal diseases, namely, enteropathogenic (EPEC) *E. coli,* enterotoxigenic (ETEC) *E. coli,* enteroinvasive (EIEC) *E. coli,* entroadhesive *(*EAEC*),* introaggregative *(*EAEC*)* and the highly pathogenic enterohaemorrhagic (EHEC) *E. coli,* exemplified by *E. coli* O157:H7, which is a newly evolved *E. coli* serotype that has become pathogenic through the acquisition of virulence factors (Smith and Francisco, 1995). This serotype is reportedly the fourth most common enteric pathogen recovered from human stool samples and results in fatal haemorrhagic colitis in infants and immuno compromised individuals (Huang and Chang, 1996). The diarrhoeal outbreaks resulting from *E. coli* contamination have been associated with the use of contaminated water and consumption of contaminated food products such as raw milk and raw or undercooked beef (Weagant *et al.,* 1995). Infections due to pathogenic *E. coli* are the commonest illnesses in developing countries and produce up to 25% of all diarrhoeal episodes (Motarjem *et al*., 1993; Abel *et al*., 2009). Therefore, *E. coli* transmission has been substantially associated with weaning foods. The severity of different pathogens associated with complementary foods in developing countries is shown in Table 1.

Table 1. Pathogens in weaning foods commonly consumed in developing countries (Muhimbula and Issa-Zacharia, 2010).

Study place	**Types of food**	**Pathogens isolated**	**Contamination level**
Tanzania	Soy-rice porridge (traditional weaning food)	Aerobic bacteria, Coliforms, Entrobacteriacae	Aerobic bacteria*:* 2.24-3.84 log cfu/ml, Coliforms: 1.71-2.4 log cfu/ml, Entrobacteriacae*:* 1.72-2.5 log cfu/ml
South Africa	Vhuswa (traditional maize based weaning food)	*Salmonella, Shigella E. coli, Camplylobacter jejui*	70%, 5%,5%, and 2% respectively
Nigeria	Fermented cereal weaning foods	Fecal coliforms	31.3% of the selected samples were contaminated with fecal coliforms

India	Rice, pulses, and banana	Aerobic mesophilic count (AMC), Yeast and mold (YM), *Staphylococcus aureus* (SA)	AMC: 4.00 log cfu/ml, 3.00 log cfu/ml, SA: 3.00 log cfu/ml
Zimbabwe	Maize porridge, fresh milk, beans, sour milk	Fecal coliforms	60% of the samples were contaminated with fecal coliforms
Egypt	Home-made weaning foods	*E. Coli, Bacillus cerus*	43.7% and 21.4% respectively

Bacillus cereus

B. cereus is a common contaminant of milk, dried milk products, dairy products and surfaces of milking equipment (Becker *et al.,* 1994) and has been isolated from contaminated rice, spices (Damgaard *et al.,* 1996), meat, cereals and dried products (Tan *et at.,* 1997). Toxins produced by *B. cereus* have been found to be responsible for two distinct food borne illnesses, one characterized by diarrhoeal symptoms and the other by emetic symptoms (Jackson, 1993). The diarrhoeal toxin is sensitive to heat and therefore can be inactivated by cooking, and is also sensitive to proteolytic enzymes and acids. The emetic toxin, however, is resistant to proteolytic enzymes and extreme pH (Tan *et al.,* 1997). The production of toxins by *B. cereus* is reportedly inhibited at low temperatures ranging from 6°C to 21°C, with amounts of toxin increasing with an increase in temperature and at pH above 5 (Becker *et al.,* 1994). *B. cereus* toxins are inactive at pH < 4 and are only produced at high bacterial densities (>10^4 cfu/ml) (Tan *et al.,* 1997). The ability of *B. cereus* to form spores has been found to allow it to survive extreme conditions such as dryness, high temperatures and low pH.

***Campylobacter* spp**.

Campylobacters have been isolated from unchlorinated water, raw milk and raw poultry. C. *jejuni* strains have reportedly been responsible for most human enteritis illnesses, resulting in diarrhoea accompanied by severe dehydration in infants and the elderly. Illnesses have been found to result from the ingestion of as few as 500-800 cells in milk. Campylobacters have been found to produce enterotoxins and cytotoxins which have

been implicated in cases of bloody diarrhoea. Growth of *Campylobacter* is inhibited at low temperature and low pH and they are unable to grow in lactic acid fermented food products. They can survive in chilled foods but fail to grow at pH 4.7 (Cruysen and Birtantie, 1988).

Vibrio cholerae

Vibrio cholerae has been frequently isolated from rivers and inshore marine waters. Contamination of fresh and cooked foods has been found to be a major factor in the spread of cholera during an epidemic as the disease is commonly associated with the ingestion of sewage contaminated drinking water and/or contaminated foods. Pathogenicity is associated with adherence of the bacteria to the small intestines where they produce enterotoxins. Symptoms include severe diarrhoea and dehydration, which are fatal if not treated, especially in malnourished individuals. Vibrios are sensitive to high temperature, acid and dry conditions and their survival in acid foods, dried foods, salted fish and fruit juices has been found to be minimal (Jay *et al*., 2005).

Listeria monocytogenes

Listeria monocytogenes has been isolated from soil, silage, sewage, food processing environments, raw meats and poultry (Johnson *et al*., 1990). These microorganisms have been found to infect a wide variety of animals including cattle, sheep, birds, rodents, fish and humans. Outbreaks of human listeriosis have been linked to the consumption of tomatoes, lettuce, unpasteurized milk, milk products and undercooked chicken. Pathogenic *L. monocytogenes* strains are hemolytic and are thought to have an infective dose of over 100 viable cells (Lovett *et al.*, 1987). Growth is reportedly very slow at O ^{o}C while it is inhibited at pH <5 and during fermentation of food products.

Clostridium perfringens

Clostridia have been isolated from dust, soil, flies and vegetation. They are transmitted by stored cooked meat, poultry and dehydrated food products. Pathogenicity involves the production of exotoxins and enterotoxins which may be produced in foods resulting in

illness, however, illnesses have been reported to result from lysis of sporulating cells in the intestine Food poisoning occurs following the consumption of a large number of clostridial cells and symptoms include diarrhoea and abdominal pains. Clostridial spores have been found to be resistant to high temperatures up to 100 °C; however, resistance to heat is decreased in acidic conditions (Kokai-Kun *et al*., 1999).

***Aeromonas* spp.**

Aeromonas are commonly isolated from water, and contaminated food. They have been associated with foods of animal origin including raw meat and milk. Although they are established fish pathogens, some species have been identified as potential human pathogens and have been associated with traveller's and infantile diarrhea. *Aeromonas* are thought to produce enterotoxins. The enterotoxins, in combination with other virulence factors associated with colonization of the intestine, are thought to be the most probable cause of diarrhoea. Although they have been found to grow rapidly at refrigeration temperatures, their growth is completely inhibited at pH 4.5 under the same conditions (Bryan, 1998).

3.4. Food-borne Diseases

Food-borne diseases manifest themselves through a wide range of symptoms and signs, such as diarrhoea, vomiting, abdominal pain, fever and jaundice. They can cause severe and/or long-lasting damage to health, including acute, watery, and bloody diarrhoea (leading to severe dehydration and ulceration), meningitis, and chronic diseases affecting the renal, cardiovascular, respiratory, and immune systems (WHO and UNICEF, 1998). The serious implications of food-borne infections are their effect on nutritional status. Food borne infections can lead to a reduction in food intake owing to anorexia (Muhimbula and Issa-Zacharia, 2010). Poor food intake, aggravated by the loss of nutrients from vomiting, diarrhoea, mal-absorption and fever over an extended period of time, will lead to nutritional deficiencies for growth and immune function of infants and children. The report of Adams and Mortarjemi (1999) indicated that an infant whose resistance is suppressed becomes vulnerable to diseases and is susceptible caught in a vicious circle of malnutrition and infection. Guerrant *et al*. (2008) suggested that a

substantial proportion of global malnutrition is due to impaired intestinal absorptive function resulting from multiple and repeated enteric infections.

