Jimma University

College of Natural Sciences

Department of biology

Prevalence and antibiotic Resistance patterns of aerobic spore forming bacteria in processed commercial and local products, and associated risk factors

By:-Rehika Abdulaziz

September, 2019

Jimma, Ethiopia

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By:-RehikaAbdulaziz

A Thesis Submitted to the Department of Biology, College of Natural Science, Jimma University, in partial fulfillment of the requirement for the Degree of Master of Science in Biology (General Biology)

Advisor: Ketema Bacha (PhD, Prof.)

Co-advisor**: Shiferaw Demissie (MSc.)**

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Approved by Examining Board

Name Signature Date

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Research advisors signature date

Advisor:- Ketema Bacha (PhD, Prof.) ---------- ----------

Co-advisor:- Shiferaw Demissie (MSc.) ---------- ----------

Examiners

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Declaration

I, the under signed, declare that this Thesis is my original work and has not been presented for any Degree in any other University and all materials used for this Research are dully acknowledged.

Name; Rehika Abdulaziz ----------

Signature; ----------

Date; ----------

This MSc. Thesis has been submitted with my Approval as supervisors.

Advisors Name; -Ketema Bacha (PhD, Prof), Signature 

Co-Advisor Name;-Shiferaw Demissie (MSc.) Signature----------

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List of Acronyms

ABIS; Online bacteria identification software

ACC; Aerobic Colony Count

CAM; Complementary and Alternative Medicine

CDC; Center for Disease Control and Prevention

EFSA; European Food Safety Authority

GRAS; Generally Recognized As Safe

HPA ; Health Protection Agency

LAB; Lactic Acid Bacteria

MERS-CoV; Middle East Respiratory Syndrome Corona Virus

NCCLS; National Committee for Clinical Laboratory Standards

SPC; Standard Plate Count

UHT; Ultra High Temperature

USEA; US Environmental Protection Agency

Abstract

The presence of aerobic spore forming bacteria and the problems linked to their presence in these processed, local foods and drinks have not been studied and documented in Jimma town. Therefore, the aim of this research was to assess the prevalence of aerobic spore forming bacteria in selected commercial and local food samples and determining the associated risk factors. A total of 50 samples of Keribo, Row milk,powderd milk, Wheat flour and Honey (10 samples each)were collected from five different sites in Jimma town (Kochi, Ajip, Bishishe, Merkato and Bochobore) and their mean aerobic spore count were compared from site to site and among the five items. After aerobic spore count, the colonies were further purified and a total of 60 aerobic spore forming bacterial isolates (12 isolates from ten samples of each food item) were characterized using conventional morphological, biochemical and physiological tests. Six tentative identities of aerobic spore forming bacteria were detected using the methods described by Bergy's manual and ABIS. B. coagulans (41.7%), B. subtilis (33.3%), B. cereus (25%) dominated Keribo samples while B, cereus & B, subtilis (33,3%, each), B, coagulans (16.7%), B, megaterium(16.7%) were among the frequently isolates species from Row milk. Bacillus subtilis (50%) dominated in Powdered milk. while B. cereus, B. coagulans, and B. subtilis were equally detected (25% each) in honey samples. Likewise, Bacillus subtilis (50%) was frequently identified from wheat flour. Sensitivity of the isolates towards six galleries of antibiotics indicated that, all the detected Bacillus strains were resistant to Ampicillin but showed similarity in their sensitivity to ciprofloxacilin, Clindomycin, Gentamycin and vancomycin except B.coagulans and B.cereus which resisted Kanamycin and Vancomycin, respectively. Almost all the strains exhibit multi drug resistance (showed resistance to two or more Antibiotics).A total of 6 MDR patterns was observed in B. subtilis, followed by 4 MDR (B. megaterium, and B. cereus), 3 MDR (B. coagulans) and 2 MDR (B. pumilus). The highest MDR patter recorded was resistance to Ampicillin, Ciprofloxacin, Clindomycin, Gentamycin, Kanamycin, and vancomycin. The detection of the six Bacillus strains in the five food items and the observed high antibiotic resistance patterns calls for regular inspection for safety of these foods and drinks.

***Key words**; Aerobic spore formers, Bacillus, Honey, Keribo, powdered milk, public health, Raw milk. Wheat flour*

I. Introduction

Converting milk and cereal wheat in to powdered milk and wheat flour decrease their water activity (a_w) and increase their shelf life and enable them to be stored for extended periods without substantial loss of quality. However, spore formers are theoretically more challenging in these powdered, heat treated and dry food products. Because the high temperature treatment eliminate other potential vegetative contaminants but favor a dormant, tough, & non reproductive structure called spores which will later germinate when the growth condition is optimum (Stenfors and Granum, 2001, McGuigganet *et al*., 2002).

Although pasteurization, UHT(ultra Heat Treatment) and refrigeration are important sterilization practices used to curb microbial proliferate in these foods, some potential pathogens especially spore formers have been reported to survive these conventional sterilization techniques (Stenfors and Granum, 2001, McGuigganet *et al*., 2002).Therefore the presence and multiplication of aerobic spore forming bacteria in raw milk (including powdered milk),Keribo, wheat powder and honey are not only of concern as a cause of economic loss through spoilage of contaminated products but also pose many time serious public health hazards by poisoning foods(Chalmers, 1955).

Since microbial contamination is an essential public health issues of developing nations including Ethiopia, strict hygienic practices and proper training about food safety issues of these commercial and local food products at all stages from point of production to the point of consumption must be given (Carlin *et al*., 2010).

As contaminated raw milk, powdered milk, Keribo, Honey and wheat powder are a source of harmful bacteria that cause different types of diseases, to safe guard and protect the public from these diseases, new scientific and efficient methods of preventing contamination must be used. Because aerobic spore forming bacteria cannot easily killed by heat, chemical treatment and by using food preservation techniques including refrigerators, a large variety of antibiotics are currently used to treat these diseases, but their efficacy has been threatened by microbial resistance(Bacha *et al*.,2010).

Currently there is concern over the possible spread of resistance determinants to antimicrobials (Florez *et al.*,2005).Thus, using this study it is hoped to know few of the effective drugs which the identified strains in these selected food products are sensitive after carrying out their total load and identifying them using conventional morphological and biochemical tests. Finally, this study could also serve as a useful informative base line data for further studies to scale up the hygienic qualities of these food products.

1.1. Statement of the problem

Hygienic (sanitation) problems, lack of education, lack of careful inspection, controlling of food safety and quality from the public health authorities, existence of infectious diseases linked to the presence of microbial agents in row and heat treated food products including the current emergence of resistance of most microbial agents to various antibiotics could be key challenges for the health of food venders, local food processors and consumers located in the data collected sites(Reda *et al.*,2012).

Though some of the food products are carefully handled and properly stored in a refrigerator, their expiry date and the ability of spore formers to overcome these challenges were also the other key problems (Stenfors and Granum, 2001, McGuiggan 2002). All of these challenges together with shortage of adequate documents on aerobic spore forming bacteria in these selected food products were problems that would be solved by this study.

1.2. Objectives

1.2.1 .General objective

The general objective of the study was To determine prevalence of Aerobic spore forming Bacteria and their drug resistance patterns in selected commercial and local food products.

1.2.1.1 Specific objectives

The specific objectives of the study were to:-

- To determine the total load of Aerobic spore forming bacteria in selected food samples.
- To characterize and identify aerobic spore forming bacteria.
- To determine antibiotic susceptibility patterns of aerobic spore formers isolated from food samples.
- To determine the risk factor for isolation of aerobic spore forming bacteria from selected food items.

1.3. Significance of the study

The information obtained from this study will help the public health authorities and institutions which are engaged in food safety and quality issues to monitor conditions of sanitation by regularly inspecting the healthy conditions of the vended sites, households, vendors and consumers.

Besides, the document will help them to give trainings and awareness on how to take measures to prevent, control and treat diseases linked to the bacteria identified in these locally prepared and commercial food products.

This study will also serve as a base line information to these who need further extend research in this area.

2. Literature Review

2.1. Microbiology of foods and beverages

Micro organisms are important in food products because they produce desirable flavor and physical characteristics in many food products during their fermentation. Food products may Become contaminated with pathogens, microbial toxins, and spoilage organisms and become vehicles for the transmission of disease to humans and other animals and many micro organisms are capable of causing off flavor and physical defects in food products. Foods and beverages may contain beneficial micro organisms called Probiotics, food spoilage micro organisms and food borne pathogen (Kinfe and Abera, 2007).

2.1.1 Probiotics

Now a day's, food is no longer considered by consumers only in terms of taste and immediate nutritional needs but also in terms of their ability to provide specific health benefits beyond their basic nutritional value (Saarela, 2002). Currently, the largest segment of the functional food market is dominated by healthy food products targeted towards improving the balance and activity of the intestinal micro flora (Saarela, 2002). Consumption of food containing live bacteria is the oldest and still most widely used way to increase the number of advantageous bacteria called Probiotics in the intestinal tract (Salminen, 2004).The use of live micro organisms for enhancement of consumers health as an aid to cure some type of gastro intestinal disorders has been recognized for almost a century since the first report by Russian scientist and novel laureate Elie Metchnikoff in 1910 (Ketema *et al*., 2009).

The term Probiotics was derived from the Greek word 'pro bios' which means 'for life' opposed to 'antibiotics' which means 'against life' (Yavuzdurmaz, 2007).Probiotics, as defined in a FAO/WHO (2002) report, are live micro organisms which when administered in adequate amount confer a health benefit on the host. They are live microorganisms which help to protect the host from various intestinal disorders while increasing the number of beneficial bacteria and making the balance steady. They are also called friendly bacteria and they can be used as complementary and alternative medicine (CAM) (Yavuzdurmaz, 2007).The Probiotics bacteria have been historically used to treat a variety of ailments, including infections of mucosal surfaces such as the female genital organs and gastrointestinal tract (FAO/WHO, 2002). However with the discovery and development of antibiotics in the twentieth century, these traditional therapies lost their value. As studied by many scientists, the health benefits of

Probiotics include, prevention and treatment of diarrheal diseases, prevention of systemic infections, management of inflammatory bowel diseases, immune modulation, prevention and treatment of allergies, anticancer effects, treatment of cholestrolaemia, alleviation of lactose intolerance, lowering blood pressure, reduction of helicobacter pylori infections, prevention of osteoporosis and prevention of urogenital infection(Çakır, 2003; Schrezenmeir and De Vrese, 2001; Dunne *et al*., 2001 Dugas *et al*., 1999).

Probiotics taken with food and drink inhibit the growth of pathogenic microbes in the intestinal tract using the mechanisms such as, the production of inhibitory substances, blocking of adhesive sites, competition with pathogens for nutrients and available energy, stimulating of immunity, degradation of toxic substances, suppression of toxic production, reduction of gut PH, enzymatic contribution to digestion and attenuation of virulence, (Çakır, 2003; Schrezenmeir and De Verse, 2001; Dunne *et al*., 2001 Dugas *et al*., 1999).These considerations were also valid for the LAB isolated from fermented food products.

It is believed that most Probiotics do not permanently adhere in the intestinal mucosa but exert their effects as they metabolize and grow during their passage through the intestine. Thus, daily consumption of these bacteria is probably the best way to maintain their effectiveness (Fuller, 1992; Medina *et al*., 2001).The most common Probiotics are Lactic acid producing Bacteria such as, Lacto bacillus, Streptococcus, Bifidobacteria, Fungi such as,Saccharomyces,Aspergellus like Aspergillusoryzae(Fuller, 1992; Medina *et al*., 2001).How ever Lactic acid bacteria have attained major attention for Probiotics activity and have generally been considered as a good Probiotics organism(Saavedra2001,Sullivan etal.,1992).The most common Probiotics are Lactic acid bacteria and Bifido bacteria. These bacteria and most Probiotics are generally recognized as Safe (GRAS).

2.1.2. Food spoilage micro organisms

As Rawat (2015) indicated, the number and type of microorganisms in a food are largely determined by the environment from which the food was obtained, micro biological quality of the foods in its raw or un processed state (intrinsic factors), handling and processing sanitation and effectiveness of packaging, handling and storage conditions in restricting microbial growth (extrinsic factors).

Food spoilage is a metabolic process that causes foods undesirable or unacceptable for human consumption due to changes in sensory characteristics. Spoiled foods may be safe to eat i.e. they may not cause illness because there are no pathogens or toxin but changes in texture, smell, taste or appearance cause them to be rejected (Rawat,2015).

When food is spoiled, it deteriorates to the point in which it is not edible to humans or its quality of edibility becomes reduced. Besides, it appears different from the food in its fresh form such as, changing colors, texture, unpleasant odor or an undesirable taste. The item may become safer than normal. If mould occurs, it is often visible externally on the item (Rawat, 2015).

Spoilage microorganisms are organisms whose growth in the food creates undesirable characteristics in that food and any microorganism which is not intentionally added in to food or intentionally allowed to grow in foods so as to impart certain qualities in that food is considered a contaminant (Kinfe and Abera, 2007).

Growth of the contaminant in that food will spoiled the food making it unfit for human consumption. The microorganisms that cause ordinary food spoilage are not necessarily harmful to us. Infact, centuries before refrigerators, the earliest sauces and seasonings were used to mask the off tastes and smells of food that had begun to spoil (Kinfe andAbera,2007).

Spoilage bacteria don't normally cause food poisoning. Typically, the microorganism that cause food borne illnesses are odor less and flavor less and other wise undetectable outside the laboratory (Kinfe and Abera, 2007).There for, it is important to note that spoiled food is not necessarily dangerous food and at the same time the bacteria may or may not be harmful, but waste products may be unpleasant to taste or may even be harmful to one's health.

