College of Natural Sciences

Department of Biology

Microbiological Quality of Homemade and Commercial Weaning Foods in Jimma Zone, South West Ethiopia

By: Gennale Dida Dhugo

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)

February, 2020

Jimma, Ethiopia

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Lata Lachisa (MSc)

Approved by:

Name	**Signature**	**Date**
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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 or the materials used for the thesis have been dully acknowledged.

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The work has been done under supervision

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2. Lata Lechisa (MSc)	______________	_____________

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ABBREVIATIONS

ASC: Aerobic Spore Count

BPW: Buffered Peptone Water

CF: Complementary Foods

FC: Fungal Count

ICDS: Integrated Child Development Schem

ICMSF: International Commission for Microbiological Specification for Foods

IDAEC: Industries of the European Communities

ISO: International Organization for Standardization

MSA: Mannitol Salt Agar

NA: Nutrient Agar

NCD: Non-Commercial Diet

NCCLS: National Committee for Clinical Laboratory Standards

PCA: Plate Count Agar

PCD: Powder Commercial Diet

PIF: Powder Infant Formula

SC: Staphylococci Count

ABSTRACT

Access to adequate nutrition during infancy and early childhood is essential to ensure growth, health, and development of children to their full potential. Weaning foods, which are introduced into the child's diet alongside breast milk, prepared under unhygienic conditions could contribute to transmission of disease causing foodborne pathogens compromising children's health. This study aimed to evaluate the microbiological quality of homemade and commercial weaning foods in Jimma Zone, South West Ethiopia. A total of 150 weaning food samples which comprises 90 commercial (30 Anchor, 30 Ayu, and 30 Baby Kin) and 60 Homemade (30 cheese and 30 firfir) were collected from different supermarkets and mothers feeding their infants with weaning foods in Jimma Zone (Jimma town, Asandabo, Oomo Nadda and Oomo Beyam). The study involved collection of relevant data using interview and laboratory analysis for microbial quality. Laboratory analysis was done for enumerating Aerobic mesophilic bacteria, Enterobacteriaceae, Staphylococci, Yeast and Mold. SPSS statistical software version 23 was employed for data analysis. The finding of the study showed that, 84(56%) of vendors were belonged to the age group between 31- 40 years and 79(52.7%) *had primary education, while 36(24 %) were uneducated. In weaning food practice, majorities, 84(56%) used cold water only to prepare weaning foods, 66(44%) of the users couldn't check the expiry date and (35.5%) of the sellers' stored weaning food in refrigerator while some (25.5%) stored in shelf. The mean count of Aerobic mesophilic bacteria was the highest (6.9 log CFUg-^{1}) in Firfir sample followed by Cheese (6.7 log CFUg^{-1}) whereas the lowest was in Ayu (4.0 log CFUg -^{1}).Of the total 901 isolates characterized, the most predominant were Bacillus spp. (34.3%) followed by Staphylococcus spp. (27.2%) and Enterobacteriaceae (15.1%). Totally, 27.2% of samples were positive for S. aureus and 9.4% samples were positive for Salmonella spp. Majorities of S. aureus were susceptible to ciprofloxacin (91.8%) followed by gentamycin (85.7%). However, they were highly resistant to penicillin G (100%). Salmonella spp, (100%) were susceptible to each of Ciprofloxacin, Gentamycin, Kanamycin, and Norflaxacin. .However, (38.1%) were resistant to tetracycline. On the other hand, E. coli was susceptible to Gentamycin (100%). In challenge study, the initial cell density of test stains separately inoculated to the samples was 3-4.5log CFUg^{-1}. There was faster growth rate of Salmonella spp., in firfir sample (4.9 Log CFUg^{-1}) followed by cheese (4.7 Log CFUg^{-1}) at 6 hrs.Most weaning foods available to the infants have high level of microbial contamination. The issue related to weaning food quality is the serious issue unless strong police created much life of infants will be lost.*

Keywords: *Antimicrobial Susceptibility, Commercial, Homemade, Weaning Food*

1. INTRODUCTION

1.1. Background of the Study

Weaning food is the introduction of solid food into a baby's diet during the first year of life while the baby will progress from breast milk or formula milk only to a fully mixed diet with foods of different textures and tastes. It is introduced into the child's diet along with breast milk when breast milk alone is no longer sufficient to meet nutritional requirements of the child (Muhimbula and Issa-Zacharia, 2010).

Baby supplementary nutrition after 6 month of birth plays vital role in determining the body defense mechanism against infection and care techniques that improve child survival by ensuring the growth, health, and development of children to their full potential (WHO, 2008). Actually, during the first 6 months of infant's life, breast milk is a sole and adequate source of nutrition (WHO, 2000). Then after, to meet their evolving nutritional requirements, infants should receive nutritionally adequate and safe weaning or complementary foods alongside with breast feeding (WHO, 2002).

Weaning food 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, 2003). Weaning period is a very critical period in the life of a child and if not well managed, might lead to malnutrition and other health implications (Ozumba *et al*., 2002). Therefore, adequate nutrition and health care during the first years of infant life is fundamental to prevent malnutrition and child death (Tunung *et al*., 2007).

In developing country, more than 10 million children die each year from lacking of appropriate weaning foods and infectious diseases associated with it. In Ghana, around 1.4 million deaths and 10% of the disease burden was reported in children younger than 5 years due to problems related with weaning foods (Tetteh *et al*., 2004). In sub Saharan African countries such as Ethiopia, there was high rate of malnutrition in children which results from the interaction between poor diet and disease (Edward and Parrett, 2003).Timely introduction of appropriate and safe weaning foods promote good nutritional status and growth of infants and young children (Saeeda *et al*., 2009).

The target age range for weaning feeding is generally 6 to 24 months of age although breast feeding may continue beyond the second year (Obi and Nwozor, 2012). So, formulation of weaning food ingredients from both animal and plant source is of vital importance to meet the requirements of the growing children (Cardinale, 2005).Development of weaning foods based on locally available cereals and legumes has been advised to combat malnutrition among children of low socio-economic groups (Imtiaz *et al.*, 2011). During formulation of weaning foods; the techniques of food preparation process, packaging, handling, storage, sanitation, sensory properties, and food quality and safety issues in general should be taken in to account (Ifediora *et al.*, 2006).This is because; weaning foods prepared under unhygienic conditions are shown to be heavily contaminated with microbes(Nkere *et al*., 2011).

Even though, there are many factors responsible for contamination of weaning food, microbial agents is the major risk factor. Other sources such as water, personal hygiene and cooking utensils contribute greatly to the contamination of weaning foods, especially among mothers who do not observe proper hygienic conditions (Hammad, 2004).Infants may be exposed to food contaminants through infant formula and complementary foods and thus subjected to the foodquality and safety problems experienced by the general population. Because infants and young children are especially susceptible to disease causing microbes, they are at a great risk of acquiring infections. Basically, weaning foods are contaminated with bacteria, parasites, viral and chemical hazard that may either be naturally present in foods or appears as contaminants as a result of pollution or poor agricultural practice. Moreover, throughout preparation, mixing, cooking, packaging, and transporting care is required to be taken to prevent contamination of the product (Onweluzo and Nwabugwu, 2009).

Contaminated complementary foods are the major route of transmission of disease among infants. For this reason, the higher incidence of disease coincides with the increase in the intake of these foods. Fortunately, the presence of microorganisms like aerobic flora, total coliforms, molds, yeast and pathogens such as salmonella, pseudomonas, bacillus and *E.coli* in the weaning food are the main concern. On the other hand, if storage facilities are inadequate and the weaning food is kept at ambient temperature for some time those few microorganisms will multiply rapidly, causing spoilage, public health hazards or both proper maternal practices regarding the

management, preparation, administration and storage of complementary foods may reduce their contamination (Temple *et al*., 1996).

In general, commercial infant food is relatively safe in compare to traditional or homemade weaning food. However, it is very expensive and unaffordable to low-income families in developing countries (Shimelis and Rakshit., 2008). Quality and nutritional issues of traditional weaning food could be improved by combining locally available foods that complement each other and need to be modified to improve quality and their nutritional status with properly managing the hygienic status (Abebe *et al*., 2006). Otherwise, homemade foods can cause disease with 6 to 24 hrs of their preparation (Nemo *et al*., 2017). Therefore, personal hygiene, environmental hygienic, preparation, processing, packaging and storage of weaning foods are crucial to prevent contamination (Satter *et al*, 2013).

In Jimma zone, even though there are commercial weaning foods such as Anchor (processed milk), Ayu (Made from sorghum, barley, maize, wheat, broad beans, chick-peas, field peas, and lentil) and baby king (Prepared from sorghum, barley, maize, wheat, emmer and wheat) and homemade weaning food such as cheese (fermented milk) and firfir (Mixture of dried Injera spicy, garlicky and tomato sauce,),there is no systematic research done yet to determine the quality of these types weaning foods. The present study was, therefore, initiated to assess the microbiological quality of homemade and commercial weaning foods in Jimma zone, south western Ethiopia.

1.2. Statement of the problem

Weaning foods are perceived to be a major public health food in view of health situations associated with sanitation condition (Dewey and Brown, 2003). There is general perception that these foods are safe ,mainly because of the environment under which they are prepared, processed, and stored ;but the source are contaminated, inappropriate processing and unhygienic packaging material and some microbes are resistant to available preservation method which expose the food to numerous potential contamination which in turn are risky for human health.

Weaning food has been reported to be contaminated with pathogen and have been implicated in food born epidemics (Gangule *et al*, 2004). In deed weaning foods are prone to spoiled because they are Prepared and sold under unfavorable condition such as high temperature. Additionally

because commercial prefer to take their products to their customers they often operate from place such as bus terminal, industrial areas market places and sub way stations.

In Ethiopia varies food were reported to care pathogen or allows the growth of pathogens. However, there is limited information on the microbial load and safety of weaning foods, in spite of the wide spread use of such food items in the country (Ashenafi, 2002). Even though there was no recorded data about magnitude of food borne illness which occurred as a result of in appropriate handling, it was observed that in different parts of the country town, there were high rate of sanitary condition of foods. This could directly or indirectly affect the safely of food (Mankee *et al.*, 2003).

Jimma zone one of the economic center of a country, it is observed that large numbers of commercial are founds in the town and the customer from the sectors are increasing due to urbanization and the movement of people from place to place(Alemu *et al*., 2011). However, the overall microbial quality of weaning foods are not known in spite of the wide spread use of weaning items in the town. Therefore these studies will assess the microbial quality and associated risk factors of weaning foods in Jimma Zone.

1.3. Significance of the study

The Significance of the study is to reveal the impact associated with the Weaning foods, Microbiological quality and Safety of Weaning foods in Jimma Zone, South West Ethiopia. Moreover the study helps Community to have better understanding and awareness about microbial quality and Safety of Weaning foods. Current information on the status microbial quality of weaning foods should be availed for consumers' protection, to improve sanitary condition in foods, handling, processing, preserving and storing.

Analyzing the microbiological quality of weaning foods is important to identify the risk of food borne disease that may arise through consumption of weaning foods. This study will provide information on microbiological quality of weaning foods and useful information for health workers involved in the quality control of weaning foods and prevention of food borne disease. Therefore, finding of this study will contribute baseline data to be used for the control of food borne disease associated with weaning foods and provide base line data that can be used as a

source of information to design monitoring the microbiological quality of weaning foods for communities.

1.4. Scope of the study

The general overview of this thesis is to assess the microbiological quality of weaning foods found in Jimma Zone, in order to ascertain the microbial quality of weaning foods. Accordingly, the following issues were addressed: determining the microbiological quality of weaning foods to evaluate the safety problems related to these foods, assessing risk factors associated with weaning foods, and determining growth potential of selected foodborne pathogen in representative weaning foods.

1.5. Basic Research Questions

- What is the level of understanding on hygienic processing and handling of weaning foods among the local community and venders of commercial weaning foods?
- What is the safety status of homemade and commercial weaning foods in the study site?
- What microbial groups dominate contaminants of homemade and commercial weaning foods availed in the study site?
- What are the risk factors for prevalence of pathogens in weaning foods?
- Which weaning foods are more prone to contamination, hence support growth of foodborne pathogens?
- How sensitive are the pathogens isolated from the weaning foods to commonly used antibiotics?

2. Objectives of the study

General objective

The general objective of this study is:

- To assess the microbial quality and safety of Homemade and Commercial weaning foods found in Jimma Zone.

Specific objectives

The specific objectives of the study are

- To document hygienic practices related to storage and handling of commercial and homemade weaning foods
- To determine the microbial load of selected homemade and commercial weaning foods
- To determine prevalence of foodborne pathogens in selected homemade and commercial weaning foods.
- To Isolate and identify foodborne pathogenic microorganisms associated with weaning foods.
- To determine the major the risk factors for prevalence of pathogens in weaning foods
- To determine sensitivity of pathogens isolated from weaning foods to commonly used antibiotics
- To evaluate growth potential of common foodborne pathogens in selected commercial and homemade weaning foods

3. LITERATURE REVIEW

Weaning food is the food give in addition to Mother breast after 6 mothers .Mothers breast (Breast milk contains 80% of water and 20 % of nutrition).Up to 6 month it is enough for infants but after six months they need another additional foods .This additional foods are called weaning foods .Many peoples considers that during weaning periods infants total leave breast milk and use an other foods whether in powder or liquid forms (WHO, 2006).However weaning periods are not about that. Weaning periods are the most remarkable periods which cover the first 24 months in the journey of life. During this period infants need another additional food as breast milks are not all supply. Weaning foods include both Commercial and homemade .This foods are exist both in powder or liquid forms. These weaning foods are Anchor, Ayu, Baby king, Cheese and Firfir and etc. Powdered infant Formula is the most important formula of (FAO and WHO, 2003).