Millions of children in the world die each year from diarrheal diseases and more suffer from persistent diarrhea leading to impairment of nutritional status. Global mortality among children under the age of 5 years is estimated at 9.7 to 10.6 million deaths each year with 18% or 1.9 million per year (over 5000 deaths daily) attributable to diarrhea (WHO and UNICEF, 1998). In developing countries, the weaning process in human infants has been associated with an increase in diarrhoeal episodes resulting from consumption of microbiologically contaminated weaning foods. It has been estimated that 1500 million infants and children under the age of 5 years suffer from diarrhoeal infections each year and in 1990, more than 3 million died as a result (Motarjemi and Nout, 1996). These diarrhoeal infections have been associated with enteric pathogens such as *Salmonella* spp. *Shigella,* spp., *Escherichia coli, Aeromonas* spp., *Campylobacter* spp., *Bacillus cereus* and *Clostridium perfringens* (Black *et al*., 1989; Gomes, 1991*).*

4. MATERIALS AND METHODS

4.1. Description of the study site

The study was conducted in Jimma University Specialized Hospital, Jimma town, located at 353 km Southwest of Addis Ababa (Figure 1). The town's geographical coordinates are approximately 7°41' N latitude and 36° 50'E longitude. The town is found in an area of average altitude of about 1780 m above sea level. The town is generally characterized by warm weather with annual mean temperature of 30°C. The annual rainfall ranges from 1138 mm to 1690 mm (Alemu *et al.*, 2011). The data obtained from Jimma University Specialized Hospital showed that the hospital provides curative and preventive service for 300-400 patients per day at its out-patient department (OPD). On the other hand, the pediatric and child health department gives 34 and 15 out-patient and in-patient services per day respectively.

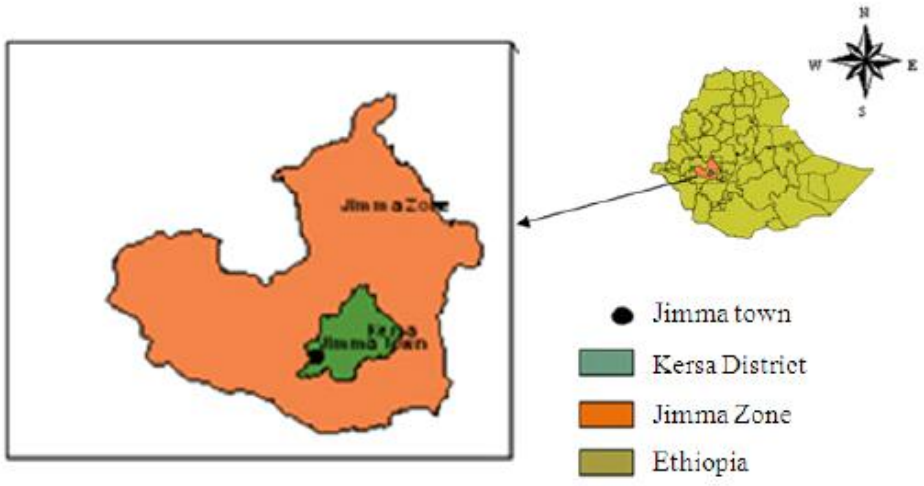

Figure 1: Geographical location of Jimma town (Alemu *et al*., 2011).

4.2. Study subjects

A total of 90 infants admitted to Jimma University Specialized Hospital, pediatrics in-patient department were investigated for microbial contamination of weaning foods.

4.3. Sample size determination

The sample size was determined based on the number of infants admitted to Jimma University Specialized Hospital. According to the data obtained from Jimma University Specialized Hospital (From August to October, 2013) average of 462 infants had been admitted to the IPD (Pediatric Inpatient Department) per month. Based on this data, the total number of population investigated during sample collection period, from January to March, 2014 (three months) was 1386. Therefore, sample size was determined using the following statistical formula (Daniel, 1983):

$$n = \frac{n_0}{1 + \frac{n_0}{N}}$$

$$n_0 = \frac{Z^2_{\alpha/2} p(1-p)}{d^2}$$

Where:

n = sample size

d= margin of error

N = total number of population

p= proportion of population

α= level of significance

Where: d = 0.1

p = 0.5

α= 0.05

$$n_0 = \frac{(1.96)^2 \times 0.5 \times 0.5}{(0.1)^2} = 96$$

Considering the population correction factor into account the sample size will be:

$$n = \frac{96}{1 + \frac{96}{1386}} = 89.7$$

≈ 90

4.4. Sampling technique

Purposive random sampling technique was used to address representative of infants aged between 6-24 months. This technique was used due to infants aged less than 6 months, above 2 years, and those did not receive weaning foods in the time they stayed in the hospital were excluded from the study.

4.5. Data collection

Data about general background of mothers, food makers, the infant, general sanitation of the mothers, duration and storage temperature before consumption, and other information were collected from mothers feeding their infants with weaning foods using structured interview. Additional data were also collected from physicians/health workers and food makers in JUSH.

4.6. Sample collection

A total of 90 traditional 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, Department of Biology, Applied Microbiology Research and Postgraduate Laboratory for analysis. The microbial analysis was conducted within an hour after collection.

4.7. Microbiological methods

4.7.1. Sample preparation

The collected food samples were analyzed for microbial quality and safety. Accordingly, 25ml 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 and incubated at appropriate temperature (32-35 °C) for 24 hours. The colonies were counted from plate containing microbial colonies between 30 and 300. The colonies were counted per sample and then expressed in log colony forming units (Log CFU).

4.7.2. Microbial Enumeration

The homogenate from sample preparation in buffered peptone water was used for the following procedures: aerobic mesophilic count, Enterobacteriaceae count, total coliform count, aerobic spore count, staphylococci count, lactic acid bacteria count, and yeast and mould counts.

4.7.2.1. Total aerobic mesophilic bacterial count

From appropriate dilutions, 0.1 ml of the aliquot was spread plated on Plate Count Agar (PCA) and the plates were incubated at 32 °C for 24 hrs. After incubation, plates with colonies between 30- 300 were counted (Weil *et al*., 2006).

4.7.2.2. Enterobacteriaceae count

To count the members of Enterobacteriaceae 0.1 ml of the aliquot was spread-plated on MacConkey agar (Oxoid) and incubated 32 ^{o}C for 24 hrs. After which, pink to red purple colonies were counted as member of a family Enterobacteriaceae (Spencer *et al*., 2007).

4.7.2.3. Coliform count

From appropriate dilutions, 0.1 ml of the aliquot was spread plated on pre-solidified surfaces of Violet Red Bile Agar (VRBA) (Oxoid) plates. Then the plates were incubated

at 32 ^{o}C for 24 hrs. Purpish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms (Weil *et al*., 2006).

4.7.2.4. Aerobic bacteria spore count

For aerobic bacterial spore counts, 10 ml of appropriate serial dilutions were heated in a water bath kept 80 °C for 10 minutes and then cooled rapidly in tap water. From appropriate dilution, 0.1 ml aliquot was spread-plated on pre-dried surface of plate count agar (PCA) and incubated at 32 ^{o}C for 72 hrs (Acco *et al*., 2003).

4.7.2.5. Staphylococci count

From appropriate dilutions, 0.1 ml of the aliquot was spread plated onto Mannitol Salt Agar (MSA) (Oxoid) by using the spread plate method and incubated at 32 °C for 48 hrs. Typical golden yellow color colonies were counted as *Staphylococcus* spp. (Acco *et al*., 2003).

4.7.2.6. Lactic acid bacteria count

From appropriate dilutions, 0.1 ml of the aliquot was spread plated 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).

4.7.2.7. Yeast and mould counts

The yeasts and moulds count of weaning food samples were determined by direct plate count using Potato Dextrose Agar (PDA) supplemented with 0.1g chloramphenicol. From appropriate dilutions, 0.1 ml aliquot was spread-plated on pre-solidified surfaces of PDA supplemented with 0.1g chloramphenicol and incubated at 25-28 ^{o}C for 5-7 days. Smooth (non-hairy) colonies without extension at periphery were counted as yeasts where as hairy colonies with extension at periphery were counted as mould (Spencer *et al*., 2007). (Spencer *et al*., 2007).

4.8. Microbial analysis

After enumeration of aerobic mesophilic bacteria, 10 to 15 colonies with distinct morphological differences such as color, size and shape were randomly picked from countable plates and aseptically transferred into a tube containing 5 ml nutrient broth, then incubated at 32 ^{o}C for 24 hrs. Cultures were purified by repeated plating and preserved on nutrient agar slants at 4 ^{o}C. Then microbes were characterized to the family and genus levels. In addition, the isolates were further identified based on their cell morphology and biochemical tests.

4.8.1. Cell morphology

The morphological study includes cell shape, cell arrangement and presence or absence of endospores. These were carried out by Gram staining techniques and observing under microscope using oil immersion objective. Schefer-Fulton endospore staining techniques were used to identify the presence or absence of endospore.

4.8.2. KOH-test (test for lipopolysaccharide)

One or two drops of 3% KOH solution were placed on a clean microscopic slide. A colony was aseptically picked from the surface of plate count agar plates using an inoculating loop and stirred in the KOH solution for 10 seconds to 2 minutes. The inoculating loop was raised slowly from the mass. Then, the thread of slime followed the loop in Gram negative bacteria. But in Gram positive bacteria, there was no thread of slime that followed the loop (Gregerson, 1978).

