Jimma University

College of Natural Sciences

Department of Biology

Microbiological Safety and Quality of homemade foods among Jimma University Community Primary School Students, Jimma town, Southwest Ethiopia

By

Tadele Abebe

A Thesis paper Submitted to 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)

October, 2013

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Advisor: Ketema Bacha (PhD) **signature_____**

Tsige Ketema (Asst. Prof) **signature_____**

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LIST OF ACRONYMS

ACFDP -Advisory Committee for Food and Dairy Products

ANZFA –Australia New Zealand Food Authority

FSAI- Food Safety Authority of Ireland

KABP-Knowledge, Attitudes, Beliefs and Practices

NIAID-National Institute of Allergy and Infectious Diseases

NIH -National Institutes of Health

NSW-New South Wales food authority guide line

USDA-ERS-United States Department of Agriculture Economic Research Service

VTEC- Verocytotoxin producing *E. coli*

USDHHS -United States Department of Health and Human Services

WHO- world health organization

XLD-Xylose Lysine Deoxycholate Agar

ABSTRACT

This study assessed the microbiological quality and safety of various ready-to-eat foods of community school students prepared at home. Microbiological analysis was conducted on 170 samples which included vegetables 18(10.58%), rice 24(14.11%), spaghetti 17(10%), firfir76 (44.70) %), legumes 26(15.29%), and meat 9(5.29%).The isolates were identified following the standard microbiological methods and data was analyzed using the one-way-ANOVA test.

Bacterial growth was present in all the food types evaluated , high mean total aerobic count were observed in meat 5.44 log CFUg^{-1}followed by vegetables, 5.27log CFUg^{-}1while rice had the lowest count(4.03logCFUg^{-1}).Bacillus(42.58%), S.aureus (15.18%),Pseudomonas,(3.79%),Micrococcus(22.41%),Entrococus(12.5%), Aeromonas (1.33%), Alcaligens (1.61%) and Acintobactor (0.56%), were microbial isolates included in this study. Fortunately, Salmonella spp. were not isolated from spaghetti, meat and rice food samples. The results indicated that most of the ready-to-eat food samples examined in this study did not meet the NSW, 2009 bacteriological quality standards, therefore posing potential risks to students. This should draw attention of the parents, the school administrators and others responsible personnels to ensure that food quality and safety standards should improved to curtain foodborne infections.

Keywords: contaminated foods, food safety, Hazards, pathogens, ready-to-eat foods,

1. INTRODUCTION

Food borne illness is usually either infectious or toxic in nature, and caused by agents that enter the body through the ingestion of food. Therefore they are responsible for high levels of morbidity and mortality in the general population, but particularly for at-risk groups, such as infants and young children, elderly and immunocompromised. Cases of foodborne diseases (FBDs) occur daily throughout the world, from the most to the least developed countries. The consumption of foods contaminated by foodborne pathogenic microorganisms and toxins produced by the microorganisms causes deaths, illnesses, hospitalization, and economic losses. In industrialized countries the percentage of the population suffering from FBDs each year has been reported to be up to 30% (WHO, 2007).

The global incidence of food borne illnesses is difficult to estimate but it has been reported that in 2000 alone 2.1 million people died from diarrheal diseases. A great proportion of these cases can be attributed to contamination of food and drinking water (Oranusi, *et al.*, 2013)

Homemade foods are often prepared at home and has a direct contact with a food makers hand, and this may lead to an increased incidence of contamination with potential food borne pathogens, such as *Staphylococcus* spp. (Colombari,*et al*., 2007).

The microbial load and the presence of the bacterial pathogens in foods are a good indication of the food quality and the potential health risk they pose to consumers. Escherichia *coli* O157:H7 and *Salmonella* spp. are among the most dangerous food borne bacterial pathogens in terms of human health and disease (Hosein,*etal*, 2008)

According to one report from the United States Department of Agriculture Economic Research Service (USDA-ERS) "Food borne illnesses account for about 1 of every 100 U.S. hospitalizations and 1 of every 500 deaths" (Buzby, *et al.,* 2001).Another study conducted by Roberts (2007) estimates that social costs of all acute food borne illness are a total of U$1.4 trillion.

The basic human requirement for the intake of food places every human being at risk of containing infection by food-borne pathogens. This fact is true not only in developing

countries but in many developed countries. The use of unhygienic utensils and materials, consumption of raw or unsafe food, as well as cross-contamination via inanimate surfaces by raw food , are some of the factors and practices that have been implicated in food-borne outbreaks(Taulo, *etal.*, 2008).

As reviewed by sudershan, *etal.,(*2009), Food borne illnesses are a widespread public health problem globally. Particularly Developing countries bear the burden of the problem due to the presence of a wide range of food-borne diseases In India an estimated 400,000 children below five years age die each year due to diarrhea. Several millions more suffer from multiple episodes of diarrhea and still others fall ill on account of hepatitis A, enteric fever, etc. caused by poor hygiene and unsafe drinking water (sudershan, *etal.*,2009). According to this review Health and nutrition are both dependant on the wholesomeness of food and its freedom from microbial and chemical contamination, as well as on its adequacy with respect to quantity and nutritive value (Sudershan, *etal.*, 2009).

On the other hand, 9.4 million people are sickened and 1350 deaths occur each year in the United States due to 31 major pathogens that contaminate food. The relative numbers of illnesses due to microorganisms makes microbiological quality to be the most important aspect of food safety. Thus, food safety primarily focuses on the control of contamination of foods by pathogens (Hanning, *etal.*, 2012).

Numerous studies in developing countries have shown that homemade foods prepared under unhygienic conditions are heavily contaminated with pathogenic agents and are a major risk factor in the transmission of diseases, especially diarrhea. It is generally recognized that contamination of foods may occur as a result of poor hygiene of food handlers, household equipments and the environment where the preparation of food takes place (Muhimbula and Issa-Zacharia, 2010).

Bacteria are the most common food poisoning agents. More than 90 percent of the cases of food poisoning each year are caused by *Staphylococcus aureus, Salmonella spp, Clostridium perfringens, Campylobacter spp, Listeriamonocytogenes, Vibrio parahaemolyticus, Bacillus cereus,* and Entero-pathogenic *Escherichia coli*. Total coliform counts (TC) and *E. coli* are used as hygiene quality parameters. Because , the presence of *E. coli* in foods is an indicator of direct or indirect fecal contamination. It is also an indicator of

the possible presence of enteric pathogens. Each year, millions of individuals become ill from food borne diseases. Thus use of treated waste water for irrigation, poor personal hygiene, improper cleaning of storage and preparation areas and unclean utensils are contributors for food contamination (Khiyami,*et al.*, 2011).

As the opinion of the ACFDP (Advisory Committee for Food and Dairy Products) that ready-to-eat foods should be free from *Salmonella* spp, *Campylobacter* spp, and *E. coli* O157 and other Verocytotoxin producing *E. coli* (VTEC). Appropriate control measures during production, adequate hygiene standards, and appropriate cooking during final preparation should ensure that the end products are free from viable organisms and that the foods are therefore of good quality. Gilbert *et al.*, 2000 mentioned that Ready-to-eat foods containing salmonellas or other pathogens may not always cause illness but there is good microbiological and epidemiological evidence that small numbers of pathogens in foods have caused illness. The ACFDP takes the view that there is no justification for processed ready-to-eat foods being contaminated with these organisms and that their presence, even in small numbers, results in such foods being of unacceptable quality/potentially hazardous.

As mentioned by Cairncross and Curties (2003) diarrheal diseases are the second most common global illness affecting young children and a major cause of death in lower income countries, moreover they are closely linked with poor sanitation, poor hygiene, and lack of access to safe and sufficient supplies of water and food. Each year, nearly two million children under the age of five die of diarrheal diseases caused by unsafe water supplies, sanitation, and hygiene. Interventions such as simple hand washing have been shown to reduce sickness from diarrheal diseases by up to 47%, and could save up to one million lives.

In most cases parents might give attention only to the availability of food but not to its safety. As a result, those children may easily be threatened by foodborne diseases of the enteric pathogens and other disease causing agents which contaminate the food. In most developing countries including Ethiopia, sufficient statistics on food borne diseases are not available due to poor or nonexistent reporting systems (Kinfe and Abera 2007).

Therefore studying the microbial load of the homemade foods and the presence of these pathogens among the tested food could have a great importance in understanding the health

risk of the children. However, no study so far has been conducted on the microbial quality of the food prepared by parents of Jimma University Community Primary School students. Thus, the present study was carried out to assess the microbiological quality of various ready-to-eat foods from randomly selected students at Jimma University Community School.

2. OBJECTIVES OF THE STUDY

2.1. General objective:

- To evaluate the microbial safety and quality of homemade food and anti microbial susceptibility of the isolates among Jimma University Community Primary School students

2.2. Specific objectives:

- To determine the microbial load of homemade food and brought by students to school.
- To isolate and characterize the microbial flora of the food samples to the genus level and various bacterial groups
- To assess the hygienic practices of the parents of the student and sanitary qualities of the school feeding rooms.
- To evaluate the growth potential of food borne pathogens isolated from food samples on some traditional sauces
- To determine antibiotic susceptibility patterns of potentially pathogenic isolates

3. LITERATURE REVIEW

3.1. Food Safety

Access to safe food is basic human rights despite many foods are frequently contaminated with naturally occurring pathogenic microorganisms. Such pathogens cannot be detected organoleptically (seen, smelled or tasted), but can cause disease of varying severity, including death specially if the way they are conserved provides conditions for those microorganisms to grow and reach considerable levels of contamination. Thus, food safety issues are of major importance to world health (Oranusi, *et al*., 2013)

The recent study states that Food safety and food security are interrelated concepts with a profound impact on quality of human life, and there are many external factors that affect both of these areas. Food safety is an umbrella term that encompasses many facets of handling, preparation and storage of food to prevent illness and injury. On the other hand food security is referring to food availability and proper utilization of the food. Pathogenic bacteria, viruses and toxins produced by microorganisms are all possible contaminants of food and impact food safety (Hanning, *etal.*, 2012).

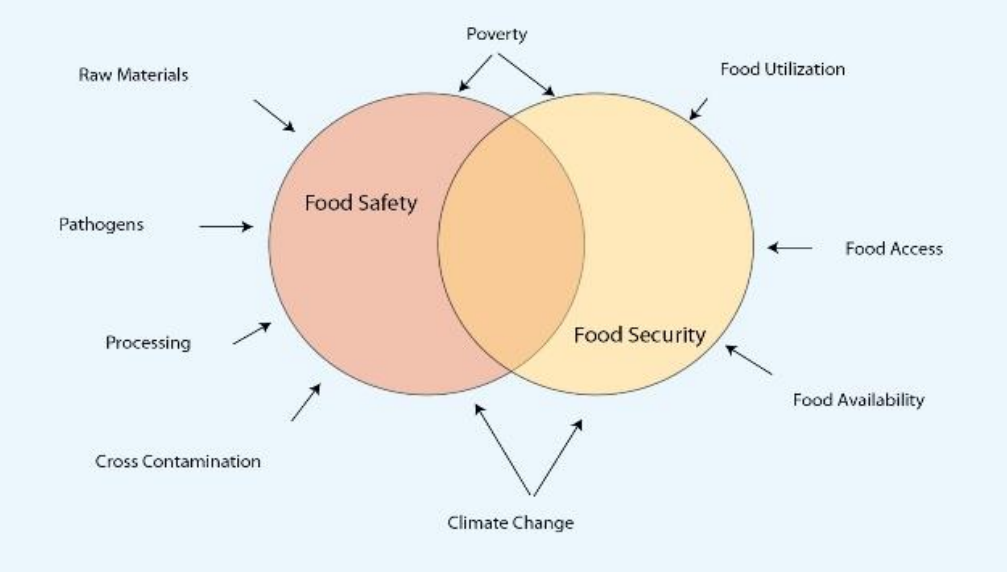

Figure 1: Interrelationship of food safety and food security (Hanning, *etal.*, 2012)

Many human illnesses are food-related. Nutritional status and economic well-being are affected by food carrying pathogenic organisms and their toxins. It is estimated that approximately 3 million children below the age of five die of diarrhea every year. About 70% of these deaths have food-borne origin. Many food products are easily contaminated when produced in an unhealthy and unclean environment (Sudershan, *etal.,* 2009).

3.2. Food safety knowledge

According to Hailessilsse, *etal.*, (2011) because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipments, and lack of education for food-handlers Food borne diseases are common in developing countries including Ethiopia.

As National Hygiene and Sanitation Strategy program report, about 60% of the disease burden was related to poor hygiene and sanitation in Ethiopia. Unsafe sources, contaminated raw food items, improper food storage, poor personal hygiene during food preparation, inadequate cooling and reheating of food items and a prolonged time lapse between preparing and consuming food items were mentioned as contributing factors for outbreak of food borne diseases (Hailessilsse,*et al.*,2011).

Most foodborne diseases are preventable with proper food preparation and handling. There for it is important to educate school children and food handlers for taking specific measures to make food and water safe. Educating school children about food safety gives them knowledge to be selective about the foods they choose to eat. Young children in particular should therefore be taught basic rules of food safety such as washing hands before eating (WHO, 2000).