Powdered infant formula (PIF) is readily available, as either a supplement or replacement for breast milk. PIF is heat-treated during processing but, unlike liquid formula products, it is not subjected to sufficient treatment to make the final packaged product commercially sterile. Salmonella and Cronobacter sakazakii (formerly classified as Enterobacter sakazakii) have been identified as the organisms of greatest concern with PIF (FAO/WHO, 2004). It has been suggested that outbreaks and sporadic cases of Salmonellosis due to PIF are likely to be under-reported (FAO/WHO, 2006). To date, there is limited public information in Ethiopia on the microbiological quality of powdered infant formula. *Bacillus cereus*, a ubiquitously distributed aerobic spore-former, tolerates adverse environmental conditions better than most other bacterial pathogens (Rowland *et al*, 2005). (Souza, 2005) reported that 54% of 261 samples of infant food distributed in 17 countries was contaminated with B. cereus reaching levels of 0•3–600 viable cells g^{-1}. Also, (Bryce *et al*.,2005), demonstrated that hospital prepared infant food may be contaminated with enter toxigenic B. cereus at levels above the Association of Dietetic Food Industries of the European Communities (IDAEC) proposed safety limit of 10^3 cfu /ml. To guard against possible deleterious effects of food-borne Gram-negative enteric bacteria, hospitals are routinely pasteurizing many milk-based products (Sudershan *et al*., 2009). Several researchers have shown, however, that counts of aerobic spore-forming bacteria in these pasteurized foods are little affected by this thermal process (Collins, 2007). Observations that psychotropic B.

cereus strains have been implicated in outbreaks of food-related illness (Turner *et al*., 2003) and are capable of producing toxins (Gong *et al*., 2004), have raised concern about their growth and enterotoxin production abilities in refrigerated foods (Song-Suk *et al*.,2005). There is insufficient evidence to establish conclusively whether B. cereus diarrheal type food intoxication results from consumption of preformed toxin (John,2007) or from production of enterotoxin by ingested cells or spores in the ileum (Yamashiro,2003). However, all of the studies on the stability of the diarrheal enterotoxins that are cited in the literature have been performed in non-food systems, and recent evidence suggests that this stability may be significantly greater when the toxin is performed in foods such as milk (Samson and Lakech, 2000). The existence is reported here of psychotropic strains of B. cereus capable of growth and enterotoxin production in reconstituted milk-based infant formulae.

Diarrhea is the second leading cause of death in children worldwide after neonatal disorders and is a leading cause of growth faltering and malnutrition. Every year, around 8.8 million children under the age of 5 years die (Marinelli *et al*., 2001). Half of these deaths are associated with malnutrition and around 2 million die as a complication of diarrhea. The highest mortality rates related to diarrhea are found in less-developed countries and the highest morbidity rates occur among malnourished children. The case fatality rate is the highest among children aged 6–12 months. Several factors may precipitate the disease condition: a not yet matured immune system, waning maternal antibodies and inappropriate complementary feeding (Black *et al*., 2003).

Beyond the age of six months, breast milk alone is no longer sufficient to meet the nutritional demands of the growing infant, and complementary foods need to be introduced (WHO, 2001). This period is critical for children in developing countries. Empirical evidence demonstrates that introduction of CF in resource-poor settings can result in diets that are nutritionally inadequate and microbiologically unsafe, leading to possible multiple nutrient deficiencies and higher exposure to foodborne pathogens and consequent to gastrointestinal illnesses, (Kimmons *et al*., 2005). Microbial contamination leading to infections and poor nutrient associated with weaning foods may contribute significantly to deaths of 13 million infants and children aged less than five years worldwide each year (Pathak *et al*., 2012).

3.1. Safety of weaning foods

Food safety is not a luxury of the rich but a right of all people (WHO, 2003). Educational programmes based on the hazard-analysis-critical-control-point approach, taking into consideration also sociocultural factors, should be integrated into all national infant-feeding or food and nutrition programmes (Blais *et al*., 2005). Food-borne infections can have dangerous and long-term effects, especially on nutritional status (Nemo *et al*., 2017). Formula fed infants usually require formula for their first year but they are introduced to other kinds of foods once they reach six months of age. Based on literature, weaning foods prepared under unhygienic conditions are frequently heavily contaminated with pathogens and may, thus, be a major factor in causing diarrhoeal diseases and associated malnutrition (Degaga *et al*., 2015). In particular, traditional gruels used in The Gambia for supplementing breast milk were found to be heavily contaminated with potentially pathogenic micro-organisms, and such supplements are important factors in weaning-related diarrhoea (Iroegbu *et al*., 2000).

3.2. Hazard analysis of Weaning foods

Educational programmes based on the hazard-analysis-critical-control-point approach, taking into consideration also sociocultural factors, should be integrated into all national infant-feeding or food and nutrition programmes (Yasin *et al*., 2012). Food-borne infections can have dangerous and long-term effects, especially on nutritional status. Weaning foods are heavily contaminated with pathogenic microorganisms because 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 (Rane, 2011). This calls for designing of strategies for community level training. Thus, there is a lack of factual knowledge about the epidemiological significance of weaning foods, poor knowledge of vendors in basic food safety measures and inadequate public awareness of hazards posed by certain foods (Desousa, 2008). The factors that should be considered for the analyzing the hazards because of foods are many. However, the major sources of contaminants of weaning foods in Ethiopia are Poor personal hygiene, improper food handling, improper processing , packaging, storage practice of foods and poor knowledge of companies towards food borne disease are expected as the associated risk factors to contamination of weaning foods in Ethiopia(Gorris, 2005).

3.3. Handling of Weaning Foods

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).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 (Lewis *et al.*, 2006).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.

3.4. Utensils and equipment

The serving utensils used for the preparation of weaning foods are often contaminated with *Staphylococcus spp.* which may have originated from Contaminated hands and cooking utensils contribute greatly to the contamination of weaning foods, especially among mothers who do not observe proper hygienic condition when they touch the food preparation areas, dishcloths and the water during dishwashing and hand washing which indicates cross contamination between dishwater, food preparation surfaces, and the food itself perceive a major health risk (Das *et al*., 2010).It has further been shown that weaning foods prepared under unhygienic conditions are frequently contaminated with enteric bacterial pathogens (Nicolas *et al*.,2007).

3.5. Weaning Food Contamination Mechanisms

The hazard analysis considered in foods showed the existing microbial analysis, food preparation, vending, serving and storage practices to identify the sources and mode of contamination (Lund, 2009).Analysis of route of contamination showed that there is the great the great interaction among the vendors, environment and sanitation. Weaning foods prepared

under unhygienic conditions are shown to be heavily contaminated with pathogenic microorganisms (Baqui and Ahmed, 2006).

Contamination of food including drinking water with microbial agents is the major risk factor in the transmission of diarrhea 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 (Michelson *et al*., 2000). Therefore, careful hygienic preparation and storage of weaning foods is crucial to prevent contamination. Personal hygiene plays an important role in feeding infants. If sanitation is not observed, weaning feeding may do more harm than good to the infant by introducing infections to the infant (Satter *et al*., 2013). It is therefore, important that all foods prepared for young infants are handled in a way that they are free from any contamination.

3.6. Weaning food and public health Problems

Poor quality of weaning foods and improper weaning practices predispose infants to malnutrition, growth retardation, infection, diseases and high mortality (Onofiok *et al*., 2005). WHO recommends a gradual weaning period from 6 months to 2 years (WHO, 2003).This allows for the child to still receive the benefits from breastfeeding, while also consuming the necessary nutrients from the complementary foods. Foods should be prepared adequately containing the required nutrients as well as appropriately with a suitable texture and temperature (WHO, 2002). Without the knowledge of proper weaning practices as well as a perception of the child's hunger needs, malnutrition and illness may ensue. The weaning period is therefore a vulnerable time when the child should be attentively cared for and observed so as to maintain health (Spencer *et al*, 2007). Complementary foods in sub-Saharan Africa and in Sudan in particular, comprise thin gruels made from maize, millet, sorghum and cassava. These gruels have low levels of energy, protein and micronutrients and high concentrations of factors inhibiting absorption of nutrients (John, 2012).

Adequate nutrition and health care during the first years of infant life is fundamental to prevent malnutrition and child death (Chukuezi, 2010). More than 10 million children die each year from 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 (Ismail, 2006). The target age range for weaning feeding is generally 6 to 24 months of age although breast feeding may continue beyond the second year (WHO, 2008; Muhimbula and Issa-Zacharia, 2010).

3.7. Prevalence of pathogens in Weaning foods

3.7.*1*. Enterobacteriaceae and Coliform

Enterobacteriaceae is a large family of bacteria that includes many of the more familiar pathogens, such as *Salmonella, E. coli,* and *Shigella,* which are commonly isolated from foods. The isolation of Enterobacteriaceae species and the high most probable number prove clearly that poor hygiene meals could be sources of food born disease (Motarjemi *et al*., 2004). Coliform is the most common contaminating microorganisms in weaning foods because food products especially contaminated coliform whose noncompliance rate was found to be as high as 8-15 %.(Fang *et al*., 2002). The fecal coliforms like *E. coli* are presently used as an indicator of the sanitary conditions. Since this microorganism is a typical component of the fecal microbiota, its detection may indicate the potential occurrence of other microorganisms which could be more pathogenic to the man (Souza, 2005).

3.7.2. *Staphylococcus aureus* and *Salmonella spp.*

Staphylococcus aureus is one of the most commonly contaminant microorganisms of weaning foods. Thus, *S. aureus* contamination can be readily avoided by heat treatment of food. Nevertheless, it remains a major cause of foodborne disease because it can contaminate food products during preparation and processing. S. aureus is indeed found in the nostrils, on the skin and hair of warm-blooded animals. Up to 30-50% of the human populations are carriers and *S.aureus* causes skin and soft tissue infections such as abscesses, bloodstream infections, pneumonia, or bone and joint infections (Schmitt *et al*., 2009). According to (Bergdoll ,2004), *S. aureus* can resist pH from 4.2 to 9.3, with an optimum of 7 to 7.5, sodium chloride concentrations (up to 15% NaCl) and can grow in a wide range of temperatures from 7° C to 48.5°C with an optimum of 30 to 37°C. These characteristics enable S. aureus to grow in a wide variety of foods and ecological niche, can easily explain their incidence in foodstuffs that require

manipulation during and after processing. *S. aureus* is an important pathogen due to a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance. Salmonella are perhaps the most relevant group of pathogens for both humans and animals, due to great existence of many strains. Certain strains of *Salmonella spp*. are harmful to man and their frequency varies from one year to another or from one country to another. In developing countries, street foods in particular have been reported to be contaminated with *Salmonella spp*. and have been implicated in a few outbreaks of foodborne diseases. S*almonella* are infectious bacteria associated with foodborne and gastrointestinal illnesses such Salmonellosis and typhoid fever (Mankee *et al*., 2003).

3.7.3. Growth Potential of Pathogenic Microorganisms

Growth potential of *Salmonella spp*. is continued in the lag phase. Otherwise, population growth should be limited to less than 1 log, following the same rationale as for enter Hemorrhagic *E. coli. Staphylococcus aureus* in challenge studies revealed there was no detectable toxin should be formed under the time/temperature (Vestergaard, 2001).This limiting potential growth level is based on an initial population of 10^3 CFU/g, and a minimum of 10^6 CFU/g to produce toxin. *Staphylococcus aureus* does not compete well with other microorganisms; therefore, it is not appropriate to consider in foods with high levels of other organisms, such as raw vegetables or properly fermented products.

Typically, in a microbiological challenge study, the levels of live challenge microorganisms are enumerated at each sampling point. The selection of enumeration media and method is dependent on the type of pathogens used in the study. If the product does not have a substantial background microfloral, non-selective media for direct enumeration may be used. In cases where toxin-producing organisms are used such as *Staphylococcus aureus*, appropriate toxin testing should be performed at each time. It is practical to analyze the product, including un-inoculated control samples, at each or selected sampling points in the study to see how the background microflora is behaving over product shelf life. For example, if a product has a high background microflora, it may suppress the growth of the challenge inoculum. In some cases, this is useful and desirable because the product spoils before pathogens can grow (Vestergaard, 2001).

3.7.4. Drug Resistant Bacteria Isolated from Selected Weaning Foods

Susceptibility patterns of bacterial species against antibiotics are different depending up on the drug used. Studies showed that multiple antibiotic resistances is observed in *E. coli* for instance ampicillin (42%) while low resistance (26%) for gentamycin and the bacterial species is an intermediate sensitivity up to 10 % for ceftriaxone (Busani *et al*., 2004). *Salmonella spp*. are the most important pathogenic bacteria implicated in food borne outbreaks with the maximum resistance (42%) for ampicillin (Centinkaya *et al*., 2008). The species also showed developing resistance for amikacin and gentamycin suggesting that these antibiotics can be least effective against Salmonella in the years to come. Farzana *et al*. (2009) reported that most of the isolates of Salmonella are higher degree of resistance to ampicillin. However, the species are sensitive to ciprofloxacin, ceftriaxone and cotrimaxazol. However, resistance to antibiotics involves different mechanisms; greater sensitivity of *Salmonella spp*. for ciprofloxacin can be recognized. The antibiotic resistance of gram-positive bacteria like *S. aureus* is evident that they resist ampicillin, ciprofloxacin and gentamycin. However, greater susceptibility of *S. aureus* for amikacin is possibly due to its minimal use (Galan and Curtiss, 2001).