Food spoilage can be caused by three types of microorganisms, these are, Bacteria, Yeasts and Moulds**.**

2.1.3. Food borne pathogens

Food borne diseases are diseases caused by eating and drinking foods and drinks containing certain chemicals and harmful microorganisms. Infectious diseases are the biggest problem in human beings and every year gastro intestinal infections are responsible for significant morbidity and mortality worldwide (Culligan *et al*., 2009). World health organization (WHO, 2002) estimated more than four billion episodes of diarrheal diseases annually while there were 2.2 million deaths attributable to enteric infections, making it the fifth leading cause of death at all

ages worldwide. The problem is very serious in rural areas of Africa particularly Ethiopia due to poor sanitary conditions and limited health facility.

It is also reported by the World Health Organization (2013) that food-borne diseases largely reduce the health and economic growth of both developed and developing countries. According to the WHO (2019),an estimated 600 million almost 1 in 10 people in the world fall after eating contaminated food and 420,000 die every year, resulting in the loss of 33 million healthy life year. Apart from diarrhea, food-borne illnesses can also trigger other serious complications such as kidney and liver failure, brain and neural disorders, reactive arthritis, cancer and death (WHO, 2013). Therefore, the causes of food-borne outbreaks need to be investigated carefully to prevent these outbreaks (CDC, 2011).

2.1.4. Spore forming bacteria associated with foods and beverages

Spore forming bacteria that are present in foods are important because the formation of spore by the bacterium allows it to be resistant to heat, freezing, chemicals, and other adverse environmental conditions that our food undergoes during processing and preparation (Causin,1989).

The production of endospores which means bacterium divides within its cell wall, and one side then engulfs the other by several bacterial genus provides them the capability to survive adverse environmental conditions, and ensures a wide dispersion through different habitats (Sneath *et al*., 1986).They are tougher than the average microscopic unicellular organisms. These species, which include the genera Bacillus, Clostridium and Sporolactobacillus, can surround themselves with durable coats of protein that allow them to survive in hostile environmental conditions by remaining dormant for years protected from stresses i.e. chemicals, heat, radiation and dehydration (Carlin *etal*.,2010).

When revived, however, these bacteria can cause a number of diseases including Botulism, anthrax, tetanus and acute food poisoning. The ubiquitous presence, their resistance to heat in common industrial processes such as, pasteurization, the adhesive characters that facilitate their attachment to processing equipment and their ability to germinate and grow in both favorable and unfavorable conditions make them potential sole surviving and growing contaminants in commercial and local food products (Carlin *et al*.,2010).

Spore formers cause two kinds of problems in the food industry. Firstly, they cause disease and secondly they cause a reduction in shelf life and food spoilage. Soil together with air, plants and their products, food utensils, food handlers, animal feeds, human and animal wastes and animal hides are sources of food contamination. Aerobic spore formers transfer from these sources to many types of commercial and local food products. Because of the complexity of food chain, particular spore forming species or types may encounter niches where proliferation occures.This can happen on the primary product ion levels, in the processing line, during distribution or in the final product (Carlin *et al*., 2010).

This proliferation steps enable the spore formers either to enter as a contaminant in a next step of the production chain or to provoke food quality or safety problem in the final product. For the food industry, it is a challenge to gain insight in to the whole contamination flow of endospore formers originating from soil as well as from other source of food contaminats.There for, measures has to be taken to control these spore forming micro organisms in each contamination flow to minimize the risk and to protect the consumers health. Spore forming bacteria include, Bacillus, clostridium, Sporolactobacillus and Sporosarcina (Carlin *et al*., 2010).

2.1.4.1. Aerobic spore forming bacteria

Some spore forming bacteria grow in aerobic condition. They are called aerobic spore forming bacteria. The spores of these bacteria present in raw milk, meat by surviving pasteurization and other food processing events such as refregiration,UHT(Stenfors and Granum, 2001, McGuiggan 2002).

The presence of aerobic mesophilic spore forming bacteria in high levels in sterilized food products will be able to cause the deterioration and or the reduction of shelf life (Fernanda *et al*.,2010).With aerobic spore forming bacteria, it is important to ensure that contamination of raw milk,powderd milk,honey,Keribo and wheat flour are minimized. To achieve this, the nature and origin of spores and in particular of spores in raw milk and meat must be understood although the incidence of bacillus species in dairy products from different geographical area has widely investigated(Montanari *et al*., 2004,Scheldeman *et al*., 2005).

The most commonly studied and researched aerobic spore forming bacteria is *bacillus cereus*

Bacillus cereus

It is an aerobic spore forming rod shaped Bacteria normally present in soil, dust and water. It is a spore forming bacteria umbiqous in the environment. It is readily isolated from soil, cereal crops

and vegetables etc. It has been reported that soil can contain approximately, 1000 to 100,000 spores per gram. Hence it is not uncommon to find this Bacterium in food especially in raw agricultural products such as raw fruit and vegetables, raw herbs. These foods usually contain less than 100 spores per gram but higher amount may be found in some herbs and spices (EFSA,2005).The bacteria is most likely to contaminate milk and meat by poor hygienic practices from the above sources.

B.cereus can form spores which are able to resist heat and survive the cooking temperature .It can either grows in the presence or absence of oxygen. The optimal growth temperature for B.cereus is around 30^0C to 37^0C (EFSA, 2005)

.At a temperature below 10^0c, B.cereus is unable to grow and produce toxin that cause vomiting (EFSA,2005) .Therefore, controlling storage temperature of food is important to prevent food born diseases caused by the bacterium.

Two types of illness have been attributed to the consumption of foods contaminated with *B. cereus*. The first and better known is characterized by abdominal pain and non-bloody diarrhea; it has an incubation period of 4-16 h following ingestion with symptoms that last for 12-24 h. The second, which is characterized by an acute attack of nausea and vomiting, occurs within 1-5 h after consumption of contaminated food; diarrhea is not a common feature in this type of illness (Stenfors *et al.*, 2008).

The MYP agar has been the standard media for plating *B. cereus*, but it has little selectivity so background flora is not inhibited and can mask the presence of *B. cereus.* Bacara is a chromogenic selective and differential agar that promotes the growth and identification of *B. cereus*, but inhibits the growth of background flora (Stenfors *et al*., 2008).

The chromogenic agar has been suggested for the enumeration of *B. cereus* group as a substitute for MYP Typical colonies will grow as pink-orange uniform colonies surrounded by a zone of precipitation. The identification would include all species from the *B. cereus* group: *B. cereus, B. thuringiensis, B. anthracis,B. mycoides,* and *B. weihenstephanensis.* Biochemical testing will be necessary to delineate to the species level. The Bacara media can be purchased as prepared plates or media in flasks to which two supplied reagents are added. The media has a proprietary formulation and cannot be purchased in a dehydrated form (Stenfors *et al*., 2008).

The effect of aerobic spore forming bacteria on food spoilage and health hazards will be studied on Five food items called raw milk, powdered milk, Keribo, wheat flour and Honey.

Milk

Milk, the first food takes on birth, is a 'treasure-trove' of more than 200 ingredients, many with unique functional and nutritional properties which modern processing is capable of isolating and refining for a multiplicity of uses in the food and related industries (Chatterjee and Acharya, 1992). Spore-forming microorganisms have a special position among total micro flora of milk with regard to their greatest ability to survive thermal treatment of milk and subsequently to propagate in final products (Mayr *et al.,* 1998; Abo-Elnaga *et al.,* 2002 and Vyletělová *et al.,* 2002)

Aerobic and facultative anaerobic spore-forming bacteria of the genus *Bacillus* causing a serious problem in milk industry, due to the heat resistance of spores and ability of vegetative cells to produce extra-cellular enzymes causing deterioration of milk and milk products. *Bacillus* spp. are quite common in the agricultural environment and may contaminate milk from various sources either during the production, storage or processing. Lowering this spore load by good hygienic measures could probably further reduce the contamination level of raw milk, in this way minimizing the aerobic spore forming bacteria that could lead to spoilage of milk and dairy products (Westhoff and Dougerty, 1981).

This study was planned to enumerate and identify the aerobic spore forming bacteria *in* heat treated milk and their significance from the public health and economic points of view. Milk is an excellent carrier of undesired microorganisms. Despite being a nutritious food for humans, it also serves as a good medium for the growth of many microorganisms, especially bacterial pathogens. High temperature used for processing of products, activates the spore germination and growth, resulting in spoilage of products (Westhoff and Dougerty, 1981).

Bacillus species are frequently present in raw milk and most common cause of sweet curdling, bitter flavor and bitty cream in pasteurized milk. These defects occur because the spores of these organisms survive pasteurization and in pasteurized products held at ambient temperatures the spores can germinate and grow to produce vegetative cells in largenumbers. These organisms gain entrance to milk from unclean utensils and the dust of hay, straw and grains(Chalmers, 1955).

Presence and multiplication of aerobic spore formers in dairy products are not only of concern as a cause of economic loss through spoilage of contaminated products but also pose many a times serious public health hazards in terms of food poisoning. In the last decades, the importance of

the psycho tolerant aerobic endospore formers for the keeping quality of milk has increased significantly, owing to extended refrigerated storage of raw milk before processing on the farm, higher pasteurization temperatures, reduction of post pasteurization contamination, and prolonged shelf-life requirements of the consumer product (Chalmers, 1955).

It should be remembered that pasteurization activates spore germination and thus enhances vegetative cell growth. Growth of *B. cereus* in pasteurized milk is considered the main limiting factor determining the shelf life of this food product. Too high levels of *B. cereus* in pasteurized milk before the end of shelf life or prolonged refrigerated storage cause common structural defects known as sweet curdling and bitty cream (Heyndrickx *et al.,* 2010).

Milk produced in udder cells is sterile but due to its high nutrient content, it can be a good growth substrate for contaminating bacteria. The quality of milk is monitored via somatic cell counts and total bacterial counts, with prescribed regulatory limits to ensure quality and safety. Bacterial contaminants can cause disease, or spoilage of milk and its secondary products. Aerobic spore-forming bacteria, such as those from the genera Sporosarcina, Paenisporosarcina, Brevibacillus, Paenibacillus, Geobacillus and Bacillus, are a particular concern in this regard as they are able to survive industrial pasteurization and form biofilms within pipes and stainless steel equipment.

Milk powder

Many types of food products are prepared from the milk. One of the products is powdered milk. Converting milk into milk powder increases its shelf life, and enables it to be stored for extended period without substantial loss of quality, even at ambient temperatures. Microbes such as Aspergillus, Bacillus, Enterococcus, Micrococcus, Mucor, Penicillium, Rhizopus, and Streptococcus can cause spoilage of dried milk powder (Chalmers, 1955).

High microbial load in milk and its products leads to spoilage, and economic losses to the producers. Hence, microbial safety is an essential public health issue of developing as well as developed nations. The pathogens such as Escherichia coli, Listeria monocytogenese, Salmonella, and Shigella must be absent in milk powder (Chalmers, 1955).

It is emphasized that good manufacturing practices with careful attention to quality of incoming milk, training of milk suppliers, and plant workers, temperature control, hygienic conditions, and sanitation of equipment and processing plants will significantly reduce the contamination with microbes. Furthermore, all government should start food safety policy with the objective of reducing food-borne illness among the consumers (Chalmers, 1955).

Keribo

Keribo is an indigenous traditional fermented beverage produced and consumed in different parts of the country, including Jimma zone. It is produced mainly from barley and sugar. Fermented Keribo constitutes a major part of the beverage being served on holy days, wedding ceremony and also as sources of income of many households in Jimma zone (Reshid, 2010). The popularity of this traditional fermented beverage is more reflected among the religious groups and those who do not like alcohol drinks. Being considered as none or low alcoholic beverage, Keribo is popular among both adults and children. It has poor keeping quality with shelf life of not more than a day or two, and it has a pronounced characteristic of the deteriorating beverage at the end of 48 hr, of fermentation (Reshid,2010).

The processes involved in Keribo production are simpler than those related traditional Ethiopian fermented beverages such as 'Tella' (Samuel and Berhanu, 1991), Shamita (Bacha *et al*., 1999), and 'Borde' (Ketema *et al*., 1998), although traditional Keribo brewers still rely upon locally available equipment. The sources of fuel are common to all these products; they all use rudimentary sources of energy which do not readily lend themselves to modernization of the process or development of local capabilities. Firewood is traditionally used for cooking of the ingredients and alternative energy sources should be sought when the production technology is upgraded. The roasting of Barley for Keribo preparation is the same as roasting of enkuro for Borde and/or Tella brewing. Roasting and fermentation afford increased energy density and reduce anti-nutritional factors such as polyphenol, phytic acid, trypsin inhibitors and lectins found in cereals (Nout, 1990).

In general, Keribo is a traditional non alcoholic dark brown colored fermented beverage commonly consumed in rural and urban areas of Jimma zone, south western of Ethiopia. With some similarity to Boza of Bulgaria, Albania, Turky and Romania (Blandino *et al.,* 2003).It is produced by an overnight fermentation of cereal (Barley) predominantly by activities of LAB like the fermentation of Shamita (Bacha *et al.,* 1999).

High count of LAB could account for acidification of the product with extention of fermentation periods. A lactic acid bacterium has been involved in the natural fermentation of many traditional Ethiopian fermented foods and beverages (Bahiru, 2000).

Honey

Honey has been used by countless cultures all around the world over the past 2,500 years. While the numerous health benefits of honey have made it an important element of traditional medicines such as Ayurvedic treatments, scientists are also researching its benefits in relation to modern medicine, particularly in the healing of wounds (Finola *et al.*, 2007).

It is known as *Honig* in German, *Miele* in Italian, *Shahad* in Hindi, *Miel* in French and Spanish, *Mel* in Portuguese, *мед* in Russian, *Honing* in Dutch, and *μελι* in Greek; there is almost no part in the world where honey is not widely used and celebrated as a part of the cultural diet(Finola *et al.*, 2007). But what makes honey so popular? Most likely, it is the ease with which it can be consumed. One can eat it directly, put it on bread like a jam, mix it with juice or any drink instead of sugar, or mix it with warm water, lime juice, cinnamon, and other herbs to make a medicine. It is savored by all due to its taste as well as health benefits, making it extremely useful and versatile. Some people have the opinion that all honey available in the market is natural and obtained from the wild. But it can also be produced scientifically controlled systems.