Many program planners believe that by enhancing knowledge or altering attitudes, they can induce behavioral change, but education alone may not result in behavioral change, and that to change most complex behaviors, multifaceted approaches are needed (Patah, *etal.,* 2009).

3.3. Sources of contamination of homemade foods

Infants and young children are very susceptible to food borne diseases and if they consume contaminated foods, they are likely to contact infections or intoxications leading to illness and often death. It is generally recognized that the possible source of contamination of foods occur as a result of poor hygiene of food handlers, household equipments and the environment where the preparation of food takes place (Sheth and Dwivedi, 2006).

As the study conducted in almost all regions of Tanzania and Zanzibar indicated water is often contaminated and this water is used for making foods (Kung'u *et al.,* 2009).

Statistics showed that Poor personal hygiene causes more than 90% of the food safety problems and improper hand washing alone accounts for more than 25% of all food borne illnesses (Patah,*etal.*, 2009).

3.4. Cross contamination

The transfer of germs from one food items to another is called cross contamination. Approximately 10 to 20% of food-borne disease outbreaks are due to contamination by the food handler (Zain and Naiming, 2002).

The common cause of cross contamination in the kitchen is because of contaminated hands and equipments used to prepare cooked and raw food at the same time. Cross contamination can also occur when uncovered raw foods are stored directly adjacent to or above ready-to-eat foods in a refrigerator or other holding equipments. In a recent review, cross-contamination was identified as an important contributory factor in 36.3% (147/405) outbreaks of food-borne disease (Patah, *et al.*, 2009).

3.5. Consumer behavior and food handling practices

There are limited studies carried out on the Knowledge, Attitudes, Beliefs and Practices (KABP) of people on food safety issues. A study carried out to assess the attitudes and practices regarding diarrhea in a rural community of India revealed that a majority of the mothers were not aware of the precautionary measures to be taken to prevent diarrhea. A striking observation during the study showed that the prevalence of diarrhea was high (23%)

among pre-school children in spite of access to safe drinking water, availability of toilet facilities in most of the households and existence of a strong health infrastructure (Sudershan, *etal.,* 2009).

Studies conducted in United Kingdom, Northern Ireland and in the United States reported that as compared with women, men are less knowledgeable about food safety and have riskier hygiene and cooking practices (Redmond, 2003).

3.6. Transmission of Food borne Diseases

Poor sanitary practices in food storage, handling, and preparation can create an environment in which bacteria such as *campylobacter, salmonella,* and other infectious agents are more easily transmitted. Moreover, transmission of intestinal parasites and enteropathogenic bacteria is affected directly or indirectly through objects contaminated with faeces. These include food, water, nails, and fingers, indicating the importance of faecal-oral, human-to-human transmission (Murat, *et al.,* 2006).

Accordingly, food-handlers with poor personal hygiene working in food serving establishments could be potential sources of infections of many intestinal helminthes, protozoa, and enteropathogenic bacteria. Food-handlers who harbour and excrete intestinal parasites and enteropathogenic bacteria may contaminate foods from their faeces via their fingers, then to food processing, and finally to healthy individuals (Gashaw,*et al.,* 2008).
In some instances, transmission occurs through close contact between infected and uninfected individuals as in infected food handlers and consumers, respectively (Donato,*et al.,* 2003).

3.7. Categories of microbiological quality

As explained by the Guidelines for the microbiological examination of ready - to - eat foods four categories of microbiological quality have been assigned based on standard plate counts, levels of indicator organisms and the number or presence of pathogens. These are satisfactory, marginal, unsatisfactory and potentially hazardous (NSW, 2009).

- **Satisfactory: -** results indicate good microbiological quality. No action required.

- **Marginal: -** results are border line in that they are within limits of acceptable microbiological quality but may indicate possible hygiene problems in the preparation of the food.

  Action: Re-sampling may be appropriate. Premises that regularly yield borderline results should have their food handling controls investigated.
- **Unsatisfactory: -** Results are outside of acceptable microbiological limits and are indicative of poor hygiene or poor food handling practices.

Action: Further sampling, including the sampling of other foods from the food premise may be required and an investigation undertaken to determine whether food handling controls and hygiene practices are adequate.

- **Potentially Hazardous: -** the levels in this range may cause food borne illness and immediate remedial action should be initiated.

Action: Consideration should be given to the withdrawal of any of the food still available for sale or distribution and, if applicable, recall action may be indicated. An investigation of food production or handling practices should be investigated to determine the source/cause of the problem so that remedial actions can commence.

Table 1. Guidelines for determining the microbiological quality of ready-to-eat foods (NSW. 2009)

	Satisfactory	Marginal	nsatisfactory	Potentially Hazardous
Standard Plate Count Level	$< 10^4$ CFU/g	10^4- 10^5 CFU/g	$\geq 10^5$CFU/g	
Indicators				
Enterobacteriaceae	$< 10^2$ CFU/g	$10^2 – 10^4$ CFU/g	$\geq 10^4$ CFU/g	
Escherichia coli	< 3 CFU/g	3 - 100 CFU/g	≥ 100 CFU/g	**
Bacillus cereus	$< 10^2$ CFU/g	$10^2 – 10^3$ CFU/g	$10^3 – 10^4$ CFU/g	$\geq 10^4$ CFU/g
Salmonella	not detected in 25g			detected

**Pathogenic strains of *E. coli* should be absent

3.8. Food quality/safety indicator tests

3.8.1. Aerobic Plate Count

The aerobic plate count (APC) or standard plate count (SPC) is important in food microbiology as an indicator of the microbiological quality as well as a measure of sanitation used during handling of a food. APC determines counts of the non-fastidious aerobic bacteria. In some foods, high APC may indicate poor quality. Higher bacterial numbers spoil the food faster and result in loss of quality. Food which appears normal may have high APC, indicating that the food is about to spoil (Jacob, 2010).

Although APC is a fast and efficient method to test the microbiological quality of the food, the test has some limitations (Yousef and Carlstrom, 2003). Fermented foods (e.g. cheddar

cheese) naturally contain a high microbial load, and in consequence APC cannot be used to evaluate their general microbiological quality. In addition, the plating medium does not support the growth of fastidious microorganisms, which result in an under representation of these microorganisms in the APC. Moreover, incubation conditions favor growth of mesophilic aerobic bacteria, and other categories such as strict anaerobes are ignored. Standards for quality indicator tests for the various foods available are very limited. There are different acceptance levels of APC for different food products. For example, sliced cooked ham has a suggested level of $< 10^6$ CFU/g as a satisfactory level, 10^6 - 10^7 CFU/g as an acceptable level and $\geq 10^7$ CFU/g as unsatisfactory level (Gilbert, *et al*., 2000).

3.8.2. Coliforms Count

The term coliform does not have taxonomic value. It represents a group of species from several bacteria namely, *Escherichia, Enterobacter, Klebsiella, Citrobacter,* and probably *Aeromonas* and *Serratia* (Feng, *et al.,* 2001). The main reason for grouping them together is their many common characteristics. They are all gram-negative, non spore forming rods; many are motile, are facultative anaerobes resistant to many surface-active agents, and ferment lactose to produce acid and gas within 48h at 32 or 35^{o}C (Yousef and Carlstrom, 2003). Some species can grow at higher temperature (44.5^{o}C), while others can grow at 4 to 5^{o}C. All species are able to grow in foods except those that are at pH $\leq$ 4.0 and water activity $\leq$ 0.92; they are sensitive to low-heat treatments and are killed by pasteurization (Yousef and Carlstrom, 2003). Since coliforms are common inhabitants of the intestinal tract, their presence in food may indicate fecal contamination. In some plants foods, they are present in very high numbers because of contamination from soil. The specificity of coliforms as an indicator of fecal contamination for raw foods is reduced since large numbers of coliforms in the food may result from growth of small non fecal inoculums. In contrast, in heat-processed (pasteurized) food products, their presence is considered as an indicator of post heat-treatment contamination from improper sanitation. Therefore, in heat-processed foods, their presence, even in small numbers, is considered more as an indicator of improper sanitation than fecal contamination (Ray, 2004).

3.8.3. *Enterobacteriaceae*

In ready-to-eat foods that are fully cooked, Enterobacteriaceae are used as an indication of either post-processing contamination or inadequate cooking. As they can be found in raw foods, their detection may not be an indication of any processing failure. As such it would be inappropriate to test ready-to-eat foods containing raw components (particularly fresh fruits and vegetables) for *Enterobacteriaceae* (NSW 2009).

3.8.4. *Staphylococcus aureus*

S.aureus is a gram-positive cocci, occur generally in bunches, and are nonmotile, noncapsular and nonsporulating (Tatini, 1973). *S. aureus* are facultative anaerobes, but grow rapidly under aerobic conditions. Most strains ferment Mannitol and produce coagulase. They are mesophiles with a growth temperature range of 7 to 48ºC, with fairly optimal growth between 20 and 37ºC. They can grow at relatively low water activity (0.86), low pH (4.8), and high salt and sugar concentrations up to 15%. Because of their ability to grow under several conditions, *S. aureus* can grow in many foods (Ray, 2004). Enterotoxin-producing *S.aureus* strains have generally been associated with *staphylococcal* food intoxication. Although strains of several other *Staphylococcus* species are known to be enterotoxins producers, their involvement in food poisoning is not fully known. *S.intermedius* is the only non-*S.aureus* species that has been clearly involved in a *staphylococcal* food poisoning outbreak (Khambaty, *et al.*, 1994).

S. aureus, along with many other *staphylococci*, are naturally present in the nose, throat, skin, and hair of healthy humans, animals, and feather of birds. *S. aureus* can be present in infections, such as cuts in skin and abscesses in humans, animals and birds. Food contamination generally occurs from these sources (Tatini, 1973).
From a study conducted by Mead, *et al.*, (1999), it is estimated that in the United States *staphylococcus* food poisoning causes approximately 185,000 illnesses every year, it was the fifth highest number of estimated cases among 19 known bacterial foodborne pathogens.

Many different foods can provide nutrients that allow growth of *S. aureus*, and have been implicated in staphylococcal food poisoning, including milk, cheeses, salads, cooked meals and sandwich fillings (Loir, *et al.*, 2003). In most of the cases, the main sources of

contamination are improper handling of food by contaminated hands from humans or when they cough or sneeze, and contamination occurs after heat treatment of the food (Loir, *et al.*, 2003).

*Staphylococca*l toxins are enteric toxins that cause gastroenteritis (Halpin-Dohnalek and Marth, 1989). The symptoms of *staphylococcal* food poisoning are abdominal cramps, salivation, nausea and vomiting, sometimes followed by diarrhea (Ray, 2004). The onset of symptoms is rapid varying from 30 minute to 8 hours and it last for less than 24 h (Loir, *et al.*, 2003).

4. Methods and materials

4.1. Description of Study Site

The study was conducted at Jimma town, Jimma University Community School, located at 353 km southwest of Addis Ababa. The study area has an average altitude of 1, 780 m above sea level. As a part of Jimma the study site has a mean annual maximum temperature of 30°C and a mean annual minimum temperature of 14 °C (Alemu *et al.*, 2011). The annual rainfall ranges from 1138-1690 mm. From a climatic point of view, abundant rain fall makes Jimma to be one of the best watered of Ethiopian high land areas, conducive for agricultural production (Alemu *et al.*, 2011).

4.2. Study design and population

Cross sectional study design was used. The total population of Jimma University Community Primary School first cycle students were 351 of these 307 brought their lunch frequently. So, the sample size was calculated by using Cochran (1977) formula .Accordingly, a total of 170 samples were included in the study. That means:

$$n = \frac{n_0}{1 + \frac{n_0}{N}} \quad \textit{WHERE} \; n_0 = \frac{Z^2_{\alpha/2} p(1-p)}{d^2}$$

d= margin of error

N = total number of the population

p= proportion of population

α= level of significance

d= 0.05, p = 0.5 and α = 0.05

$$n_0 = \frac{(1.96)^2 \times 0.5 \times 0.5}{(0.05)^2} = 384$$

Considering the population correction factor into account the sample size were:

$$n = \frac{384}{1 + \frac{384}{307}} \approx 170.$$

4.3. Sampling technique

A systematic random sampling technique was used to address representatives of each grade (grade 1 to grade 4) of Jimma University Community primary School first cycle students. By using proportional calculation the appropriate sample were taken from the strata.

4.4. Data collection

To examine the academic and the monthly income of students parents, a well organized questionnaires' were sent to them and other Socio-demographic characteristics were collected from the students using structured questionnaire during the time of sample collection.

4.5. Sample collection

A total of 170 samples were collected from Jimma University community school students between March, 2013–June, 2013. The samples were consisted of 76 firfir, 17 spaghetti, 9 meat, 26 legume, 24 rice and 18 vegetables. All samples were collected aseptically and immediately brought to the Microbiology laboratory Department of Biology, Jimma University for analysis. Microbiological analysis was conducted within an hour of sample collection. The food samples were kept in refrigerator at 4 ^{0}C until microbial analysis was conducted.

4.6. Microbiological analyses:

4.6.1. Sample preparation and Microbial Enumeration

Twenty five grams of each sample was weighed and homogenized by blending in 225 mL of sterile buffered peptone water. One milliliter of the homogenate was introduced into 9 mL of the buffered peptone water in a test tube, labelled 1:10 dilution and serially diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. One hundred microliters of each of the diluted sample was spread-plated on a pre-sterilized and surface dried agar plates. Accordingly, appropriate dilutions were inoculated on PCA plates for the count aerobic mesophilic bacteria. The plates were incubated aerobically for 24 h at 37 °C. All discrete colonies were counted and expressed as the log10 of colony forming units per gram (CFU g^{-1}) (Weil, *et al*., 2006).