3.7.5. General guidelines to ensure safety and quality of weaning foods

According to the guidelines of the Protein Advisory Group, weaning foods should have a protein content of at least 20% (on a dry weight basis), a fat content of 10%, a moisture content of 5% to 10%, and a total ash content of not more than 5%. Thus, weaning food should be rich in calories, good-quality protein, vitamins, & minerals, and also easily digestible, low in indigestible fiber and free from anti nutritional factors (Lai, 2001).

Food should be precooked and predigested or processed in such a way that it needs minimum preparation prior to feeding and when stirred with cold or warm water or milk it should form a slurry or semisolid mass of soft consistency, enabling the child to swallow it easily. It is advisable not to add artificial colors and flavors to weaning foods, and the composition of the food must follow the guidelines and standards recommended by competent agencies, such as the Bureau of Indian Standards (Van Acker *et al*., 2001). The current recommendations regarding the frequency of meals with complementary foods for breastfed infants result from theoretical estimates based on the energy provided by complementary foods, assuming a gastric capacity of

30g/kg and an energy intake of at least 0.8 kcal/g. Since an infant has small stomach, so it is advisable to feed the child frequently. The WHO recommends that, 2-3 meals per day for infants aged 6-8 months, 3-4 meals per day for infants aged 9-11 months and children 12-24 months (WHO, 2002).

Weaning food or complementary food is normally a semi-solid food that is used in addition to breast milk and not only to replace with mostly prepared homemade (Rapley, 2006). Food should be rich in calories and adequate in good-quality protein, vitamins, and minerals enabling the child to swallow it easily. The food should be precooked and predigested or processed to minimum preparation prior to feeding.

4. MATERIALS AND METHODS

4.1. Descriptions of Study Area

The study was conducted in Jimma Zone, particularly at Jimma town, Asandabo, Oomo Nadda and Oomo Beyam. Jimma is located 353 km southwest of Addis Ababa, the capital city of Ethiopia. The town's geographical coordinates are 7°41'N latitude and 36°50'E longitude. The study area has an average altitude of 1, 780 m above sea level. It lies in the climatic zone locally known as "Woyna Daga" (1,500-2,400 masl) which is considered ideal for agriculture as well as human settlement. The town is generally characterized by warm weather with a mean annual maximum and minimum temperature of 30°C and 14°C, respectively(Alemu *et al*., 2011). The annual rainfall ranges between1138-1690 mm. The maximum precipitation occurs during the three months period (June, July and August), with minimum rainfall occurring in December and January. From a climatic point of view, abundant rain fall makes this area one of the best watered regions of Ethiopian high lands, conducive for agricultural production (Alemu *et al*., 2011) (Fig.1). Asandabo is one of the Kebeles in Jimma Zone, located 302 km from the center (Addis Ababa) and 85-90km east of Jimma town. This town is characterized with both warm and cold weather conditions. Because of its suitability for both agro economies and Pastoral activity, the morbidity of people is high towards it. In addition to the above two areas, the current study was also conducted in other two Woredas (districts) of Jimma Zone, namely Oomo Nadda and Oomo Beyam. These two places are far away from the center (324 km and 347, respectively) with moderate weather condition (Fig. 1).

4.2. Study Design, Sample Size and Sampling techniques

Cross sectional study design was employed. A total of 150 weaning food samples (30 Ankor, 30 Ayu, and 30 Baby from among commercial weaning foods, and 30 cheese and 30 firfir from among homemade weaning foods) were collected from different supermarkets and mothers feeding their infants with weaning foods in Jimma Zone (Jimma town, Asandabo, Oomo Nadda and Oomo Beyam).Particular supermarkets selling weaning foods among other products and homemade weaning food houses were selected based on the availability and willingness of

retailer to sell their products for laboratory analysis. The samples were collected following simple random sampling method.

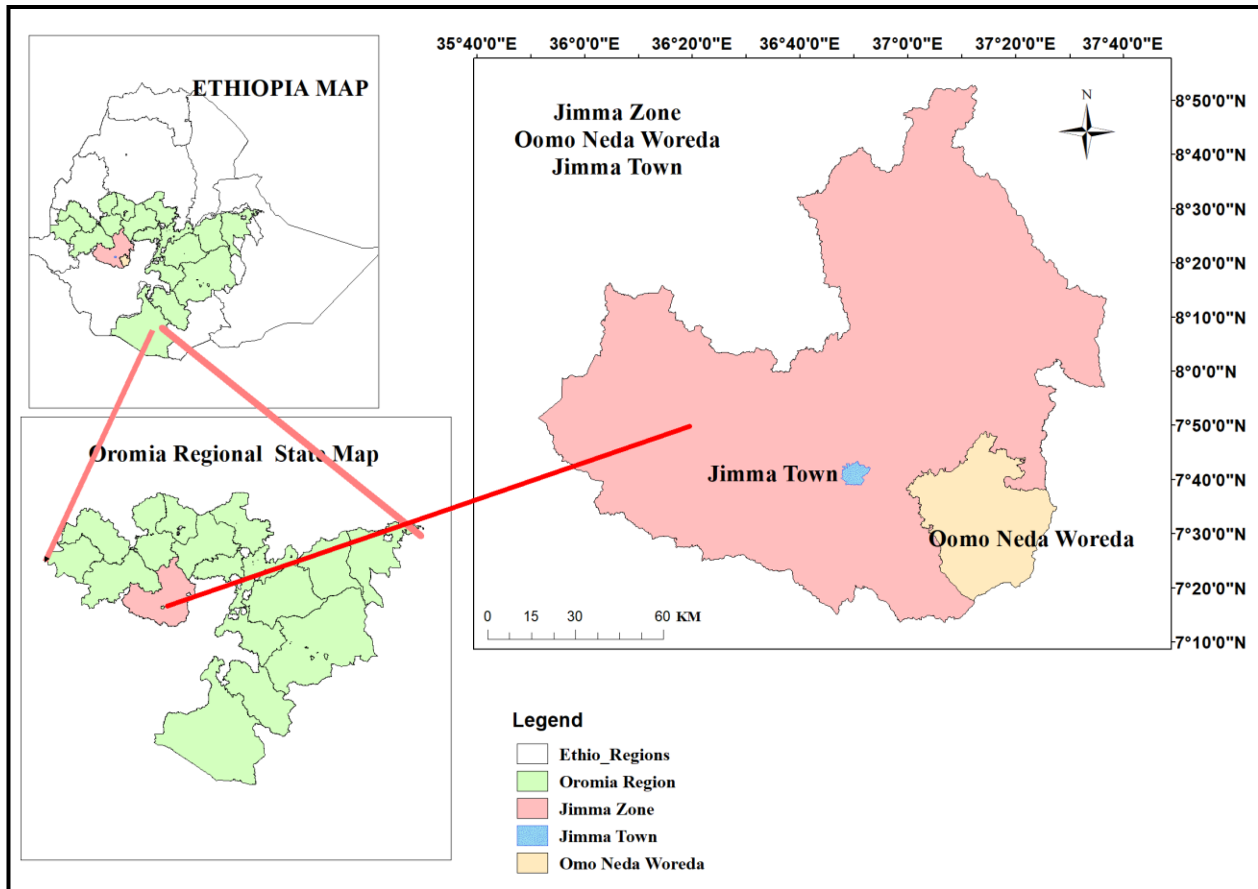


Figure 1 Map of the study area (ArcGIS, 2019)

4.4. Socio-demographic data collection

Data about people engaged on selling/vending of the weaning foods (commercial and/or homemade), and general sanitation of the work environment were collected from the different Super markets and Mothers preparing food for their infants at home using interview and onsite observation (Appendix 1).

4.5. Sample collection

A total of 150 weaning food samples (250g each), constituting 30 Ankor, 30 Ayu, and 30 Baby from commercial, and 30 cheese & 30 firfir from home-made, were collected from different supermarkets and mothers feeding their infants with weaning foods in Jimma Zone (Jimma town,

Asandabo, Oomo Nadda and Oomo Beyam).Each sample was collected using sterile aluminum sheet and transported to laboratory using an icebox. All weaning food samples were transported to Research and Postgraduate Laboratory, Department of Biology, College of Natural Sciences, Jimma University, within less than three hours of collection; all samples were processed for microbial and physicochemical analysis within one to three hours of collection. The weaning food samples were kept in the refrigerator at 4 ^{o}C until processed.

4.6. Microbiological Methods

4.6.1. Sample preparation

Approximately 250 g each of weaning food sample (commercial and homemade) was collected from supermarkets and homes prepared foods. The collected food samples were analyzed for microbiological quality. Accordingly, 25 g each of weaning food sample was mixed in 225 ml buffered peptone water (BPW) and homogenized in a flask for five minutes using shaker at 160 rpm. After homogenization, 1 ml of each food sample was transferred aseptically into 9 ml of BPW, and was mixed thoroughly using vortex. The homogenate was serially diluted from 10^{-1} to 10^{-6} and a volume of 0.1 ml aliquot of appropriate dilution was spread-plated on pre-solidified plates and incubated at appropriate temperature and time for enumeration of different microbial groups including aerobic mesophilic bacteria, Enterobacteriaceae, coliform, aerobic spore forming bacteria, staphylococci, yeasts and moulds. The colonies were counted from plate containing microbial colonies between 30 and 300. The counted colonies were expressed in colony forming unit per gram ($CFUg^{-1}$) and was converted to log $CFUg^{-1}$.

4.6.2. Microbial Enumeration

4.6.2.1. Aerobic Mesophilic Bacterial Count

From appropriate dilutions, 0.1 ml of the aliquot was spread plated on Plate Count Agar (PCA) (Oxoid) and the plate was incubated at 32°C for 48 hrs (Weil *et al*., 2006).

4.6.2.2. Enterobacteriaceae Count

From appropriate dilutions, 0.1 ml of the aliquot was spread-plated on MacConkey agar (Oxoid) and was incubated at 32 ^{o}C for 18 - 24 hrs. After which, pink to red purple colonies were counted as member of the family Enterobacteriaceae (Spencer *et al*., 2007).

4.6.2.3. 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 plate was incubated at 32 ^{o}C for 18 - 24 hrs. After this, purplish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms (Weil *et al*., 2006).

4.6.2.4. Aerobic Bacterial Spore Count

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

4.6.2.5. Staphylococci Count

From appropriate dilutions, 0.1 ml of the aliquot was spread plated onto Mannitol Salt Agar (MSA) (Oxoid) and was incubated at 32 °C for 48 hrs (Acco *et al*., 2003).

4.6.2.6. Yeast and Molds Counts

From appropriate dilutions, 0.1 ml aliquot was spread-plated on pre-solidified surfaces of Potato Dextrose agar supplemented with 0.1g Chloramphenicol and incubated at 25-28 ^{o}C for 5-7 days Spencer *et al*., 2007). Smooth (non-hairy) colonies without extension at periphery (margin) was be counted as yeasts. Hairy colonies with extension at periphery was counted as molds.

4.7. Microbial Analysis

After enumeration of aerobic mesophilic bacteria, 10 to 15 colonies with distinct morphological differences such as color, size and shape was randomly picked from countable plates and aseptically was transferred in to a tube containing 5 ml nutrient broth (Oxoid). The inoculated cultures were incubated at 32 ^{o}C for 24 hrs. Cultures were purified by repeated plating and was preserved on slants at 4 ^{o}C for a month. Finally, the obtained organisms were characterized to genus and family levels. The characterization of isolates was done based on John (2012) bacterial classification manual.

4.7.1. Cell Morphology

In order to assess the cell morphology of the pure culture, gram staining, motility test and endospore test was used. The morphological study includes cell shape, cell arrangement, presence or absence of endospore and motility.

4.7.1.1. Gram staining

A smear of pure isolates was prepared on a clean slide and allowed to air-dry and heat-fix. The heat fixed smear was be flooded with crystal violet dye for 1 minute and rinsed under tap water for 3 seconds. Then, the slide was be flooded with iodine solution for 1 minute and rinsed under tap water for 3 seconds. After rinsing, the smear was decolorized with 96 % of ethanol for 20 seconds and was washed slide gently under tap water for 3 seconds. Thereafter, the smear was counterstained by safranin and was dried by absorbent paper. Finally, the air-dried smear was observed under oil immersion objective. At the completion of the Gram Staining, gram-negative bacteria was stained pink/red and gram-positive bacteria was stained blue/purple (Gram, 1884).

4.7.1.2. Motility Test

A motility medium was prepared using a test tube. A purified broth culture was taken by a sterile needle and stabbed straight vertically into a test tube containing motility medium to the bottom of the tube and was incubated at 35°C for 24 hours. A positive motility test was indicated by a red turbid area diffusing away from the line of inoculation and a negative test will be indicated by red growth along the inoculation line only but no further (Shields and Cathcart, 2012).

4.7.1.3. Endospore Test

Endospore test was done according to Schaeffer and Fulton (2003) method. A smear of isolates was prepared on a clean glass slide and allowed to air-dry. The air-dried smear was heat fixed. Heat fixed smear was flooded with 0.5 % (w/v) malachite green solution and steamed using cotton dipped in 96 % ethanol for 5 minutes. After cooling, the slide was washed with tap water and counterstained with safranin for 30 seconds. The slide was washed with tap water and air dried/ blotted to be observed under the oil immersion lens (×1000) to check the presence of endospore.

4.7.2. Biochemical Test

4.7.2.1. KOH-test (Test for Lipopolysaccharide)

Two drops of 3 % KOH solution was placed on a clean microscopic slide. A colony was aseptically picked from the surface of nutrient agar using an inculcating loop and stirred in the KOH solution for 10 seconds to 2 minutes. The inoculating loop was raised slowly from the mass when the KOH solution become viscous, the thread of slime followed the loop for 0.5 to 2 cm or more in gram-negative bacteria. In case of no slime and watery suspension do not follow the loop, the reaction was considered negative and the isolate was considered as gram positive bacteria (Gregerson, 2004).