4.8.3. Oxidation Fermentation (O/F) Test

This test was used to assess the ability of the isolate to utilize glucose and to determine the metabolic path way (i.e. fermentation or oxidation). Ingredients (g/l): Peptone, 2 g; yeast extract, 1 g; NaCl, 5 g; K_2HPO_4, 0.2 g; glucose,10 g; bromothymol blue, 0.08 g; agar, 2.5 g; distilled water, 1000 ml; pH, 7.10. Accordingly, test tubes containing 15 ml of freshly prepared medium for O/F test were autoclaved and immediately cooled under tap water to avoid dissolution of oxygen in the medium. Then, the broth cultures were inoculated into the medium by stabbing with a sterile straight wire to the bottom. An

organism with oxidative metabolism displayed yellow in the upper half of the tube and green in the lower half. An organism with fermentative metabolism displayed yellow in both halves of the tube. Acid formation and growth regions were interpreted after 2 to 5 days of incubation at 32 ^{o}C (Hugh and Leifson, 1953).

4.8.4. Catalase Test

Catalase test was used to identify organisms that produce the catalase, an enzyme which converts hydrogen peroxide (H_2O_2) to water and O_2. This test was carried out after young colonies flooded with a 3% solution of H_2O_2. The formation of bubbles indicated the presence of catalase enzyme (MacFaddin, 1980).

4.8.5. Cytochrome Oxidase test

This test was conducted following the method outlined by Kovacs (1956). Freshly prepared reagent A and B were mixed in the ratio of 2:3 immediately before use. Reagents: A, 1% α – naphthalene absolute ethanol and B, 1% N, N – dimethyl –p – phenylene diammonium chloride in distilled water. About 2 – 3 drops of the oxidase reagent was added to the surface of the culture of isolated colonies of each test bacterium. The appearance of a blue color on the colonies was observed. Color changes occur, if any, within 20 – 30 seconds. Color changes after 20 – 30 seconds were disregarded, since the reagent begins to change color with time due to auto-oxidation.

4.9. Detection of Pathogens

4.9.1. *Salmonella* and *Shigella*

For the detection of *Salmonella and Shigella* spp., 25ml of weaning food samples were mixed with 225 ml of Buffered Peptone Water (BPW) and incubated at 37 °C for the metabolic recovery and proliferation of cells which could be injured during processing or to bring the number of target organisms to a detectable level. Then 1 ml pre-enrichment broth culture was added to 10 ml of Selenite Cystein Broth (Oxoid) and again incubated at 37 ^{o}C for 24 hrs. Thereafter, a loopful of suspension from a tube was streaked onto Xylose Lysine Deoxycholate Agar (XLD) (Arvanitidou *et al*., 2005).

The plates with typical colonies of *Salmonella*, i.e. red colonies with black centers on XLD agar, and typical colonies of *Shigella*, i.e. red colonies on XLD agar were examined. These cultures were analyzed by the following standard biochemical tests.

Triple Sugar Iron Agar (Oxoid)

The butt was stabbed and the slant was streaked and incubated at 37 ^{o}C for 24 hrs to detect fermentation of glucose, sucrose and lactose as well as production of H_2S. The presence of alkaline (red) slant and acid (yellow) butt, with production of H_2S was considered as presumptive for *Salmonella* spp. An alkaline (red) slant and an acid (yellow) butt with no gas production was a presumptive for *Shigella spp*.

Lysine Iron Agar (Oxoid)

The butt was stabbed and the slant was streaked and incubated at 37 ^{o}C for 24 hrs. Then, the production of an alkaline reaction (purple color) throughout the medium was presumptive for *Salmonella* spp. Due to the production of H_2S, an intense blackening of the medium was positive reaction for *Salmonella* but negative result for *Shigella* spp.

Urea Agar (Oxoid)

The slant was streaked and incubated at 37 ^{o}C for 24 hrs to assess the hydrolysis of urea. No color change in the slant was considered as negative and thus presumptive for both *Salmonella* and *Shigella* spp.

Simmons Citrate Agar (Oxoid)

The slant was streaked and incubated at 37 ^{o} C for 24 hrs to determine citrate utilization as a sole source of carbon. The presence of growth and color change from green to blue was considered as presumptive for *Salmonella. Shigella* is citrate negative; so no color change in the medium is presumtive result for *Shgella* spp.

Sulfide Indole Motility (SIM) Medium (Oxoid)

The SIM medium was stabbed to the bottom and incubated at 37 ^{o}C for 24 hrs for determination of H_2S production, indole production and motility. Diffused growth away from stab line or turbidity throughout the medium shows positive motility for *Salmonella*.

Production of indole was investigated by adding Kovac's reagent (HCl, 250 ml, amyl alcohol, 750 ml and para-dimethyl-amino-benzaldehyde 50 g /l) to growth in this culture medium. Diffused growth and non-utilization of indole (absence of deep red color at the surface of agar) was considered as presumptive for *Salmonella* spp. Absence of diffused growth is a presumptive for *Shigella* species.

4.9.2. *Staphylococcus aureus*

After counting staphylococci, golden yellow colonies on the Mannitol Salt Agar plates were aseptically picked and transferred into 5ml nutrient broth and incubated at 37 ^{o}C for 24 hrs for further purification .Then, a loopful of culture from the nutrient broth was streaked on nutrient agar and incubated at 37 ^{o}C for 24 hrs. Finally, the distinct colonies were characterized using the established microbiological methods (Acco *et al*., 2003). Gram-positive, cocci with cluster arrangement under the microscope were subjected to preliminary biochemical tests (oxidase, catalase and coagulase tests).

Coagulase test

Coagulase test was done according to Cheesbrough (2006) using slide test procedures. Accordingly, colonies of the purified isolates were emulsified in a drop of distilled water on two ends of clean glass slide to make thick suspensions. One was labeled as test and the other as control. The control suspension was served to rule out false positivity due to auto-agglutination. A loopful of human blood plasma was added to one of the suspensions and mixed gently. Clumping within 10 seconds was observed for coagulase positive organism (*Staphylococcus aureus).*

4.10. Statistical analysis

Statistical analysis was performed by using SPSS soft ware version 16.0. The bacterial counts were expressed as mean ± Standard deviation. Mean values of weaning food samples were compared using one way ANOVA. The mean difference was considered significant level at $p < 0.05$ (95% confident interval).S

4.11. Ethical consideration

Ethical clearance was obtained from Research Review and Ethical committee of College of Natural sciences, Jimma University. Respondents and concerned physicians and health workers were informed about the purpose of the study. Food samples were collected after obtaining consent from each participant.

5. RESULTS

5.1. Socio-demographic characteristics

Of the 90 parents of infants' interviewed in this study, a significant number (85.6%) of the respondents were mothers (Table 2). The majority (56.7%) of the respondents were within an age group of 30 to 39 years. With respect to the educational status, 65.6%, 26.7%, 5.6%, 2.2% of the respondents were illiterate, elementary school, high school, and above grade 12 respectively. Occupationally, 75.6%, 8.9%, 4.4%, and 1.1% of the respondents were unemployed, private workers, farmers, and civil servants respectively.

Table 2. Socio-demographic characteristics of in-patient infants' parents in Jimma University Specialized Hospital, Jimma town, Southwestern Ethiopia, 2014

Characteristics		Number of respondents (N=90)	
		Frequency	Percent (%)
Parents/care givers	-Mother -Father	77 13	85.6 14.4
Parents' residence	-Urban -Rural	13 77	14.4 85.6
Age	<20 20-29 30-39 40-49	1 30 51 8	1.1 33.3 56.7 8.9
Academic status	-Illiterate -Grade 1-4 -Grade 5-8 -Grade 9-12 -Above grade 12	59 15 9 5 2	65.6 16.7 10.0 5.6 2.2
Occupation	-Unemployed/house wife -Private sector/business -Farmer -Civil servant	68 8 13 1	75.6 8.9 4.4 1.1

5.2. Description of weaning food types and infants

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). Gruel is made from flour of cereal blends boiled into thin, smooth porridge; whereas besso is prepared from flour of roasted barely. To make besso, the flour is mixed gently with water and sugar for few minutes and ready for consumption thenafter.