For count of *Entrobacteriacea*, macconkey Agar (Oxoid) was used and plates were incubated at 37°C for 24 hours. All purple colonies were counted as members of *Entrobacteriacea* (Spencer,*et al*., 2007).

Coliforms were counted on Violet Red Bile Agar (Oxoid) after incubating plates at 37°C for 24 hours. Red to pink colonies, surrounded by precipitated bile, was counted as coliforms (Weil *et al*., 2006).

For count of *staphylococci*, Mannitol Salt Agar (Oxoid) was surface plated and incubated at 37°C for 36 hours. Yellow colonies were counted as *staphylococci* (Acco, *et al*., 2003).

Bacterial spores were counted after heating the suspension for ten minutes in water bath (80°C) and spread-plating 0.1 ml of appropriate dilutions on the pre dried surface of PC plates. Colonies were counted after incubation at 37° C for 24 hours (Acco, *et al*., 2003).

From appropriate dilutions, 0.1 ml of the aliquot was spread plated on de Mann Rogosa Sharpe (MRS) agar media and incubated at 37 °C for 48 hrs under anaerobic condition using anaerobic jar, and the colonies are counted as lactic acid bacteria (Patra, 2011).

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

4.6. Microbial Identification

The identification of a bacterial species is based on many factors, including cell colony, morphology, Chemical composition of cell walls, biochemical activities, and nutritional requirements (Christopher and Bruno, 2003). In this study10 to 15 colonies with distinct morphological differences were randomly picked from countable plates and Cultures were purified by repeated plating and organisms were identified depending up on their shape, cell arrangement, gram staining, endospores staining, and biochemical tests.

4.6.1. Cell morphology

In order to assess the cell morphology of the pure culture, gram staining and wet mount were used. The morphological study includes cell shape, cell arrangement, presence or absence of endospores and motility.

4.6.2. Gram staining

A smear of pure isolates were prepared on a clean slide and allowed to air-dry and heat-fix. The heat fixed smear was flooded with crystal violet dye for 1 minute and rinsed under tap water for 3 seconds. Then, the slide was flooded with iodine solution for 1 minute and rinsed under tap water for 3 seconds. After rinsing, the smear was decolorized with 95% of ethanol for 20 seconds and rinsed slides gently under tap water for 3 seconds. Thereafter, the smear was counter stained by safranin and 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 were stained pink/red and gram-positive bacteria were stained blue/purple (Elmanama, 2009).

4.6.3. 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 incubated at 35°C for 24 hours. A positive motility test is indicated by a red turbid area diffusing away from the line of inoculation and a negative test was indicated by red growth along the inoculation line only but no further (Shields and Cathcart, 2012).

4.6.4. Endospore test

Endospore test was done according to Schaeffer and Fulton (1933) method. A smear of isolates were prepared on a clean glass slide and allowed to air-dry. The air-dried smear is 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 rinsed with tap water and counterstained with safranin for 30 seconds. The slide was rinsed

with tap water and air-dried; finally under the oil immersion lens (100X) the presence of endospores was examined. Endospores are bright green and vegetative cells are brownish red to pink

4.6.5. KOH Test

Two drops of 3% KOH solution was placed on a clean microscopic slide. A colony was aseptically picked from the surface of PCA using an inculcating loop and stirred in the KOH solution for 10 seconds to 2 minutes. The inoculating loop was rose 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. If there was no slime, but a watery suspension that do not follow the loop, the reaction was considered negative and the isolate was considered as gram positive bacteria (Gregerson, 1978).

4.6.6. Oxidation fermentation test (O/F)

This test is used to assess the ability of the isolate to utilize glucose and determine the metabolic way that is fermentation or oxidation. Ingredients (g/l): Peptone, 2g; yeast extract, 1 g; NaCl, 5g; K_2HPO_4, 0.2g; 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 was immediately cooled under tap water to avoid dissolution of oxygen in the medium. Then, the broth cultures were inoculated into the medium by stabbing with a sterile straight wire to the bottom. Acid formation and growth regions were interpreted after 2 to 5 days of incubation at 32 ^oC (Hugh and Leifson, 1953).

4.6.7. Catalase test

Catalase test was carried out by flooding young colonies with a 3% solution of hydrogen peroxide (H_2O_2). The formation of bubbles indicates the presence of catalase (McFadden, 1980).

4.6.8. Cytochrome oxidase test

This test was conducted using the method out lined by Kovacs (1956). Accordingly, freshly prepared reagent A and B will be mixed in the ratio of 2:3 immediately before use. Reagents: A, 1% naphthalene absolute ethanol, B, 1% N, N – dimethyl –p- phenylenedi ammonium chloride in distilled water. Three drops of the oxidase reagent was added on to the surface of the freshly drown colonies of test bacterium. The appearance of a blue color on the colonies was observed within 30 seconds. Blue Color appeared in the presence of oxidase enzyme.

4.7. Isolation of Salmonella Spp.

For the detection of *Salmonella* spp., 25g of food samples was weighed and mixed with 225 ml of BPW and incubated at 37 °C for 24 hrs. Then after, 1 ml pre-enrichment broth culture was added to 10 ml of selenite cystein broth (Oxoid) and again incubated at 37 ^{o}C for 24 hrs. Thereafter, a loopful of suspension from a tube was streaked onto Xylose Lysine Deoxycholate Agar (XLD) (Oxoid). The presumptive *Salmonella* colonies (black colony surrounded by red color) was picked and transferred to 5 ml nutrient broth (Oxoid) and incubated at 37 ^{o}C for 24 hrs followed by streaking onto Nutrient Agar (Oxoid) for purity and incubated at 37 °C for 24 hrs (Arvanitidou, *et al*., 2005). The presumptive *Salmonella* was characterized by standard biochemical test. The biochemical testes were done according to the procedure of Johnson and Case (2007).

4.7. 1. Triple Sugar Iron Agar (Oxoid)

The butt was stabbed and the slant was streak 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.

4.7.2. Lysine Iron Agar (Oxoid)

The butt was stabbed and the slant was streak and incubated at 37 ^{o}C for 24 hrs. *Salmonella* produces the enzyme lysine decarboxylase that produces an alkaline reaction (purple color)

throughout the medium. Due to the production of H_2S, an intense blackening of the medium was observed as a positive reaction for *Salmonella* spp.

4.7.3. Urea Agar (Oxoid)

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

4.7. 4. Simmons Citrate Agar (Oxoid)

The slant was streaked on Simmons Citrate Agar 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.

4.7. 5. Sulfide Indole Motility test medium (oxoid)

The SIM medium was stabbed to the bottom and incubated at 37 ^{o}C for 24 hrs for the determination of H_2S production and motility. Production of Indole was investigated by adding Kovac's reagent 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.

4.8. Isolation of staphylococcus aureus

After counting *staphylococci*, golden yellow colonies on MSA plates were aseptically picked and transferred into 5ml nutrient broth (Oxoid) and incubated at 37 ^{o}C for 24-36 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 were characterized using the established microbiological methods (Acco, *et al.*, 2003). Gram-positive cocci with clustered arrangement under the microscope were subjected to preliminary biochemical tests (oxidase, catalase and coagulase tests).

4.8.1. Coagulase test

Coagulase test was done according to Cheesbrough (2006) using slide test procedures. Accordingly, 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 as control. The control suspension was served to rule out false positivity due to auto-agglutination. A loopful of human blood plasma was added to one of the suspensions and mixed gently. Clumping within 10 seconds was observed for coagulase positive organisms.

4.9. Antibiotic sensitivity test

Antimicrobial susceptibility of 41 *S.aures spp.* and 13 *salmonella spp*. were carried out by disc diffusion method on Mueller-Hinton agar using commercial antibiotics. The results were 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*., 1966). McFarland is a Barium Sulphate standard against which the turbidity of the test and control inoculums was compared. This standard was prepared by mixing two solutions; solution A and solution B. Solution A is 1 % v/v solution of sulphuric acid (H_2SO_4) and solution B is 1 % w/v solution of barium chloride ($BaCl_2$). To get 0.5 McFarland standard, concentration equivalents to cell density of about 10^7- 10^8 $CFUg^{-1}$, 0.5 ml $BaCl_2$ 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 inocula. Culture containing test tube with approximately equal concentration or density with 0.5 McFarland standards. The standard was used immediately and stored in a well sealed container in a dark place at room temperature (20 - 28 ^oC). When matched with the standard, the inocula were swabbed by cotton swab onto the 90mm diameter plate starting at the top of the Mueller-Hinton agar plate and swabbed back and forth from edge to edge. To ensure the inoculum is evenly distributed. The plate was Rotated approximately 60° and the swabbing procedure was repeated for three times in similar fashioned and allowed to dry (Bauer, *et al*., 1966). Thereafter, the antibiotic discs were placed using forceps on the medium and incubated at 37°C for 18 hrs and the zones of inhibition were measured manually with a transparent ruler (NCCLS, 2007). The results of

the antimicrobial susceptibility were interpreted based on the guidance of National Committee for Clinical Laboratory Standards (NCCLS, 2007). Finally, the isolates were classified as sensitive, intermediate, or resistant. Intermediates were considered as resistant for purpose of analysis. The concentrations of Antibiotics for S.aures used were as follows: ampicilin (10 μg), amoxicillin (10 μg), ceftriaxone (30 μg), chloroanphinicol (30 μg), gentamycin (10 μg), kanamycin (30 μg), streptomycin (10 μg), and tetracycline (30 μg). The concentrations of Antibiotics for Salmonella spp. used were as follows: ampicilin (10 μg), amoxicillin (10 μg), ceftriaxone (30 μg), chloroanphinicol (30 μg), streptomycin (10 μg), and tetracycline (30 μg). *Salmonella typhimurium* (ATCC13311*)* and *S. aureus* (ATCC25923) were used as a control organism. (Khiyami,*et al*., 2011)

4.10. Growth potential of some food borne pathogens

The growth potential of *Salmonella typhimurium* (ATCC13311*), S.aureus* (ATCC25923) and *E. coli* (ATCC25922) were assessed in three different sauces (meat, shiro and cabbage) with injera by which the majority of the students consumed for their lunch. Three hundred grams of each food items was separately homogenized using food processor (NM-343.Nima LTD Osaka, Japan) and steamed at 80 ^{o}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 inoculums level of 10^2-10^3 CFUg^{-1}. The challenged food was incubated at 37^{o}C for 24 hrs. To determine the initial inoculum level, 10 g of each freshly inoculated food was homogenized in 90 ml of BPW and 0.1 ml of appropriate dilution was spread plated on XLD for *S.typhimurium*, 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, while assessing growth potential, the pH of each food sample was measured using digital pH meter from 0 hr to 24 hrs at an interval of 6 hrs (Muleta and Ashenafi, 2001)

4.11. Statistical analysis

Statistical analysis was performed by using SPSS software version 16.0. The bacterial counts were expressed as mean ± Standard deviation. Mean values of food samples from community school students were compared using one way ANOVA. The mean difference was considered significant at $p < 0.05$ or 95% confident interval.

4.12. Ethical consideration

Ethical clearance was obtained from Research and Ethical Review Committee of College of Natural science, Jimma University. Students and concerned school administrators were informed about the purpose of the study prior to data collection.

5. Result

5.1. Sociodemographic characteristics of the students

Sociodemographic characteristics of the students showed that, of the total 170 students, 97 (57.05%) were females and 73 (42.95) were males. The age of the student was found ranging from 5-10 years (Table2).

Table 2 .Sociodemographic characteristics of study population among Jimma University community school students. Jimma town south west Ethiopia, 2013

Character		Number of respondent	Percentage (%)
Grade	1	44	25.82
	2	42	24.70
	3	42	24.70
	4	42	24.70
Sex	male	73	42.94
	female	97	57.06
Age	5	31	18.23
	6	55	32.35
	7	23	13.53
	8	37	21.76
	9	22	12.94
	≥10	2	1.17

Nearly 77% of the students brought their lunch which were prepared by their mothers (78%) and 90.58% of the food were prepared early in the morning, 9.42% foods were prepared in the evening, of which 7.64%were stored in refrigerator while 1.78% were left on the shelves (Table 3). Educationally, none of the mothers of these students took a special

training related to food hygiene and safety, although the majorities of students mothers (35.29%) were trained to high school level followed by diploma (34.11%), and 20% of the mothers having first degree and above. The habit of wearing overcoat by all food makers was very poor although their habit of wearing hair cover and hand washing practices were very good (Table 3).Moreover,159 students use a metallic lunch box, while 11 uses a plastic lunch box (Table-4).