4.7.2.2. Oxidation Fermentation (O/ F) Test

This test is used to assess the ability of the isolate to utilize glucose and to determine the metabolic 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 will be autoclaved and immediately cooled under tap water to avoid dissolution of oxygen in the medium. Then, the broth cultures was 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 was interpreted after 2 to 5 days of incubation at 32 ^{o}C (Hugh and Leifson, 1953).

4.7.2.3. Catalase Test

Catalase 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 (MacFaddin, 1995).

4.7.2.4. Coagulase test

Coagulase test was done using slide test and tube test procedures (Cheesbrough, 2006). In slide test, a colony of the purified isolates was 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 was as controlled. 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 organisms. On the

other hand, Coagulase test was done using tube test. Accordingly, three test tubes was taken and labeled as test, negative control and positive control. Each tube was filled with 0.5 ml of 1 in 10 diluted human's plasma. To the tube labeled test, 0.1 ml of overnight broth culture of test bacteria was added. To the tube labeled positive control, 0.1 ml of overnight broth culture of known *S. aureus* was added and to the tube labeled negative control, 0.1 ml of sterile broth was added. All the tubes were incubated at 37 ^{o} C and observed up to four hrs. Positive result was indicated by gelling of the plasma, which remains in place even after inverting the tube.

4.8. Isolation of *Staphylococcus aureus*

After counting *Staphylococci*, golden yellow colonies on MSA plates was aseptically picked and transferred into 5 ml 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 supplemented with 0.75% NaCl and again incubated at 37 ^{o}C for 24 hrs. Finally, the distinct colonies was characterized using the established microbiological methods (Acco *et al.,* 2003). Finlay, isolation and characterization of *S.aureus* was done following the above mentioned activities (section 4.7) including catalase and coagulase tests. Strain with gram-positive cocci with clustered arrangement, catalase and coagulase positive were selected as presumptive *S.aureus.*

4.8. Isolation of *Salmonella spp.*

For the detection of *Salmonella spp*., 25 g of food samples were mixed with 225 ml of BPW and incubated at 37 °C for 24 hrs. Then 1 ml pre-enrichment broth culture was added to 10 ml of selenite cysteine broth and again incubated at 37 oC for 24 hrs. Thereafter, a loopful of suspension from a tube was streaked onto Xylose Lysine Deoxy cholate Agar (XLD). The presumptive *Salmonella* colonies (black colony surrounded by red color) were picked off, transferred to 5 ml nutrient broth, incubated at 37 ^{o}C for 24 hrs, then streaked onto Nutrient Agar for purity, and incubated at 37 °C for 24 hrs (Arvanitidou *et al*., 1998). The presumptive *Salmonella spp*. were characterized by standard biochemical tests. The biochemical testes were done according to the procedure of Johnson and Case (2007).

Triple Sugar Iron Agar

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 or without production of H_2S was considered as presumptive for *Salmonella spp.*

Lysine Iron Agar

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.*

Urea Agar

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

Simmons Citrate Agar

The slant was streaked and the tube was 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 spp.*

Sulfide Indole Motility (SIM) Medium)

The SIM medium was stabbed to the bottom and incubated at 37 ^{o}C for 24 hrs for the determination of H_2S production, indole production and motility. Production of indole was investigated by adding Kovac's reagent (HCl, 250 ml, amyl alcohol, 750 ml and paradimethyla mino-benzaldehyde 50 g /l) to growth in this culture medium. The non-utilization of indole and absence of deep red color at the surface of agar was considered as presumptive for *Salmonella spp.*

4.9. Antimicrobial Susceptibility Testing for Some Pathogens

Antimicrobial susceptibility testing for pathogens isolated from some Homemade and Commercial weaning foods was performed using the disk diffusion method and the results was

interpreted as per the criteria of the National Committee for Clinical Laboratory Standards (NCCLS, 2007). A standardized suspension of the bacterial isolates was prepared and the turbidity of the inoculum was matched with the turbidity standard 0.5 McFarland (Bauer *et al*., 2006). McFarland is a Barium Sulphate standard against which the turbidity of the test and control inoculum was compared. This standard was prepared by mixing two solutions; solution A and solution B. Solution A was 1 % v/v solution of sulphuric acid (H_2SO_4) and solution B was 1 % w/v solution of barium chloride (BaCl2). To get 0.5 McFarland standard, concentration equivalents to cell density an amount of 0.5 ml BaCl2 of 1 % solution A was mixed with 99.5 ml H_2SO_4 of 1 % solution B. A small volume of the turbid solution was transferred to a screw-cap bottle of the same types as used for preparing test and control inoculums. Culture containing test tube with approximately equal concentration or density with 0.5 McFarland standards was used for inoculation of media. The standard was used after shaking immediately before use; and stored in a well-sealed container in a dark place at room temperature (20 - 28 ^oC) when not used. When matched with the standard, the inocula was confluent growth. Then, the standardized suspension was swabbed by cotton swab onto the Muller-Hinton Agar (Oxoid) and allowed to dry. Thereafter, the antibiotic discs was placed using forceps on the medium and incubated at 37°C for 18 hrs and the zones of inhibition was measured manually with a transparent ruler. The results of the antimicrobial susceptibility was interpreted based on the guidance of National Committee for Clinical Laboratory Standards (NCCLS, 2007). Finally, the isolates was classified as sensitive, intermediate, or resistant. Intermediates was considered as resistant for purpose of analysis. The following standard drug discs (Oxoid) and their potency (µgml-1) was used depending up on the antibacterial spectrum, toxicity, effectiveness and availability (Vlkova *et al.,* 2006). As a result, ciprofloxacin (5), gentamycin (10), kanamycin (30), norflaxacin (10), streptomycin (10) and tetracycline (30) for *Salmonella spp*. and, ciprofloxacin (5), erythromycin (15), gentamycin (10), kanamycin (30), penicillin G (10), streptomycin (10) and tetracycline (30) for Staphylococcus aureus. The reference strains was *Salmonella typhimurium* and *Staphylococcus aureus.*

4.10. Growth Potential of Some Foodborne Pathogens

The growth potential of *Salmonella, Staphylococcus aureus* and *E. coli* was assessed in food samples of Anchor, Ayu, Baby king, Cheese and Firfir. Two hundred grams of each food items

was separately homogenized using food processor (NM-343) and steamed at 80°C for 10 minutes to kill any vegetative cells that might be present in the food items. One hundred grams of each food items was challenged separately with 1 ml overnight culture of the test strains to bring the final inoculum levelof 10^{2}-10^{3}CFUg^{-1}. The challenged food was incubated at 37 ^{o} C for 24 hrs. C for 24 hrs. To determine the initial inoculum level, 10 g of each freshly inoculated food was be homogenized in 90 ml of BPW and 0.1 ml of appropriate dilution was be spread plated on XLD for *Salmonella*, MSA for *S.aureus* and VRBA for *E. coli*. A portion of food sample was further sampled aseptically at 6 hrs interval from 0-24 hrs (Muleta and Ashenafi, 2001a). While assessing growth potential, the pH of each food sample was measured using digital pH meter from 0 to 24 hrs at an interval of 6 hrs.

4.11. Data Analysis

The Percentage of Coefficient of variation (% CV) was calculated to see if there is significant variation in counts within the food samples analyzed. The data obtained from the respondents were analyzed using SPSS software version 23.0 Mean values of food samples from different sites were compared using one way ANOVA and the significance of differences were considered at 95% confidence interval ($P < 0.05$).

5. RESULTS

5.1. Socio-demographic Characteristics of Vendors and Producers

Majorities117 (78%) of venders and producers of the weaning foods were females. Furthermore, 84(56%) of the respondents were aged between 31-40 years, with only 2(1.33%) aged below 20 years. Educationally, 79(52.7 %) had primary education, while 36(24 %) were uneducated (Table 1).

Table 1 Socio-demographic characteristics of venders and producers of weaning foods, Jimma Zone, 2019

Parameters	Number of Respondents(N=150)	Percentage (%)
Sex		
Male	33	22.00
Female	117	78.00
Age		
<20	2	1.33
20-30	15	10
31- 40	84	56
>40	49	32.67
Academic Status		
Uneducated	36	24.00
Primary Education	79	52.70
Secondary Education	35	23.30
Marital Status		
Unmarried	8	5.30
Married	121	80.70
Divorced	12	8.00
Widow	9	6.00

5.2. General hygiene of weaning foods

Data on the status of the general hygiene of weaning foods and food handler practices were collected through interview and onsite observation. Accordingly, majorities 84(56%) of the

respondents used cold water only to prepare weaning foods and 112(74.7%) feed their infants using spoon. For commercial weaning food, 66(44%) of the users didn't check the expiry date of the products when they buy it. Moreover, majorities 53(35.5%) of the retailers' have stored weaning foods in refrigerator while some 38(25.5%) stored it in shelf (Table 2).

Table 2 Weaningpractices of infants in Jimma Zone, Southwestern Ethiopia, 2019.

Characteristics	Category	Number of Respondents (N=150)	Percentage (%)
Breast feeding	Yes	106	70.7
	No	44	29.3
Age of feeding started	>6	97	64.7
	< 6	53	35.3
Methods of feeding	With Spoon	112	74.7
	with their hands	38	25.3
Source of water used for preparation of foods	Cold water only	84	56
	Warm water only	18	12
	Cold water with soap	35	23.3
	Warm water with soap	13	8.7
Place of commercial weaning foods bought	Directly from industry	-	-
	From Super Markets	58	38.7
	From Mini shop	83	55.3
	From whole seller	9	6
Check expired date of weaning food	Yes	66	44
	No	84	56
Storage place of weaning foods	In refrigerator(4 ^{o}C)	53	35.5
	On shelf	38	25.5
	Another places	59	39.3
Storage time after weaning food prepared	>6 hours	121	80.7
	< 6 hours	29	19.3
Types of weaning foods used	Commercial	101	67.3
	Non-commercial	49	32.7

5.3. Microbial load

The mean microbial load (log CFU g^{-1}) of commercial and homemade weaning food ranged between 4±0.5 to 5.5±0.6. The maximum mean count of 5.5±0.6 log CFU g^{-1}) was recorded for AMB, and the minimum count (4±0.5 log CFU g^{-1}) was noted for mould. Among the collected food samples, firfir sample had harboured higher number of bacterial colonies that ranged between 5.3 log CFU g^{-1} to 6.9 log CFU^{-1}followed by cheese with bacteria population ranging between 4 log CFU g^{-1} to 6.7 log CFU g^{-1} whereas the lowest was recorded for ayu (3.5 log $CFUg^{-1}$to 4.0 log $CFUg^{-1}$) (Table 3).

The mean count of AMB was the highest (6.9 log $CFUg^{-1}$) in firfir sample followed by cheese (6.7 log CFUg-1) whereas the lowest was recorded in ayu (4.0 log $CFUg^{-1}$). The mean count of Enterobacteriaceae was the highest in cheese (6.3 log $CFUg^{-1}$) with closer mean counts observed in firfir (6.2 log $CFUg^{-1}$) and baby king (4.5 log $CFUg^{-1}$). Likewise, the mean counts (log $CFUg^{-1}$) of coliform and yeast, were the highest in firfir (6.3 and 5.2, respectively).Furthermore, the mean counts (log $CFUg^{-1}$) of staphylococci were the highest (6.6) in firfir followed by cheese (6.5). However, it was relatively lower in ayu (3.9) and anchor (4.3). Generally, the mean counts of all microbes in all food samples were above detectable level except in ayu samples, whereas the mean counts of mould and coliform were below detectable level (Table 3).

Table 3 Total mean microbial counts (log $CFUg^{-1}$) of weaning food, Jimma Zone, 2019

Food Type	Sample size	AMB	Entero	Coli	ABS	Staph	Yeast	Mold
Anchor	30	4.4±0.5	4.2±0.6	4.0±0.4	3.9±0.5	4.3±0.6	3.9±0.4	3.4±0.5
Ayu	30	4.0±0.4	3.5±0.6	3.7±0.3	3.5±0.6	3.9±0.5	3.2±0.7	3.0±0.4
Baby king	30	5.4±0.7	4.5±0.4	4.3±0.6	4.0±0.4	4.9±0.5	4.1±0.7	3.8±0.3
Cheese	30	6.7±0.5	6.3±0.7	6.0±0.5	6.1±0.6	6.5±0.4	5.2±0.5	4.0±0.4
Firfir	30	6.9±0.7	6.2±0.4	6.3± 0.6	5.3±0.5	6.6±0.7	5.2±0.6	3.7±0.5
Total	150	5.5±0.6	5.0±0.5	4.9±0.5	4.6±0.5	5.2±0.5	4.3±0.6	4.0±0.5
p-value		0.003	0.000	0.000	0.003	0.005	0.005	0.004

AMB = Aerobic Mesophilic Bacteria, Entero = Enterobacteriaceae, ABS = Aerobic Bacterial Spore, Staph =Staphylococci, Coli = Coliform

With regards to microbial load of weaning foods collected from specific sites, the highest mean counts of AMB (5.4log $CFUg^{-1}$) was recorded from Anchor collected from Oomo Nadda. The mean microbial load of Ayu ranged between 3.2± 0.6 to 4.3± 0.6 $CFUg^{-1}$, with the maximum count observed for yeast (Table 4). In baby king and firfir, AMB were dominant with counts in the range of 4.3± 0.5 and 7 ± 0.3 $CFUg^{-1}$, respectively. The maximum AMB count of baby king was observed in samples from Oomo Beyam and that of firfir from Oomo Nada. There was statistically significant difference ($p \leq 0.05$) among the mean counts of all microbes (Table 4).