Honey is a sweet and flavorful product which has been consumed as a high nutritive value food. It is essentially composed of a complex mixture of carbohydrates (of which fructose and glucose accounts for nearly 85-95%) and other minor substances, such as organic acids, amino acids, proteins, minerals, vitamins, and lipids (White, 1975). Honey is a global food that is known also for its healing, antiseptic, antioxidant and anti bacterial properties (Aboud *et al.*, 2011).

The quality of honey is mainly determined by its sensorial, chemical, physical and microbiological characteristics (Finola *et al.*, 2007). Microorganisms in honey may influence the stability of the products and its hygienic quality. Honey has several sources of microbial contamination. Primary sources include pollen, the digestive tracts of honey bees, dust, air, soil and nectar, and are somewhat difficult to eliminate. On the other hand, secondary sources, due to honey handlers and processing, are easier to control by the application of good manufacturing

practices(Snowdon and Cliver,1996).Bacillus; Clostridium, Penicillium, Mucor, Saccharomyces, Schizosaccharomyces and Torula are the microorganisms of concern in honey (Collins *et al.*, 1999; Migdal *et al*., 2000; Finola *et al*., 2007). Sulfite reducing Clostridia is an indicator organism, whose presence in honey provides evidence of contamination or pollution (Collins *et al.,* 1999). The presence of spores of Clostridium is especially dangerous for infants and small children (Centorbi *et al*., 1999). Infant botulism is mainly caused by the consumption of honey contaminated with C. botulinum (Finola *et al*., 2007).

Wheat powder (flour)

Clostridium, salmonella spp,shigellaspp,Staphylococcusaureaus,Listeriamonocytogens,Yeastes Lactic acid bacteria and moulds are found in wheat flour in different counts(Berghofer *et al*.,2003) Most frequent counts in traditional and industrial wheat flour were total aerobic mesophilic bacteria with the average $4x10^4$ and $2.5x10^4$cfu/g respectively .The less moisture and water content (a_w) makes wheat powder to have long extended shelf life although spore formers may exist in it(Berghofer *et al*.,2003).

2.2. Antibiotic susceptibility of Bacteria

Antibiotics are one of the great discoveries against bacterial infections. Unfortunately; bacteria can fight back by being resistant towards antibiotics. From the human perspective, résistance to antibiotics is an undesirable ability of micro organisms, including spore forming bacteria. A bacterium with antibiotic resistance that becomes an opportunistic pathogen could be highly detrimental to the infected host. Furthermore, a bacterium with antibiotic resistance and the ability to transfer the resistance to pathogenic bacteria could cause great harm to humans and animals (Teuber *et al*., 1999; Saarela *et al.*, 2000).

A bacteria should preferably not posses any antibiotic resistance and if does, it should be unable to transfer the antibiotic resistance genes. These considerations will also be valid for the Bacteria which will be isolated from raw milk, powdered milk, meat, wheat powder and honey. They should not carry transmissible antibiotic resistance genes (Saarela *et al*., 2000)

Safety is one of the recommended attributes in the FAO/WHO guidelines (2002) on evaluation for bacteria. The safety of the food products is appraised with the phenotypic and genotypic characteristics and the statistics of used micro organisms (Cakir, 2003).The main requirement of

safety aspect of bacteria is, They should not carry transmissible antibiotic resistance genes (Saarela *et al.*,2000).An important safety aspect in assaying for bacteria is antibiotic resistance. This is because antibiotic resistant genes, especially those encoded by plasmids could be transferred between microorganisms (Cakir, 2003).

The potential strains need to be assayed for their antibiotic resistance to prevent the undesirable transfer of resistance to other endogenous bacteria. The risk of gene transfer depends on the nature of the genetic material (plasmids, transposons), the nature and concentrations of the donor and recipient strains and their interactions and the environmental conditions. The presence of an antibiotics may facilitate the growth of antibiotic resistant mutants (Marteu, 2001).

3. Materials and methods

3.1. Description of the study area and period

The study was conducted in Jimma town located at 353km south west of Addis Ababa the capital city of Ethiopia. The towns geographical coordinates are 70 41ˆN latitude and $36^{0}50$ˆ E longitude. The study area has an average altitude of 1780 m above sea level (Reda *et al.*, 2012). The microbial analysis of the five food items were conducted in Jimma University, Biology department, Microbiology laboratory from September 2018 to August 2019.

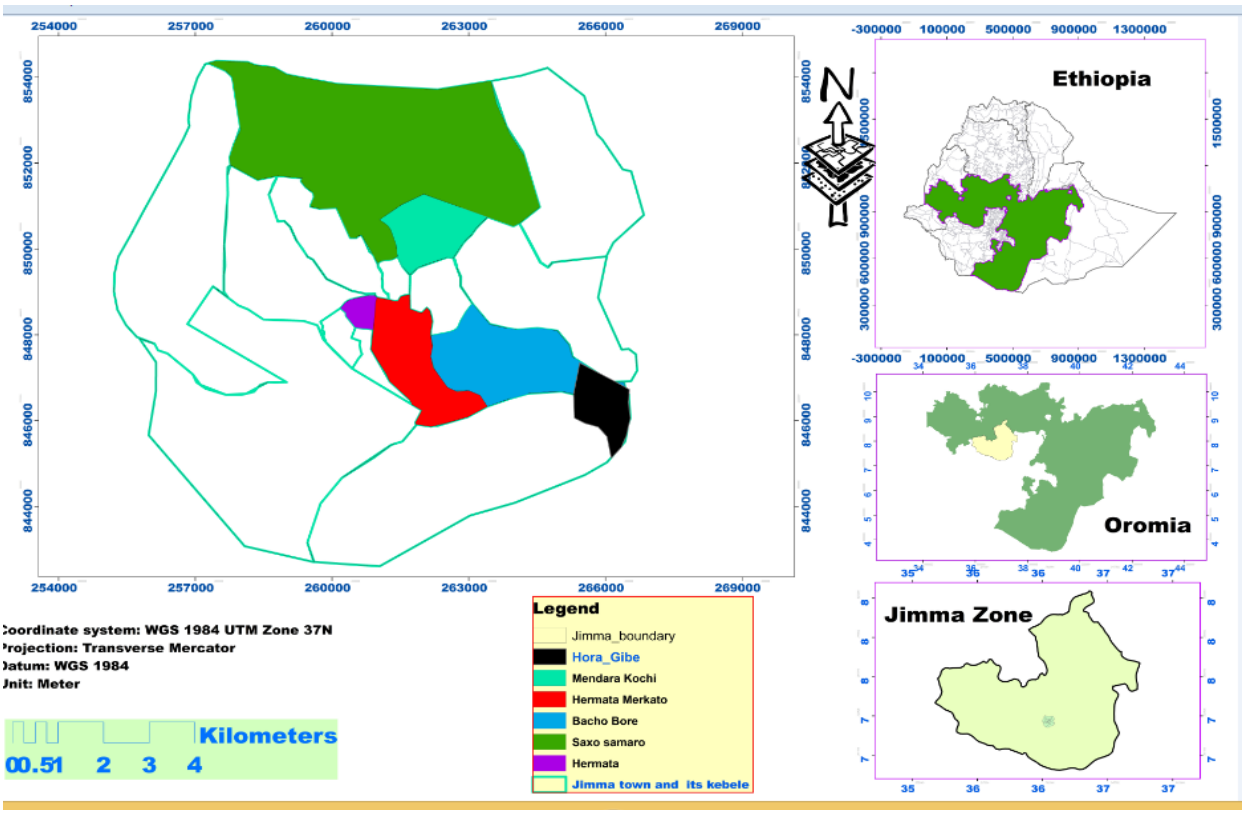

Figure 1 Map of the study area (ArchGis, 2019)

3.2. Study design and population

A cross sectional and experimental study design was used. The total population of food product vendors and households (who prepared food for their own consumption) of the five study sites were 50.These were, 10 households and vendors in each of the five sites of Jimma town.

3.3 Sample size and technique

A total of 50 questionnaires were formulated and a simple random sampling technique was used to get information to address representative individuals from the whole population and 50 respondents (10 respondents from each site) were randomly selected to fill the questionnaire. Furthermore, in addition to these tools, observation was done to assess the sanitation of the sites and food samples.

The questionnaire used to gather information included issues related to hygienic condition and sanitation of the vended sites, vendor, water used to wash and rinse the utensils, use of detergents to wash hands, carefulness of the food vendors including households when handling money, practices (awareness) they get from health authorities relating to food safety and quality including shelf life of the food product.

The food samples needed to the study were also collected. A total of 50 food samples of row milk, powdered milk, wheat flour, Honey and Keribo(10 samples each) were collected from the five site of Jimma town namely Ajip, Bishishe, Bochobore, Kochi and Merkato using random sampling technique .Some of the food samples were purchased from vendors and other food samples were obtained freely from certain households.

3.4. Sample collection and preparation

The fifty samples (ten samples from each) of the five food items (500gm or ml each) collected from the five sites were transferred alternatively (ten samples of food item at a time) to Jimma University Micro biology Laboratory for bacteriological examination using sterile equipments or ice box. Upon arrival to the laboratory, the samples were analyzed as rapidly as possible. Twenty five gm of sample from each five food items was separately mixed with 225ml sterile 0.1% buffered peptone water and homogenized in sterilized flasks for 30 seconds using a shaker at normal speed. The homogenate was then serially diluted by transferring 1ml from each homogenized sample and pippeted asceptically in to 9ml of sterile saline solution. A dilution series of 10^{-2} to 10^{-5} was made in sterile saline solution. The remained part of the arrived food samples were stored at 4^0C to be used for further analysis. Unlike other food samples, Keribo is rarely accessed from market. But then, it was obtained in the above sites from households who prepared it during religious festivals for family consumption. The other food items were purchased from households, shops and supermarkets.

3.5. Isolation and estimation of aerobic spore count

To kill the vegetative and none spore forming Bacteria, The fifty serially diluted Food samples were subjected to heat treatment at 80^0C for 10 minutes (Abo-Elnaga *et al.,* 2002). The samples were then cooled to room temperature and aliquots of 0.1ml from the appropriate dilutions were spread plated in duplicate on pre dried and appropriately marked standard plate count agar and finally incubated at 32^0C for 72hrs.All the isolates on each agar plates were counted using a marker pen. The viable bacterial colony in each serial dilution were counted and expressed in colony forming units per milliliter ($CFUml^{-1}$). I.e, the number of Aerobic spore forming bacteria from each duplicated countable plates were reported as mean CFU/ml (CFU/gm) calculated from Arithmetic mean of total samples. Those dilution factors which contain more colonies were selected for the mean count and Only countable plates (30-300 colonies) were considered for enumeration of the cell density. The number of colonies on each agar as well as relevant dilutions of agar plates has to be noted down.

3. 6.Purification of isolates

Individual colonies with distinct morphological characteristics such as shape, size, margin, elevation, pigmentation were randomly picked from each countable agar plates as aerobic spore forming bacteria isolates and purified by transferring to the nutrient broth.

The purified strains were verified by three subcultures from a single colony. That is, each aerobic spore forming bacteria isolates (with its own codes) was purified by repeated streak plating on nutrient agar for three times. Thereafter, a single colony was transferred in to 5ml of nutrient broth and allowed to grow by incubating at 32^0C for 72hrs.From the broth culture, a loopful was streak plated on to pre solidified nutrient agar and pure colonies from each nutrient agar plates were maintained on the nutrient agar slants at 4^0C for further characterization and identification (Magula *et al.*, 2001).

3.7. Characterization and identification of aerobic spore forming bacteria

All the aerobic spore forming bacteria isolates were characterized using compound light microscope (1000X) and conventional biochemical and physiological tests. All the isolates of bacteria were separately activated in nutrient broth. Young (overnight and active) aerobically incubated cells were used for all tests. Since Bacillus cereus is dominantly found in most food samples (velusamy *al et*, 2010), the purified and stored colonies of aerobic spore forming

bacteria were also activated and inoculated on Bacara agar (a selective and diagnostic medium for the isolation and enumeration of *B. cereus*) and incubated at 37^0C for 24hrs to confirm its presence.

3.7.1. Morphological test.

Morphological identification of the pure cultures of aerobic spore forming bacteria was conducted microscopically under oil emersion objective using gram staining technique. Therefore, cell shape, motility, cell grouping were the basic features evaluated during morphological observation.

Cell shape and spore formation test

Shape was identified by gram staining techniques.

spore formation test

Smear 4-5 days old bacteria culture was placed on clean glass slide and heat fixed. Then, the smear was covered with a piece of absorbent paper and placed on the wire gauze on a ring stand. The paper was saturated with malachite green and placed near the Bunsen burner until steam was rinsing from the surface. The slide was reheated to keep it steaming for about 3 minute. The paper was removed with forceps and the slide was rinsed thoroughly with tap water and then drained and counter stained for 45 seconds with 0.5% safranin. Finally, it was washed, dried and examined under a microscope. The vegetative cell would appear red and spores would appear green (Tambeker and Bhutada,2010).

The spore formers which appeared green were screened for biochemical and physiological test(Tambeker and Bhutada,2010).

Motility test

A needle containing fresh active bacterial culture was inoculated by making a single stab about two thirds down the motility agar medium (HI medium) and the needle was pulled up along the same path. The inoculated agar medium was incubated at 32^0C for 72hrs and examined for the type of growth. Motile organisms produce diffuse growth out in to the agar medium away from

each stab. Non motile organisms produced growth only in and along the stab line (Cruickshank *et al.,* 1975).