Table 3. Hygienic practice and educational status of food makers. Jimma town south west Ethiopia, 2013

Character	alternatives	Number of respondent	Percentage (%)
Food maker/handler	Mother	132	77.64
	Sister	11	6.47
	helper	27	15.88
When is the food prepared	At the evening	16	9.41
	in the morning	154	90.58
Educational status of food maker	elementary	18	10.58
	high school	60	35.29
	Diploma	58	34.11
	degree and above	34	20
Habit of Wearing overcoat	Yes	0	
	no	170	100
Habit of Wearing hair cover and hand washing	yes	170	100
	no	0	
Are nails of food makers short, trimmed and clean	Yes	41	24.11
	no	129	75.89
Occupation of the parents	Government employee	156	91.76
	Merchant	14	8.24
Monthly income of the parents	451-900	13	7.64
	901-1617	29	17.05
	1618-2500	46	27.05
	>2501	82	48.23

5.2. Physical Conditions of the Dining Room

Although the school dining room was in good condition, constructed of bricks/ceramics with smooth surfaced wall, it gives no service and students are obliged to use their class rooms for storage and tree shades and class rooms for dinning, Foods prepared in the evening were stored in the fridge (8%) and 2% uses their shelves for storing of the food, with complaints of absence of water for hand washing by majority (70%) of the respondents (Table.4).

Table 4. Physical conditions of storages environment at home and school. Jimma town, southwest Ethiopia, 2013

character	Physical condition	Number of respondent	Percentage (%)
Storage at home	Refrigerator	13	8
	shelf	3	2
Lunch box	Metallic	159	93.53
	plastic	11	6.47
Storage in the school	Store house	0	
	class room	170	100
Availability of Dining hall	Yes	0	
	no	170	100
Availability of hand washing facility for dining	yes	51	30
	no	119	70

5.3. Food makers' knowledge about food borne diseases and source of Information

School was the first source of information followed by mass media while information from health professionals was the least, accounting for 123 (72%), 29 (17%) and 18 (11%), respectively. As presented in Table 5, all respondents (100%,n=170) knows the major routes through which a food borne disease can be transmitted.

Table 5. Awareness on food borne disease among food handlers, Jimma University community school, Jimma town, Ethiopia, 2013

characteristics	Source	Number of respondent	Percentage (%)
source of information about food borne disease	• School	123	72.35
	• Health professionals	18	10.58
	• Mass Medias	29	17.05
Source of contamination and Transmission route of food borne disease	✓ Contaminated food	170	
	✓ Contaminated hand	170	100
	✓ Contaminated water	170	100
	✓ Vectors	170	100
	✓ Exposure to flies	170	100
	✓ contaminated environment	170	100
	✓ dirt equipment	170	100
	✓ Dirt hands	170	100
	✓ Using the same container for cooked and raw foods	170	100

5.4. Microbiological enumeration

The mean bacterial count of the isolates in the food samples were expressed as $\log_{10}$ CFU $g^{-1}$for easy computation. The mean value of aerobic bacterial count on firfir, spaghetti, rice, legume, vegetables and meat were 4.71, 4.34, 5.03, 4.9, 5.27 and 5.44 $\log_{10}$ CFU g^{-1}respectively (Table 6).

There was statistically significance difference ($p<0.05$) among the mean count of AMB, *Enterobacteriaceae*, Aerobic bacterial spore (ABS), *Staphylococci*, *Yeasts* and *Molds* in all food samples However no significant difference was observed in mean count of LAB and coliforms ($p>0.05$)(annex-4).

Plate count of aerobic mesophilic microorganisms found in foods is one of the microbiological indicators for food quality. The presence of aerobic organisms reflects existence of favorable conditions for the multiplication of microorganisms. In this study, of all the evaluated samples rice, vegetable and meat had mean contamination levels of ≥ 5.0 $\log_{10}$ CFU g^{-1} (table 6).The mean counts of Staphylococci in meat sauce and spaghetti were 3.06 and 2.84 log CFU g^{-1}.

The mean count of *Enterobacteriaceae* was highest (3.42logs $CFUg^{-1}$) in spaghetti followed by legumes (2.36logs $CFUg^{-1}$). With all food items tested having mean microbial count above the acceptable level. The mean count of *staphylococcus* was higher in vegetable followed by meat (Table 6).Even if the isolated *S.aures* in the samples were few but their mean counts were above the detectable level. Except in vegetables the mean count of aerobic spore former bacteria was below log 4 (Table 6).

Table 6. Mean counts (log10 CFU g^{-1}) of microbial groups in different food samples, Jimma University Community School, Jimma town, Ethiopia, 2013.

Food item	AMB	ENTRO	Coliforms	ABS	STAPH	LAB	Yeast	Mold
Firfir	4.71	2.95	2.02	3.13	2.85	2.85	2.25	2.04
Spaghetti	4.34	2.91	3.42	3.54	2.84	3.16	1.23	1.23
Rice	4.03	2.46	1.66	2.63	3.23	3.60	1.64	1.72
Legume	4.9	3.23	2.36	3.81	3.05	3.28	2.14	1.96
Vegetable	5.27	3.39	2.34	4.07	3.65	2.62	2.62	1.90
Meat	5.44	2.73	1.89	3.95	3.06	3.92	2.76	1.32

Where: AMB=Aerobic Mesophilic Bacteria; ENTRO=Enterobacteriaceae; ABS=Aerobic Bacteria Spore; STAPH=Staphylococci; LAB=Lactic acid bacteria

Firfir- stands to mean food (Injera or bread) broken into smaller pieces and mixed with spice or sauce. Usually it is fast meal made of *injera* (pancake like bread) mixed with different sauce

* The word legume in the present study stands for Ethiopian popular sauce shiro wat and lentil (misir wat) which was made from the family of legumes.

5.5.Food category

The food samples obtained from the students were catagorized as satisfactory, intermidiate ,unsatisfactory and potentially hazardous based on the standared manual(Table.1).Thus 76 (44.70)%), 17(10%), 9(5.29), 26(15.29%) , 18(10.58%),and 24(14.11%)were satisfactory for firfir, spaghetti, meat, legume, vegetables and rice respectively. From the total of 170 samples, *Salmonella spp* were detected in 13 samples (Table.7).

Table 7. Food safety categories of samples collected from Jimma University community primary school students. Jimma town, Ethiopia, 2013

Food item	Number No(%)	Satisfactory No (%)	Intermediate No (%)	Unsatisfactory No (%)	Hazardous No (%)
Firfir	76(44.70)	63(37.05)	4(2.35)	3(1.76)	6(3.52)
Spaghetti	17(10)	14(8.23)	2(1.17)	1(0.58)	0(0)
Meat	9(5.29)	6(3.52)	0(0)	3(1.76)	0(0)
Legume	26(15.29)	17(10)	4(2.35)	3(1.76)	2(1.17)
Vegetable	18(10.58)	6(3.52)	3(1.76)	4(2.35)	5(2.94)
Rice	24(14.11)	21(12.35)	1(0.58)	2(1.17)	0(0)

5.6. Microbiological analysis

From the total of food samples analyzed, 1423 bacterial isolates were characterized and grouped into their respective genera Using standard manual (John, 2012). The aerobic micro flora of firfir was dominated by a variety of Gram positive and Gram negative bacterial groups. Among gram positives *Bacillus* and *Micrococcus* species were the dominant

microflora. Bacillus was dominant in firfir samples followed by legumes than in other samples. In contrary, Gram negative isolates dominated more of the spaghetti samples. *Pseudomonas* isolates were the dominant among Gram negative isolates (Table 9)

Table 8.Frequency distribution of dominant bacteria in some homemade foods obtained from Jimma University Community School. Jimma town, Southwest Ethiopia, 2013

Food Type	No of isolate	*Bacillus*	staph	Micrococcus	entro	Pseudomonas	Aeromonas	Acintobactor	Alkaligens
Firfir	655	271	110	159	91	15	2	0	7
Spaghetti	80	34	26	7	3	3	4	0	3
Meat	135	59	23	29	11	9	3	1	0
Legume	260	113	17	85	26	13	1	4	1
Vegetable	145	77	19	16	18	4	0	2	9
Rice	148	52	21	23	29	10	9	1	3
Total	1423	606	216	319	178	54	19	8	23
percentage	100	42.58	15.18	22.41	12.50	3.80	0.63	0.56	1.62

5.7. The Prevalence of *Staphylococcus* and *Salmonella* species

From the overall food samples analyezed in this study 41 (24.11%) were positive for *S.aureus*,but the frequency distribution varied among the food samples. Its prevalence was hihgest in firfir 17 (41.46%),followed by vegitable11 (26.82 %),and the least was in spagetti 2 (4.87 %) accordingly,it was 5 (12.19%) in legume and 3 (7.31 %) in both meat and rice(Table 10.).On the other hand 13 (7.64%) were positive for *salmonella spp*. However the frequency distribution varied among the food samples. Its prevalence was the highest in firfir 6 (46.16%) folloed by vegetables 5 (38.46%) and the least in legumes 2 (15.38%). However, S*almonella* spp.were not isolated from rice,meat and spaghetti food samples (Table.9).

Table 9. Frequency of isolation of *Staphylococcus spp* and *Salmonella spp.* from different food item, Jimma town,Southwest, Ethiopia, 2013.

Food Item	*Staphylococcus sp.*	*Salmonella spp.*
	Frequency(%)	Frequency(%)
Firfir	17(41.46)	6(46.16)
Spaghetti	2(4.87)	0(0)
Legume	5(12.1)	2(15.38)
Vegetable	11(26.82)	5(38.46)
Meat	5(7.31)	0(0)
Rice	3(7.31)	0(0)

5.8. Antimicrobial Susceptibility Test and Multi Drug Resistance Profiles of *S. aures* and *salmonella spp.*

In this study all of isolates of *S.aures* were susceptible to Gentamycin followed by Chloroanphinicol, streptomycin, vancomycine and kanamycin. In contrast all isolate were resistant against Methicilin and Ampicilin followed by Amoxicillin, Cephtriaxone and Tetracycline (Table 10)

Table 10. Antibiotic susceptibility pattern of *S.aures* and *Salmonella Spp* isolated from foods, Jimma University Community Primary School, Jimma town, Southwest Ethiopia, 2013

		S.aures(n=41)			*Salmonella*(n=13)		
Drug	D/potency (µg/ml)	R(%)	I(%)	S(%)	R(%)	I(%)	S (%)
Streptomycin	10	1(2.43)	4(9.75)	36(87.80)	3(23.07)	1(7.69)	9(69.23)
Tetracycline	30	20(48.78)	0(0)	21(51.21)	4(30.76)	3(23.07)	6(46.15)
Amoxicillin	10	37(90.24)	0(0)	4(9.75)	11(84.61)	0(0)	2(15.38)
Cephtriaxone	30	30(73.17)	1(2.43)	10(24.39)	7(53.84)	5(12.19)	1(7.69)
Methicilin	5	41(100)	0(0)	0(0)			
Vancomycine	30	12(29.26)	0(0)	29(70.73)			
Kanamycin	30	2(4.88)	17(41.46)	23(56.09)	0(0)	0(0)	13(100)
Chloroanphinicol	30	3(7.31)	0(0)	38(92.68)	1(7.69)	0(0)	12(92.30)
Ampicilin	10	41(100)	0(0)	0(0)	13(100)	0(0)	0(0)
Gentamycin	10	0(0)	0(0)	41(100)	1(7.69)	0(0)	12(92.30)

R-Resistant, I-Intermediate, S-Sensitive,

Among the ten antimicrobial drugs tested Mueller-Hinton agar *S.aures* showed multiple drug resistance to four of them, and the highest MDR frequency were noted for AMX/CRO/MET/AMP which accounted for 61.53% of the observed MDR. The maximum MRD recorded for *S. aureus* was resistance to six antibiotics, of which the pattern TET/AMX/CRO/MET/VAN/AMP was the dominant one accounting for about 13.5% (Table 11).

Table 11.Multi drug resistant pattern of S*taphylococcus aures* isolated from foods, Jimma University Community Primary school, Jimma town, Southwest, Ethiopia, 2013

No of resistance	MDR pattern	Frequency(%)
Two	MET/AMP	1(2.70)
Three	AMX/MET/AMP	3(8.10)
	TET/MET/AMP	2(5.40)
Four	TET/AMX/MET/AMP	1(2.70)
	AMX/CRO/MET/AMP	8(21.62)
	STR/AMX/MET/AMP	1(2.70)
Five	AMX/CRO/MET/VA/CHL	1(2.70)
	TET/AMX/MET/VAN/AMP	1(2.70)
	TET/AMX/CRO/MET/AMP	5(13.51)
	AMX/CRO/MET/VAN/AMP	5(13.51)
	AMX/CRO/MET/CHL/AMP	1(2.70)
Six	TET/AMX/CRO/MET/AMP/KAN	2(5.40)
	TET/AMX/CRO/MET/CHL/AMP	1(2.70)
	TET/AMX/CRO/MET/VAN/AMP	5(13.51)

TET-tetracycline, AMX-amoxicillin, CRO-cephtriaxone, MET-methicilin, VAN-vancomycine, AMP- ampicilin, CHL- Chloranphinicol, STR-streptomycin, KAN-Kanamycin,

Furthermore the result of this study shows that *Salmonella* isolated from the samples were mostly resistant to both Ampicilin (100%) and Methicilin (100%) followed by Amoxicillin (90.24%)(Table 12.). The highest sensitivity was observed for Gentamycin (100%) followed by Kanamycin (97.5%) and Streptomycin (96.8%). All antimicrobial resistance profiles in *Salmonella* isolated from each sample are presented in Table 12.