Table 4 Mean microbial counts (log CFUg^{-1} ± SD) of weaning foods with their specific sites, Jimma zone, 2019

Food Sample	Site	Mean microbial counts (log CFUg-1 ± SD)							
		AMB	Entero	Coli	ABS	Staph	Mould	Yeast	p-value
Anchor	Jimma	4.8±0.4	4.0±0.4	3.2±0.8	4.0±0.3	4.6±0.5	3.6±0.4	5.1±0.4	0.005
	Asandabo	4.5±0.7	5.1±0.8	4.1±0.4	4.5±0.2	4.3±0.7	3.5±0.9	5.8±0.9	
	Nadda	5.4±0.6	4.7±0.4	4.1±0.4	4.2±0.7	4.7±0.7	4.2±0.2	5.7±0.3	
	Beyam	5.3±0.3	4.9±0.6	4.6±0.5	4.9±0.6	4.9±0.4	3.9±0.5	5.4±0.6	
	Average	5.0±0.5	4.7±0.6	4.0±0.5	4.4±0.5	4.6±0.6	3.8±0.5	5.5±0.6	
Ayu	Jimma	3.9±0.4	3.0±0.7	3.3±0.7	3.4±0.3	3.7±0.5	3.0±0.6	4.3±0.5	0.003
	Asandabo	3.5±0.3	3.1±0.5	3.2±0.4	3.3±0.5	3.4±0.6	3.2±0.6	4.9±0.5	
	Nadda	3.3±0.2	3.4±0.6	3.1±0.6	3.6±0.4	3.5±0.4	3.1±0.6	4.6±0.7	
	Beyam	3.0±0.6	3.7±0.4	3.5±0.3	3.5±0.4	3.9±0.5	3.3±0.6	3.5±0.6	
	Average	3.4±0.4	3.3±0.6	3.3±0.5	3.5±0.4	3.6±0.5	3.2±0.6	4.3±0.6	
Baby king	Jimma	4.4±0.7	3.7±0.2	3.2±0.4	2.9±0.6	4.1±0.4	3.1±0.4	4.2±0.6	0.005
	Asandabo	4.2±0.5	3.5±0.8	3.0±0.6	2.4±0.7	3.9±0.4	3.0±0.5	3.0±0.6	
	Nadda	4.0±0.4	3.9±0.4	3.0±0.7	2.8±0.6	3.6±0.7	3.4±0.4	3.4±0.4	
	Beyam	4.5±0.3	3.1±0.6	3.6±0.6	3.2±0.8	4.3±0.3	2.9±0.3	3.9±0.7	
	Average	4.3±0.5	3.6±0.5	3.1±0.6	2.8±0.7	4.0±0.5	3.1±0.4	3.8±0.6	
Cheese	Jimma	6.8±0.5	6.2±0.3	4.1±0.3	2.7±0.5	6.4±0.2	3.8±0.7	5.5±0.2	
	Asandabo	7.0±0.4	6.3±0.3	4.4±0.7	3.3±0.5	6.6±0.3	3.5±0.5	5.6±0.3	0.005
	Nadda	7.2±0.3	6.0±0.5	4.2±0.4	3.5±0.6	6.2±0.7	3.4±0.6	4.1±0.3	
	Beyam	6.3±0.6	5.4±0.3	4.1±0.6	3.1±0.3	6.7±0.6	3.1±0.6	5.7±0.3	
	Average	6.8±0.5	6.0±0.4	4.2±0.5	3.2±0.5	6.5±0.5	3.5±0.6	5.2±0.2	
Firfir	Jimma	6.3±0.4	6.6±0.4	5.0±0.3	4.2±0.7	5.8±0.5	4.0±0.7	3.9±0.6	0.004
	Asandabo	7.3±0.3	6.5±0.6	5.5±0.7	3.9±0.4	5.5±0.6	4.3±0.3	5.8±0.6	
	Nadda	7.5±0.3	6.9±0.3	5.3±0.5	5.1±0.4	6.7±0.4	3.8±0.5	5.4±0.6	
	Beyam	7.0±0.2	6.2±0.6	4.9±0.5	3.9±0.4	6.5±0.6	3.8±0.5	4.9±0.6	
	Average	7.0±0.3	6.6±0.5	5.2±0.5	4.2±0.5	6.1±0.5	4.0±0.5	5.0±0.6	

Where = AMB = Aerobic Mesophilic Bacteria, Entero = Enterobacteriaceae, ABS = Aerobic Bacterial Spore, Staph =Staphylococci, Coli = Coliform

5.4. Microbial analysis of weaning foods

From the 150 food samples analyzed, a total of 901 bacterial isolates were identified. Of which 242 bacterial were isolated from firfir, 193 from cheese, 186 from anchor, 174 from Baby king and 106 from ayu (Table 5)**.**All the 901 bacterial isolates were characterized and grouped into one family and six genera based on John's (2012) bacterial classification. Generally, Gram-positive bacteria (75.5%) dominated the Gram-negative bacteria (24.5%). Among the isolates, *Bacillus spp*. were dominant in all food samples accounting for 43.0% in Firfir, 34.9 % in Ayu, 31.2% in Anchor, 27.0% in baby king, and 32.6% in cheese. *Staphylococcus spp*. was the second most dominant bacteria in Firfir (32.2%), Anchor (23.1%) and Ayu (29.2%), Baby King (25.3) and Cheese (25.4). Generally, among the total isolates, *Bacillus spp*. (34.3%) were dominant in weaning food samples followed by *Staphylococcus spp*. (27.2%) (Table 5)**.**

Table 5 Frequency distribution of dominant bacteria in some weaning food, Jimma Zone, 2019

Sample source	Bacterial isolates	*Bacillus spp*	*Staphylococcus spp.*	Enterobacteriaceae	*Salmonella spp*	*Micrococcus spp.*	Other Gram +ve bacteria
Anchor	186	58(31.2)	43(23.1)	29(15.6)	21(11.3)	20(10.8)	15(8.1)
Ayu	106	37(34.9)	31(29.2)	13(12.3)	9(8.5)	10(9.4)	6(5.7)
Baby king	174	47(27.0)	44(25.3)	31(17.8)	23(13.2)	17(9.8)	12(6.9)
Cheese	193	63(32.6)	49(25.4)	30(15.5)	20(10.4)	21(10.9)	10(5.2)
Firfir	242	104(43.0)	78(32.2)	33(13.6)	12(5.0)	10(4.1)	5(2.1)
Total	901	309(34.3)	245(27.2)	136(15.1)	85(9.4)	78(8.7)	48(5.3)

5.10. Prevalence of *S. aureus* and *Salmonella spps.*

Overall, 32.7% of the samples were positive for *S. aureus*. However, its frequency distribution varied among the various weaning food samples. Accordingly, the prevalence was 70%in firfir samples whereas the lowest prevalence was observed in ayu(3.3%).Likewise, 28% of the samples were positive for *Salmonella spp*. with the highest prevalence (60%)recorded in firfir samples(Table 6).

Table 6 Prevalence of *S. aureus* and *Salmonella spp*.in weaning foods, in Jimma Zone, 2019

Sample	Sample Size	No. *S. aureus* positive samples (%)	No. *Salmonella spp*. Positive samples (%)
Anchor	30	6(20)	3(10)
Ayu	30	1(3.3)	2(6.7)
Baby king	30	8(26.7)	6(20)
Cheese	30	13(43.3)	13(43.3)
Firfir	30	21(70)	18(60)
Total	150	49(32.7)	42(28)

5.11. Antimicrobial Susceptibility patterns of some pathogenic Bacteria

Out of a total of 49 isolates of *S. aureus* tested for drug susceptibility, majorities were susceptible to ciprofloxacin (91.8%) and gentamycin (85.7%) followed by kanamycin (81.6%). However, the isolates were highly resistant to penicillin G (100%) followed by moderate resistance to tetracycline (36.7%).Similarly, from a total 49 isolates of *B.cereus*, majorities were susceptible to kanamycin (91.84%) and ciprofloxacin (85.6%).However, all isolates (100%) were resistant to penicillin G (Table 7).On the other hand, all the 42 isolates of *Salmonella* (100%) were susceptible to ciprofloxacin, gentamycin, kanamycin and norflaxacin. However, some isolates were resistant to tetracycline (38.1%) and streptomycin (35.1%). Likewise, all 42 isolates of *E. coli* (100%) were susceptible to gentamycin but few were resistant to tetracycline (38.1%) and Streptomycin (19.05%) (Table 7).

Table 7 Antimicrobial susceptibility patterns of pathogens isolated from weaning foods in Jimma Zone, southwestern Ethiopia, 2019

Antimicrobial Agents	Disc potency (µg/ml)	*S. aureus*			*Bacillus cereus*			*Salmonella spp*			*E. coli*		
		R	I	S	R	I	S	R	I	S	R	I	S
		F (%)	F (%)	F (%)	F (%)	F (%)	F (%)	F (%)	F (%)	F (%)	F (%)	F (%)	F (%)
Ciprofloxacin (CIP)	5	-	4(8.2)	45 (91.8)	-	7(14.3)	42 (85.6)	-	-	42 (100)	-	2(4.76)	40(95.24)
Erythromycin (E)	15	13 (26.5)	20(40.82)	16(32.7)	11(22.45)	17(34.7)	21 (42.86)	ND	ND	ND	ND	ND	ND
Gentamycin (CN)	10	-	7 (14.3)	43 (85.7)	-	14(28.6)	35 (71.43)	-	-	42 (100)	-	-	42(100)
Kanamycin (K)	30	-	9 (18.4)	40 (81.6)	-	4(8.2)	45 (91.84)	-	10(23.8)	32 (76.2)	-	13(30.95)	29(69.01)
Penicillin G (P)	10	49 (100)	-	-	49 (100)	-	-	ND	ND	ND	ND	ND	ND
Streptomycin (S)	10	16 (32.7)	20 (40.82)	13 (26.5)	10 (20.41)	18 (36.7)	21 (42.86)	15 (35.7)	12 (28.6)	15 (35.7)	8 (19.05)	14(33.3)	20(47.62)
Tetracycline (TE)	30	18 (36.7)	7(14.3)	24 (49)	15(30.6)	10 (20.41)	24 (49)	16 (38.1)	-	26 (61.9)	16 (38.1)	10(23.81)	16(38.1)
Norfloxacin (NOR)	10	ND	ND	ND	ND	ND	ND	-	-	42 (100)	-	9(21.43)	33(78.6)

Where: R= Resistance I= Intermediate S=Susceptible F=Frequency

5.12. MDR patterns of *S. aureus* and *B. cereus* isolated from Weaning foods

The multi-drug resistance (MDR) patterns of *S.aureus* revealed that, 30.6 % of the isolates were resistant to 3 antibiotics (mainly P/E/TE and E/P/K combinations) (resistance to Penicillin, Erythromycin, and Tetracycline; and resistance to Erythromycin, Penicillin, and Kanamycin) followed by 26.50 % resistance to 5 antibiotics and 20.41 % to 6 antibiotics (Table 8). The highest MDR in *S.aureus* (17.02 %) was recorded for four antibiotics (mainly P/E/TE/S). The maximum number of antibiotics resisted by *S.aureus* was six antibiotics with total proportion of 20.41 %. Totally, MDR to three and five antibiotics dominated the resistance pattern (Table 8).

The MDR patterns of *B.cereus*revealed that, 24.6 % of the isolates were resistant to 3 antibiotics (mainly E/P/TE and E/P/S) followed by 22.45 % to 5 antibiotics and 20.49 % to 6 antibiotics. The highest MDR in *B.cereus* (18.4% and 14.3%) were for two and four antibiotics mainly P/S and CIP/E/K/P/S/TE, respectively. The maximum numbers of antibiotics resisted by *B.cereus*was six antibiotics with total proportion of 20.49 %. Generally, MDR to three and five antibiotics dominated the resistance pattern (Table 8).