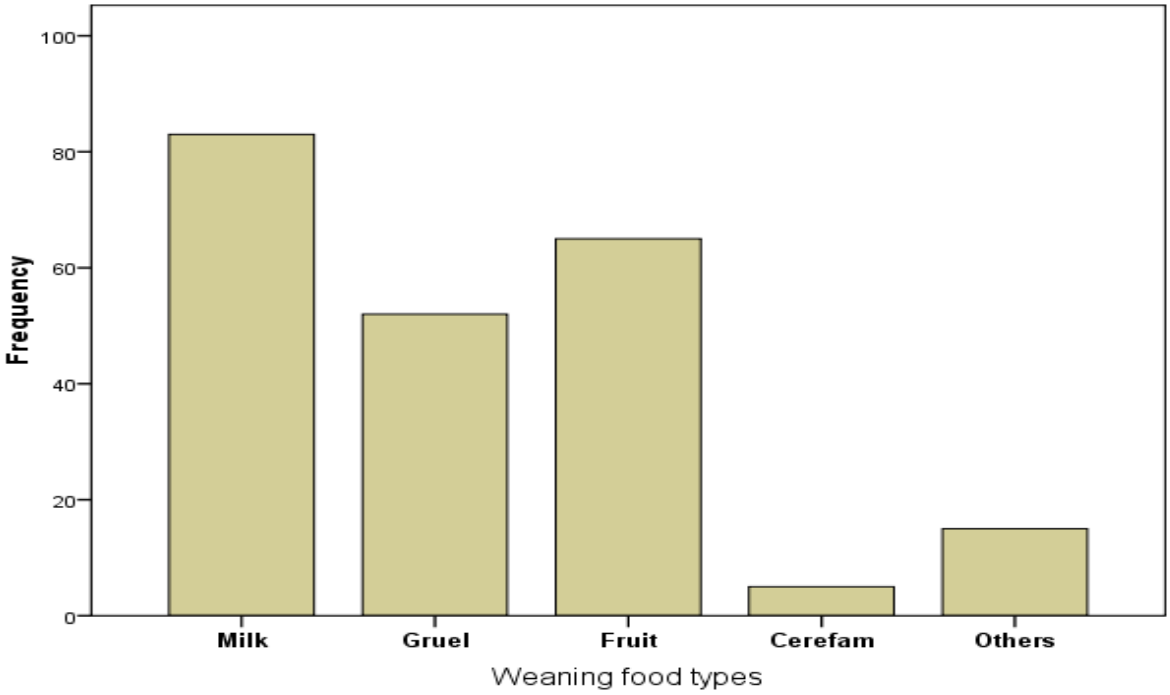

Figure 2: Weaning food types of in-patient infants in Jimma University Specialized Hospital, 2014

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 3). Among bottle-feeding infant 93% used a single bottle throughout the feeding period but few (7%) used more than two bottles and/or exchanging teats of the bottle after every feeding.

Table 3. 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	72	80.0
	No	18	20.0
Age when feeding on weaning foods started	<6 month	33	36.7
	≥6 month	57	63.3
Methods of feeding infants	-Bottle-feeding	28	31.1
	-Feeding with spoon/cup	62	68.9
Bottle washing practices	-Cold water	7	25.0
	-Cold water and soap	13	46.4
	-Warm water and soap	8	28.6
No. and frequency of use of bottles	-Using more than one bottle exchanging one after the other	2	7.0
	-A single bottle all the time	26	93.0

5.3. Hygienic practices of the parents

Parents tend to cool their infant foods either by transferring from one utensil to the other (79.6%) or leaving it open (16.7%) for a while until it cools down. Only 7.8% of the parents have the chance of using the refrigerator. On the other hand, most (86.7%) of the parents have the experience of storing food for more than six hours (Table 4).

Table 4. General sanitary practices of in-patient infants' parents in Jimma University Specialized Hospital, Jimma town, Southwestern Ethiopia, 2014

Characteristics		Number of respondents (N=90)	
		Frequency	Percent
Sources of water	-Tap water	43	47.8
	-Well water	29	32.2
	-River	16	17.8
	-Pond	2	2.2
Cooling cooked/boiled food	-Periodic transfer from one utensil to the other until it cools	68	75.6
	-Making the utensils open to cool	15	16.7
	-Using both methods	7	7.8
Hand washing practice after toilet	-Yes	46	51.1
	-No	44	48.9
Hand washing practice before preparing food	-Yes	89	98.9
	-No	1	1.1
Hand washing status	-Using water only	85	94.4
	-With water and soap	5	5.6
The status of cleaning utensils	-Using water only	60	66.7
	-With water and soap	30	33.3
Food storage place	-Shelf or others	83	92.2
	-Refrigerator	7	7.8
Food storage time	<6 hours	12	13.3
	≥6 hours	78	86.7
Training on hygienic preparation of foods	-Yes	3	3.3
	-No	87	96.7

5.4. Microbiological load of weaning foods

The mean microbial counts for selected weaning foods of in-patient infants in Jimma University Specialized Hospital were shown below (Table 5). 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. In the present study higher count of AMB (6.60 log CFU/ml) was detected in milk. Weaning cereals and fruit juices had closer coliform counts (2.25 and 2.02 log CFU/ml respectively). The mean count of *Staphylococcus* spp. was highest (4.60 log CFU/ml) in weaning cereals and below detectable level (1.78 log CFU/ml) in fruit juices. On the other hand the mean counts of LAB were lowest (2.84 log CFU/ml) in fruit juices than other weaning food samples. The count of Enterobacteriaceae and coliforms was comparable in fruit juices. All weaning food samples had higher aerobic mesophilic bacteria count than other microbial groups. The mean counts of most microbial groups were highest in weaning milk. On the other hand the mean counts of yeasts were < log1 CFU/ml in all the three weaning food types (Table 5). Moulds were also below detectable level, and even not detected in many of the weaning food samples.

The minimum and maximum microbial counts of all weaning food samples were shown in (Appendix 1). Accordingly, the maximum counts (log CFU/ml) of aerobic mesophilic bacteria, aerobic spore former, LAB, and staphylococci were observed in cereals with values as high as 8.93, 5.93, and 8.83, and 6.93 respectively. On the other hand, the maximum counts (log CFU/ml) of Enterobacteriacae and coliforms were observed in fruit juices, with readings of 7.66, and 7.58 respectively. Whereas the maximum count of yeasts (4.89 log CFU/ml) were observed in milk samples.

Table 5. 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 ($P>0.05$) (Appendix 2).

Analysis of the microbial load of weaning foods based on source and type revealed that 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 3).

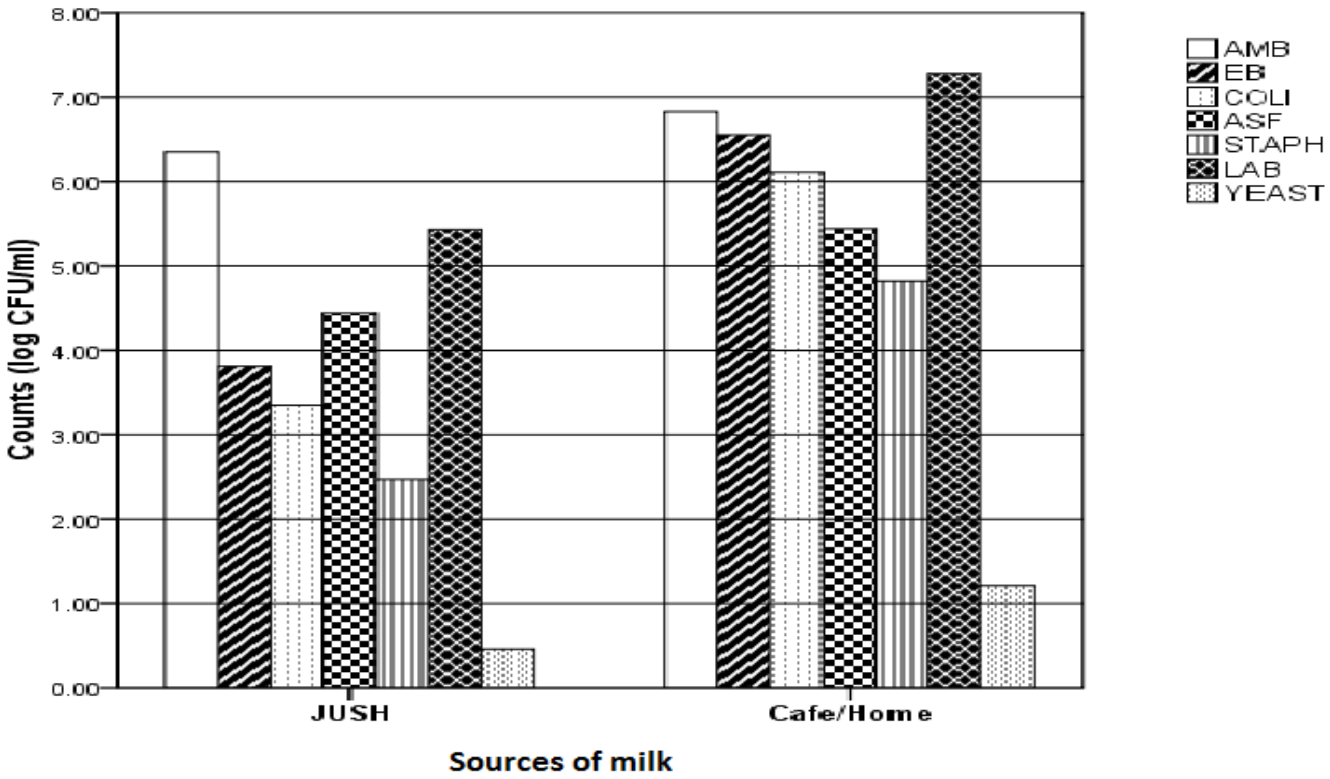

Figure 3: Mean microbial load of weaning milk of in-patient infants in Jimma University Specialized Hospital, 2014