In addition, all *Salmonella* isolated were analyzed for multiple drugs resistance profiles, and it was revealed that except one isolates others exhibited resistance to two or more antimicrobial drugs. The maximum number of antibiotics resisted by salmonella with the only pattern of STR/TET/MET/AMP was accounted for 7.69%. Generally, MDR to the combination of two to three antibiotics were dominated the resistance pattern (Table.12).

Table 12. Multi drug resistant pattern of *salmonella spp.* isolated from Jimma University Community Students. Jimma town, Southwest Ethiopia, 2013

No of resistance	MDR pattern	Frequency (%)
One	AMP	1(7.69)
Two	CHL/AMP	2(15.38)
	MET/AMP	3(23.07)
	TET/AMP	3(23.07)
Three	STR/MET/AMP	2(15.38)
	TET/MET/AMP	1(7.69)
Four	STR/TET/ /MET/AMP	1(7.69)

5.9. Growth potential of some standard bacteria on some traditional sauces

5.9.1. Growth potential and pH of some selected traditional sauces challenged with *S. aures.*

The mean count *staphylococcus aures* in each food sample was 1.8 to 3.29 logs $CFUg^{-1}$ at 0 hr. At 6 hour there was a slight increase rate in all foods but the growth was faster in shiro followed by meat. At 18 hrs the highest growth was in meat and shiro (5.42 and 5.14 log $CFUg^{-1}$) followed by cabbage (4.61$CFUg^{-1}$) (Fig 2.)

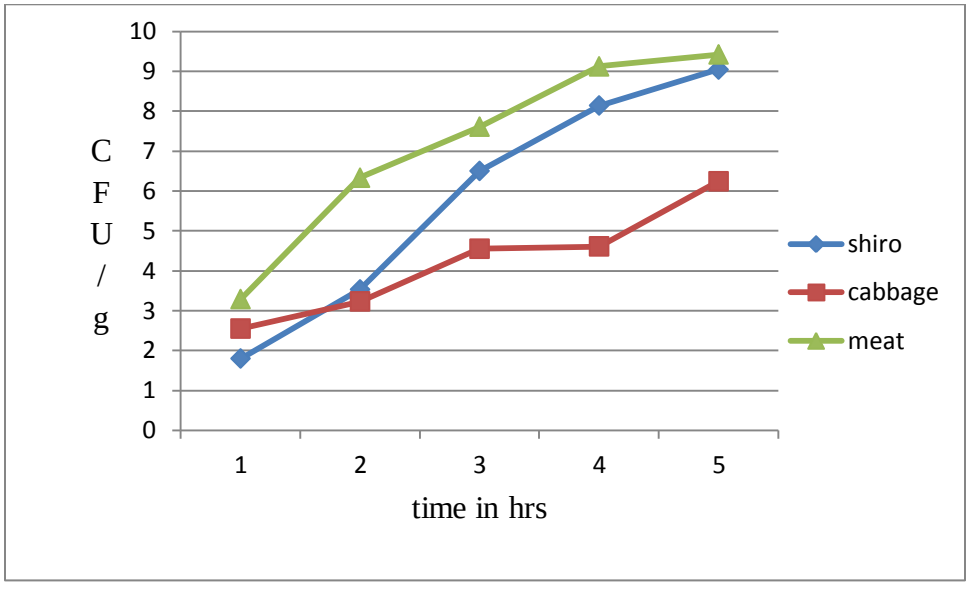

Fig.2. Growth potential of *S aures* in some selected traditional sauces Jimma town, 2013

The pH of food samples challenged with the isolates of *S.aures* was above 5.5 at 0 hour. In the next two 6 hours it was increased in all food items. The pH dropped down below 7 at 18 hrs except shiro. In the last 24 hours the pH of all the food items was approaches to neutral (Fig.3)

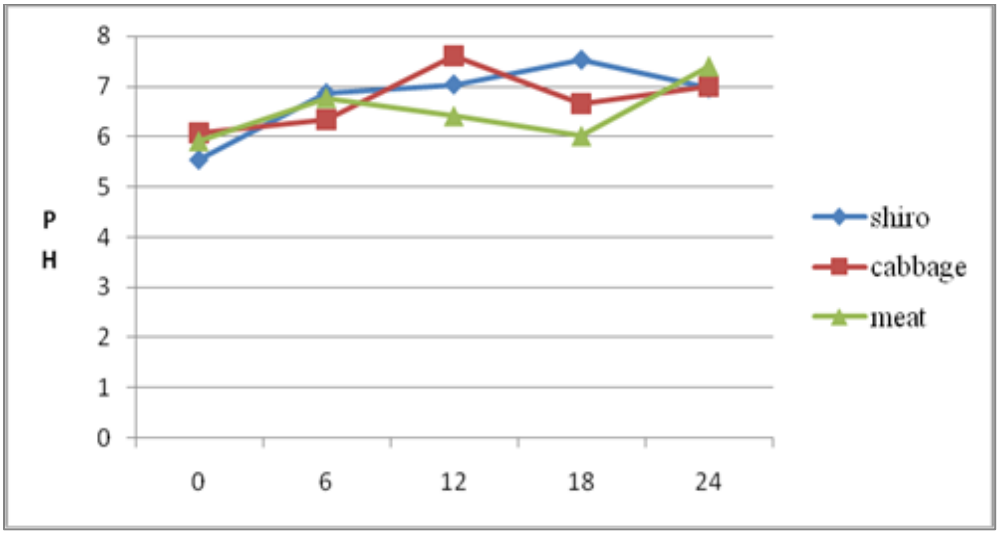

Fig.3. Change in pH of some traditional foods challenged with *S.aureus*, Jimma town, Southwest Ethiopia, 2013

5.9.2. Growth potential and pH of some selected traditional sauces challenged with *S.typhimurium.*

The growth potential of *S.typhimurium* was analyzed in shiro, meat and cabbage over a period of 24 hours. In this study, the highest count of *S.typhimurium* were 8.9 log $CFUg^{-1}$ with in 24hrs in meat sample (Fig. 4) and the lowest were 6.5 log $CFUg^{-1}$ in cabbage with in 24 hrs. The mean count of test strains were increased by >2 log units in the first 6 hrs in shiro and meat. In the third 6 hrs, the growth rate of test strains were increased by three logs $CFUg^{-1}$ in cabbage followed by meat and shiro (Fig.4).

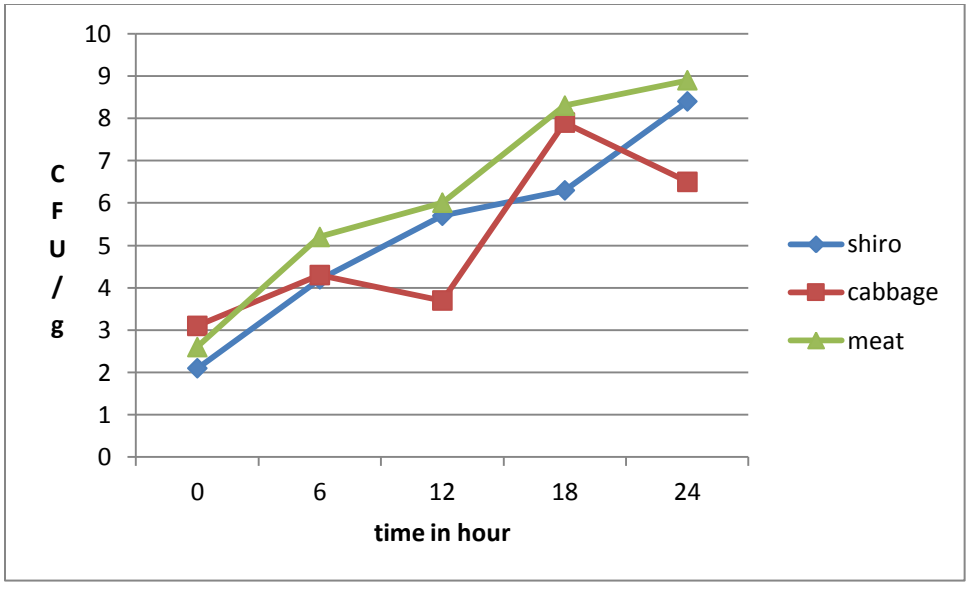

Fig.4. . The growth potential of *S.typhimurium* in traditional sauces, Jimma town, Southwest Ethiopia, 2013

The pH of meat challenged with *S.typhimurium* was 5.21 at 0 hour which was the lowest pH as compared as cabbage and shiro (Fig.4) in addition there were a fluctuation of pH throughout the period of 24 hours. Furthermore the pH was relatively stable in shiro remaining almost around neutrality (Fig. 5)

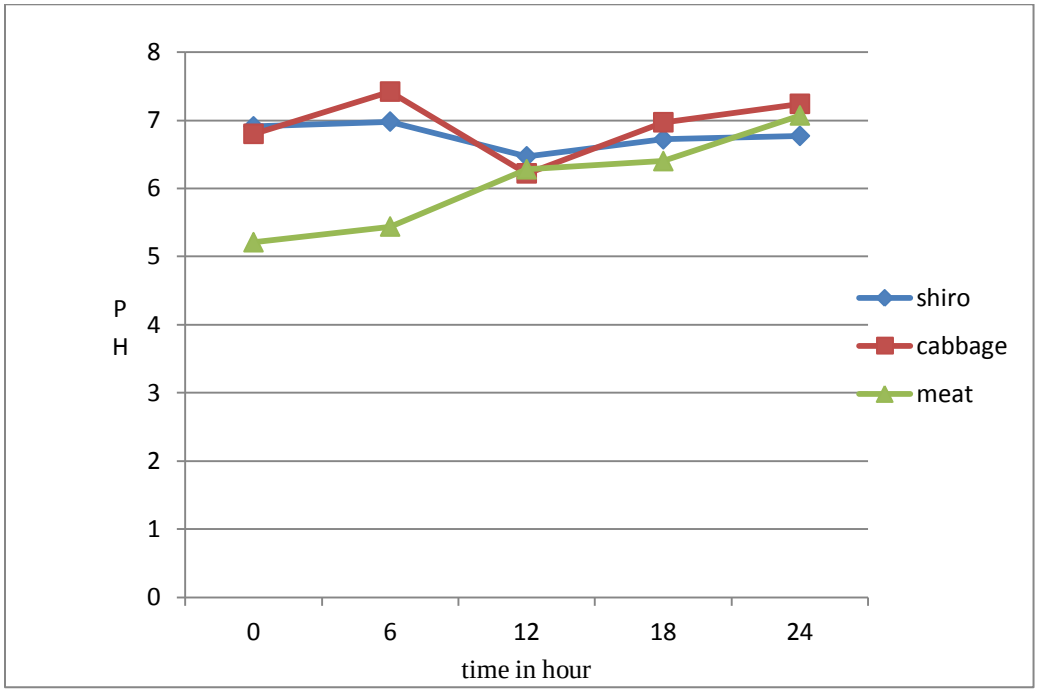

Fig.5 Change in pH of some traditional foods challenged with *S.typhimurium*, Jimma town, Southwest Ethiopia, 2013

5.9.3. Growth potential and ph of some selected traditional sauces challenged with *E.coli*

Although the pattern was the same for all sauces, the growth of *E. coli* in the first 6 hrs was higher in cabbage. In the second 6 hrs (at 12hrs) the growth rate increased by greater than 2 log units in meat sauce followed by shiro, but in cabbage its growth decreases by 0.5log $CFUg^{-1}$. at 24 hr the growth was very high in meat ($9CFUg^{-1}$). Accordingly mean colony forming unit in all food items were >7log $CFUg^{-1}$(Fig.6)

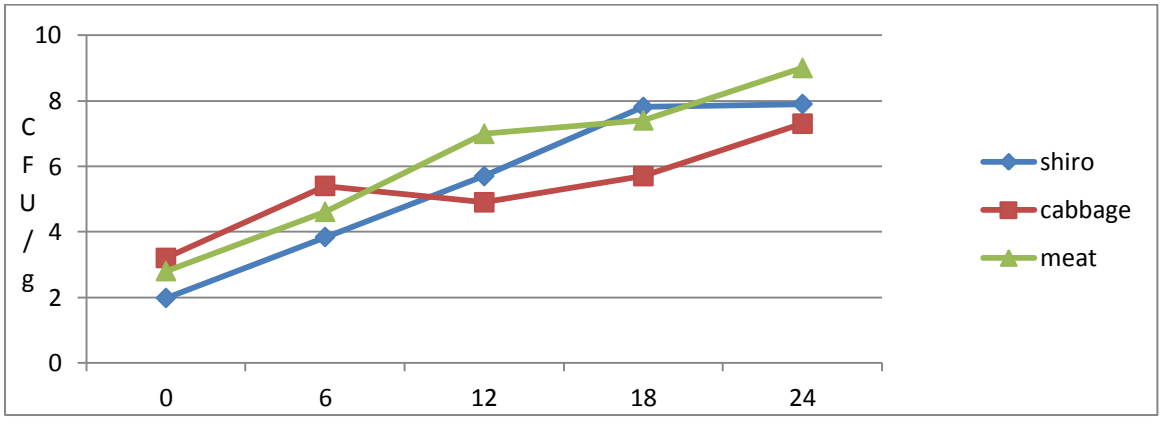

Fig.6. The growth potential of *E.coli* in traditional sauces, Jimma town, Southwest Ethiopia, 2013

At the beginning (0 hour) the pH of meat challenged with the test strain *E.coli* was 5.69 in the next two 6 hrs the pH falls down to 4.11and 4.01at six and 12 hrs respectively. Finally it rise up and approaches to neutral. Accordingly the pH of shiro and cabbage was around neutral throughout a period of 24 hrs (Fig 7).