Table 8 MDR patterns of *S. aureus* and *B. cereus* from weaning foods, Jimma Zone, 2019

No. of antimicrobial (%) resistance	*S. aureus*			*B. cereus*		
	Antimicrobial resistance patterns	No. of isolates (%	Total	Antimicrobial resistance patterns	No. of isolates (%)	Total
Two	E/P,	2(4.1)	3(6.12)	E / P	6(12.24)	9(18.36)
	P/TE,/	1(2.04)		P/S	3(6.12)	
Three	E/P/K,	4(12.77)	15(30.6)	E/P/S	1(2.04)	12(24.6)
	E/P/TE	3(4.26)		E/P/TE	3(6.12)	
	P/E/TE	1(2.13)		E/P/TE	1(2.04)	
	P/K/TE,	3(4.26)		,E P/S	3(6.12)	
	E/P/CN	3(12.77)		P/TE/CN	1(2.04)	
	P/E/CIP	1(2.13)		E / P/ TE	3(6.12)	
Four	E/P/K/S	2(2.13)	8(16.33)	CIP/E//K/TE	2(4.1)	7(14.3)
	E/P/K/TE	1(4.26)		CN/P/K/TE	2(4.1)	
	P/E/TE/S	5(17.02)		E/P/TE /S	3(6.12)	
Five	S/P/E/K/TE	3(8.51)	13(26.5)	CN/P/E/S/TE	2(4.1)	11(22.45)
	CIP/P/E/S/TE	5(2.13)		E/K/P/S/TE	1(2.04)	
	S/P/E/CIP/TE	4(2.13)		CIP/K/P/S/TE	4(8.2)	
	S/CIP/K/TE/PE	1(2.13)		CN//E/K/P/S	2(4.1)	
				CIP /CN/E/P/TE	2(4.1)	
Six	S/P/E/CN/TE/S	3(2.13)	10(20.41)	CIP/E/K/P/S/TE	5(10.2)	10(20.41)
	P/E/K/S/TE/CN	5(2.13),		CN/E/K/P/S/TE	3(6.1)	
	P/E/CIP/S/TE/CN	2(2.23)		CIP/CN/E/P/S/TE	2(4.1)	

Where, CIP= Ciprofloxacin, E= Erythromycin, K= Kanamycin, N= Gentamycin, P= Penicillin, S= Streptomycin, TE= Tetracycline

5.13. MDR patterns of *Salmonella spp.,* and *E.coli* isolated from Weaning foods

Salmonella *spp* and *E.coli spp*s were the highest resistance (59.52 %) towards three antibiotics followed by four antibiotics (21.43 % each) (Table 9). In case of *Salmonella spp*., the maximum number of antibiotics resisted was four antibiotics. However, the highest MDR (59.52 %) was observed to (E /NOR/TE) (resistance to Erythromycin, Norfloxacin, and Tetracycline).Generally, MDR to three antibiotics dominated the resistance pattern (59.52 %) and The MDR profile of *E.coli spp.* showed that the highest resistance (52.4 %) towards three antibiotics followed by four antibiotics (38.1 %). The maximum number of antibiotics resisted by *E.coli spp*., was four anti

biotics. However, the highest MDR (16.7 %) was observed for four antibiotics with combinations of CIP/K/TE/S. Generally, MDR to three antibiotics dominated the resistance pattern (52.4 %) (Table 9).

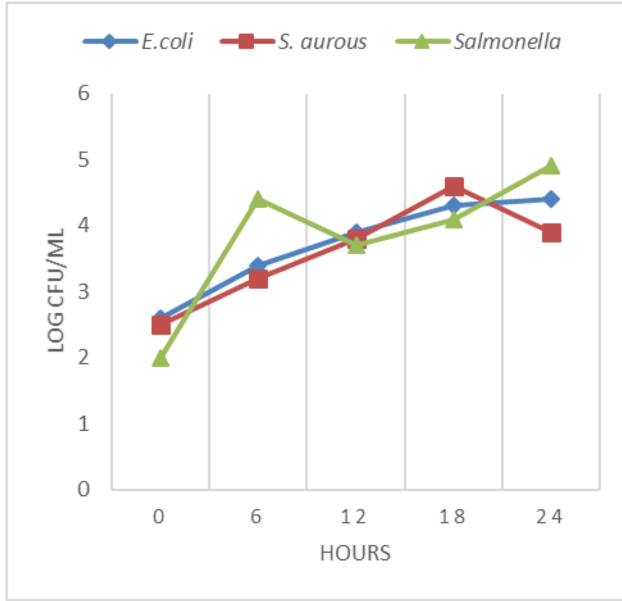
Table 9 MDR patterns of *Salmonella spp. and E. coli* isolated from weaning foods, Jimma zone, and 2019

No. of antimicrobial (%) resistance	*Salmonella spp.*			*E. coli*		
	Antimicrobial resistance patterns	No. of isolates (%	Total	Antimicrobial resistance patterns	No. of isolates (%	Total
Two	E/NOR	5(11.9),	8(19.04)	NOR/S	1(2.4)	4(9.52)
	NOR/S	3(7.14)		E / NOR	3(7.14)	
Three	E/NOR/S	5(11.9),	25(59.52)	E/NOR/S	5(11.9)	22(52.4)
	E/NOR/S	2(4.76),		E/CIP/ TE	4(9.52),	
	E/NOR/TE	4(9.52),		K/NOR/S,	2(4.8),	
	E/NOR/TE	4(9.52),		E/NOR/ TE	2(4.8)	
	E / NOR/ TE	6(14.3)		NOR/TE/CN,	6(14.29),	
	NOR / TE/CN	4(9.52),		CN/NOR/TE,	3(7.14)	
Four	CN/NOR/K/TE	3(7.14)	9(21.43)	CIP/E//K/TE	4(9.52),	16(38.1)
	CIP/E/K/TE	1(2.4),		CIP/K/TE /S	7(16.7)	
	E/NOR/TE /S	5(11.9)		CN/NOR/K/TE	5(11.9),	

Where**,** CIP= Ciprofloxacin, E= Erythromycin, K= Kanamycin, N= Gentamycin, NOR= Norfloxacin, S= Streptomycin, TE= Tetracycline

5.14. Growth Potential of Some Foodborne Pathogens

The initial cell density of test stains separately inoculated to the samples was 3-4.5log CFUg^{-1}. There was faster growth rate of *Salmonella spp., in* firfir sample (4.9 Log CFUg^{-1}) followed by cheese (4.7 Log CFUg^{-1}) at 6 hrs. At 18 hrs, the growth rate of *Salmonella* was increased by greater than 1 log CFUg^{-1} in firfir (6.3 log CFUg^{-1}). On the other hand, *S.aureus* dropped down to the minimum growth of 3.2 log CFUg-^{1}at 6 hrs in anchor (Figure 2).



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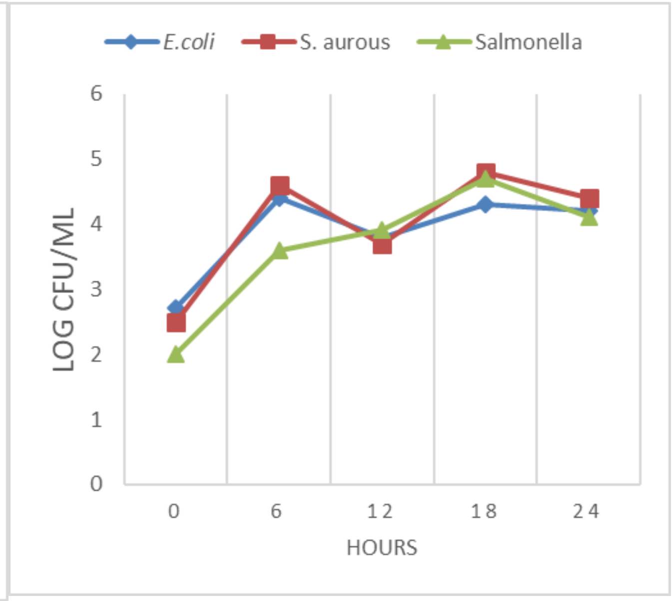

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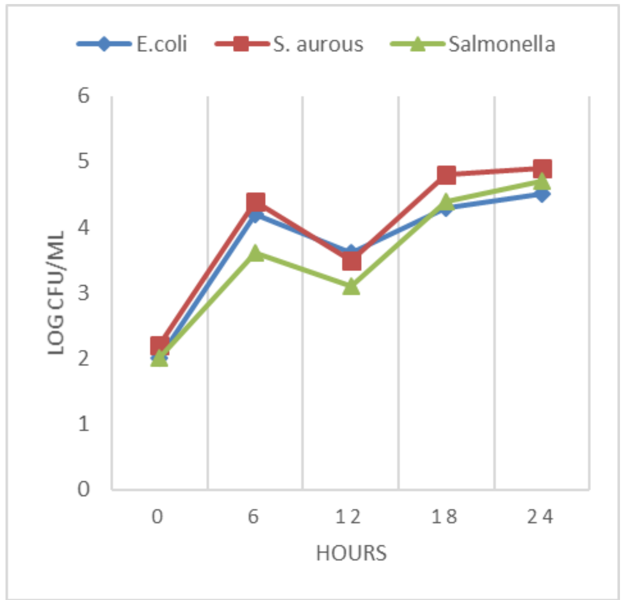

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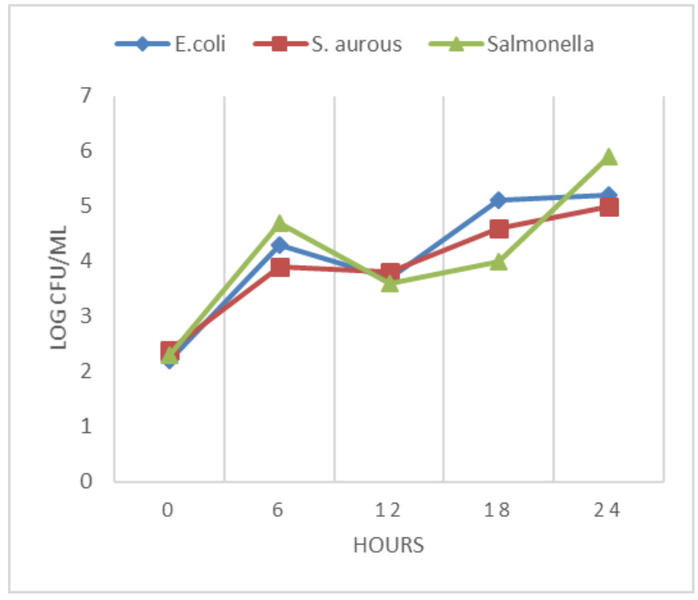

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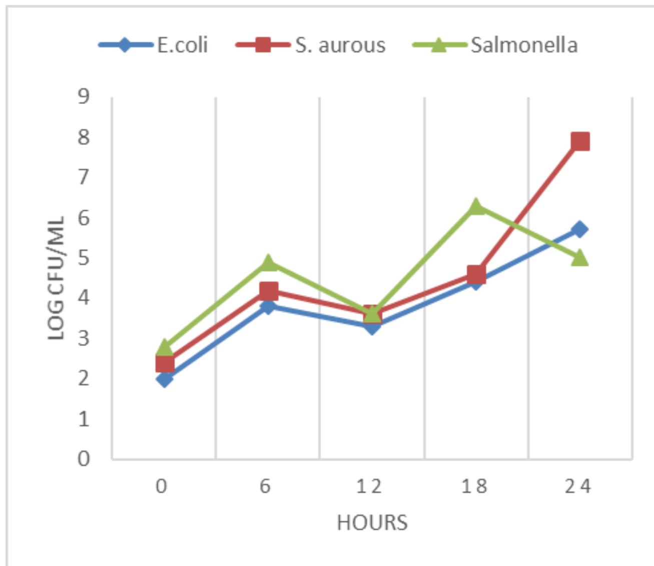
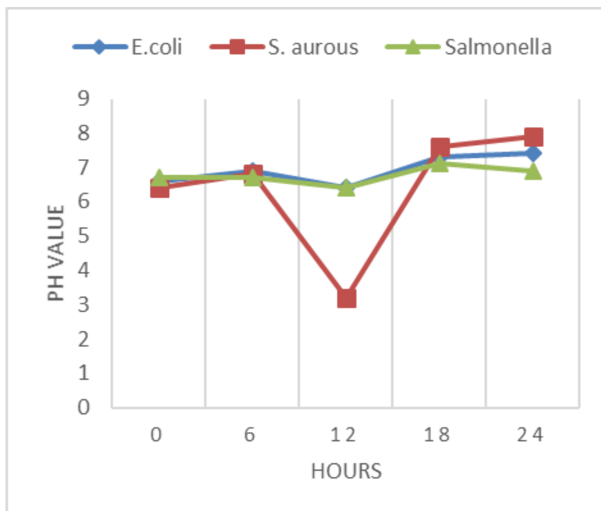


e

Figure 2 Growth potential of *E. coli*, *S. Thyphimurium*, and *S. aureus* in anchor (a), ayu (b), Baby king (c) Cheese (d) and Firfir (e)

The pH value of *E. coli*, *S.aureus* and S. *Thyphimurium* in all food samples revealed that there was little difference in reading among a food sample as time goes from 0 hr to 24 hrs. Accordingly, at 0hr the pH of all Food Samples are in the interval of 6.6-6.7 and as time goes from 6-24 hrs, pH was closed to neutral. At 6 hrs, almost all samples revealed rise in pH. In contrast, the pH value of food samples dropped at 12 hrs followed by rise thereafter until 24 hrs (Figure 3).