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

In cereal-based weaning foods, higher counts of aerobic mesophilic bacteria, Entrobactericae, coliforms, aerobic spore former, LAB, and yeasts were observed in besso (non-heat treated cereals). However, counts of staphylococci were higher in gruel (heat-treated cereals) than in besso samples (Figure 4). In weaning fruit juices, the counts of aerobic mesophilic bacteria, Enterobacteriacae, and staphylococci were higher in orange juice samples than rani and mango juices (Figure 5). Rani juice contains the same ingredients as mango juice except some additional ingredients in the former. Yeasts were the least detected in fruit juices and totally absent and gruel samples.

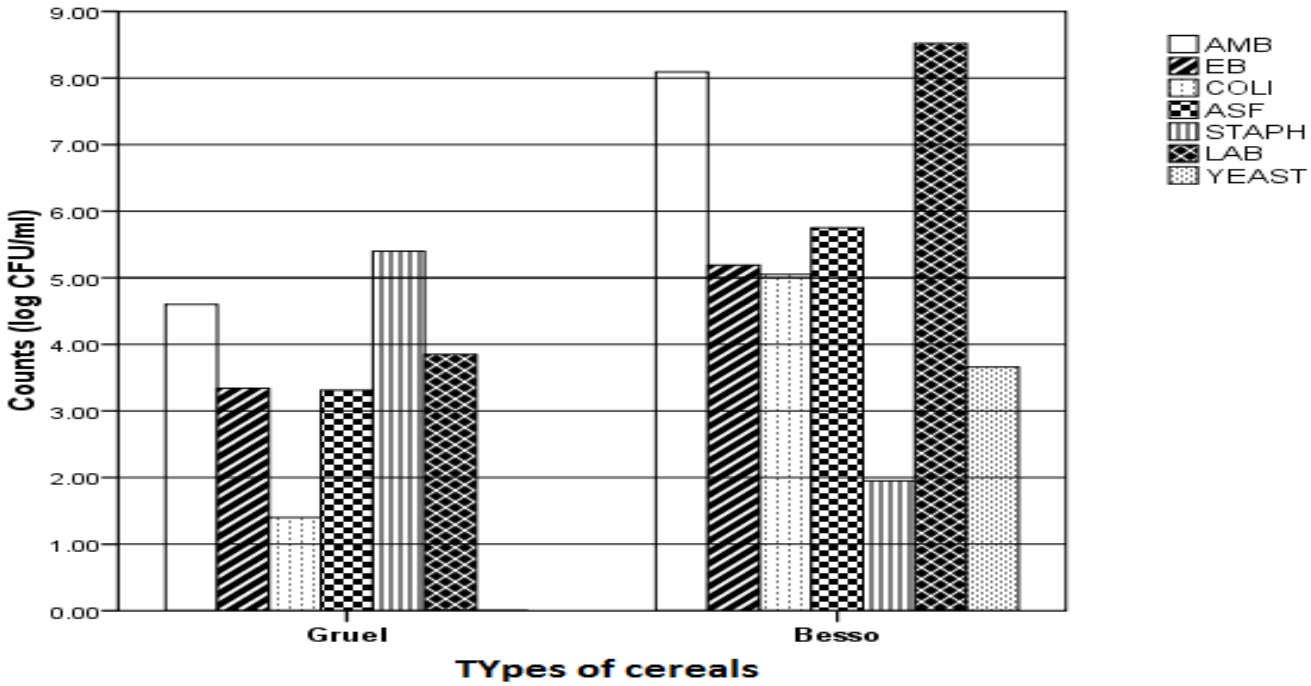

Figure 4: Mean microbial load of weaning cereals of in-patient infants in Jimma University Specialized Hospital, 2014

Where; AMB: Aerobic mesophilic bacteria, COLI: Coliforms, EB: Enterobacteriaceae, STAPH: *Staphylococcus* spp , ASF: Aerobic spore formers, LAB: Lactic acid bacteria

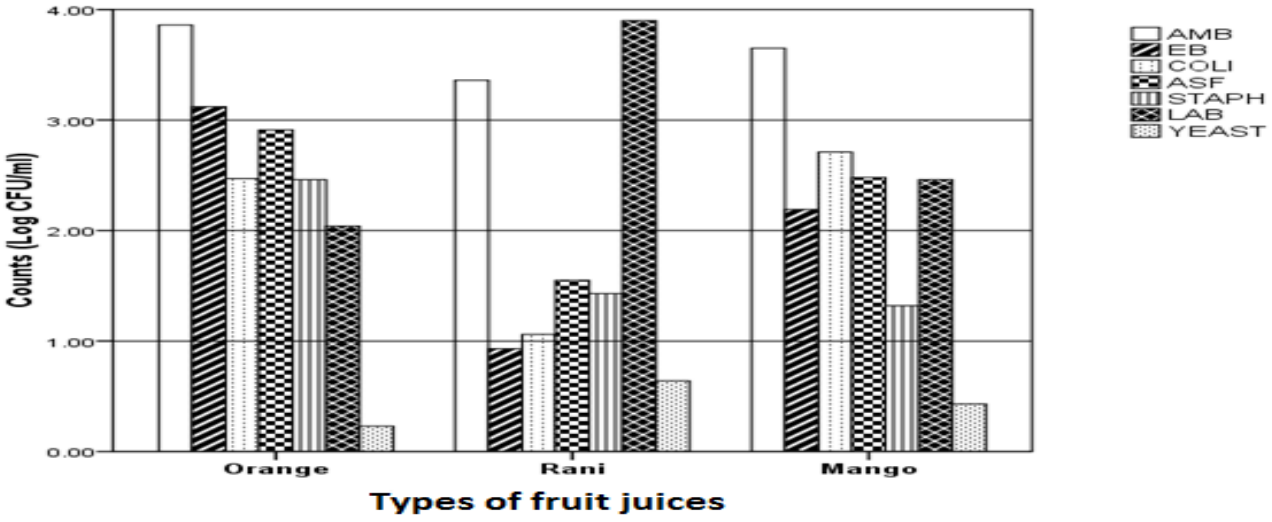

Figure 5: Mean microbial load of weaning fruit juices of in-patient infants in Jimma University Specialized Hospital, 2014

Where; AMB: Aerobic mesophilic bacteria, COLI: Coliforms, EB: Enterobacteriaceae, STAPH: *Staphylococcus* spp, ASF: Aerobic spore formers, LAB: Lactic acid bacteria

5.5. Microflora 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 6). Staphylococci (21.4%) were the most frequently isolated microorganisms from all weaning food samples followed by *Baclillus* (18.9%), *Lactobacillus* (16.5%), *Micrococcus* (15.9%), Enterobateriacae (12%), *Pseudomanas* (6.6%), other Gram positive (6.6%), and *Aeromonas* were the least isolated (2%). The most dominant bacterial groups isolated from weaning cereals were staphylococci (27.7%) followed by *Bacillus* (24.7%) and *Micrococcus* (22.1%). However, milk samples were dominated by *Bacillus* (29.4%) followed by staphylococci (18.2%) and *Lactobacillus* (16.7%). Similarly, in fruit juices *Lactobacillus* (27.6%) were the most dominant microflora followed by staphylococci (18.1%) and Enterobacteriacae (15.2%) (Table 6).

Table 6. 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 + bacteria	15 (5.9)	13 (5.5)	18 (8.6)	46 (6.6)
Total	**252 (36)**	**235 (34)**	**210 (30)**	**697 (100)**

5.6. 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. On the other hand, *Salmonella* was isolated from 3 (10 %) of cereal samples. However, fruit juices contain the least *Salmonella* isolates 1 (3.3 %) as compared to milk and cereals. *Shigella* was not detected in any of the weaning food samples (Table 7).