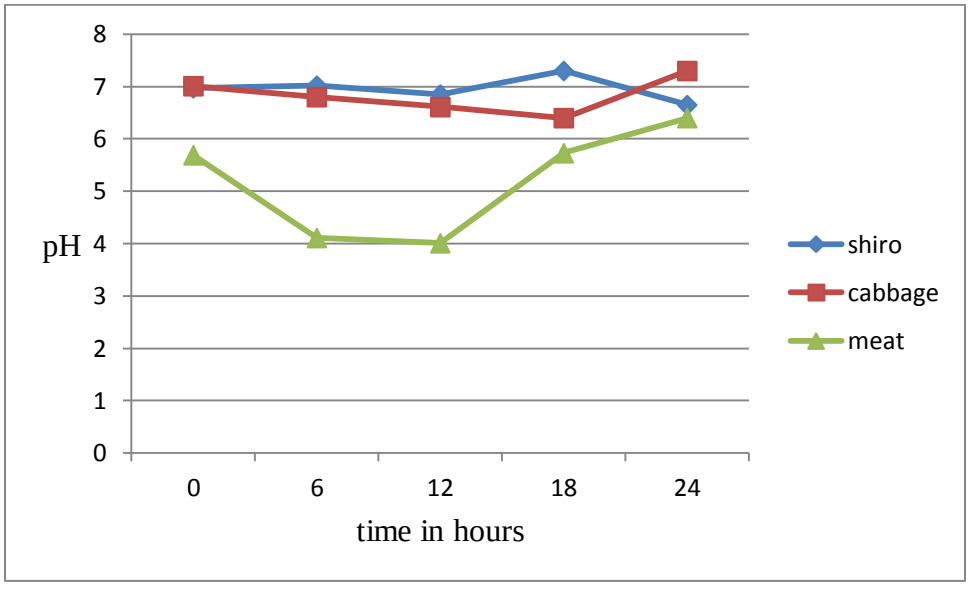

Fig.7. Change in pH of some traditional foods challenged with *E.coli*, Jimma town, Southwest Ethiopia, 2013

6. DISCUSSION

Hand-processed foods are among the most consumed products worldwide. They are also liable to heavy microbiological contamination due to their manufacturing process. One of the most basic ways to show that we care about children is to feed them nourishing safe food (Benjamin, 2012). Because feeding children healthy food is important for a number of reasons: for instance, Food gives children the energy and nutrients they need to be active, to think, and to grow. Therefore safely prepared food helps children avoid food borne illness and they can develop lifetime habits through what they eat in childhood. In addition when children eat with others; they develop social and communication skills. Therefore feeding microbially safe food should be the thing that parents think first (Benjamin, 2012).

In agreement with report of Chukuezei (2010), the socio demographic characteristics of students and their parents in the present study revealed that all (100%) of the food makers at home were females. This is probably due to culture of the society that gives role for women to take care and prepare food for children. In addition several studies on domestic food handling practices conducted in United Kingdom, Northern Ireland and in United States, showed that as compared as women, men are less knowledgeable about food safety and have riskier hygiene and cooking practices (Jacob, 2010).

The overall strategy for reducing food borne illness is to place emphasis on education about proper food storage and preparation practice along with strict and more targeted enforcement (Dugassa, 2007). From response of the participants, it was found out that there were no illiterate among food makers and the highest proportion (35.29%) were at the level of high school followed by diploma holder (34.11%) and graduate of bachelor degree (20%). This finding shows a better educational status of the food makers' when compared with those in Bangkok (Cuprasitrut.*etal.* 2011).This might be because of about 95% of the students were come from educated parents who were a member of academic or administrative staff of Jimma University.

Limited studies have been examining whether there may be disparities in trends of food borne illnesses for populations of different income levels. As Borgnolo, *et al.,* (1996) a case-control study in north-east Italy food borne illness is directly associated to those with "low social class" with incidence of the disease. In addition, Bytzer, *et al*., (2001) studied 9,000

Australian adults and the finding showed the prevalence rates increase with decreasing social class. Likewise research conducted in Denmark from 1993 to 2004, found higher incidence rateratios (IRR) of infection with *entrobacteriacea* is low in high income groups. The highest income groups (≥400,000 Danish crowns/year) showed IRRs of 1.5, 2.7, 1.18 and 1.17 for *Campylobacter, Shigella, Salmonella* Enteritidis and *Yersinia enterocolitica* infections respectively. It is incomparable with the present study in which about 48%of the sample population had above 2500 monthly income. This shows that income can affect the quality of food (Jacob, 2010).

The category ready-to-eat food can be considered as high risk foods because they do not require any heating or process prior to consumption. Therefore it has to be safe, but food workers may transmit pathogens to food from a contaminated surface, from another food, or from hands contaminated with organisms from their gastrointestinal tract **(**Weil, *etal.,* 2006). Food workers' poor personal hygiene is an important contributor to foodborne illness outbreaks. For example Guzewich and Ross found that in 89% of outbreaks caused by food contaminated by food workers, pathogens were transferred to food by workers' hands (Laura, *et al.,* 2007). When food handlers do not practice proper personal hygiene or correct food preparation, they may become vehicles for microorganisms, through their hands, cuts or sores, mouth, skin and hair, among others. In this study food workers had a habit of hand washing but the presence of fecal coliforms in tested food samples indicates a post-sanitization or post-process contamination, often caused by a lack of hand hygiene on the part of food handlers (Ana, *et al.,* 2008).

Numeration of the total aerobic bacteria on food samples examined in the present investigation showed high microbial contamination in some foods. Cenci-Goga, *et al*., (2005) pointed out that the total aerobic bacteria count was a good indicator of food safety. A similar study was carried out in Lagos (Uzeh, *et al*., 2009) and the total aerobic bacteria count ranged from $3.3x10^3$to $5.9x10^6 CFUg^{-1}$.These reflect the existence of favorable conditions for the multiplication of microorganisms. In this study, of all the food sample types tested vegetable and meat had mean contamination levels of ≥5.0 log10 CFU g^{-1}. The New South Wales Food Authority (NSW, 2009) recommends the standard limit for bacterial count of fully cooked ready-to-eat foods to be <5.0 log10 CFU g^{-1}. Hence these foods could be of high risk in transmitting enteric pathogens. These findings corroborate previous works (Bukar, *etal.,* 2010). In their study, Mensah, *et al*., 2002, found a bacterial count of 6.3 ±

0.78 in ready to eat food of Accra, which is greater than this finding(5.27log CFU g^{-1}) .This study is in line with the study conducted in Nigeria which was less than 6.3 log CFU g^{-1}(Odu and Ameweiye, 2013). But a contrast count (8.4 log CFU g^{-1}), was reported from Misurata City, Libya (Abdalhamid, *etal.*, 2013)

According to food guide line all food samples investigated in the present study belongs to level one, which means all food samples are fully cooked. Specifically the mean count of AMB in vegetable and meat samples in the present study were>5log10CFU g^{-1}.Hence they belong to unsatisfactory, but a comparison made from the results, shows that meat sample had more bacterial contamination than the pastry. This may be because meat offers a rich nutrient media for microbial growth (Clarence, *etal*., 2009).

The counts of coliforms varied between 1.66 – 3.42 logs CFU g^{-1}. This is in line with a research conducted in Tirumala, India which was between 0.28-3.99 logs CFU g^{-1} (Suneetha, *etal*., 2011). According to the NSW guide line the mean count of coliforms <2 log CFU $g^{-1}$is considered as acceptable. In the present study the mean count of coliforms in all foods except meat and rice were above this standard. Thus most of the food items tested was in unacceptable level. Existence of coliforms on ready to eat food products reflected the recontamination caused by secondary processing and poor personal hygiene, or probably due to water used for cooking and serving which could contaminated with fecal coliforms **(**Weil, *etal.,* 2006). This implies that contamination was mainly due to poor quality of water used for preparation as well as prevailing unhygienic conditions related to improper washing of utensils, inadequate storage of these at ambient temperatures in unhygienic places, and personal hygiene of food makers (Suneetha, *etal.*, 2011).

Most ready-to-eat foods in Kumasi, Ghana were reported to be contaminated with enteric bacteria, and had bacterial counts higher than the acceptable levels (Feglo and Sakyi, 2012).In contrast the mean count of *entrobacteriacea* in this study was in between 2.46 and 3.39 log $CFUg^{-1}$which was below the standard level of the guide line; therefore all the food items in relation to the present study were considered as satisfactory (NWS, 2009).

S.aureus is found on the skin, nose and throat of most healthy people; they are also widespread in untreated water, raw milk and sewage. When *S.aureus* is allowed to grow in foods, it can produce a toxin that causes illness. Although, cooking destroys the bacteria, the

toxin produced by *S.aureus* is heat stable and may not be destroyed even by heating, let alone by refrigeration. Foods that are handled frequently during preparation are prime targets for *Staphylococci* contamination (Ghosh, *etal*., 2004)

In this study the highest and the lowest *S. aureus* count detected were 3.65log10 $CFUg^{-1}$and 2.84log10 $CFUg^{-1}$. This result is in agreement with the finding of Sina, *et al*., (2011) who reported 3.5 log10 $CFUg^{-1}$ and 2.9 log10 $CFUg^{-1}$ the highest and the lowest respectively. In contrast to this investigation Alyaaqoubi *etal*., (2009) reported in forty eight ready-to-eat foods studied in Malaysia there was no *S.aureus.* Generally the presence of *S.aures* in these foods is probably because the hands which, handled them were contaminated (Amissah and Owusu, 2012).Thus the presence of *S. aureus* in food is an indication that such food is potentially hazardous (Amissah and Owusu, 2012). Moreover, these organisms may take the chance to multiply in the product during storage & produce their enterotoxins which constitute a public health hazard (Staphylococcal food poisoning) to the consumers (Abdalhamid, 2013).

The mean aerobic spore count of the present study is higher in vegetables (4.07 log $CFUg^{-1}$) and lower in rice (2.63 logs $CFUg^{-1}$) on the other hand firfir, spaghetti, legume and meat had 3.13 log $CFUg^{-1}$, 3.54 log $CFUg^{-1}$, 3.81 log $CFUg^{-1}$ and 3.95 log $CFUg^{-1}$ respectively. In many types of food soil can be considered as the initial source of contamination for spore formers. Usually, when direct transfer from soil is involved, levels of these spore formers in foods, ingredients, or feeds are too low to cause problems but spores can germinate and grow during storage, which leads to enzyme formation and metabolism. Microbial spoilage enzymes such as proteases, lipases and lecithinases are often responsible for off-flavour and structural defects (Witthuhn,*etal.*,2011).This can happen on the primary production level, in the processing line, during distribution. These proliferation steps enable the endospore former such as *B. cereus* provokes food quality or safety problems (Heyndrickx, 2011). For instance in England and Wales in the period 1992–2006, 4% of the outbreaks associated with prepared salads were caused by *Bacillus spp*(Little and Gillespie,2008).The presence of these spore formers in this study is probably due to contamination during preparation, storage at home or it may be in the school because of the absence of an appropriate storage.

The presence of *Salmonella* in 25 g of a sample examined is regarded as potentially hazardous to consumers, and is unacceptable for consumption. Rajkowski and Fan (2008)

also isolated *Salmonella* from vegetable samples and suggested that contamination with human pathogen could occur when bovine manure used as fertilizer, contaminated water or cross contamination.

Even though ready-to-eat foods containing *Salmonella* or other pathogens may not always cause illness, there is good microbiological and epidemiological evidence that small numbers of pathogens in foods causes illness (Amissah and Owusu, 2012). Therefore according to Gilbert *et al.*,(2000) the presence of this organisms, even in small amounts, they make the food unacceptable. Therefore, in this study absence of *Salmonella* in spaghetti, rice and meat makes these foods acceptable (Foskett*et al*., 2003). This is in agreement with the study reported by Soriano *et al.*, (2001) who found no *Salmonella* in read to eat food samples from Valencia, Spain. In addition, no *salmonella* was also reported from Hulu, Malaysia (Alyaaqoubi, *etal*., 2009).The absence of *Salmonella* indicated that good handling practices during the cooking process and good storage facilities were available when these food items (spaghetti, rice and meat) were used. However a total mean count of 1.08 $\log CFUg^{-1}$ of *Salmonella* was found in firfir, vegetable and legumes which were 7.64% of the overall samples tested in this study. This is in line with Sudershan, *etal.*, (2012) who found 2.6 log10 $CFUg^{-1}$*Salmonella* from chicken. Bukar, *et al.*, (2010), also reported that about 10.0% *salmonella* positive ready–to–eat foods from Kanometropolis, Nigeria. Thus these food items are considered as potentially hazard (Abdalhamid, 2013).