3.7.2. Biochemical and Physiological test

The isolates were further characterized using conventional biochemical and physiological tests including tests for Gram reaction (KOH test), Catalase test; carbohydrate fermentation,starch hydrolysis, salt tolerance test, acid tolerance test. Since Bacillus Cereus is common aerobic spore forming bacterial contaminant in most studied food samples, its presence was confirmed by using Bacara agar. Bacterial strains were designated by letters and numbers in their order corresponding to the name of the food sample from which they were first isolated.

KOH test

The Gram reaction test was done according to rapid method proposed by Gregerson (1978). Briefly, two drops of 3%KOH solution were placed on a clean microscope slide. An overnight culture was scrapped from the plate aseptically with a wire loop and stirred in to KOH solution. After 10 seconds, the inoculating loop was raised slowly from the mass. When KOH solution become viscous and threads of slime were following the loop for 0.5 to 2cm, the reaction was considered positive and the isolates were considered Gram negative. Absence of such slime was an indication of the presence of gram positive Bacteria.

Catalase test

A drop of 3% hydrogen peroxide was placed on a clean glass slide. With a wire loop, overnight culture of the isolate from nutrient agar plat was picked and transferred in to the drop of hydrogen peroxide. Both were mixed and observed for gas bubbles production. Gas production was considered as positive test for Catalase enzyme production (Tambekar and Bhutada, 2010).

Carbohydrate fermentation test

This test was used to determine the ability of an organism to ferment a specific carbohydrate with or without the production of gas. Phenol red was used as an indicator in the media. An inverted Durham tube in the broth captures some of the gas the organism produces allowing production to be seen. The test was done using three sugars, lactose, glucose and maltose (Tambekar and Bhutada, 2010).

Gas production from glucose

To determine the homo fermentative and hetro fermentative characterization of isolates, CO_2 production from glucose was evaluated using inverted Durham tube.Accordingly.Durham tube was placed in a test tube containing phenol red broth base medium in to which glucose (1%) was added and mixed with distilled water. The tube was placed in an inverted position. After sterilization in autoclave, each test tube was inoculated with a single colony of the bacterial culture under study and incubated at 32^0C for 24 hrs.The isolates were designated as homo fermentative or hetero fermentative by observing the presence or absence of gas in Durham tube (Tambekar and Bhutada,2010).

Starch hydrolysis

Freshly activated young Bacterial cultures were streaked on starch agar plates and incubated at 32^0C for 24 hrs. Finally, drops of gram iodine (indicator) was added on the plates and rotated gently for any clear zone to be observed.

3.7.3. Physiological test

Temperature tolerance

To detect the growth temperature of the strains, Bacterial cultures were grown at varying temperatures I.e. 20^0C, 45^0C and 60^0C for 72hrs in nutrient broth. Then 0.1ml inoculums was transferred to nutrient agar plates by pour plate method and incubated at 32^0C for 72hrs.The growth of aerobic spore forming bacteria on nutrient agar plates was used to designate isolates as temperature tolerant (Tambekar and Bhutada, 2010).

Salt tolerance test

Isolates from each sample were tested for their tolerance against different Nacl concentration. For this purpose 5%,6% and 8% Nacl concentrations were selected .Each of these different salt concentrations were added in a test tube containing nutrient broth and inoculated with fresh overnight culture of the test organism and incubated at 32^0C for 72hrs.After 72hrs of incubation,0.1ml inoculums was transferred to nutrient agar plates by pour plate method and incubated at 32^0C for 72hrs.The growth of bacteria on nutrient agar plates was used to designate isolates as salt tolerant (Tambekar and Bhutada,2010)

Acid Tolerance test

The isolated bacterial cultures were inoculated in to sterile nutrient broth tube of varying p^H of 3,5,8 and incubated at 32^0C for 72 hrs. Then 0.1ml inoculums form each broth were poured to nutrient agar plats by pour plate method and incubated at 32^0Cfor 72 hrs.The growth of bacteria in the agar plates were used to designate isolates as P^H tolerance (Tambekar and Bhutada, 2010).

3.8. Antibiotics susceptibility test.

The antibiotic sensitivity of the aerobic spore forming bacterial isolates were assessed using disk diffusion (Kirby Bauer) test. Overnight active aerobic spore forming bacterial Inocula which were prepared in Pepton water by adjusting its turbidity equivalent to 0.5 McFarland units (approximately 10^7-10^8CFU/ml) were spread plated on the Muller Hinton agar. The antibiotic discs were placed on the surface of the agar plates and the plates were kept at 4^0C for 1hr for diffusion and then incubated at 37^0Cfor 24hrs.

Zone of suppression of growth were assessed against 6 standard antibiotic discs (Oxoid) were used depending up on the antibacterial spectrum, toxicity, effectiveness and availability (Vlkova *et al*., 2006), namely Ampicillin, ciprofloxacilin, Clindomycin, Gentamycin, Kanamycin and vancomycin. The zone size (mm) interpretive chart for antibiotics was measured according to performance standard for antimicrobial disc susceptibility tests as described by (Bauer *et al,* 1966)

3.9. Risk Factor Analysis

Possible factors that contributed to prevalence of aerobic spore formers were assessed using observational check list.

3.10. Statistical analysis

Data was entered to micro soft excel and analyzed using SPSS soft ware package(version 20).Descriptive statistics was used to describe data on microbial counts, biochemical analysis and antibiotic susceptibility tests. Results were presented using tables. Relationship between parameters were analyzed using one–way ANOVA and differences were determined by Duncan's multiple range test ($p<0.05$).All statistical results with $p<0.05$ were considered to be statistically significant.

3.7. Ethical consideration

Ethical clearance was obtained from Research and Postgraduate Coordinator Office, College of Natural science, Jimma University. Respondents and concerned officials were informed about the purpose of the study. The consent was obtained from food venders after a brief explanation of the objectives and benefits of the study.

4. Result

4.1. Socio -demographic character of respondents

Most of the vendors (96%) engaged in the business are females and aged between 31-40 years (80%) (Table1). With regards to education status, the vendors are mostly (90%) attendees of primary education with very few (10%) had chance of attending secondary school.

Table 1. Socio-demographic charact
eristics of vendors/ respondents in Jimma town, south western Ethiopia, 2019

Parameters		Number of respondents (N=50)	Percentage %
SEX :	Male	2	4.0
	Female	48	96
Age :	<20	-	0.0
	20-30	6	12
	31-40	40	80
	>40	4	8.0
Academic status :	illiterate	-	0.0
	Primary education	45	90
	Secondary education	5	10
	Certificate & the above	-	0.0

Physical observation on the hygienic status and appropriateness of the sites for vending revealed lots of limitations. There were hygienic inadequacy with respect to the sanitation of vending sites, households and food venders that may be a key factor for microbial load of the studied food products contributing to the safety and quality problems. The hygienic condition of sites, vendors, and households including their processing, preparing and handling materials were not acceptable .The respondents lack knowledge or awareness on the effect of microorganisms on these food products. They did not take education from the local health authorities on food safety and quality (data not given). All of these Problems question the sanitation of the local and commercial food products selected for this study.

The highest counts (CFU/ml or gm) of Aerobic spore forming bacteria recorded in Keribo from Qochi, Row milk from Ajip, powdered milk from Qochi, wheat flour from Merkato and Honey from Qochi were $2.95x10^5$, $1.51x10^5$, $2.99x10^5$, $1,8.2x10^4$ and $5.9x10^4$, respectively. The mean colony count was a bit higher in powdered milk samples with a minimum count in Honey food samples with mean count (log cfu/ml or gm) of 4.31 and 3.24, respectively ((Table 2)). Deep

roasting of the cereal and boiling at about 65-70^{0}C for 15 to 20 minutes during Keribo preparation, low water activity of powdered milk and wheat flour together with high temperature treatment (80^{o}C) of the five food items, potentially eliminated the vegetative and none spore forming bacteria and favored the growth of Aerobic spore forming bacteria later on during lab assessment of the products. The mean Aerobic spore count (log CFU/ml or gm) compared among five food items of (Honey, Keribo, powdered milk, Row milk, and Wheat flour)are as depicted in Table 2 below, with highest mean count recorded for sample from powdered milk (Table 2) .

Table 2 Mean count (log cfu/ml) of aerobic spore forming bacteria in selected commercial and local food products, in Jimma town, 2019.

Sample source	Number of samples	Mean count	P value
Honey	10	3.24	0.51
Keribo	10	4.05	
Powdered milk	10	4.31	
Row milk	10	3.54	
Wheat flour	10	2.74	

ASC-Aerobic spore count; CFU-colony form units

As one way ANOVA analysis showed, there was no significant difference ($p>0.05$) in mean aerobic spore counts when the five food items are compared among themselves. Furthermore, the mean Aerobic spore count of each food items when compared among the five sites of Jimma town showed that there was no significant difference among each ten food samples of five food items ($P>0.05$) collected from the five sites of Jimma town (Table 3)..The similarities of the storage temperature, moisture content of the place, sanitation (hygienic condition) of the vendor and water used for washing and rinsing the utensils, the use of detergents to wash hands, containers and carefulness of the vendor when handling money probably contributed to the observed relatively lower mean counts.

Table 3 Mean counts (log CFU/ml) of aerobic spore forming bacteria associated with commercial and local products, in Jimma town, 2019.

Food type	Number of sample	Sample site and mean count(X ±SD)					P.Value
		Ajip	Bishishe	Bochobore	Kochi	Merkato	
Honey	10	0.0±0.00	4.60 ±0.14	4.56 ±0.09	4.70 ±0.09	2.33 ±3.30	0.08
Keribo	10	2.73±3.86	5.30±0.24	5.09±0.16	2.27 ±3.21	4.86 ±0.18	0.56
Milk powder	10	4.84 ±0.46	2.40 ±3.40	4.55 ±0.05	5.09 ±0.54	4.67 ±0.14	0.49
Row milk	10	5.14±0.08	2.50 ±3.54	5.09 ±0.07	0.00 ±0.00	4.95 ±0.03	0.07
Wheat flour	10	0.00 ±0.00	4.50 ±0.01	2.26 ±3.20	4.51 ±0.02	2.45 ±3.47	0.31

X=Mean; SD=Standard deviation

After Aerobic spore count, the representative colonies from countable plates of plate count agar were picked based on their shape, size, margin, elevation, color; and inoculated into the nutrient broth(5ml each) and were purified by repeated plating. A total of 60 Aerobic spore forming bacteria (twelve isolates from each) were isolated from the nutrient agar plates.

The morphological identification of cultures was carried out microscopically after gram staining. The cultures biochemical and physiological characteristics were also determined. The morphological, biochemical, and physiological characterization of Aerobic spore forming bacteria isolated from Keribo, Row Milk, powdered Milk, Wheat flour and Honey were as depicted in Tables 4 below. Observation of the Gram stained cells indicated that all the strains were rod shaped and were seen scattered randomly.

The probable identities of morphologically, biochemically and physiologically identified Aerobic spore forming bacteria were determined based on the methods described in Bergey's Manual of Determinative Bacteriology and ABIS (online bacteria identification software).Thus, Kr7b,Kr8, Kr9b from Keribo; H_1a,H_5b,H_7a from Honey; Wf_1b,Wf_4a,Wf_8a from wheat flour M_3b, M_4f, M_5b, M_7a from milk samples and Mp_1a,Mp_3b Mp_6a from milk powder are *Bacillus cereus* (Table 4). Kr_2, Kr_3, Kr_6, Kr_{10}a, kr_{10}c from Keribo; H_{2b}, $H_{5a,}$ H_7b from honey, M_3a, M_{10}a from milk sample, Mp_9a, from powdered milk are *Bacillus Coagulans*. Kr_3, Kr_7a, Kr_9a, Kr_{10}b, from Keribo; H_2a, H_6b,H_7c from Honey; Wf_1a,Wf_1c,Wf_5a,Wf_7a,Wf_{10a},Wf_{10}b from Wheat flour; M_3c, M_5a, M_8a, M_9a from Milk sample; Mp_2a, Mp_2b, Mp_3a, Mp_4a, Mp_4b, Mp7a from Milk powder are *Bacillus subtilis*. H_1b, H_6a, H_6c, from Honey; and M_3d, M_3e, from Milk sample are *Bacillus Magaterum.*

Wf_1e, Wf_5b, Wf_6a from Wheat flour are *Bacillus pumilus*. Mp_8a, $Mp_{10}a$ from powdered milk are *Bacillus licheniformis*. Since previous studies indicated that *Bacillus cereus* is the most common strain in food products, its presence was confirmed by Bacara agar.

Morphological, Biochemical and physiological characteristics of the sixty isolates with tentative identities are depicted in Table 4 below.

Table 4 Morphological, Biochemical and physiological characterization of isolates, Jimma town, 2019.