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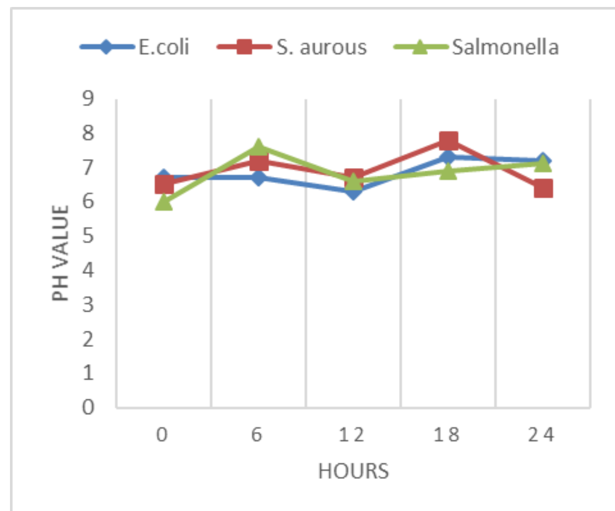

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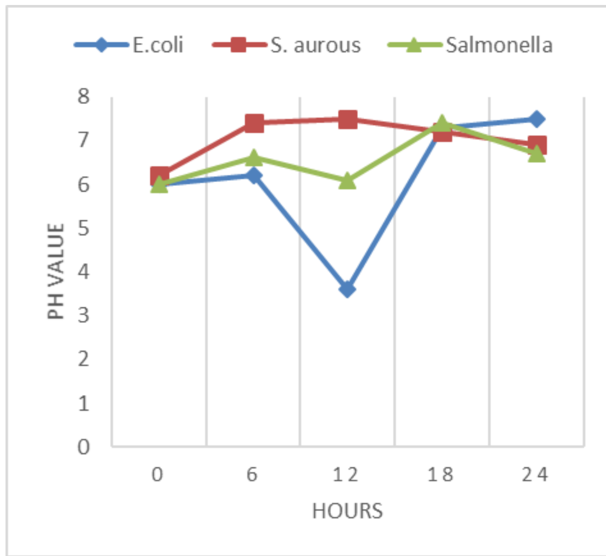

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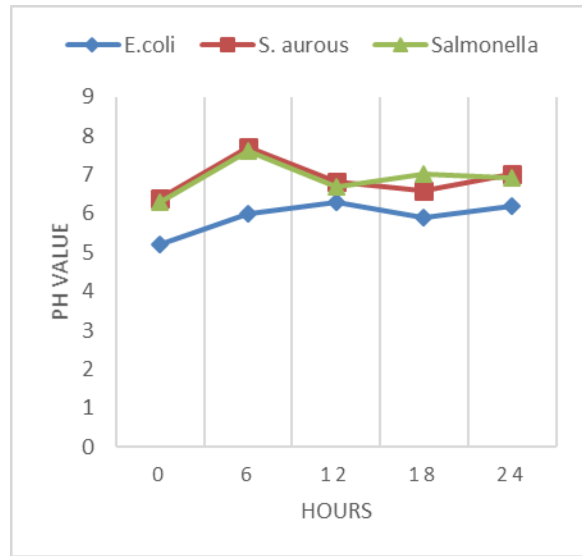

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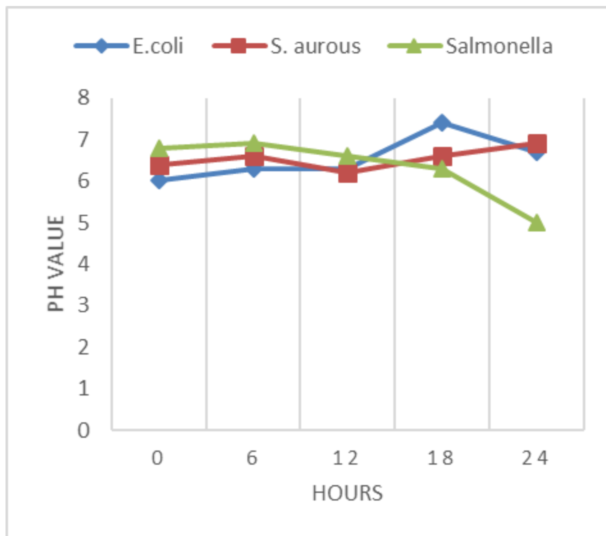

e

Figure 3pH of *E. coli (ATCC25922)*, *S. Thyphimurium (ATCC13311)*, and *S. aureus* in Anchor (a), Ayu (b), Baby King (c), Cheese (d) and Firfir (e) Jimma Zone, southwestern Ethiopia.

6. DISCUSSION

The result of the present study showed that most of parents had no training on important hygienic practices to be followed during food preparation and had no adequate awareness to give special attention to the microbial safety of weaning foods. Hence, the hygienic practices and handling of weaning foods related to storage was found very poor. In developing countries, up to 70% of diarrhoeal episodes are traced to pathogens transmitted from weaning food (Motarjemi *et al*., 2004). Fecal-contaminated water, an important vehicle for transmitting pathogenic microorganisms, may account for a high degree of morbidity and mortality. Traditional weaning foods in East Africa are known to be of low nutritive value (Guiro *et al*., 2013) and are characterized by low protein, low energy density, and high bulk density.

In this study, about 56% of the parents used cold, tap or well water only to prepare homemade weaning foods. According to the report of WHO (2000), microbial contamination of weaning foods can be overcame by using safe water for preparation, frequent washing of hands and proper storage. On the other hand, in this study, 80.7% of the parents stored food for more than six hours. Our observation was similar to the study conducted in Nigeria in which 90.3% of mothers stored weaning foods for more than Six hours (Omemu and Omeike, 2010).

Concerning the microbiological quality of weaning foods, the mean counts of Aerobic mesophilic bacteria ranged between 4.0 log CFU/g (Ayu) to 6.9 log CFU/g (Firfir). Similarly, the study conducted in Lagos, Nigeria, revealed that the total aerobic mesophilic bacteria count ranged from 3.5 l to 6.8 log CFU/ml ((Uzeh *et al*., 2009). The overall mean count of 5.5 log CFU/g recorded for AMB for all weaning foods was slightly lower than that reported from Nigerian (Nwogwugwu *et al*., 2012) who reported the counts between 7.0 to 7.3 logs CFU/g in Nigerian novel weaning food (DUPAP).The mean counts of Enterobacteriaceae in the present study was 5.0 log CFU/g which is higher in compared to study conducted in zanbar (Kungu *et al*. ,2009), reported mean countEnterobactericae2.54 log CFU/g in weaning porridge samples from Zanzibar. According to the guideline, the mean counts (CFUg-1) of Enterobacteriaceae in Anchor (4.2), ayu (3.5), Baby king (4.5), Cheese (6.3) and Firfir (6.2) revealed unsatisfactory level ($\geq$ 4 log $CFUg^{-1}$). Enterobacteriaceae and the high number clearly prove that poor hygiene meals that could be a source of foodborne disease (Motarjemi *et al*., 2009). The presence of

Enterobacteriaceae could be due to contaminants from utensils, environment, vendors and holding food in open market without appropriate sanitation and due to their ability to colonize both wet and dry environments and excellent indicators of equipment contamination from environmental source and long holding of food at ambient temperature that would be easy for Enterobacteriaceae to dominate the flora (Muleta and Ashenafi, 2001b).

With respect to the mean count of Coliforms, general the highest microbial counts were recorded and it accounted about 4.90 log CFU/g). The result of this study is much higher than the result reported 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. But, the maximum mean count (6.9 log CFU/g) were identified particularly from firfir samples. In contrast to this study, Hussein (2016) reported higher number (5.6 log $CFUg^{-1}$) from Assiut, Egypt. Here, the high count of coliforms in firfir in the study could be revealed to unclean utensils such as feeding materials or probably due to initial contamination of different sources. General lack of hygienic practices showed a strong likelihood of cross-contamination between unclean utensils, and the weaning foods (Desausa, 2008).The permissible level for coliform is similar Enterobacteriaceae. Hence, the counts of Coliform in Firfir (4.9 log $CFUg^{-1}$) were unsatisfactory whereas Anchor (4.0 log $CFUg^{-1}$) and Ayu (3.7 log $CFUg^{-1}$) were in acceptable level (2 to < 4 log $CFUg^{-1}$). However, the count of Coliform in Baby king (4.3 log $CFUg^{-1}$) belonged to the group with satisfactory level ($<$ 2 log $CFUg^{-1}$).The presence of Coliform in the present study could be due to fecal contamination of food or water after preparation. Of course, once introduced into the food samples and the foods left at ambient temperature for a while, the contaminating Coliform would multiply to higher counts (Tomkins, 2001). The higher counts in the present study could be due to the contamination of food by the heat resistant spores, which have survived cooking temperature because of temperature / time abuse during preparation of food.

The mean Aerobic Spore Forming of the present study was higher in firfir (5.3 log CFU/g) and lower in ayu (3.5 log CFU/g). The result of the present study is much higher than the study report by (Mosupye and Van Holy, 2009) where they reported the bacterial counts ranged from 1.2 to 2.0 log $CFUg^{-1}$ from the Firfir samples collected from (Johannesburg) South Africa. Accordingly, mean counts of ABS obtained from this study is not in line with the guideline (4.3 log $CFUg^{-1}$) were of unsatisfactory level (>4 log $CFUg^{-1}$). Generally, the higher counts in the

present study could be due to the contamination of weening food by the heat resistant spores, which had survived cooking temperature because of temperature / time inadequacy during preparation of food.

The mean counts of Staphylococci in the present study were 5.2log $CFUg^{-1}$. This is in agreement with the microbiological studies made in weaning foods in India (Mohapatra *et al*., 2002), whose counts were greater than 3 log $CFUg^{-1}$. The higher Staphylococci in the present study could be due to unhygienic handling of the foods'. Personal hygiene since the interview results revealed 69 % of the vendors handled foods in unappropriate ways. According to (Mensah, 2002), the uses of a fork or spoon to serve food reduced the level of contamination. Staphylococci exist in air, dust, sewage, water and food or on food equipment and environmental surfaces. The presence of Staphylococci in food is indication that such food is potentially hazardous (Amissah and Owusu, 2012).

The predominant micro floral of weaning foods in the present study was generally *Bacillus spp*. (34.3 %) followed by *Staphylococcus spp*. (27.2 %), and *Micrococcus spp*. (8.7 %). The prevalence of *S. aureus* in the present study was 27.2%. As the study conducted by (Ikeh *et al.,* 2001) have been reported some enteric bacterial pathogens, such as *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* and *Shigella*, were isolated and found in weaning foods in many developing countries. The presence of these pathogens in weaning foods is dangerous to consumers and the problems are severe in infants and young children. Higher number of *S. aureus* was isolated from Firfir (32.2%). This is probably due to cross contamination and lack of personal hygiene. In the present study, the prevalence of *Salmonella spp.* were 9.4%. The presence of these organisms indicates poor food preparation such as inadequate cooking and unclean utensils which contribute to cross contamination (Tunung *et al*., 2007). The report of (Potgieter *et al*., 2005) showed that in South Africa 3.2% of Vhuswa (local weaning food) samples were contaminated with *Shigella*. However, in the present study, *Shigella spp*. was not isolated from any of the weaning food samples. This is because of *Shigella* are not as persistent as *Salmonella* (Cetinkaya *et al*., 2008) or most likely killed during cooking process (Muleta and Ashenafi, 2001). Here the majority of the people use Commercial weaning foods for their children which considered as safe by many including who are educated. Even though there was no recorded data about magnitude of food borne illness which occurred as a result of in

appropriate handling, it was observed that in different parts of the country town, there were high rate of sanitary condition of foods. This could directly or indirectly affect the safely of food (Martins, 2006). Transportation is also matter for the less quality of weaning foods when foods travel from one place to another the quality of the foods may be affected with either biological or chemical hazards.

Regarding to the antimicrobial susceptibility pattern of bacteria pathogens isolated from the present study, majority of *S. aureus* were susceptible to ciprofloxacin and gentamycin followed by kanamycin (81.6%), while they were highly resistant to penicillin G (100%) followed by tetracycline. this result is exactly similar with the study conducted by (Sina *et al*., 2011) who reported that, 100% of the isolates were resistant to Penicillin G. This resistance of *S. aureus* to penicillin G may be due to the production of penicillinase enzyme that hydrolyzed the beta lactam ring of penicillin (Lowy, 2003).

As the study conducted by (Akbarmehr, 2012) in Sarab showed that *Salmonella spp*. were highly susceptible to ciprofloxacin and gentamycin (91.89% each). However, isolates of *Salmonella spp*. exhibited resistance to streptomycin and tetracycline (29.72% each). In the present study, high number of *Salmonella spp*. was susceptible to ciprofloxacin and gentamycin (100%) and they had showed highly resistance tetracycline (38.1%) followed by Streptomycin and this study is similar with the above study conducted in Sarab. Antimicrobial resistance emerges from the use of antimicrobials in animals and human, and the subsequent transfer of resistance genes and bacteria among animals, humans, animal products and the environment (Schroeder *et al*., 2008). Hence, as the result of present study revealed that *S. aureus* was the most susceptible to ciprofloxacin and gentamycin followed by kanamycin. Generally, MDR to three and five antibiotics dominated the resistance pattern. Multi Drug Resistance is defined as resistance of an isolate to two or more than two antimicrobial agents used for test (Bartoloni *et al*., 2005). *Salmonella spp* and *E.coli spp* showed that the highest resistance of the isolates towards three antibiotics and followed by four antibiotics.

In challenge study, the initial inoculums for all selected samples were 2-3 log CFU$g^{-1}$of the test strains. Accordingly, the growth potential was ranged from4.2 -5.7log CFUg^{-1}in*E.coli,* 4.1 – 5.9 log CFUg^{-1}in *S. Typhimurium*and3.9 – 7.9 log CFUg^{-1}in *S. aureus* in all food samples at 24 hr. But Generation time and Growth phase for each are different. *S. Typhimurium* occurs at lag

phase while all the rest shows their own generation time and phase. Accordingly, in current study, in all commercial foods, pathogens couldn't reach the infectious dose within 24 hours. However, the homemade food reached the infective dose with few hours of challenged pathogens. This could be due the nature of food, processing, and storage time. Generally, in challenging test the counts of all microorganisms were higher in Firfir and lower in Ayu. Lowest counts in Ayu could be due to the very low pH, which inhibits the growth of pathogens. Moreover, growth of challenged pathogens increased as pH reached to the neutral whereas their counts decreased as pH lowered from the neutral (Don, 2008). The change in pH could be changing of source of carbon and nitrogen. Some microbial cultures generate enzymes to utilize a new carbon and energy substrate when a small amount of the original carbon and energy substrate is present (Lee, 2011). However, pH is not the only required for the growth microorganisms but also other intrinsic parameters and extrinsic parameters (Jay *et al*, 2005). In general, the prevalence of *Salmonella* spp. and *S.aureus* in the present study was high as time increased from 24-48 hrs. This could be due to, personal hygiene and lack of awareness. Even though, there was high number of foodborne pathogens, yet there is no report on the outbreaks of disease. On the other hand, the isolates in the present study identified by Conventional methods. Thus, such type identification of the pathogens could not show the exact identity.