In the present study, 31.1% samples were positive for *S. aureus*. However, its frequency distribution varied among weaning food samples. Accordingly, higher prevalence of *S. aureus* (46.7%) was observed in cereal-based weaning foods, followed by milk (36.7%). In fruit juices the prevalence is relatively lower (10.0%) (Table 7).

Table 7. 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)**

6. DISCUSSION

Feeding infants healthy food is important for a number of reasons. Food gives infants and young children the energy and nutrients they need and to grow (Mamiro *et al*., 2005). Adequate nutrition during infancy and early childhood is fundamental to child development. Rapid growth of infants during the first two years of life requires an adequate supply of nutrients to cope with the rapid development of body muscles and other tissues (Grummer-Strawn *et al*., 2008). Therefore, weaning period is important for the promotion for optimal growth, health and behavioral development of infants (Obi and Nwozor, 2012). However, in developing countries, the beginning of the weaning process in human infants has been associated with an increase in diarrheal episodes as a result of consumption of contaminated weaning foods (Omemu and Omeike, 2010). Therefore, feeding microbially safe food should be the thing that parents think first.

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. The report by Cuprasitrut *et al*. (2011) indicated that giving training to all the communities are very important at all levels of preparation because food safety is critical to the public health, consumers, and government. Food safety is the set of conditions and practices of protecting foods from pathogenic microorganisms, and toxic compounds produced during preparation, processing, and storage (Mortarjemi *et al*., 1993). The overall strategy for reducing food-borne illness is to give emphasis to education about proper food storage and preparation practice along with strict and more targeted enforcement. Therefore, food handlers should have necessary knowledge and skills to handle food hygienically (FAO, 1998). However, findings of the current study indicated poor educational status of parents and calls for designing of strategies for community level training as suggested by Cuprasitrut *et al*. (2011). The in-patient infants included in this study were found in the age between 6-24 months. Breast feeding infants were 80% and most (63.3%) of the in-patient infants began feeding with weaning foods after six months. Similar report by Omemu and Omeike (2010) indicated that none of the mothers introduced weaning food to the babies

before 4 months of age and most (87.5%) of the mothers introduced weaning foods between 4-6 months. In another report Satter *et al*. (2013) showed that 42% of infants aged less than six months are exclusively breastfed. In the present study most of the infants (68.9%) fed with spoon and/or cup but the rest (31.1%) were bottle-feeding; out of which 93% used a single bottle and teats throughout the feeding period. Pathak *et al*. (2012) reported that mothers with several bottles were significantly protected against microbial contamination compared with those who owned fewer. Report by Morterjemi *et al*. (1993) showed that unclean pots, cooking utensils, baby bottles and teats are potential sources of microbial contamination. Moreover, the report of Obi and Nwozor (2012) indicated that using clean feeding utensils, and avoiding the use of feeding bottles and teats play crucial role in preventing microbial contamination of weaning foods.

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. Omemu and Omeike (2010) from Nigeria reported 96.2% of mothers prefer feeding their infants with food that has been prepared and served from their kitchen. In the present study 52.2% of the parents did not use tap water which is critical raw material for food preparation. According to the report of WHO (2000) microbial contamination of weaning foods can be prevented by using safe water for preparation and frequent washing of hands. Water could be contaminated with biological, chemical, and physical hazards. Contaminated water creates public health if it is incorporated into food as an ingredient processing of food or used in the washing of utensils. Therefore, water supply needs closer attention in food operations.

Simple processing and handling techniques, such as storing non-perishable items in safe place (e.g., clean, and closed containers); consuming the cooked foods shortly after preparation or, if possible, storing at cold (<10 ^{o}C) temperatures are also important to prevent microbial contamination. However, in low income settings adequate food handling may 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 and only 7.8% used refrigerator for storage. Similar to this

study, Omemu and Omeike (2010) from Nigeria reported that 90.3% mothers stored weaning foods for more than six hours. Another report by Kungu *et al*. (2009) from Zanzibar indicated that high bacterial numbers in infant porridge were detected after held for four hours preparation as compared to freshly prepared ones which suggest possible household contamination during this storage time. Therefore, 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. Enumeration of total aerobic mesophilc bacteria on food samples examined in the present study indicated high microbial contamination in weaning foods as they were good indicators of food safety (Cenci-Goga *et al*., 2005). However, 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). In this study, weaning milk and cereals had mean contamination levels of ≥5.0 log CFU/ml. Greater contamination was detected in non-heat treated cereals and milk obtained from cafe and home. However, 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). According to the guideline, the mean counts (log CFU/ml) of Enterobacteriacae in milk (5.12 log CFU/ml) revealed unsatisfactory level (≥4 log CFU/ml), where as weaning cereals (3.78 log CFU/ml) and fruit juices (2.07 log CFU/ml) belonged to the group with acceptable level (< 4 log CFU/ml). Fruit juices processed under hygienic condition could play important role in enhancing consumers' health through inhibition of breast cancer, congestive heart failure, and urinary tract infection (Ketema *et al*., 2008).

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. Welearegay *et al*. (2012) reported that unhygienic conditions of milking, unclean milk handling equipment and the use of contaminated cleaning water were among the important sources of milk contamination. Another report by Pathak *et al*. (2012) showed that the possible source of contamination for heat processed food samples could be post preparation processes such as unclean utensils used for storage. Of course, once introduced into the food samples and the food left at ambient temperature for a while, the contaminating coliforms would multiply to higher counts. In the present study, a 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. Low levels of spore formers in foods can cause a problem when the spores germinate and grow during storage, which leads to enzyme formation and metabolism. Microbial spoilage enzymes such as proteases, lipases, and lecithinases are often responsible for off-flavor and structural defects. These enable endospore formers such as

B. cerus to provoke food quality and safety (Witthuhn *et al*., 2011). 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. Therefore, some *Bacillus* spores survived in milk even after heat treatment (Pathak *et al*., 2012).

Staphylococcal food poisoning is a major form of food-borne illness and appears to continue so as time goes on and the environment conditions are favorable for growth and multiplication. *Staphylococcus* spp. produce a protein toxins and virulence factors thought to contribute to the pathogencity of the organism. Although, sufficient cooking destroys the bacteria, the toxin produced by *S. aureus* is heat stable and may not be destroyed even by heating (Ghosh *et al*., 2004). On the other hand, Obi and Nwozor (2012) showed that insufficient cooking which does not involve long period of heating enables the survival of many pathogens in the weaning foods. The mean counts of staphylococci in the present study were 3.26 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). Moreover, these organisms may get chance to multiply in the product during storage and produce their enterotoxins which constitute a staphylococcal food poisoning, which is public health hazard to the consumers (Ghosh *et al*., 2004). It has been reported that production of enterotoxin occurs when the counts of *S. aureus* reach 6 log CFU/ml (Schelin *et al*., 2011).

In the present study, the mean count of LAB was 4.65 log CFU/ml. In agreement with the present 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. LABS are the most important groups of bacteria in the food industry which are used in making starter culture for fermented food products (Seo *et al*., 2010). It has also been recognized that LAB are capable of producing inhibitory substances that are antagonistic toward other microorganisms (Ennahar *et al*., 2000). This inhibition is may be due to the production of many metabolites such as organic acids (lactic and acetic acid), hydrogen peroxide, diacetyl and bacteriocins (Lasagno *et al*., 2002). The primary antimicrobial effect exerted

by LAB against spoilage and pathogenic bacteria is the production of lactic acid and reduction of pH. However, improper storage of food products at household level before cooking can encourage growth of pathogenic microorganisms (Omemu and Omeike, 2010).

Yeasts and moulds are common contaminants in food. Although yeast does not result in food poisoning, it can cause to spoil. A number of moulds produce toxic substances designated as mycotoxins. Some mycotoxins are mutagenic and carcinogenic (Seo *et al*., 2010). However, in the present study, in most of weaning food samples yeasts (>83%), and moulds (100%) were not detected. Similar results obtained by Satter *et al*. (2013) also revealed that total yeasts and moulds count per gram were absolutely nil/g in the all weaning foods analyzed. However, the study of Braide *et al*. (2012) showed 42.8% fungal isolates from industrially processed fruit juices. Ejechi *et al*. (1998) reported that heating foods at temperatures of 70-75 ^{o}C is effective to inactivate yeasts and the spores of common contaminant fungi.

