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MICROBIOLOGICAL QUALITY OF ERGO COLLECTED FROM BAHIR DAR TOWN

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By

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As a thesis research advisor, I here by certify that I have read and evaluated this thesis prepared under my guidance, by Abdurkadir Beyan entitled as "Microbiological quality of ergo from Cafes and Restaurants in Bahir Dar town. I am recommending the paper to be submitted as fulfilling the requirement for the Degree of Master of Science in Biology (Applied Microbiology).

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As members of the board of examiners for the MSc thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Abdurkadir Beyan and examined the candidate. We recommended the thesis to be accepted as Fulfillment for the Requirements of the Degree of Master of Science in Biology (Applied Microbiology).

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LIST OF ABBREVIATIONS

AMB- Aerobic Mesophilc Bacteria

ANRS- Amhara National Regional State

Cfu- Colony forming unit

FAO- Food and Agricultural Organization

GIT- Gastro intestinal tract

HACCP- Hazard Analysis and Critical Control Points

Km- Kilometer

l- Liter

LAB- Lactic acid bacteria

MRS-de Mann Rogosa Sharp Agar

MSA- Manitol Salt Agar

ND- Not detected

NFM-Naturally fermented milk

PCA-Plate Count Agar

SDA-Sabouraud Dextrose Agar

SSA- Salmonella Shigella Agar

TSI- Triple Sugar Iron Agar

WHO- World Health Organization

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ABSTRACT

Ergo is a traditional Ethiopian fermented milk produced by spontaneous fermentation using traditional utensils and fermented at room temperature. Ergo can be a potential health hazard if prepared from milk contaminated with pathogenic bacteria such as *Staphylococcu* and *Salmonella.* This study was conducted to evaluate microbial quality of Ergo from cafeterias and restaurants in Bahir Dar town. Thirty samples were collected and subjected to microbiological examination (aerobic mesophilic bacteria, *Staphylococcus aureus*, *Salmonella* spp., lactic acid bacteria and yeasts and moulds). The results showed that aerobic mesophilic bacteria, *Salmonella* lactic acid bacteria, *S.aureus*, and yeasts and molds counts were high. From a total of 30 samples, 26 (86.66%) of aerobic mesophilic bacteria count exceed the guide line ($<10^5$ cfu/ml) for ready to eat food while, 4 (13.33%) of AMB was below the guideline. *Salmonella* was detected in 23 (76.66%) samples. Similarly, *Satphylococus aureus* was detected in 19 (63.33%). All the tests in bacterial counts indicated that the microbiological quality of ergo prepared for consumption in cafeteria and restaurants of Bahir Dar town was hygienically poor and this call for careful hygienic measures during production and handling of milk.

Key words: Fermented milk, *Ergo,* lactic acid bacteria, Pathogenic bacteria, *Salmonella, S. aureus.*

1. INTRODUCTION

Fermented foods and beverages constitute a major portion of peoples' diets all over the world and provide 20 – 40% of the total food supply (Campbell-Platt, 1994). Apart from providing variety to foods, fermented foods have the advantage of prolonged shelf-life due to organic acids such as lactic acid, acetic acid and other acids which is produced by lactic acid bacteria during fermentation which lowers the pH thus inhibiting the growth of spoilage microorganisms (Fields *et al*,. 1981). It also provide other benefits for lactose intolerant consumers due to the presence of lactase enzyme (β-galactosidase) and other inhibitory compounds effective against several pathogenic and spoilage bacteria (Nout,1994).

In fermentation, the raw materials are converted by microorganisms (bacteria, yeast and molds) to products that have acceptable qualities of food. In common fermented products such as yogurt, lactic acid is produced by the starter culture bacteria to prevent the growth of undesirable microorganisms (Ray and Daeschel, 1992). Food fermentations have a great economic value and it has been accepted that these products contribute in improving human health (Soomro *et al*., 2002).