Isolates cod	*Sample source*	*Morphological Characterization*	*Biochemical characterization*							*Physiological characterization*									*Isolates Tentative Identity*
										Temperature Tolerance			Acid Tolerance			Salt Tolerance			
		Cell morphology	*KOH test*	*Catalase*	*Motility*	*Fermentation type*	*Lactose*	*Maltose*	*Starch*	20^0C	45^0C	60^0C	3	5	8	5%	6%	8%	
Kr_2	Keribo	Rod	-	+	+	+/-	+	+	+	+	+	+	-	+	+	+	-	-	*B.coagulans*
Kr_3	Keribo	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
Kr_3	Keribo	Rod	-	+	+	+/-	+	+	+	+	+	+	-	+	+	+	-	-	*B.coagulans*
Kr_6	Keribo	Rod	-	+	+	+/-	+	+	+	+	+	+	-	+	+	+	-	-	*B.coagulans*
Kr_{7a}	Keribo	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
Kr_{7b}	Keribo	Rod	-	+	+	+/+	+	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
Kr_8	Keribo	Rod	-	+	+	+/+	+	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
Kr_{9a}	Keribo	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
Kr_{9b}	Keribo	Rod	-	+	+	+/+	+	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
Kr_{10a}	Keribo	Rod	-	+	+	+/-	+	+	+	+	+	+	-	+	+	+	-	-	*B.coagulans*
Kr_{10b}	Keribo	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
Kr_{10c}	Keribo	Rod	-	+	+	+/-	+	+	+	+	+	+	-	+	+	+	-	-	*B.coagules*
H_{1a}	Honey	Rod	-	+	+	+/+	-	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
H_{1b}	Honey	Rod	-	+	+	+/-	+	-	+	+	+	-	-	+	+	+	+	-	*B.magaterum*
H_{2a}	Honey	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
H_{2b}	Honey	Rod	-	+	+	+/-	+	+	+	+	+	+	-	+	+	+	-	-	*B.coagulans*
H_{5a}	Honey	Rod	-	+	+	+/-	+	+	+	+	+	+	-	+	+	+	-	-	*B.coagulans*
H_{5b}	Honey	Rod	-	+	+	+/+	+	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*

Isolates cod	*Sample source*	*Morphological characterization*	*Biochemical characterization*							*Physiological characterization*									*Isolates Tentative Identity*
										Temperature Tolerance			Acid Tolerance			Salt Tolerance			
		Cell morphology	*KOH test*	*Catalase*	*Motility*	*Fermentation type*	*Lactose*	*Maltose*	*Starch*	20^0C	45^0C	60^0C	3	5	8	5%	6%	8%	
H_{6a}	Honey	Rod	-	+	+	+/+	+	-	+	+	+	-	-	+	+	+	+	-	*B.magaterum*
H_{6b}	Honey	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
H_{6c}	Honey	Rod	-	+	+	+/-	+	-	+	+	+	-	-	+	+	+	+	-	*B.magaterum*
H_{7a}	Honey	Rod	-	+	+	+/+	-	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
H_{7b}	Honey	Rod	-	+	+	+/-	+	+	+	+	+	+	-	+	+	+	-	-	*B.coagulans*
H_{7c}	Honey	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
Wf_1a	W.flour	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
Wf_1b	W.flour	Rod	-	+	+	+/+	-	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
Wf_1c	W.flour	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
Wf_1e	W.flour	Rod	-	+	+	+/-	-	-	-	+	+	-	-	+	+	+	+	+	*B.pumilus*
Wf_4a	W.flour	Rod	-	+	+	+/+	-	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
Wf_5a	W.flour	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
Wf_5b	W.flour	Rod	-	+	+	+/-	-	-	-	+	+	-	-	+	+	+	+	+	*B.pumilus*
Wf_6a	W.flour	Rod	-	+	+	+/-	-	-	-	+	+	-	-	+	+	+	+	+	*B.pumilus*
Wf_8a	W.flour	Rod	-	+	+	+/+	-	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
Wf_7a	W.flour	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
$Wf_{10}a$	W.flour	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
$Wf_{10}b$	W.flour	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*

Isolates cod	*Sample source*	*Morphological characterization*	*Biochemical characterization*							*Physiological characterization*									*Isolates Tentative Identity*
										Temperature Tolerance			Acid Tolerance			Salt Tolerance			
		Cell morphology	*KOH test*	*Catalase*	*Motility*	*Fermentation type*	*Lactose*	*Maltose*	*Starch*	20^0C	45^0C	60^0C	3	5	8	5%	6%	8%	
M_3a	Milk	Rod	-	+	+	+/-	+	+	+	+	+	+	-	+	+	+	-	-	*B.coagulans*
M_3b	Milk	R0d	-	+	+	+/+	-	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
M_3c	Milk	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
M_3d	Milk	Rod	-	+	+	+/-	+	-	+	+	+	-	-	+	+	+	+	-	*B.megaterium*
M_3e	Milk	Rod	-	+	+	+/-	+	-	+	+	+	-	-	+	+	+	+	-	*B.megaterium*
M_4f	Milk	Rod	-	+	+	+/+	-	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
M_5a	Milk	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
M_5b	Milk	Rod	-	+	+	+/+	-	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
M_7a	Milk	Rod	-	+	+	+/+	-	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
M_8a	Milk	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
M_9a	Milk	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
M_{10}a	Milk	Rod	-	+	+	+/-	+	+	+	+	+	+	-	+	+	+	-	-	*B.coagulans*
Mp_1a	Milk.p	Rod	-	+	+	+/+	-	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
Mp_2a	Milk.p	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
Mp_2b	Milk.p	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
Mp_3a	Milk.p	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
Mp_3b	Milk.p	Rod	-	+	+	+/+	-	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
Mp_4a	Milk.p	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*

Isolates cod	*Sample source*	*Morphological characterization*	*Biochemical characterization*							*Physiological characterization*									*Isolates Tentative Identity*
										Temperature Tolerance			Acid Tolerance			Salt Tolerance			
		Cell morphology	*KOH test*	*Catalase*	*Motility*	*Fermentation type*	*Lactose*	*Maltose*	*Starch*	20^0C	45^0C	60^0C	3	5	8	5%	6%	8%	
Mp_4b	Milk.p	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
Mp_6a	Milk.p	Rod	-	+	+	+/+	-	+	+	+	+	-	-	+	-	+	+	-	*B.cereus*
Mp_7a	Milk.p	Rod	-	+	+	+/-	-	+	+	+	+	-	-	+	+	+	+	-	*B.subtilis*
Mp_8a	Milk.p	Rod	-	+	+	+/-	+	+	+	+	+	-	-	+	-	+	+	-	*B.lichenoforms*
Mp_9a	Milk.p	Rod	-	+	+	+/-	+	+	+	+	+	+	-	+	+	+	-	-	*B·coagulans*
$Mp_{10}a$	Milk.p	Rod	-	+	+	+/-	+	+	+	+	+	-	-	+	-	+	+	-	*B.lichenoforms*

+(Growth) --(No growth)

The frequency distribution of the identified aerobic spore forming bacilli in the five food items was as summarized here under (Table 5). Their frequency distribution varied in each of the five food items. In the present study, *B.cereus, B.coagulans* and *B.subtilis* were identified in Keribo samples with the prevalence of *B.subtilis* recorded the highest (41.7%).

Table 5 Frequency distribution of isolates of Aerobic spore formers in different products

Product	Tentative isolates	Number of isolates	Frequency of isolation (%)
Keribo	*Bacillus cereus*	3	25
	Bacillus coagulans	5	41.7
	Bacillus subtilis	4	33.3
Honey	*Bacillus cereus*	3	25
	Bacillus coagulans	3	25
	Bacillus subtilis	3	25
	Bacillus B.megaterium	3	25
Wheat fiour	*Bacillus cereus*	3	25
	Bacillus subtilis	6	50
	Bacillus pumilus	3	25
Milk	*Bacillus cereus*	4	33.3
	Bacillus coagulans	2	16.7
	Bacillus subtilis	4	33.3
	Bacillus megaterium	2	16.7
Milk powder	*Bacillus cereus*	3	25
	Bacillus coagulans	1	8.3
	Bacillus subtilis	6	50
	Bacillus lichenoforms	2	16.7

Four bacillus strains namely, *B.coagulans, B.cereus, B.subtilis* and *B.megaterium* with equal prevalence (25%) were identified in Honey food samples. Likewise, four bacilli strains including B.coagulans, *B.megaterium*, *B.cereus* and *B.subtilis,* with the last two having higher prevalence (33.3% each) were identified from row milk samples. Similarily, *B.cereus, B.pumilus* and *B.subtilis* (50%) were identified in Wheat flour samples. *B.cereus, B.coagulans, B.lichenoforms* and B.subtilis, with 50% prevalence of the later were bacilli encountered in Milk powder samples. The frequency distribution of *B.pumilus* and *B.lichenoforms* were lower compared to the other strains. *B.pumilus* was obtained only in Wheat flour and *B.lichenoforms* was obtained only in Milk powder food samples (Table 5).

Results of the susceptibility tests of Aerobic spore forming bacilli against six types of anti microbial agents revealed that, all the identified isolates were resistant to Ampicillin but sharing similarity in their lower number of resistance to Ciprofloxacin, clindomycin and Gentamycin (Table 6).

Table 6 Antibiotic susceptibility patterns of *Bacillus* species isolated from different food items in Jimma town, south western Ethiopia, 2019.

Drugs	*B.cereus* (12 isolates)		*B.coagulans* (6 isolates)		*B.subtilis* (14isolates)		*B.megaterium* (5 isolates)		*B.pumilus* (3 isolates)		*B.lichenoforms* (2 isolates)	
	R	S	R	S	R	S	R	S	R	S	R	S
Ampicillin	12	0	6	0	14	0	5	0	3	0	2	0
Ciprofloxacin	8	4	0	6	2	12	0	5	0	3	0	2
Clindomycin	11	1	2	4	13	1	3	2	2	1	1	1
Gentamycin	4	8	0	6	1	13	1	4	0	3	1	1
Kanamycin	12	0	3	3	12	2	3	2	2	1	1	1
Vancomycin	12	0	4	2	9	5	3	2	1	2	1	1

S-sensitive; R-resistant

Four bacilli strains namely, *B.coagulans, B.megaterium*, *B.pumilus* and *B.lichenoforms* showed no resistant to Ciprofloxacin. All the members of *B.coagulans* and *B.pumilus* were susceptible to Gentamycin (Table 6). Although the number of anti microbial agents used is only six, almost all the identified aerobic spore forming strains exhibited multi drug resistance as they resist two or more antibiotics. Antimicrobial drugs showing intermediate zone of inhibition were considered resistance by the bacteria.

Table 7 Distribution of MDR patterns among various species of Aerobic spore forming bacteria.

Species	Number of tested isolates	MDR patterns	Number showing the patterns
B.cereus	12	AMP,CIP,CD,GM,K,VA	3
		AMP,CIP,CD,K,VA	5
		AMP,CD,K,VA	3
		AMP,K,VA	1
B.coagulans	6	AMP,CD,K,VA	2
		AMP,K,V	1
		AMP,V	2
		Amp	1
B.subtilis	14	AMP,CIP,CD,GM,K,VA	1
		AMP,CD,K,VA	6
		AMP,CIP,CD,K	1
		AMP,CD,VA	2
		AMP,CD,K	3
		AMP,K	1
B.megaterium	5	AMP,CD,GM,K,VA	1
		AMP,CD,K,VA	1
		AMP,CD,VA	1
		AMP,K,	1
		AMP	1
B.pumilus	3	AMP,CIP,CD.K,	1
		AMP,CD,VA	1
		AMP	1
B.lichenoforms	2	AMP,CD,GM ,VA	1
		AMP,K,	1

AMP-Ampicillin; CD-Clindomycin; CIP-Ciprofloxacilin; GM-Gentamycin; K-Kanamycin; VA-Vancomycin

Almost all the identified aerobic spore forming bacilli were susceptible to Ciprofloxacin and Gentamycin (Table 7). Furthermore, five isolates from *B.cereus* (41.66%) and 1 isolate from *B.megaterium* (20%) showed resistance to five antimicrobial agents namely Ampicillin, Ciprofloxacin, Clindomycin, Kanamycin and Vancomycin. Almost all the identified strains exhibited higher resistance to Ampicilin, Kenamycin and Vancomycin. In addition, three isolates of *B.cereus* , two isolates of *B.coagulans*, six isolates of *B.subtilis* and one isolate of *B.megaterium* showed resistance to four antibiotics namely, Ampicillin, Clindomycin, Kanamycin and Vancomycin.

5. Discussion

Aerobic and facultative anaerobic spore forming bacteria of the genus bacillus are widely distributed in nature. There is a clear association between these endospore forming strains and food contamination. This is mainly because the spores which are formed at the end of growth phase within the vegetative mother cell acting as sporangium get released into the environment as survival structure (Janstova and Lukasova, 2001). Therefore, the ability of their spores to survive under different environments enable the spore formers to resist heat, attach to processing equipments (due to their adhesive characteristics) and germinate (grow) under favorable condition making them major contaminant of food from various sources during production, processing and storage (Janstova and Lukasova, 2001).

Although aerobic spore formers have been associated with the spoilage of food products, recently they have been linked to potential food poisoning microbes (Redriguez-lozan *et al.*, 2010).This research evaluated the prevalence of aerobic spore forming bacteria in row Milk, powdered milk, Keribo, Wheat powder and Honey collected from five locations of the study site (namely Bishishe, Merkato, Bochobore, Kochi and Ajip) in Jimma town and assessed the risk factors associated with the presence of these bacteria in food.

Milk samples collected from Ajip contained the largest number of aerobic spore forming bacterial strains and the least numbers were observed in the sample collected from Qochi. The powdered Milk sample collected from Qochi contained the largest number of aerobic spore forming bacilli and the smallest number was obtained in the sample collected from Bechobore. Therefore, the mean aerobic spore forming bacterial count of Keribo and row milk samples collected from Ajip, powdered milk and Honey samples collected from Qochi, Wheat flour samples collected from Merkato were higher. The presence of large number of aerobic spore forming bacteria in samples collected from the above sites were due to poor handling and sanitation, lack of training (orientation) about food safety issues, poor personal hygiene including their surroundings. This was in agreement with Janstova and Lukas ova (2001).