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%). Similar to this finding a research conducted in Nigeria revealed that *Staphylococcus* spp. (35.7%) was the first dominant bacterial isolate followed by *Bacillus (*25.0%) (Odu and Amewaiye, 2013). In another report Nwogwugwu *et al*. (2012) showed that *Staphylococcus* and *Bacillus* occur in 100% weaning food samples. Staphylococci may be expected to exist, at least in low numbers, in any or all food products that are of animal origin or in those that are handled directly by humans, unless heat-processing steps are applied to effect their destruction. Moreover, staphylococci exist in air, dust, sewage, water, or on food equipment, and environmental surfaces (Jay *et al*., 2005). On the other hand, the presence *Bacillus* spp. in ready to eat foods is used as an indication of either post processing contamination or inadequate cooking (Pathak *et al*., 2012). Moreover, Suneetha *et al*. (2011) showed that the predominance of *Bacillus cerus* in food samples implicated the ubiquitous nature of bacterial spores and its presence in raw material. *Lactobacillus* spp. was the third dominant microflora isolated from weaning food samples. According to the study conducted by Farah and Zinsstsg (2003)

Lactobacillus spp. which was isolated from milk and fermented products represents more than 60% of the population.

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. *S. aureus* is a dangerous pathogen and one of the most causative agents of hospital infectious (nosocomial infections) in human beings. In this study, 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. Consequently, depending on the extent of the initial contamination and the duration of cooking, a number of pathogens may survive the cooking process (Motarjemi *et al*., 1993).

In the present study, the prevalence of *Salmonella* spp. were 10% in which more prevalence (16.7%) was observed in milk followed by cereal-based weaning foods. 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 presence of *Salmonella* in 25 g/ml of sample examined is regarded as potentially hazardous, and is unacceptable for consumption. Hence, ready to eat foods should be free of *Salmonella* as consumption of food containing this pathogen may result in food-borne illness (Cheung *et al*., 2007).

According to Motarjemi *et al*. (1993) *Shigella* spp. are also a major health problem in developing countries and causes 10-15% of acute diarrhoea in children less than 5 years of age. 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, it is interesting that *Shigella* spp. were 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).

7. CONCLUSION

- The results obtained in this study indicate that most weaning foods available to the infants have high level of microbial contamination that do 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 possibly due to good manufacturing practices and quality control measures employed by manufacturers.

- The most predominant microbial groups isolated from weaning food samples in this study were *Staphylococcus* spp., *Bacillus* spp., *Lactobacillus* spp., and *Micrococcus* spp.

- Out of 90 weaning food samples *Salmonella* isolates were found from 9 samples with more prevalence in milk. Likewise, *Staphylococcus aureus* were encountered from 28 samples with more prevalence in weaning cereals. However, *Shigella* spp., yeasts and moulds were not the common contaminants of weaning foods investigated in this study.

8. RECOMMENDATIONS

- Since the nutrition of infants and young children depend closely on the education of their mothers or caregivers on food safety, programmes to educate mothers on food safety principles should therefore be considered as an integral part of primary health care programme. Jimma University Specialized Hospital (JUSH) should have regular program of teaching both in the out-patient, and in-patient departments. The hospital should also have regular follow-up program to the hygienic condition of the mothers and the cleanness of the utensils used to store weaning foods during the period they stay in the hospital.

- Foods should be prepared hygienically, not stored for longer time or stored safely until consumption by giving greater emphasis to infants' foods. Boiling water and sufficient heating foods before consumption, avoiding feeding infants with bottle and teats could reduce the risk of contracting food and water-borne pathogens.

- Feeding infants with bottled fruit juices which showed lower microbial load and microbiologically safer than other weaning food types. The parents should also encourage their infants to receive other commercial weaning foods available in the hospital which are recommended and freely donated by WHO/UNICEF.

- Government should work more for rural communities to improve facilities like pure water supply, and general hygiene practices. Reducing rates of child mortality and creating healthy society by accelerating the implementation of health extension program.

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10. APPENDICES

Appendix 1: Microbial load of weaning foods

Weaning food types	Microbial counts in log CFU/ml							
		AMB	EB	COLI	ASF	STAPH	LAB	YEAST
Milk	Min	4.58	0.00	0.00	3.56	0.00	3.58	0.00
	Max	7.91	6.93	6.85	5.89	5.79	7.93	4.89
	Mean	6.60	5.12	4.45	4.84	3.41	6.17	0.76
	S.D	0.74	2.43	2.26	0.69	2.16	1.26	1.74
	%C.V	11.21	47.46	50.78	14.25	63.34	20.42	229
Cereals	Min	3.81	0.00	0.00	0.00	0.00	0.00	0.00
	Max	8.93	5.99	6.76	5.93	6.93	8.83	3.93
	Mean	5.42	3.78	2.25	3.88	4.60	4.94	0.85
	S.D	1.52	1.64	2.30	1.39	2.51	2.22	1.57
	%C.V	28.04	43.38	102.22	35.82	54.56	44.94	187.70
Fruit juices	Min	2.50	0.00	0.00	0.00	0.00	0.00	0.00
	Max	5.80	7.66	7.58	3.68	6.68	5.81	3.56
	Mean	3.62	2.07	2.02	2.29	1.78	2.84	0.35
	S.D	0.51	2.71	2.66	1.11	2.48	2.31	1.07
	%C.V	14.08	130.9	131.68	48.47	139.32	81.33	305.7

Where; Min: Minimum, Max: Maximum, SD: Standard Deviation, CV: Coefficient of variation, CFU: Colony forming unit

N.B: Zero (0) indicates the count is below detectable level or even not detected at all.

Appendix 2: Statistical analysis based on weaning food types

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
AMB	Between Groups	129.770	2	64.885	59.725	0.000
	Within Groups	94.517	87	1.086		
	Total	224.287	89			
EB	Between Groups	122.248	2	61.124	10.900	0.000
	Within Groups	487.856	87	5.608		
	Total	610.105	89			
COLI	Between Groups	108.221	2	54.110	9.252	0.000
	Within Groups	508.825	87	5.849		
	Total	617.046	89			
ASF	Between Groups	99.402	2	49.701	40.483	0.000
	Within Groups	106.809	87	1.228		
	Total	206.211	89			
STAPH	Between Groups	120.057	2	60.029	10.473	0.000
	Within Groups	498.665	87	5.732		
	Total	618.722	89			
LAB	Between Groups	170.474	2	85.237	21.442	0.000
	Within Groups	345.846	87	3.975		
	Total	516.320	89			
YEAST	Between Groups	4.263	2	2.131	.958	0.388
	Within Groups	193.497	87	2.224		
	Total	197.760	89			

Where; AMB: Aerobic Mesophilic Bacteria, COLI: Coliforms, EB: Enterobacteriaceae, STAPH: Staphylococci, ASF: Aerobic spore formers, LAB: Lactic acid bacteria

Post Hoc Tests (Multiple Comparisons)

Tukey HSD							
Dependent Variable	(I) type	(J) type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
AMB	1	2	1.11785*	.26912	.000	.4761	1.7596
		3	2.91505*	.26912	.000	2.2733	3.5568
	2	1	-1.11785*	.26912	.000	-1.7596	-.4761
		3	1.79720*	.26912	.000	1.1555	2.4389
	3	1	-2.91505*	.26912	.000	-3.5568	-2.2733
		2	-1.79720*	.26912	.000	-2.4389	-1.1555
EB	1	2	1.12575	.61142	.162	-.3322	2.5837
		3	2.83486*	.61142	.000	1.3769	4.2928
	2	1	-1.12575	.61142	.162	-2.5837	.3322
		3	1.70911*	.61142	.017	.2512	3.1670
	3	1	-2.83486*	.61142	.000	-4.2928	-1.3769
		2	-1.70911*	.61142	.017	-3.1670	-.2512
COLI	1	2	2.20170*	.62442	.002	.7128	3.6906
		3	2.43330*	.62442	.001	.9444	3.9222
	2	1	-2.20170*	.62442	.002	-3.6906	-.7128
		3	.23160	.62442	.927	-1.2573	1.7205
	3	1	-2.43330*	.62442	.001	-3.9222	-.9444
		2	-.23160	.62442	.927	-1.7205	1.2573
ASF	1	2	.95801*	.28609	.003	.2758	1.6402
		3	2.54824*	.28609	.000	1.8661	3.2304
	2	1	-.95801*	.28609	.003	-1.6402	-.2758
		3	1.59023*	.28609	.000	.9081	2.2724
	3	1	-2.54824*	.28609	.000	-3.2304	-1.8661
		2	-1.59023*	.28609	.000	-2.2724	-.9081
STAPH	1	2	-1.19043	.61816	.138	-2.6644	.2836
		3	1.62740*	.61816	.027	.1534	3.1014
	2	1	1.19043	.61816	.138	-.2836	2.6644
		3	2.81783*	.61816	.000	1.3438	4.2918
	3	1	-1.62740*	.61816	.027	-3.1014	-.1534
		2	-2.81783*	.61816	.000	-4.2918	-1.3438
LAB	1	2	1.22902*	.51480	.050	.0015	2.4565

		3	3.33312*	.51480	.000	2.1056	4.5606
	2	1	-1.22902*	.51480	.050	-2.4565	-.0015
		3	2.10410*	.51480	.000	.8766	3.3316
	3	1	-3.33312*	.51480	.000	-4.5606	-2.1056
		2	-2.10410*	.51480	.000	-3.3316	-.8766
YEAST	1	2	-.09333	.38506	.968	-1.0115	.8248
		3	.40788	.38506	.542	-.5103	1.3261
	2	1	.09333	.38506	.968	-.8248	1.0115
		3	.50121	.38506	.398	-.4170	1.4194
	3	1	-.40788	.38506	.542	-1.3261	.5103
		2	-.50121	.38506	.398	-1.4194	.4170
*. The mean difference is significant at the 0.05 level.							