In the present study the mean count of LAB in firfir, spaghetti, rice, legume, vegetable and meat was 2.85, 3.16, 3.60, and 3.28, 2.62 and 3.92$\log CFUg^{-1}$ respectively. Lactic acid bacteria (LAB) are a group of related bacteria producing lactic acid as the result of carbohydrate fermentation (Ali *et al.*, 2009). In addition to flavor development and food preservation, they also produce variety of compounds with antimicrobial activity, including organic acids, hydrogen peroxide and bacteriocin. Bacteriocin produced by LAB could inhibit not only closely related species but also the growth of pathogenic bacteria (Hajar and Hamid, 2013). Many LABs have important roles in the production of fermented foods, and some of the bacteria were capable of inhibiting the growth of a wide variety of food spoilage microorganisms (Lindgren and Dobrogosz, 2006). Thus, LABs are an attractive source of inhibitory compounds with promising natural food preservatives for improved food quality

and safety. In contrast to the present study, the study conducted in ready to eat food for infants from Nigeria reported that the count of LAB ranging between 4.5 to 9.2logCFUg^{-1}

In the present study the mean count of molds and yeasts were ranging between 1.23 and 2.04 logs CFUg^{-1} and 1.23 and 2.76 log CFUg^{-1} respectively. It was the lowest microbial load for fungi when compared to the result obtained from Benign, Nigeria (Wogu, *etal.,* 2011).The highest food item contaminated by these fungus groups were vegetables and firfir. In Brazil cooked foods were considered as acceptable when the mean count of molds and yeasts is$\leq 5 \times 10^4$ CFU g^{-1} in line to this guide all food items in this study could in acceptable range (Beatriz and Eliana., 2000). As Momoh, *etal*.,(2011) presented, Yeasts and molds are problematic in foods in that they discolor food surfaces, cause off odors and off flavors in certain instances. Contamination of foods by yeasts and molds can result in substantial economic losses to producer, processor, and consumer. Several foodborne molds, and possibly yeasts, may also be hazardous to human or animal health because of their ability to produce toxic metabolites known as mycotoxins. Most mycotoxins are stable compounds that are not destroyed during food processing or home cooking. Although most foodborne fungi are not infectious, some species can cause infection, especially in immunocompromised populations (Valerie, *etal.,* 2001) and some others are known to cause allergies when they are able to produce large numbers of conidia (Seo, *etal.,* 2010).

The microorganism in ready to eat homemade food in the present study was dominated by bacillus (42.58 %) followed by micrococcus (22.41 %) and staphylococcus (15.17%).In contrast to this finding a research conducted in Nigeria revealed that *Bacillus sp*(25.0%) was second dominant bacterial isolates, next to *S.aureus*(35.7%) (Odu and Ameweiye, 2013).In ready-to-eat foods that are fully cooked, the presence of these microbes are used as an indication of either post processing contamination or inadequate cooking (NSW, 2009). The presence of *bacillus cereus* from food samples implicated the ubiquitous nature of bacterial spores. The predominance of *Bacillus* isolates on aerobic plate count plates was possibly due to the presence of spores in the raw material as they are spore forming bacteria. In general, the presence of *Bacillus cereus* in food is of great significance since this organism produces heat-sensitive (diarrheal) and heat- stable (emetic) toxins associated with food poisoning (Suneetha, *etal.,* 2011).In line with this study similar findings by Hanashiro suggested that ready to eat foods sold on the street of São Paulo city, Brazil, were

considered unsuitable for consumption due to higher load of *Bacillus cereus* (Hanashiro,*et al.*,2005).

The problems attributed to *S.aures* and *Salmonella* infection have increased significantly, in terms of both incidence and severity (Oranusi,*etal*.,2013).Furthermore, an increase of antimicrobial resistance in these pathogen makes the treatment of infection more difficult that probably results in death. Therefore, epidemiological information and monitoring systems are necessary to control *S.aures* and *Salmonella* infection in public health sector (Vindigni, *etal.*, 2007). In this study, antimicrobial susceptibility patterns of *S.aures* and *Salmonella* revealed that almost all *S.aures* and *Salmonella* isolated from all foods were sensitive to kanamycin and Gentamycin (100%) and all are resistant to Amoxicillin, Ampicilin and methicilin (100%), a similar report made by Guchi and Ashenafi (2010) shows that most of the *salmonella* isolates were resistant to amoxicillin, ampicilin and penicillin. On the other hand both isolates in this study were relatively sensitive to tetracycline, streptomycin, vancomycine and chloroanphinicol. This is in line with the study conducted in Iran which were sensitive to the above drugs (Akhbarmer, 2012).Now a day due to its remarkable capacity to be resistant to a wide range of antibiotics, *S. aureus* strains resistant to methicilin (MRSA), vancomycine (VISA/VRSA), and too many other antibiotics represent an urgent problem in both community- and hospital-acquired infections (Abdalhamid, 2013).

The growth potential of standard strains of *S.aures*, *Salmonella* and *E.coli* examined in this study showed that the maximum count was recorded in meat challenged with the standard strains within 24 hours. This is similar as that of the result reported by Muleta and Ashenafi, (2001).This is probably due to meat provides bacteria with an ideal medium on which they can grow. Because it has ample nutrients, available water and a moderate pH. The infective dose for *S.aures* is 6 log $CFUg^{-1}$(schelien, *etal.,* 2011). In this study the growth potential of *S. aures* reached to this infective dose within 6 hours, 12 hours and 24 hours in meat shiro and cabbage respectively. In this infective dose the *S.aures* populations may produce toxins which may produce illness to consumers (Bent and Monday, 2003).

The maximum bacterial count (>8log $CFUg^{-1}$) of *S. typhimurium* was recorded at the last 18 hrs in meat and 24hrs in shiro and cabbage. The pH increased as the time increased in meat till reaching neutral but almost similar in shiro and cabbage throughout 24 hrs. The change

in pH could be because of changing of source of carbon and nitrogen. As Lee, (2011) explanation, some microbial cultures generate enzymes to utilize new carbon and energy substrate when a small amount of the original carbon and energy substrate is present. This is not saying that pH is the only required criteria for the growth of organisms but other intrinsic and extrinsic parameters should be full filled (Jay, *etal*., 2005).

For causing illness the least infective dose for *salmonella typhimurium* is log 5(Toder, 2005).In the present study *salmonella* reaches to this dose within 6 hours in meat , 12 and18 hours in shiro and cabbage respectively.

On the other hand the growth potential of *E.coli* was also examined in the present study. The infectious dose and the dose response are dependent upon the strains used, and the age and physical condition of the individuals, and can therefore show wide variations and the infectious dose for *E. coli* was large (>5log $CFUg^{-1}$).The result from this challenged study revealed that *E.coli* reached to the infective dose (>5log $CFUg^{-1}$) at 6hrs in cabbage and at 12 hrs in meat and shiro (Mahendra,*etal.*,2007).

7. CONCLUSION

- In conclusion, Firfir which is the main food that students used for their daily lunch is the most contaminated foodstuff. Although food is cooked (boiled) at high temperature which could be enough to inactivate pathogens, post-contamination and cross-contamination that could be appeared due to unhygienic food handling, and incorrect storage practices might made the food unsafe or potential hazardous.
- In this study high percentage of indicator organisms as well as food borne pathogens were identified, which showed unhygienic condition of handling and processing of food at household level.
- In addition, this study confirmed the presence of drug resistant food borne pathogens; particularly *S.aureus* which are methicilin resistant and other multidrug resistant isolates.
- Even if, the knowledge and practice of food handlers towards foodborne disease, personal hygiene and food handling practices were found to be satisfactory among most of the food makers, the relative heavy microbial counts in some of the foods indicate the poor implementation of their knowledge. .
- The predominance of *Staphylococcus* species and the presence of other microorganisms might leads to unexpected foodborne diseases outbreaks unless the sanitary facilities of the school and personal hygiene of the food makers are improved.
- The isolation of *enterobacteriaceae* in cooked ready to eat foods is good indicator of the risk of transmission of fecally contaminated pathogens from infected individuals to the healthy children.

8. RECOMMENDATION

- These findings demonstrated that homemade food prepared for students at home constitutes a likely potential hazard to human health. The isolation of *Enterobacteriaceae* in ready-to-eat foods that are fully cooked is a good indicator of post-processing contamination or inadequate cooking. Therefore parent discussion should be prepared on personal hygiene, and how to improve food quality and safety?
- The study has shown that infected food handlers may be at risk of developing illness themselves, and may pose a threat to the health of the children. For instance all bacteria discussed in this study are transmitted via the fecal-oral route, direct contact with infected food handlers or by cross contamination to healthy children. Thus food makers should have to improve food processing, preparing, and storing practice.
- The school should create conducive environment for dinning and establish appropriate rooms to be used for storing food.
- Health sectors, municipality and Non Governmental Organizations should work together to improve the sanitary facilities of the community and in turn protect the health of children to reduce the public and economic burden posed due to foodborne diseases.
- The food makers,those having direct contact with foods need to have periodic medical examinations including stool test for enteric pathogens at least twice a year to prevent the problems they might face and avoid transmission of these pathogens through contaminated foods.
- Parents, The food makers and the responsible school administrators need to have training on basic principles of food handling and preparation practices.

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APPENDICE

Annex 1-John's bacterial identification

gram reaction (young culture)	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–
shape	coccus (clusters)	coccus (clusters)	coccus (chains)	coccus (tetrads)	rod	rod	irreg. rod	rod	rod	rod	rod	rod	rod	rod	rod	coccus (pairs)
aerobic growth	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
anaerobic growth	–	+	+	+	+	–	–	+	+	–	–	–	+	+	+	–
endospores	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–
motility (Motility Medium)	–	–	–	–	–	+	–	+ or –	+ or –	+ or –	+ or –	+ or –	–	+	+	–
catalase reaction	+	+	–	–	–	+	+	–	+	+	+	+	+	+	+	+
oxidase reaction	+	–	–	–	–	–	–	–	+ or –	+ or –	+	+	–	–	+	+
glucose fermentation to acid **or** to acid+gas	–	+	+	+	+	–	–	+ (or –)	+	–	–	–	+	+	+	–
Glucose O/F Medium											–	O	F	F	F	O
Micrococcus	**X**															
Staphylococcus		**X**														
Enterococcus			**X**													
Bacillus									**X**	**X**						
Alcaligenes											**X**					
Pseudomonas												**X**				
Escherichia														**X**		
most other enteric genera														**X**		
Aeromonas															**X**	

Annex -2- Questioner

Jimma University

College of Natural Sciences

Department of Biology

English Version Questionnaire for Data Collection

Verbal consent form before conducting interview

Introduction

My name is Tadele Abebe. I am a post graduate student in Jimma University and working on my thesis, entitled microbiological safety and quality of homemade food among Jimma University Community primary school students. Thus, the objective of this questionnaire is to collect preliminary information on food handling, sanitation, and other related factors. So that, you are kindly requested to respond honestly for interview questions and I need your willingness to take a sample from your food. I assured that your response is completely confidential; your name will not be written and the information collected from you will be only used for research purpose, and will not be given to a third body. Your genuine response to the question has significant impact on the quality of data and result.

Are you willing to participate?

1. If yes, proceed to the next page,
2. If no pass to the next participant.

Questioner prepared for students

General information

Code ☐

1. Grade 1-4 5-8 ☐ 9-12 ☐ ☐

2. Sex male ☐ female ☐

3. Age 5-12 ☐ 13-17 ☐ 17-20 ☐ >20 ☐

4. Who made food for you?

Mother ☐ sister ☐ on your own ☐ servant (worker) ☐ if other, specify_____

5. When your food is prepared

At the evening ☐ early in the morning ☐

6. How the food stored at home before coming to school if prepared at evening

Refrigerator ☐ on the shelf ☐ if other specify (_____)

7. What kind of material do you use to bring your food?

Metal lunch box ☐ plastic ☐ other ☐

8. Where do you keep your food in the school?

Store in the class ☐ other ☐ ☐

9. Is there dining hall in your school?

Yes ☐ no ☐

10. If yes what type of floor is that?

Tile ☐ Concrete ☐ Wooden ☐ Plastered stone/bricks Earthen. ☐

11. Educational status of food maker

Illiterate ☐ elementary (1-8) ☐ high school (9-12) ☐ special training ☐

12. Does the food maker wear appropriate over coat? Yes ☐ No ☐

13. Does the food maker wear appropriate hair cover? Yes ☐ No ☐

14. Does the food maker nails are short, trimmed and clean? Yes ☐ No ☐

Knowledge related questions to students

15 - Have you ever heard about food borne disease? Yes ☐ No ☐

16 - If you heard about food borne disease, what is your source of information?

a. school ☐

b. Health professionals ☐

c. Mass Medias ☐

d. Others (specify_____)

17 - How can food borne disease be transmitted?