A total of 60 isolates (12 isolates from each food item) were isolated and characterized by using morphological, biochemical and physiological tests. Accordingly, examination of isolates from 50 samples of the five food items revealed that 11 of the isolates from four food items (Keribo, Row Milk, powdered Milk and Honey) were identified as *Bacillus Coagulans*,23 of the isolates

were identified as *Bacillus subtilis*,16 isolates were identified as *Bacillus cereus*, while isolates from row Milk and Honey were identified as *Bacillus megaterium*, while 3 isolates from wheat powder were identified as *Bacillus pumilus, although* 2 isolates from powdered milk were identified as *Bacillus licheniformis.* Accordingly, *Bacillus cereus, Bacillus Coagulans, Bacillus subtilis, Bacillus megaterium, Bacillus pumilus* and *Bacillus licheniformis* were amog the dominant bacilli identified from the examined food items

The prevalence of *Bacillus cereus* and *Bacillus subtilis* in food samples studied in this research could be due to handling of food with bare hand, washing of the utensils with unclean water and the presence of litter and dust at the vending site which lead to cross contamination from vendor, dust to the food (Sina*etal.*,2011). From the food safety point of view, *Bacillus cereus* is the most important species known for its ability to form toxins causing food borne illnesses. The United States environmental protection agency (USEA, 2009 and 2010) has reported that *Bacillus subtilis* and *Bacillus licheniformis* are not considered as human pathogenic organisms. In contrast, the health protection agency in the United Kingdom (HPA, 2010) has reported a total of 17 outbreaks of gastro-enteritis attributed to *Bacillus subtilis* and five other out breaks attributed to other *Bacillus* species. Previous reports by HPA (2010) also include several cases of food borne illnesses associated with *Bacillus subtilis* and *Bacillus licheniformis.* According to Martimer (1975) and Turbull (2009), the production of pumilacidins by *Bacillus pumilus* strain was implicated in a small food borne out break related to the consumption of rice in a chine's restaurant. *Bacillus licheniformis, Bacillus subtilis, Bacillus pumilus* together with *Bacillus cereus* are able to grow in heat treated foods and drinks (Christiansson,1992).

Information on risk factors associated with varied foods which could affect quality and safety of food items were collected from all the five sites of Jimma town by direct observation and interviewing of vendors, local processors and consumers have revealed that vendors and local processors did not keep their personal hygiene and pay attention to the sanitation of their surroundings both during production and sales. They have not been trained (oriented) on how to handle and process food and how to keep the storage environment, Equipments, water and processed materials properly. Besides, the moisture content, storage temperature of the environment and expire date of food items were not carefully monitored and controlled. The vended foods were not properly covered; rather they were exposed to open air and surrounding

dust. All of these events encouraged contamination and lead to high microbial load (mean aerobic spore count) and compromised quality and safety of food. The risk factor assessment results were the same in all the collected sites although the bacterial load of samples collected in some sites were a bit higher.

In this study, the majority of households and food product vendors had no education or training about the hygiene of food and many had no information about food and water borne diseases. Education and training are key for households sanitation, vendors and even to consumers to prevent cross contamination and mishandling of foods at home and vending sites (Reda *et al.*,2012).Hence training and sharing of information to vendors are critical at all levels of preparation(Collins,1997). FAO (1998) suggested that the food handlers should have the necessary knowledge and skills to handle food hygienically

The antibiotic susceptibility and resistance pattern of the identified aerobic spore forming bacilli revealed that almost all the identified *Bacillus* species were resistant to Ampicillin, Kanamycin and Clindomycin. Sensitivity to both Gentamycin and ciprofloxacin was observed in most of the bacilli isolates possibly because of the presence of potentially transferable genes conferring resistance to one or more of the antibiotics (Bacha *et al.*,2010).

Thus, the presence of strains resistant to these antibiotics among our isolates was an indication that these bacterial populations possessed or already acquired the resistant genes. Variations among our isolates in degree of resistance to different antibiotics could also be explained in terms of natural presence of resistance genes, mutation rate and reception of transferable resistance gene from other microbes (Bacha *et al.*,2010).

Moreover, the level of susceptibility of the strains to various anti microbial agents could be species dependant (Danielson and Wind, 2003).The intrinsic resistance of bacillus strains to many antibiotics may be considered as advantageous for those with Probiotics potential (Bacha *et al.*,2010).This would help to sustainably utilize the strains in the intestine to maintain the natural balance of intestinal micro flora during antibiotic treatment. However, there is the danger of transferring multiple drug resistance to pathogens in the intestinal environment. Although the number of anti microbial agents used is only six, almost all the identified aerobic spore forming

strains exhibited multiple drug resistance as they resist two or more antibiotics (Bacha *et al.*, 2010).

The resistant of bacterial strains to antibiotics have recently emerged worldwide and causing great concern. With that increase the risk to public health has also increased. This is particularly serious in undeveloped countries where bacterial infections remain among the major cause of death (Bartiloni *et al.*, 2005). The co existence of resistant genes with mobile elements such as plasmids and transposons facilitate the rapid spread of antibiotic resistance genes among bacteria (Sunde,2005).

Since the six identified bacterial strains are linked to food poisoning, during production and sales, vendors and local processors must always keep their personal hygiene to discourage contamination. Sellers should also ensure that they don't expose the food and beverages to the air and outside dust during display. Vendors should wash hands before and after preparation and storage. The public health authority and vendors themselves should monitor conditions of sanitation and hygiene establishment. The regional health bureau and Jimma town health and authorities must made an effort to regularly inspect the healthy conditions of the vended sites and they must give training (orientation) to households and food sellers on how to keep their personal hygiene and how to keep their environments safe.

6. Conclusion

This research demonstrated that all the food items contained aerobic spore forming bacilli namely, *B.cereus, B.coagulans, B.subtilis, B.megaterium, B.pumilus and B.lichenoforms*. Isolation of Potential pathogenic bacteria in this study are evident that the five food items collected in Jimma town in different localities could contribute to the major public health problems in the study area.

Absent of regular inspection for safety of these foods and drinks, lack of training (orientation) on proper handling, processing, unhygienic surroundings, unclean storage places including processing and preparing materials could be the possible factors for the presence of food poisoning strains in these food items. Therefore, the detection of these aerobic spore forming bacilli calls for regular inspection for safety of these food items.

Antibiotics such as Ciprofloxacin and Gentamycin were seen highly effective in inhibiting the growth of all the six bacilli strains. But all the isolated bacilli showed resistance to two or more of the other four antimicrobial drugs. Accordingly, there could be risk of dissemination of drug resistance bacteria unless regular monitoring of the status of drug resistance among microbes associated with foods (Honey, Keribo, Powdered milk Raw milk and Wheat flour) are in place.

7. Recommendation

- In the current study, isolates were identified to their respective species tentatively using morphological, biochemical and physiological tests. For more reliable identification, molecular characterization of the isolates is recommended.
- Comprehensive evaluation of large samples involving more food items are recommended to generate conclusive information about prevalence and drug resistance patterns of aerobic spore forming bacteria in these food items.
- To improve the poor hygienic practices observed at least during vending of the food items calls for awareness raising training to food handlers besides working towards improvement of infrastructure around the food vending environment.

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9. Annex

9.1 Aerobic spore count from serially diluted plates

Table.1 The number of colonies on the nutrient Agar in duplicate from serial dilution (10^{-2}-10^{-4}) of Keribo sample

Sample	Serial dilution						Average number of colonies		
	10^{-2}	10^{-2}	10^{-3}	10^{-3}	10-^{4}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
K_s1	_	_	_	_	_	_	TLTC	_	_
K_s2	40	30	36	32	_	_	35	34	_
K_s3	295	285	167	153	113	91	290	160	102
K_s4	_	_	_	_	_	_	TLTC	_	_
K_s5	145	123	133	119	133	93	134	126	113
K_s6	297	287	209	193	200	90	292	201	195
K_s7	97	93	75	53	62	50	95	64	56
K_s8	170	156	176	126	127	119	163	151	123
K_s9	117	77	75	61	30	64	97	68	47
K_s10	64	44	62	46	_	_	54	38	_

_ (too little to count)

Table.1 The number of colonies on the nutrient Agar in duplicate from serial dilution (10^{-2}-10^{-4}) of milk sample

Sample	Serial dilution						Average number of colonies		
	10^{-2}	10^{-2}	10^{-3}	10^{-3}	10^{-4}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
Ms1	36	22	29	15	29	9	29	22	19
Ms2	35	15	26	14	19	13	25	20	16
Ms3	171	131	143	137	126	106	159	140	116
Ms4	129	115	119	105	112	106	122	112	109
Ms5	114	92	97	83	80	70	103	90	75
Ms6	22	8	_	_	_	_	15	_	_
Ms7	90	104	77	47	63	49	87	62	56
Ms8	103	91	80	74	73	61	97	77	67
Ms9	116	104	43	157	92	82	110	100	87
Ms10	145	135	133	111	124	106	140	122	115

_ (too little to count)

Table.1 The number of colonies on the nutrient Agar in duplicate from serial dilution (10^{-2}-10^{-4}) of powdered milk sample

Sample	Serial dilution						Average number of colonies		
	10^{-2}	10^{-2}	10^{-3}	10^{-3}	10^{-4}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
Mps1	59	45	66	50	53	39	52	58	46
Mps2	300	298	186	176	177	157	299	181	167
Mps3	153	147	149	109	122	110	150	129	116
Mps4	35	31	31	17	24	8	33	24	16
Mps5	30	26	22	8	17	7	28	15	12
Mps6	69	61	55	39	40	28	65	47	34
Mps7	43	33	39	21	27	15	38	30	21
Mps8	78	40	54	50	44	38	59	52	41
Mps9	40	26	31	8	25	9	33	19	17
Mps10	51	27	45	37	43	19	39	41	31

Table.1 The number of colonies on the nutrient Agar in duplicate from serial dilution (10^{-2}-10^{-4}) of Wheat flour sample

Sample	Serial dilution						Average number of colonies		
	10^{-2}	10^{-2}	10^{-3}	10^{-3}	10^{-4}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
Wfs1	32	31	_	_	_	_	31	_	_
Wfs2	38	30	_	_	_	_	34	_	_
Wfs3	_	_	_	_	_	_	_	_	_
Wfs4	_	_	_	_	_	_	_	_	_
Wfs5	34	30	_	_	_	_	32	_	_
Wfs6	35	31	_	_	_	_	33	_	_
Wfs7	96	68	62	40	39	33	82	51	36
Wfs8	_	_	_	_	_	_	_	_	_
Wfs9	_	_	_	_	_		_	_	_
Wfs10	37	31	_	_	_	_	34	_	_

_ (too little to count)

Table.1 The number of colonies on the nutrient Agar in duplicate from serial dilution (10^{-2}-10^{-4}) of Honey sample

Sample	Serial dilution						Average number of colonies		
	10^{-2}	10^{-2}	10^{-3}	10^{-3}	10-^{4}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
Hs1	69	49	37	55	45	39	59	46	42
Hs2	54	32	45	33	40	30	43	39	35
Hs3	_	_	_	_	_	_	TLTC	_	_
Hs4	_	_	_	_	_	_	TLTC	_	_
Hs5	32	32	_	_	_	_	32	_	_
Hs6	59	43	37	37	37	31	51	37	34
Hs7	_	_	_	_	_	_	TLTC	_	_
Hs8	58	36	38	42	40	32	47	40	36
Hs9	30	32	_	_	_	_	31	_	_
Hs10	53	33	30	44	31	31	43	37	31

_ (too little to count)

Table 2..Mean Aerobic spore count of Keribo collected at different sites of Jimma town, 2019

Sample Source	Collection Area	No of food samples	Mean Aerobic spore count(Cfu/ml)
Keribo	Qochi	1	TLTC
		2	$3.5x10^{4}$
	Ajip	3	$2.95x10^{5}$
		4	TLTC
	Bishishe	5	$1.34x10^{5}$
		6	$2.92x10^{5}$
	Merkato	7	$9.5x10^{4}$
		8	$1.63x10^{5}$
	Bocho bore	9	$9.7x10^{4}$
		10	$5.4x10^{4}$

* CFU = Colony forming units

*TLTC=To little to count (<30 colonies)

Table 2.Mean Aerobic spore count of Row milk collected at different sites of Jimma town. 2019

Sample Source	Collection Area	No of food samples	Mean Aerobic spore count(Cfu/ml)
Row Milk	Qochi	1	TLTC
		2	TLTC
	Ajip	3	$1.51x10^5$
		4	$1.22x10^5$
	Bishishe	5	$1.03x10^5$
		6	TLTC
	Merkato	7	$8.7x10^4$
		8	$9.7x10^4$
	Bocho bore	9	$1.1x10^5$
		10	$1.4x10^5$

*CFU = Colony forming units

*TLTC, to little to count (<30 colonies)

Table.2.Mean Aerobic spore count of powdered milk collected at different sites of Jimma town. 2019

Sample Source	Collection Area	No of food samples	Mean Aerobic spore count(Cfu/ml)
Powdered milk	Qochi	1	$5.2x10^4$
		2	$2.99x10^5$
	Ajip	3	$1.5x10^5$
		4	$3.3x10^4$
	Bishishe	5	TLTC
		6	$6.5x10^4$
	Merkato	7	$3.8x10^4$
		8	$5.9x10^4$
	Bocho bore	9	$3.3x10^4$
		10	$3.9x10^4$

* CFU = Colony forming units

*TLTC, to little to count (<30 colonies)

Table.2.Mean Aerobic spore count of Wheat powder collected at different sites of Jimma town, 2019

Sample Source	Collection Area	No of food samples	Mean Aerobic spore count(Cfu/ml)
Wheat powder	Qochi	1	$3.1x10^4$
		2	$3.4x10^4$
	Ajip	3	TLTC
		4	TLTC
	Bishishe	5	$3.2x10^4$
		6	$3.3x10^4$
	Merkato	7	$8.2x10^4$
		8	TLTC
	Bocho bore	9	TLTC
		10	$3.4x10^4$

*CFU (colony forming units)

*TLTC (To little to count, <30 colonies)

Table.2.Mean Aerobic spore count of Honey collected at different sites of Jimma town. 2019

Sample Source	Collection Area	No of food samples	Mean Aerobic spore count(Cfu/ml)
Honey	Qochi	1	$5.9x10^4$
		2	$4.3x10^4$
	Ajip	3	TLTC
		4	TLTC
	Bishishe	5	$3.2x10^4$
		6	$5.1x10^4$
	Merkato	7	TLTC
		8	$4.7x10^4$
	Bocho bore	9	$3.1x10^4$
		10	$4.3x10^4$

* CFU (colony forming units)