Where; AMB: Aerobic Mesophilic Bacteria, COLI: Coliforms, EB: Enterobacteriaceae, STAPH: *Staphylococci* , ASF: Aerobic spore formers, LAB: Lactic acid bacteria, 1: Milk 2: Cereals 3: Fruits

Appendix 3: Interview for physicians/health workers and food handlers

Jimma University

College of Natural Sciences

Department of Biology (Applied Microbiology)

Interview designed for Physicians (nurses and health workers) and food handlers for identification of microbial quality and safety of traditional weaning foods of In-patient infants in Jimma University Specialized Hospital in Jimma town, Jimma zone, Southwest Ethiopia.

Dear respondent,

This interview was designed for evaluation of microbial quality and safety of some traditional weaning foods. Your response to the questions has significant impact on the quality of data and result. Thus, you are kindly requested to respond genuinely.

Age_____ Sex_____ Responsibility_____

1. Who prepares foods for In-patient infants in Jimma University Specialized Hospital (JUSH)?

A. Parents B. JUSH C. Both

2. What type of weaning foods, JUSH prepares for the infants?

_____

3. Are the foods prepared by JUSH freshly available for the infants for every feeding time?

Yes/No

If yes how often does the hospital prepare food for the infants?_____

4. Are the prepared foods well preserved (for example, in the refrigerator) until use? Yes/No

5. Are the utensils used to prepare and store foods well cleaned with water and soap?

Yes/No

6. Is there regular supervision for the hygienic conditions of food-makers during food preparation processes?

Yes/No

7. Is JUSH gives commercial weaning foods to in-patient infants, which are recommended by WHO/UNICEF?

Yes/No

If yes would you list them?_____

8. Is the Pediatric In-patient department has regular program to teach parents about general sanitation, food quality, and safety issues?

Yes/No

Thank you for your cooperation!

Appendix 4: General responses of health workers and food handlers during preliminary survey of Jimma University Specialized Hospital

Characteristics	No. of respondents (N=7)		
		Frequency	Percent
Who prepares food for the in-patient infants?	Parents JUSH Both	- - 7	- - 100
What type of weaning food JUSH prepares for the infants?	Milk Others	7 -	100 -
How often does the hospital prepare fresh food for infants?	Once a day Twice a day Three times a day	- - 7	- - 100
Are the prepared foods stored in the refrigerator until use?	Yes No	- 7	- 100
Are the utensils used to prepare and store foods well cleaned with water and soap?	Yes No	7 -	100 -
Is there regular supervision for the hygienic conditions of food-makers during food preparation processes?	Yes No	5 2	71 29
Is JUSH gives commercial weaning foods to In-patient infants, which are recommended by WHO/UNICEF?	Yes No	7 -	100 -
8. Is the pediatric in-patient department has regular program to teach parents about general sanitation, food quality, and safety issues?	Yes No	- 7	- 100

Appendix 5: Interview for the parents

Jimma University

College of Natural Sciences

Department of Biology (Applied Microbiology)

Interview designed for parents for identification of microbial quality and safety of traditional weaning foods of In-patient infants in Jimma University Specialized Hospital in Jimma town, Jimma zone, Southwest Ethiopia.

Dear respondent,

This interview was designed for evaluation of microbial quality and safety of some traditional weaning foods. Your response to the questions has significant impact on the quality of data and result. Thus, you are kindly requested to respond genuinely.

Part I. Background Information of Respondents

Code: _____

1. Infant's parent responding to questionnaire A. Mother B. Father

   C. Others_____

2. Address: Urban_____ Rural_____

3. Age _____

4. Academic status A. Illiterate B. 1- 4 C. 5- 8 D. 9- 12 E. Above 12

5. Occupation A. Unemployed/House wife B. Private sector/Business C. Farmer D. Civil servant E. other_____

Part II. Main Information

1. Age of the infant _____ Sex _____

2. Is your infant breast feeding? A. Yes B. No

3. At what age, your infant began feeding with weaning food? _____

4. What type of weaning foods you feed your infant while you are in Jimma University Specialized Hospital (JUSH)? A. Milk B. Gruel C. Fruit juice D. Cerefam (commercial cereal and fruit blend) E. Others

4.1 If your answer for question no. 4 is A, what type of milk you feed your infant?

A. Raw milk B. Boiled milk C. Pasteurized milk D. Others_____

4.2 What is the source of the milk? A. Cow milk B. Milk powder

C. Other_____

5. If your answer for question number 6 is C, what type of fruit juice you frequently feed your infant?

A. Orange B. Rani C. Avocado D. Mango E. Other_____

6. What is your feeding status of your infant?

A. Bottle-feeding B. Spoon-feeding C. Feeding with hand

D. Others_____

7. If yes, how do you wash the bottle? Using:

A. Cold water only B. Cold water and soap C. warm water and soap D. Others

8. What is the status of using the bottle?

A. Changing a teat of a bottle after every feeding B. Using 2 or 3 bottles exchanging one after the other C. Using a single bottle all the time D. Others

9. What type of water do you use for preparation of food?

A. Tap water B. Well water C. River D. Pond water

E. Others_____

10. How do you cool, boiled/cooked ready to drink/eat weaning foods such as milk and gruel for your infant?

A. periodic transferring from one glass/cup to the other

B. Making the lid of the utensil open until it cools

C. By using both methods

D. In the refrigerator

E. Others_____

11. Do you wash your hand?

11.1 After toilet use? A. Yes B. No

11.2 Before preparing food? A. yes B. No

11.3 Before feeding the infant? A. yes B. No

12. How do you wash your hand? A. Using water only B. Using water and soap

13. Are the utensils used to store weaning foods properly washed?

A. Yes B. No

14. How do you wash the utensils?

A. Using water only B. Using water and soap

15. Where do you store the prepared weaning foods of your infants?

A. Any place in the home/hospital B. In the shelf prepared for this purpose

C. In the refrigerator D. Others_____

16. How long does the prepared weaning food stored before consumption?

A. <6hrs B. >6hrs

17. Have you experienced reheating of already prepared food? A. Yes B. No

18. Have you got education and training on general sanitation and special care given for the children by some concerned bodies such as health extension workers and other physicians?

A. Yes B. No

19. How often do the health workers and other concerned bodies visit you to give assistance on health related problems?

A. Once a week B. Once a month

C. Others_____

20. Do you have any information about food-borne diseases?

A. Yes B. No

21. If your answer for question number 20 is **yes**, can you list some food-borne diseases?

_____

22. What are the method of transmission and prevention of these diseases?

A. Transmission

_____

_____

B. Prevention

_____

_____

Ref RPG/28/06
Date 07 FEB/2014

From: Research and Postgraduate Programs Coordinating Office
College of Natural Sciences
Jimma University

To: Jimma University Specialized Hospital

Subject: Ethical clearance for Postgraduate Student Research Project

A Postgraduate student named **Birhanu Degaga** is working his student research project to finalize his partial fulfillment of the postgraduate training entitled ' *Microbiological quality and safety of weaning foods of in-patient infants in Jimma University Specialized Hospital'* under biology department in college of natural sciences in which the proposal formally processed and ethically cleared at college level.

We therefore, request your organization to support him by providing the necessary information that will help the research project. We appreciate your cooperation beforehand.

Sincerely!
Kasahun Melese Tegegne
Signed
Research and Postgraduate Programs Coordinator

Declaration

I, the under signed, declare that this is my original work and has not been presented for seeking a degree in any university and that all resources of the materials used for the thesis have been dully acknowledged.

Name: Birhanu Degaga

Signature .....

The work has been done under advisors.

Name	Signature	Date
1. Ketema Bacha (PhD)	.....	.....
2. Delelegn Woyessa (M.Sc)	.....	.....