Contaminated food Contaminated hand I don't know ☐

Contaminated water Vectors ☐

If, other (specify _____)

18. How can food are contaminated

a. Exposure to flies ☐

b. Handling in contaminated environment (area) ☐

c. Using of dirt equipment ☐

d. Using of contaminated water for equipment washing and preparation ☐

e. Dirt hands ☐

f. Using the same container for cooked and raw foods. ☐

g. Other (specify_____)

Hand washing facility for dining

19. Is hand washing facility present in your school?

Yes ☐ No ☐

20. If present, type of hand washing facility?

Piped fountains ☐ Welded metals ☐

Other (specify_____)

21. Is soap provided for hand washing?

Yes ☐ No ☐

22. Occupation of your family Government employ ☐ Merchant ☐

If other specify_____

23. Monthly income

<450 ☐ 451-900 ☐ 901-1617 ☐ 1618-2500 ☐ >2501 ☐

ANNEX-3

Mean microbial count and standard deviation of homemade foods in jimma university community school. Jimma, Ethiopia.2013.

sample		amb	entro	ecoli	abs	staph	lab	yeast	mold
firfir	Mean	4.7182	2.9532	2.0266	3.1328	2.8504	2.8543	2.2503	2.0476
	N	76	76	76	76	76	76	76	76
	Std. Deviation	0.58251	0.31718	0.96755	0.96817	0.67731	.61611	.84202	.72871
sipageti	Mean	4.3494	2.9117	3.4283	3.5433	2.8494	3.1667	1.2394	1.2372
	N	18	18	18	18	18	18	18	18
	Std. Deviation	.71692	.59422	.77930	.78324	.72579	.51240	.62456	.31496
rice	Mean	5.0392	2.4646	1.6621	2.6312	3.2379	3.6071	1.6483	1.7229
	N	24	24	24	24	24	24	24	24
	Std. Deviation	.80071	.30411	.22297	.73878	.04428	.52715	.96203	.86051
legume	Mean	4.9085	3.2346	2.3685	3.8119	3.0354	3.2869	2.1438	1.9685
	N	26	26	26	26	26	26	26	26
	Std. Deviation	.41629	.61619	.17713	.43625	.58392	.58392	.57600	.52735
vegitable	Mean	5.2772	3.3917	2.3406	4.0789	3.6528	2.6222	2.6256	1.9094
	N	18	18	18	18	18	18	18	18
	Std. Deviation	.48905	.72068	.28445	.61635	.14055	.71903	.47150	.62183
meat	Mean	5.4400	2.7314	1.8986	3.9571	3.6514	3.9214	2.7671	1.3214
	N	7	7	7	7	7	7	7	7
	Std. Deviation	.46206	.90639	.49343	.83812	.78826	.32718	.28050	.31271
Total	Mean	4.8477	2.9428	2.2136	3.3440	3.0616	3.0828	2.1110	1.8473
	N	170	170	170	170	170	170	170	170
	Std. Deviation	1.56651	2.17161	2.17061	1.92091	1.75105	2.22058	1.76582	1.65418

Annex-4 .One way ANOVA result among jimma university community school students

		Sum of Squares	df	Mean Square	F	Sig.
AMB	Between Groups	34.829	5	6.966	4.442	.001
	Within Groups	257.191	164	1.568		
	Total	292.020	170			
Entro	Between Groups	98.717	5	19.743	5.224	.000
	Within Groups	619.827	164	3.779		
	Total	718.544	169			
colifor m	Between Groups	46.604	5	9.321	2.041	.076
	Within Groups	749.013	164	4.567		
	Total	795.616	169			
ABS	Between Groups	50.529	5	10.106	3.107	.010
	Within Groups	533.450	164	3.253		
	Total	583.979	169			
staph	Between Groups	56.039	5	11.208	4.478	.001
	Within Groups	410.426	164	2.503		
	Total	466.465	169			
LAB	Between Groups	32.628	5	6.526	1.644	.151
	Within Groups	650.777	164	3.968		
	Total	683.405	169			
yeast	Between Groups	51.334	5	10.267	3.356	.007
	Within Groups	501.651	164	3.059		
	Total	552.984	169			
Mold	Between Groups	49.309	5	9.862	3.493	.005
	Within Groups	463.076	164	2.824		
	Total	512.385	169			

Where: AMB=aerobic mesophilic bacteria, Entro=*entrobacteriacea*, ABS= aerobic bacterial spore Staph=*staphylococci*, LAB=lactic acid bacteria

Annex-5 -- Questioner

Jimma University

College of Natural Sciences

Department of Biology

Amharic Version of the Questionnaire

ቃለመጠይቁ ከመደረጉ በፊት የተሳታፊዎች ፈቃደኝነት መጠየቂያ ቅፅ

ሰላምታ፡ - ስሜ ታደለ አበበ እባላለሁ፤፤በጂማ ዩኒቨርሲቲ የተፈጥሮ ሳይንስ ኮሌጅ በባዮሎጂ ት/ክፍል በማይክሮባዮሎጂ መርኀግብር የድኅረ ምረቃ ተማሪ ስሆን የመመረቂያ የምርምር ሥራዬን microbiological safety and quality of homemade food among Jimma University Community primary school students በሚል ርዕስ በመሥራት ላይ እገኛለሁ፡፡ እርስዎም በጥናቴ ውስጥ እንዲካተቱ ተመርጠዋል፡፡ በዚህ መሠረት ለምግብነት የተዘጋጀውን ምግብ ደህንነት እንዲሁም ከምግብ ዝግጅት ጋር በተያያዘ ያለውን የንፅህና ሁኔታ ለማጥናት ይህ መጠይቅ ተዘጋጅቶአል፡፡ በመሆኑም የጥናቱ ዉጤት የምግብ ዝግጅትና አጠቃላይ የንፅሃና ሁኔታ የሚሻሻልበትን መንገድ ለመፈለግና በንፅህና ጉድለት በተመጋቢዎችም ሆነ በምግብ ዝግጅት ሂደት ላይ ሊከሰቱ የሚችሉትን ምግብ ወለድ በሽታዎች ለመከላከልና ለመቀነስ እንዲያገለግል ያለመ ነው፡፡ ስለዚህ የጥናቱን ዓላማ እርስዎን በሚመለከት ለሚነሱ ጥያቄዎች ትክክለኛ መልስ በመስጠት ቀና ትብብር ያደርጉልኝ ዘንድ በትህትና እጠይቃለሁ፡፡ እርስዎን በሚመለከት የሚገኘው ማንኛውም መረጃ ለማንኛውም ሦስተኛ አካል/ወገን የማይሰጥና በምሥጢር የሚያዝ መሆኑን ግልጽ ማድረግ እወዳለሁ፡፡ ለሚጠየቁት ጥያቄም መልስ ያለመስጠት መብት አከብራለሁ፡፡

በዚህ መሠረት የሚቀርቡልዎትን ጥያቄዎች ለመመለስ ፈቃደኛ ነዎትን ;

1- አዎን ከሆነ መልሳቸው ወደሚቀጥለው ገፅ ይለፉ /ይሂዱ
2- መልሳቸው የለም ከሆነ ወደሚቀጥለው ተሳታፊ

1- ጠቅላላ መረጃ

□ሚስ□ር ቁ□ር

1. **□□ክ** 1-4 5-8 9-12
2. **□ኑ ወንድ** ☐ **ሴት** ☐
3. **□□አድሜ** 5-12 ☐ 13-17 ☐ 17-20 ☐ >20 ☐
4. **ምግብህ/ሽን የሚያዘጋጀው ማን ነው?**

አናተ ☐ **□አህት** ☐ **በ□ክ** ☐ **የቤት ሰራተኛ** ☐ **ሌላ ናስ ይገለጽ**

5. **ምግብ የሚዘጋጀው መቼነው? በጠዋት** ☐ **ማተ** ☐
6. **ምግብ የሚዘጋጀው ማተ ከሆነ የሚቀመጥበት ቦተ**

በማቀዝቀዣ ☐ በመደርደሪያ☐ ልላ ከስ ይገስጽ ☐

7.ም☐ብ ☐ሚመጣበት አቃ ከብረት የተሰራ ☐ ፕላሰቲክ ☐ልላ ከስ ይገስጽ----------

8.ት/ት ቤት ውስጥ ምግብ የሚቀመጥበት ቦ☐ የት ነው

መጋዘን ውስጥ ☐ ክፍል ውስጥ ☐ ልላ ከስ ይገስጽ----------

9.በት/ትቤት ውስጥ የመመገቢያ ክፍል አስ? አዎ ☐ የስም ☐

10.ከላይመልስህ/ሽ አዎ ከሆነ መሬቱ ምን አይነት ነው?

ሸክላ ☐ ሲሚንቶ☐ ጣውላ ☐ ሴራሚክ ☐ አፈር

11.ምግቡን የሚያዘጋጀው ሰው የት/ት ሁኔተ

ያልተማረ☐ አንደኛ ☐ረ☐(1-8☐ ሁስተኛ ☐ረ☐(9-12☐ ዲፕሎማ☐ ዲገሪና በላይ☐ ሌላ ከስ

12.ምግብ አዘጋጁ ጋዋን የመልበስ ልምድ አለው? አዎ ☐የለውም ☐

13. የምግብ አዘጋጁ ጸጉር የመሸፈን ልምድ? አ- ☐ ☐ስ☐ም☐

14. የምግብ አዘጋጁ ጥፍር አጭርና ንጹህ ነው? አዎ☐ የለውም ☐

15.ከዚህ በፊት ስለ ምግብ ወለድ በሽተ ሰምተህ/ህ ተውቃስህ? አዎ ☐ አላወቅም ☐

16.ሰምተህ/ህ ከሆነ መረጃውን ከየት አገኛችሁት?

ከት/ት ቤት ☐ ከጤና ባለሙያ☐ ከዜና ማሰራጫ☐ ሌላ ከስ ይገስጽ

17.ምግብ ወለድ በሽተ የሚተላለፈው ዕነዴት ነው?

ምተበከለ ምግብ☐ በተበከለ አጅ ☐ በበሽተ ተሸከሚዎች☐በተበከ ውሃ☐ አላውቅም☐ ሌላ

18.ምግብ የሚበከለው አንዴት ነው?

ምዝንቦች☐ በተበከለ ላከባቢ ሲሰራ ☐ ንጹህ ባልሆነ አቃ☐ በተበከለ ውሃ ☐ ንጹህ ባልሆነ አጅ ☐
ሌላ

19.በት/ቤት ውስጥ ምቹ የአጅ መተጠቢያ አለ? አዎ ☐ የለም ☐

20.ከለ ምን አይነት ነው?

☐ቧንቧ☐ ከብረት የተሰራ ማጠራቀሚያ ☐ ሌላ

21.አጃችሁን የምትተጠቡት በሳሙና ነው? አዎ ☐ አየደለም ☐

22. የቤተሰብ የስራ ሁኔተ

የመንግስት ተቀጣሪ ☐ ነጋዴ ☐ ሌላ

23. ☐☐ር ☐ሪ <150☐ 151-650 ☐ 651-1400☐ 1401-2350 ☐ 2351-3550 ☐
3551-5000 ☐ >5000 ☐

Annex -6– consent form

Jimma University

College of Natural Sciences

Department of Biology

Amharic Version of the Student parents consent form

ጥናቱ ከመጀመሩ በፊት ከተማሪዎች ወላጆች ፈቃደኛ መሆናቸውን መጠየቂያ ቅፅ

ለተማሪ----------ወላጆች

ሰላምታ፡ - ስሜ ታደለ አበበ እባላለሁ። በጂማ ዩኒቨርሲቲ የተፈጥሮ ሳይንስ ኮሌጅ በባዮሎጂ ት/ክፍል በማይክሮባዮሎጂ መርኀግብር የድኀረ ምረቃ ተማሪ ስሆን የመመረቂያ የምርምር ሥራዬን "microbiological safety and quality of homemade food among Jimma University Community primary school students "በሚል ርዕስ በመሥራት ላይ እገኛለሁ፡፡የእርስዎም ልጅ በጥናቴ ውስጥ እንዲካተት/እንድትካተት ተመርጧል፡፡ በዚህ መሠረት ለምግብነት የተዘጋጀውን ምግብ ደህንነት እንዲሁም ከምግብ ዝግጅት ጋር በተያያዘ ያለውን የንፅህና ሁኔታ ለማጥናት የምግብ ናሙና ከልጅዎ ምግብ ላይ እንድወስድ ፍቃድዎን ስጠይቅ ከጥናቱ የሚገኘው ማንኛውም መረጃ ለማንኛውም ሦስተኛ አካል/ወገን የማይሰጥና በምሥጢር የሚያዝ መሆኑን ግልጽ ማድረግ እወዳለሁ፡፡

Annex 7– consent form

Jimma University

College of Natural Sciences

Department of Biology

English Version of the Student parents consent form

To----------

My name is Tadele Abebe. I am a post graduate student in Jimma University and working on my thesis, entitled "microbiological safety and quality of homemade food among Jimma University Community primary school students". Thus, your son/daughter has been selected to be included in this study. So that, you are kindly requested to give me a permission for taking a sample from your sons/daughters food. I assured that your name will not be written and the information collected from your sons/daughters will be only used for research purpose, and will not be given to a third body.

Declaration

I hereby declare that,

The research works presented in this thesis entitle **"**Microbiological Safety and Quality of homemade foods among Jimma University Community Primary School Students, Jimma town, Southwest Ethiopia". has not been submitted for any degree in any other university on any occasion.

To the best of my knowledge no work of this type has been reported on the above subject.

All the work presented in this thesis is original and wherever references have been made, it has been clearly indicated.

Name Tadele Abebe

Signature_____date_____

The work has been done on under the supervision and approved for final submission by

1. Ketema Bacha (PhD, Associate professor)

Signature_____date_____

2. Tsige Ketema (Msc.PHD.candidate)

Signature_____date_____