*TLTC (To little to count, <30 colonies)

Descriptive Analysis

1. Raw milk

Descriptives

ASC

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Kochi	2	.0000	.00000	.00000	.0000	.0000	.00	.00
Agip	2	5.1400	.08485	.06000	4.3776	5.9024	5.08	5.20
Bishishe	2	2.5050	3.54260	2.50500	-29.3240	34.3340	.00	5.01
Bacho Bore	2	5.0900	.07071	.05000	4.4547	5.7253	5.04	5.14
Markato	2	4.9550	.03536	.02500	4.6373	5.2727	4.93	4.98
Total	10	3.5380	2.44264	.77243	1.7906	5.2854	.00	5.20

ANOVA

ASC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	41.135	4	10.284	4.093	.077
Within Groups	12.564	5	2.513		
Total	53.699	9			

2. Powdered milk

Descriptives

ASC

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Kochi	2	5.0900	.53740	.38000	.2616	9.9184	4.71	5.47
Agip	2	4.8400	.46669	.33000	.6470	9.0330	4.51	5.17
Bishishe	2	2.4050	3.40118	2.40500	-28.1534	32.9634	.00	4.81
Bacho Bore	2	4.5500	.05657	.04000	4.0418	5.0582	4.51	4.59
Markato	2	4.6700	.14142	.10000	3.3994	5.9406	4.57	4.77
Total	10	4.3110	1.54592	.48886	3.2051	5.4169	.00	5.47

ANOVA

ASC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.411	4	2.353	.972	.497
Within Groups	12.098	5	2.420		
Total	21.509	9			

3. Honey

Descriptives

ASC

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Kochi	2	4.7000	.09899	.07000	3.8106	5.5894	4.63	4.77
Agip	2	.0000	.00000	.00000	.0000	.0000	.00	.00
Bishishe	2	4.6000	.14142	.10000	3.3294	5.8706	4.50	4.70
Bacho Bore	2	4.5600	.09899	.07000	3.6706	5.4494	4.49	4.63
Markato	2	2.3350	3.30219	2.33500	-27.3340	32.0040	.00	4.67
Total	10	3.2390	2.23668	.70730	1.6390	4.8390	.00	4.77

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	34.080	4	8.520	3.893	.084
Within Groups	10.944	5	2.189		
Total	45.024	9			

4. Wheat flour

Descriptives

ASC

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Kochi	2	4.5100	.02828	.02000	4.2559	4.7641	4.49	4.53
Agip	2	.0000	.00000	.00000	.0000	.0000	.00	.00
Bishishe	2	4.5050	.00707	.00500	4.4415	4.5685	4.50	4.51
Bacho Bore	2	2.2650	3.20319	2.26500	-26.5146	31.0446	.00	4.53
Markato	2	2.4550	3.47189	2.45500	-28.7387	33.6487	.00	4.91
Total	10	2.7470	2.36737	.74863	1.0535	4.4405	.00	4.91

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28.125	4	7.031	1.575	.312
Within Groups	22.315	5	4.463		
Total	50.440	9			

5.keribo

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Kochi	2	2.2700	3.21026	2.27000	-26.5731	31.1131	.00	4.54
Agip	2	2.7300	3.86080	2.73000	-31.9579	37.4179	.00	5.46
Bishishe	2	5.3000	.24042	.17000	3.1399	7.4601	5.13	5.47
Bacho Bore	2	5.0950	.16263	.11500	3.6338	6.5562	4.98	5.21
Markato	2	4.8600	.18385	.13000	3.2082	6.5118	4.73	4.99
Total	10	4.0510	2.15439	.68128	2.5098	5.5922	.00	5.47

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16.443	4	4.111	.811	.568
Within Groups	25.330	5	5.066		
Total	41.772	9			

Total descriptive analysis

Descriptives

ASC

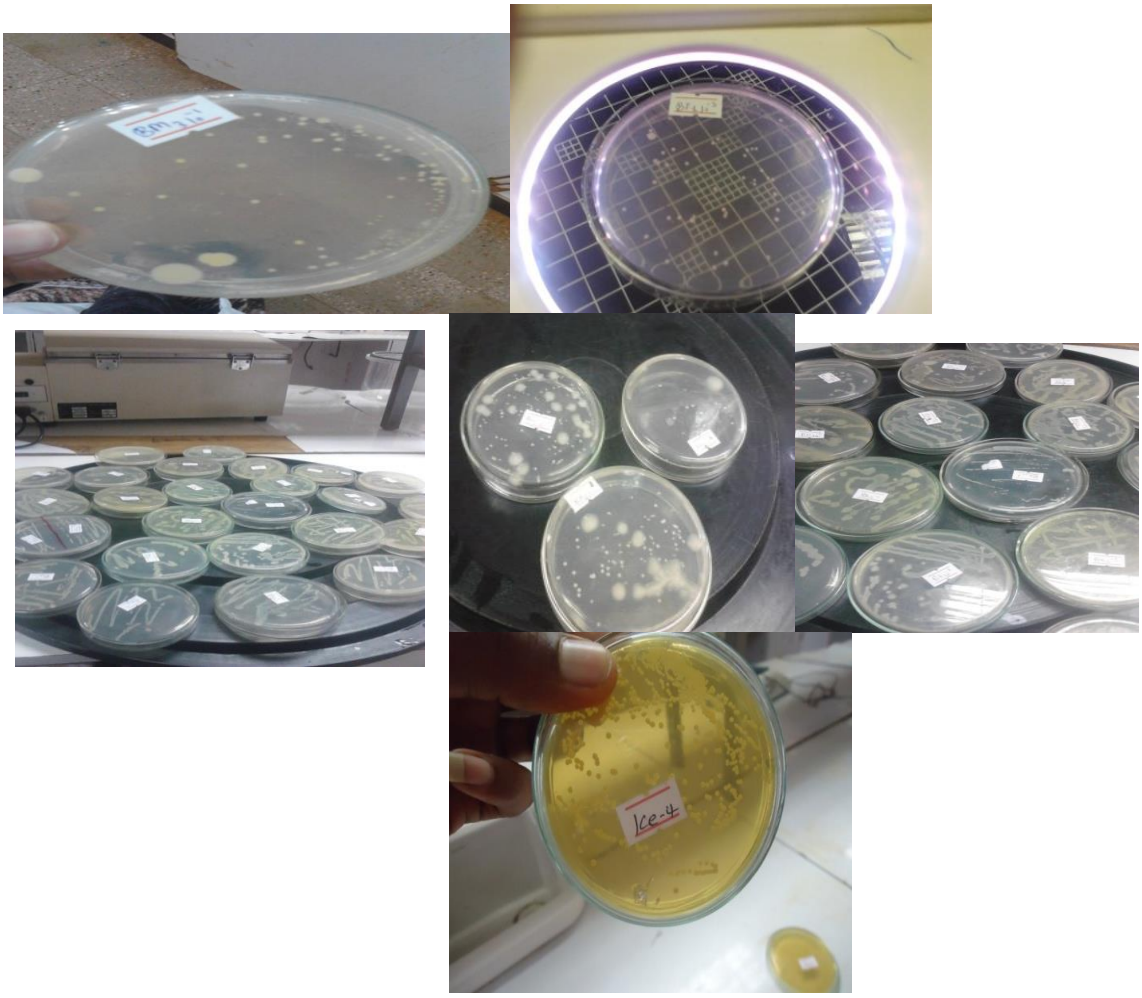
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Karibo	10	4.0510	2.15439	.68128	2.5098	5.5922	.00	5.47
Raw Milk	10	3.5380	2.44264	.77243	1.7906	5.2854	.00	5.20
Po wder Milk	10	4.3110	1.54592	.48886	3.2051	5.4169	.00	5.47
Wheat flour	10	2.7470	2.36737	.74863	1.0535	4.4405	.00	4.91
Honey	10	3.2390	2.23668	.70730	1.6390	4.8390	.00	4.77
Total	50	3.5772	2.15769	.30514	2.9640	4.1904	.00	5.47

ANOVA

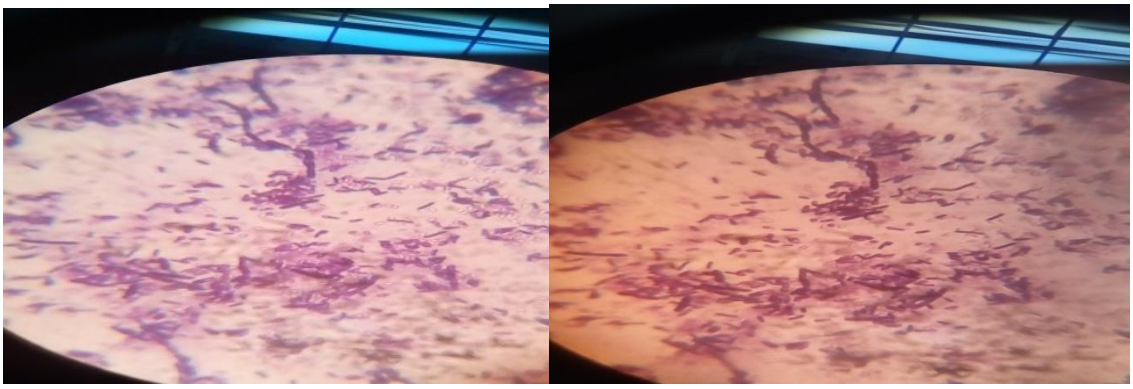
ASC

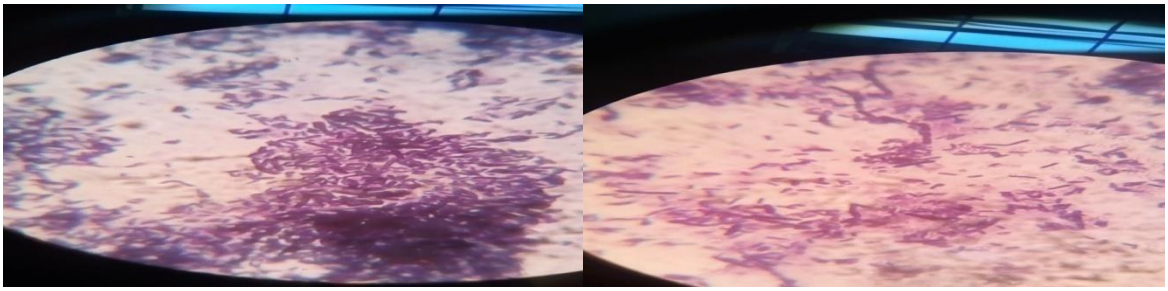
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.681	4	3.920	.830	.513
Within Groups	212.444	45	4.721		
Total	228.125	49			

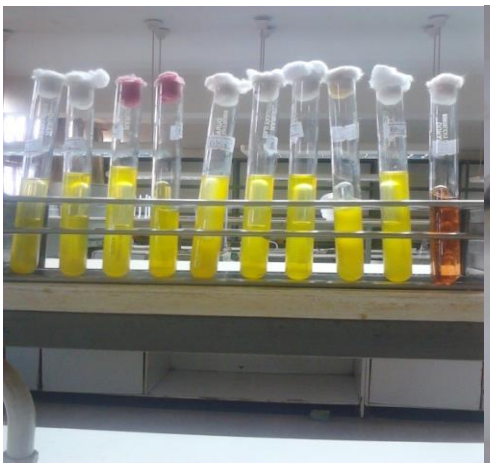
9.2 Photo of isolates of aerobic spore forming bacteria isolated from five food samples

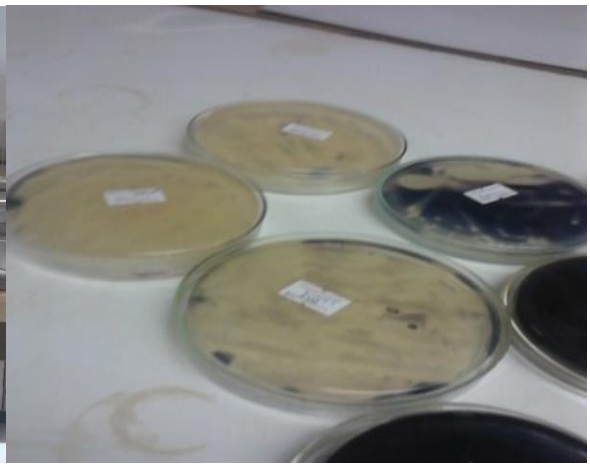


9.3. Photos collected from morphological amdbiocmemical tests









9.4 Antibiotic sensitivity test (raw data)

Table.3. Results of a sample Kirby Bauer test, Zone sizes(mm) of B.cereus against six antibiotics

Drug	Susceptibility(S)	Resistance (R)	Actual result(mm) for B.cereus												Evaluation
			Kr9b	Kr7b	Kr8	H1a	H5b	Mp1a	Wf4a	Wf8a	M3b	M4f	Mp3b	Mp6a	
Ampicillin	>14	<11	-	-	-	-	-	-	-	-	-	-	-	-	
ciprofloxacilin	>21	<15	24	24	20	20	30	16	15	20	16	15	20	30	
Clindomycin	>21	<14	22	18	10	16	-	-	7	12	7	7	16	18	
gentamycin	>13	<12	16	18	16	18	18	9	8	16	7	7	16	16	
kanamycin	>18	<13	14	14	11	12	17	8	10	14	7	8	10	11	
vancomycin	>12	<9	10	7	8	7	-	-	7	8	7	-	8	7	

R. - Resistance; S, - Sensitive

Table.3.Results of a sample Kirby Bauer test, Zone sizes(mm) of Bacilus.coagulens against six antibiotics

Drug	Susceptibility(S)	Resistance(R)	Actual result(mm) for B.coagulans							Evaluation
			Kr3	Kr2	H2b	M3a	Mp9a	H5a		
Ampicillin	>14	<11	-	-	-	-	-	-		
Ciprofloxacilin	>21	<15	23	22	25	22	22	22		
Clindomycin	>21	<14	22	22	25	18	20	22		
Gentamycin	>13	<12	19	19	20	25	20	20		
Kanamycin	>18	<13	19	18	15	12	15	18		
Vancomycin	>12	<9	12	12	10	8	8	7		

R. – Resistance S,-susceptible

Table.3. Results of a sample Kirby Bauer test, Zone sizes(mm) of Bacilus.subtilis against six antibiotics.