7. Conclusion

- The results obtained in this study indicate that most weaning foods available to the infants have high level of microbial loads that does not meet the international standards. This could be due to unhygienic preparation and improper storage.
- Firfir was the most contaminated food observed in this study, the unusually high microbial load of firfir calls for regular inspection for safety of weaning foods.
- The lowest microbial load was observed in Ayu and Anchor respectively.
- The most predominant microbial groups isolated from weaning food samples in this study were *Staphylococcus spp*., *Bacillus spp*., *Salmonella* and *E.coli*.
- *Salmonella* isolates were found more prevalence in Firfir and *Staphylococcus aureus* in Commercial weaning foods.
- *S.aureus* isolates were susceptible to Kanamycin gentamycin and ciprofloxacin but resistant to penicillin G. On the other hand, Salmonella spp. was susceptible to ciprofloxacin and gentamycin. The consumption of un hygienically prepared and contaminated weaning food could lead to the dissemination of drug resistant bacteria such as *Salmonella* (ATCC13311) and *S. aureus*.
- *Salmonella* (ATCC13311), *E. coli* (ATCC25922) and S. *aureus* were challenged on to the samples from “0” hrs to 24 hrs and none found growing to infectious dose within 24 hours. Hence it could be possible to minimize the risk associated with consumption of Commercial and homemade weaning foods contaminated with pathogens provided that the storage time is less than 24 hours. The shorter the storage time, the better it will be.
- Therefore, foods should be prepared hygienically, not stored for longer time or stored safely until consumption by giving greater emphasis to infants’ foods.

8. Recommendations

- Awareness development training should be given to the Weaning food vendors/users letting them maintain safety and quality of Commercial and Homemade weaning foods with due consideration to the nature storage place (temperature, humidity), and cautious about the products expiry dates.
- Government has to create strong police who always follows who is the producer of weaning foods, what is the personal hygiene, what steps followed during the process, is that procedures are the right procedures.
- Health workers and nutritionists can educate urban and rural mothers about the importance of adequate weaning foods and practices, infant health, host defense system, home scale drying and processing.
- Eventually, awareness creation of mothers in weaning food hygiene and continues supervision of supermarkets by stake holders specially sanitarians to improve the quality of weaning food that sell in different supermarkets.
- Where possible, implementation of HACCP principle could enhance the products safety as the principle gives due attention to safety of not only the raw materials used for the making of packed products but also ensures safety of the processing line and final product.

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Appendix1. Interview designed for Mothers feeding their infants with weaning foods in Jimma Zone, southwestern Ethiopia.

This interview was designed for evaluation of microbiological safety and quality of Weaning foods vended in Jimma zone, south west Ethiopia**.**

- Your response to the question 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. Age:

A. < 25

B .25- 30

C. 30-35

D. 35-40

E. > 40

2. Sex:

A. Male

B. Female

3. Academic status A. un able to read and write B. primary education D. secondary education

E. Others (Please Specify) _____________

4. Religion: A. Muslim B. Christian C. Others (Please Specify) _____________

5. Marital status: A. Married B. Divorced C. Widowed

6. How many years experience do you have in preparing weaning foods?

A. < 5 B. 6-10 C. 11-15 D. 16-20 E. > 20

Part II. Main Information

7. How do you prepare the weaning foods?

A. prepare separate meals/food for baby.Bprepare your baby's food in batches and freeze portions. C. Store prepared food in a sealed container in the fridge or freezer

D. frequently washing of your hands and proper storage.E .All

8. How long do you store prepared weaning foods?

A.< 6 hrs B.> 6 hrs C. unlimitted

9.Do you wash your hands before preparing food? A.YesB.No

10.Do you clean utensils and work surfaces before preparing food?A.YesB.No

11.Do you Store prepared food in a sealed container in the

fridge or freezer ?A.YesB.No

12. Do you Re-heat pre-prepared food thoroughly before cooling it down to give your baby?A.YesB.No

13. If your answer for question 12 is yes, how many times?

A.OnceB.Twice C. Three times

14. How do you Keep pets away from food ?

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

A. Tap B. Well C. River D. Spring E. Others (Please Specify) _____________

16. What type of water do you use for cleaning utensils?

A. Tap B. Well C. River D. spring E. Others (Please Specify) _____________

17. How do you clean the utensils?

A. By hand using water only B. With warm soapy water

C. With cold soapy water D. Others (Please Specify) ______________________

18. With what types of water do you clean your baby's bowls, spoons and feeding cups?

A. Tap B.Hot C. soapy water D.B and C

19. Did you get education about personal hygiene & handling of weaning foods? A. Yes B. No

20. If your answer for question 20 is yes, how often?

A. Weekly B. Monthly C. Yearly D. Others (Please Specify) __________________________

21. Do you have any information about food born disease? A. Yes B. No

22. If your answer for Q 22 is yes,

A. What types of food born disease do you know?

23. do you have any piece of information about a source of food-borne illness?

A. yes B. No

If yes List them

____

____

____

____

24.Do you make sure that a commercial weaning foods you use for your babies are within date and that the seal has not been broken ? A.yes B. No

25. Have you encountered any problem after feeding your baby with food from commercial weaning foods?   Yes/ No

26. If yes to Q 26 above, what was the cause for the observed health problem?

27. If you have any comment related with the use and problems of weaning foods, please feel free and list them down. ---
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Thank you for your time and genuine response

Appendix 2 Total mean microbial counts (log CFUg-1) of weaning food, Jimma Zone, 2019

Food Type	Sample size	AMB	Entero	Coli	ABS	Staph	Yeast	Mold
Anchor	30	4.4±0.5	4.2±0.6	4.0±0.4	3.9±0.5	4.3±0.6	3.9±0.4	3.4±0.5
Ayu	30	4.0±0.4	3.5±0.6	3.7±0.3	3.5±0.6	3.9±0.5	3.2±0.7	3.0±0.4
Baby king	30	5.4±0.7	4.5±0.4	4.3±0.6	4.0±0.4	4.9±0.5	4.1±0.7	3.8±0.3
Cheese	30	6.7±0.5	6.3±0.7	6.0±0.5	6.1±0.6	6.5±0.4	5.2±0.5	4.0±0.4
Firfir	30	6.9±0.7	6.2±0.4	6.3± 0.6	5.3±0.5	6.6±0.7	5.2±0.6	3.7±0.5
Total	150	5.5±0.6	5.0±0.5	4.9±0.5	4.6±0.5	5.2±0.5	4.3±0.6	4.0±0.5
p-value		0.003	0.000	0.000	0.003	0.005	0.005	0.004

AMB = Aerobic Mesophilic Bacteria, Entero = Enterobacteriaceae, ABS = Aerobic Bacterial Spore, Staph =*Staphylococci,*

Coli = Coliform**,**%cv

Appendix 3 Mean microbial counts (log CFUg^{-1} ± SD) of weaning foods with their specific sites, Jimma zone, 2019

Food Sample	Site	Mean microbial counts (log CFUg-1 ± SD)							
		AMB	Entero	Coli	ABS	Staph	Mould	Yeast	p-value
Anchor	Jimma	4.8±0.4	4.0±0.4	3.2±0.8	4.0±0.3	4.6±0.5	3.6±0.4	5.1±0.4	0.005
	Asandabo	4.5±0.7	5.1±0.8	4.1±0.4	4.5±0.2	4.3±0.7	3.5±0.9	5.8±0.9	
	Nadda	5.4±0.6	4.7±0.4	4.1±0.4	4.2±0.7	4.7±0.7	4.2±0.2	5.7±0.3	
	Beyam	5.3±0.3	4.9±0.6	4.6±0.5	4.9±0.6	4.9±0.4	3.9±0.5	5.4±0.6	
	Average	5.0±0.5	4.7±0.6	4.0±0.5	4.4±0.5	4.6±0.6	3.8±0.5	5.5±0.6	
Ayu	Jimma	3.9±0.4	3.0±0.7	3.3±0.7	3.4±0.3	3.7±0.5	3.0±0.6	4.3±0.5	0.003
	Asandabo	3.5±0.3	3.1±0.5	3.2±0.4	3.3±0.5	3.4±0.6	3.2±0.6	4.9±0.5	
	Nadda	3.3±0.2	3.4±0.6	3.1±0.6	3.6±0.4	3.5±0.4	3.1±0.6	4.6±0.7	
	Beyam	3.0±0.6	3.7±0.4	3.5±0.3	3.5±0.4	3.9±0.5	3.3±0.6	3.5±0.6	
	Average	3.4±0.4	3.3±0.6	3.3±0.5	3.5±0.4	3.6±0.5	3.2±0.6	4.3±0.6	
Baby king	Jimma	4.4±0.7	3.7±0.2	3.2±0.4	2.9±0.6	4.1±0.4	3.1±0.4	4.2±0.6	0.005
	Asandabo	4.2±0.5	3.5±0.8	3.0±0.6	2.4±0.7	3.9±0.4	3.0±0.5	3.0±0.6	
	Nadda	4.0±0.4	3.9±0.4	3.0±0.7	2.8±0.6	3.6±0.7	3.4±0.4	3.4±0.4	
	Beyam	4.5±0.3	3.1±0.6	3.6±0.6	3.2±0.8	4.3±0.3	2.9±0.3	3.9±0.7	
	Average	4.3±0.5	3.6±0.5	3.1±0.6	2.8±0.7	4.0±0.5	3.1±0.4	3.8±0.6	
Cheese	Jimma	6.8±0.5	6.2±0.3	4.1±0.3	2.7±0.5	6.4±0.2	3.8±0.7	5.5±0.2	
	Asandabo	7.0±0.4	6.3±0.3	4.4±0.7	3.3±0.5	6.6±0.3	3.5±0.5	5.6±0.3	0.005
	Nadda	7.2±0.3	6.0±0.5	4.2±0.4	3.5±0.6	6.2±0.7	3.4±0.6	4.1±0.3	
	Beyam	6.3±0.6	5.4±0.3	4.1±0.6	3.1±0.3	6.7±0.6	3.1±0.6	5.7±0.3	
	Average	6.8±0.5	6.0±0.4	4.2±0.5	3.2±0.5	6.5±0.5	3.5±0.6	5.2±0.2	
Firfir	Jimma	6.3±0.4	6.6±0.4	5.0±0.3	4.2±0.7	5.8±0.5	4.0±0.7	3.9±0.6	0.004
	Asandabo	7.3±0.3	6.5±0.6	5.5±0.7	3.9±0.4	5.5±0.6	4.3±0.3	5.8±0.6	
	Nadda	7.5±0.3	6.9±0.3	5.3±0.5	5.1±0.4	6.7±0.4	3.8±0.5	5.4±0.6	
	Beyam	7.0±0.2	6.2±0.6	4.9±0.5	3.9±0.4	6.5±0.6	3.8±0.5	4.9±0.6	
	Average	7.0±0.3	6.6±0.5	5.2±0.5	4.2±0.5	6.1±0.5	4.0±0.5	5.0±0.6	

Appendix4 One way ANOVA result among different microbial groups founds in different Weaning foods and, Jimma zone, 2019

ANOVA Table

		Sum of Squares	Df	Mean Square	F	Sig.
AMB	Between Groups	9.473	4	2.37	76.452	.00
	Within Groups	.276	9	.031		
	Total	9.749	13			
Entero.	Between Groups	6.376	4	1.6	32.483	.00
	Within Groups	.487	9	.05		
	Total	6.863	13			
Coliform	Between Groups	7.947	4	2.00	40.123	.00
	Within Groups	.436	9	.05		
	Total	8.383	13			
ABS	Between Groups	.495	4	.12	3.568	.005
	Within Groups	.326	9	.04		
	Total	.821	13			
Staphylococci	Between Groups	8.567	4	2.14	26.751	.004
	Within Groups	.743	9	.08		
	Total	9.31	13			
Yeast	Between Groups	15.678	4	5.23	87.167	.470
	Within Groups	0.555	9	0.06		
	Total	16.233	13			

Appendix5 John's bacterial identification

Gram reaction (young culture)	+	+	+	+	+	-	+	+	+	+	–	–	–	–	–
Shape	CCl	CCl	CCh	CT	Rod	Rod	IR	rod	rod	rod	rod	rod	rod	Rod	Rod
Aerobic growth	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Anaerobic growth	-	+	+	+	+	-	-	+	+	-	-	-	+	+	+
Endospore	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
Motility	-	-	-	-	-	-	-	+/-	+/-	+/-	+/-	+/-	-	+	+
Catalase reaction	+	+	-	-	-	+	+	-	+	+	+	+	+	+	+
Oxidase reaction	+	-	-	-	-	-	-	-	+/-	+/-	+/-	+/-	-	-	+
OF Test	-	+	+	+	+	-	-	+	+	-	+	-	+	+	+
Micrococcus	X														
Staphylococcus		X													
Streptococcus			x												
Lactococcus			x												
Enterococcus			x												
Leuconostoc			x												
Pediococcus			x	X											
Aerococcus				X											
Lactobacillus					X										
Acinetobacter						X									
Arthrobacter							x								
Clostridium								x							
Bacillus									x	x					
Alcaligenes											x				
Pseudomonas												x			
Klebsiella													x		
Shigella													x		
Salmonella														X	
Escherichia														X	
Other enteric Genera														X	
Aeromona															X

Where; CCl= Coccus (clusters), CCh =Coccus (chains), CT= Coccus (tetrad), IR= Irregular Rod

Declaration

I 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.

Gennale Dida Dugo ____________________ __________________

Signature Date

The work has been done under supervision

Name	Signature	Date
1. Ketema Bacha (PhD,Prof)	____________________	__________________
2. Lata Lachisa (MSc.)	____________________	__________________