Drug	Susceptibility(S)	Resistance(R)	Actual result(mm) for B.subtilis											
			Kr9a	Kr7a	H2a	H6b	Wf1a	Wf1c	M3c	M5a	Mp2a	Mp2b	Mp7b	Kr3
Ampicillin	>14	<11	-	-	-	-	-	-	-	-	-	-	-	-
ciprofloxacilin	>21	<15	24	17	24	23	29	30	20	21	30	26	21	30
Clindomycin	>21	<14	24	-	-	-	-	7	-	9	-	-	7	7
gentamycin	>13	<12	17	-	20	20	21	18	20	18	20	18	20	20
kanamycin	>18	<13	16	9	16	19	17	16	17	7	18	14	11	16
vancomycin	>12	<9	13	-	12	-	-	-	19	11	-	13	7	-

R. Resistance I, Intermediate S, Sensitive

Table.3. Results of a sample Kirby Bauer test, Zone sizes(mm) of Bacilus.megaterium against six antibiotics

Drug	Susceptibility(S)	Resistance(R)	Actual result(mm) for B.megaterium						Evaluation
			H6a	H1b	H6c	M3d	M3e		
Ampicillin	>14	<11	-	-	-	-	-		
ciprofloxacilin	>21	<15	27	32	27	30	26		
Clindomycin	>21	<14	25	7	-	-	29		
gentamycin	>13	<12	20	20	20	7	20		
kanamycin	>18	<13	16	17	21	-	19		
vancomycin	>12	<9	12	-	-	-	16		

R. Resistance S, Sensitive

Table.3. Results of a sample Kirby Bauer test, Zone sizes(mm) of Bacilus.pumilus against six antibiotics

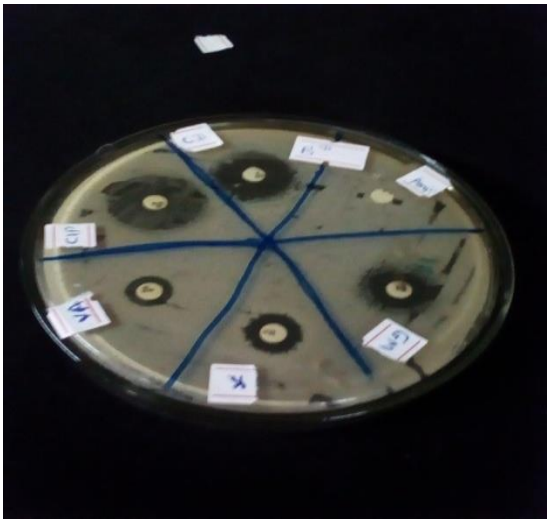
Drug	Susceptibility(S)	Resistance(R)	Actual result(mm) for B.pumilus				Evaluation
			Wf5b	Wf1e	Wf6a		
Ampicillin	>14	<11	-	-	-		
Ciprofloxacilin	>21	<15	29	35	20		
Clindomycin	>21	<14	30	-	19		
Gentamycin	>13	<12	20	24	20		
kanamycin	>18	<13	15	18	13		
vancomycin	>12	<9	14	-	12		

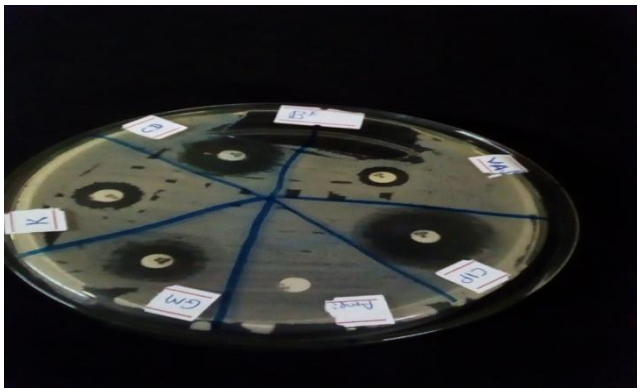
R. Resistance I, Intermediate S, Sensitive

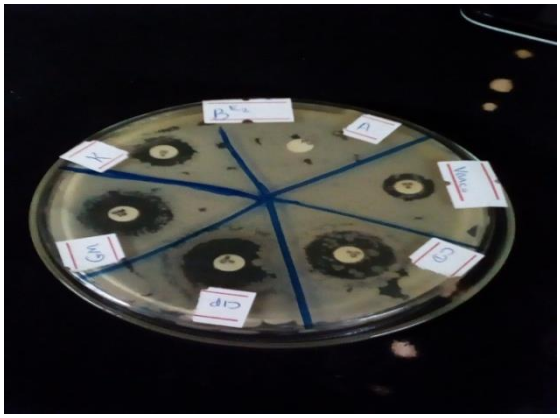
Table.3. Results of a sample Kirby Bauer test, Zone sizes (mm) of Bacilus.licheniform against six antibiotics

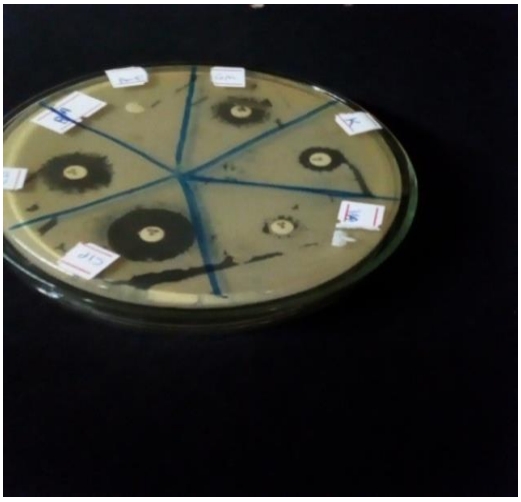
Drug	Susceptibility(S)	Resistance(R)	Actual result(mm) for B.lichenoforms					Evaluation
			Mp10a		Mp8a			
Ampicillin	>14	<11	-		-			
ciprofloxacilin	>21	<15	30		32			
Clindomycin	>21	<14	25		7			
gentamycin	>13	<12	20		10			
kanamycin	>18	<13	15		18			
vancomycin	>12	<9	12		7			

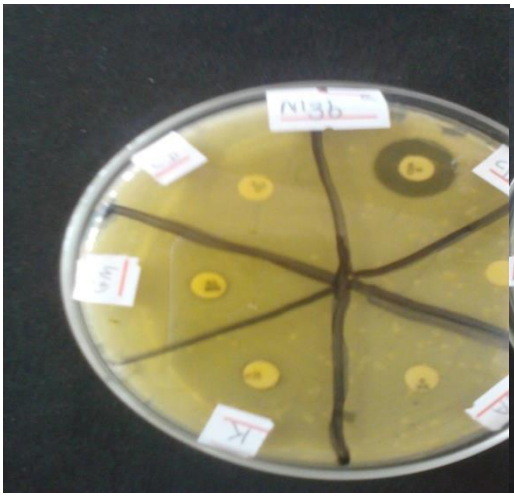
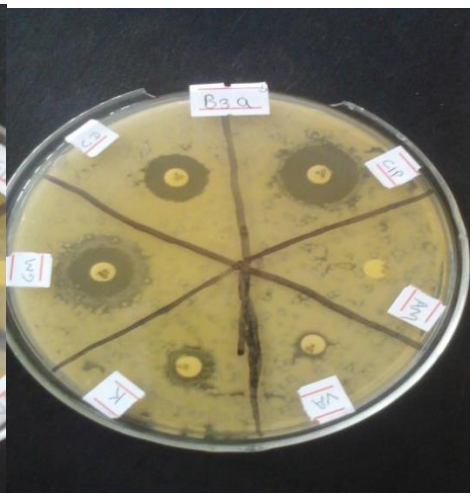
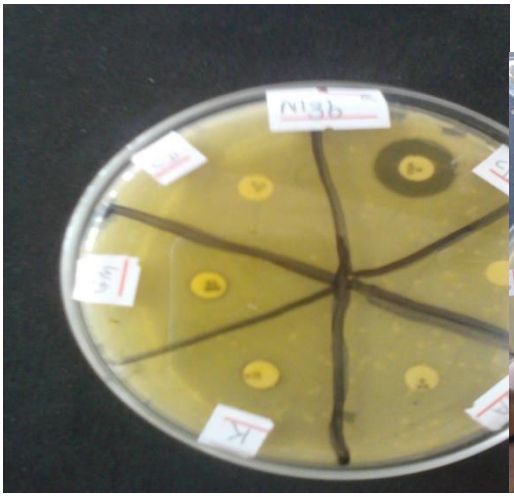
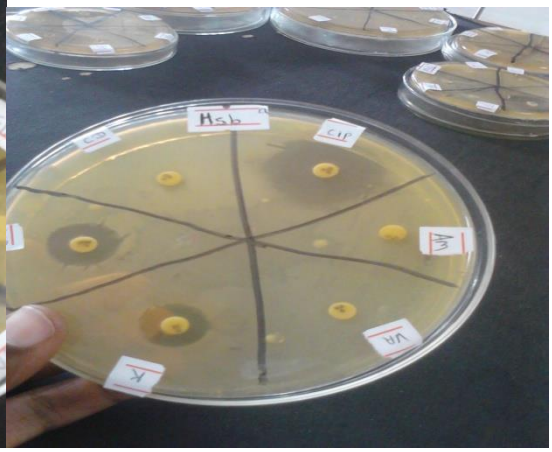
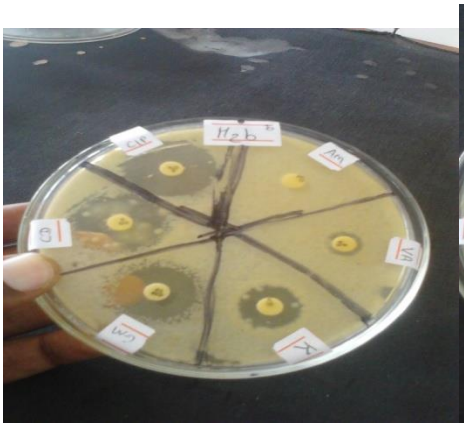
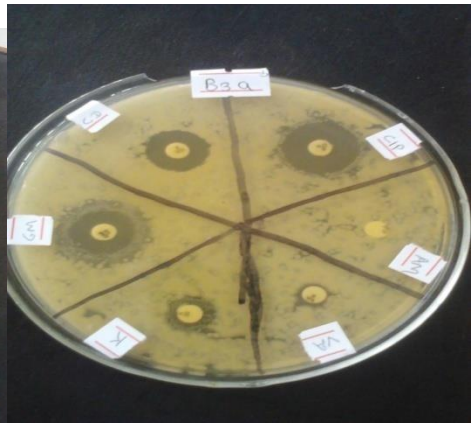
9.5.Photos collected from Antibiotic Susceptibility test

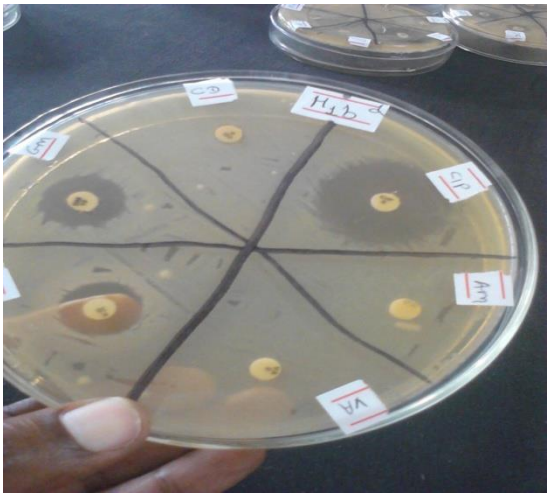










9.6. Questioners

College of Natural Science

Post Graduate Studies

Department of Biology

The following quesionairy is prepared to gather information about the sanitation of vended raw milk, powdered milk, Keribo, wheat powder and honey in streets, open markets, shops, and super markets in different sites of Jimma town. The information is collected partially by observation and partially by interview. The data is gathered carefully and genuinely as it has value on the output of the research by influencing the outcome of the laboratory results.

Instruction. I. Put X in the appropriate box

1. Vended food

Raw milk ☐ powdered milk ☐ Keribo ☐ wheat powder ☐

Honey ☐

2. The place where the food vended Street ☐ Open market ☐

Super market Shop ☐ House hold ☐ ☐

3. Vendor (Food seller)

i. Age**:** <20 ☐ 21-30 ☐ 31-40 ☐ >40 ☐

ii. Sex; Male ☐ Female ☐

iii. Academic status Illiterate ☐ 1-8 ☐ 9-12 ☐ Certificate and above ☐

II. Please tick (x) in the appropriate column

Key. 1 (poor) 2(fair) 3(good) 4(very good) 5(excellent)

Risk factors	1	2	3	4	5	comment
Storage temperature						
Moisture content of the place						
Hygienic condition and sanitation of the place						
Hygienic condition of the vendor						
Sanitation of water used for washing and rinsing the utensils						
Use of detergents to wash hands, food containers						
Carefulness of the vender when handling money						

III.Suggesions

1. Are there vectors (house fly) in and around the vended food house?

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2. Has the vender taken formal training on how to make food safe?

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3. Is there any way that the vendor knows how to make food safe (unspoiled)?

If yes, what are they? ----------

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4. For how many days the food product kept in the vended house?

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5. How is the vendor's skill in food processing and handling?

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6. Any comment about the general condition of the vended food, vendor and the place where the food is sold, the personal hygiene of the employees and the cleaning and sanitation of equipments.