The nature of fermented dairy products is different from one region to another depending on the local indigenous microflora. For example, *Leuconostoc* is responsible for traditional fermentation of milk in temperate climates, *Lactobacillus* While *Streptococcus* is responsible for fermentation in tropical and subtropical climates (Kurmann, 1994). Environmental conditions in each country affect the properties of the predominant native microflora limiting the use of some universal starters, and the rational solution is the selection of starter cultures from the native flora that could be used successfully in the dairy industry (Abdalla and Hussain, 2010).

"*Ergo*" is a traditional naturally fermented milk product which has some similarity to yoghurt. It is thick, smooth and of uniform appearance and usually has a white milk color when prepared carefully (Almaz Gonfa, 2001). In most cases, household preparation of "*ergo*" requires one way incubation at ambient temperatures. The milk coagulates within 24 hrs and preferably consumed at this time due to its good flavor. But longer keeping is not advantageous because further drop in pH will result in increased wheying off, which in turn results in loss of protein as whey

(Mogossie Ashenafi, 2006). *Ergo* is the most popular dairy product throughout the country which is consumed by every member of the family and considered as spatial food which serves as a basis for further processing the milk in to different dairy products. It also used as nutritional support to sick peoples, children, pregnant and lactating mothers of the family (O'Conner, 1994).

The souring process of ergo was carried out by *Lactococcus*, *Streptococcus*, *Leuconostoc* and *Lactobacillus* (Almaz Gonfa *et al.* 1999). Fairly high numbers of *Micrococci*, spore formers and coliforms were identified during the first 14 to 16 hours of fermentation. The *Lactococci,* which is the most dominant group throughout the fermentation, reached counts as high as 10^9cfu/ml at the end of the fermentation while, aerobic mesophilic bacteria also had similar counts and the yeast population increased to10^5 cfu/ml at 24 hours (Almaz Gonfa *et al.*, 1999).

Most of the indigenous fermented milk products are prepared by traditional methods. Such methods often bring about contamination of various microorganisms including pathogens (Kumbhar *et al.*, 2009). Many pathogenic microorganisms such as, *S. aureus*, *Bacillus cereus*, *Klebsiella*, coliforms were isolated from traditionally fermented dairy products of different parts of the world (Beukes *et al.*, 2001).. Isolation of pathogenic organisms from traditionally fermented dairy products depends on the method of manufacture which involves the use of unpasteurized milk and poor handling practice (Abdalla and Hussain, 2010).

Although lactic acid bacteria (LAB) have shown to provide measurable defense against food-borne pathogens, a broad spectrum of microbial pathogens can contaminate human food and water supplies and cause illness after they or their toxins are consumed. These include a variety of enteric bacteria, aerobes and anaerobes, viral pathogens and yeasts. During past decades, microorganisms such as *Staphylococcus aureus, Salmonella* spp., *Escherichia coli* 0157:H7, *Shigella* spp., *Listeria monocytogenes* and *Yersinia enterocolitica*, were reported as the most common foodborne pathogens that are present in many foods and able to survive in milk and fermented milk products (Tekinşen and Özdemir, 2006).

The survival of food-borne pathogens in yoghurt and other dairy products are supported by their ability to adapt to the environmental factors. During lactic acid fermentation, yoghurt bacteria metabolise lactose to produce lactic acid and hence a low pH. Many pathogens are capable to

adapt and survive this environmental stresses. Leyer and Johnson (1992) demonstrated that acid adaptation of *E. coli* O157:H7 increased its survival rate in acid foods while, *L. monocytogenes* can resist several environmental stresses including low pH in foods containing lactic acid such as yoghurt. *Salmonella* spp. and *Staphylococcus aureus* are also known to adapt to acidic environments and this promotes their survival in dairy products (Pazakova *et al.*, 1997).

Since ergo is preferably consumed soon after (24 h) fermentation, traditional fermentation of ergo would not guarantee that pathogenic bacteria such as *E. coli* 0157:H7 can be controlled and, therefore, Ergo can be a potential health hazard if prepared from milk contaminated with *E. coli* 0157:H7. So, despite the general assumption that low pH in Ergo control the proliferation of undesirable microorganisms, the dangers of listeriosis or salmonellosis from fresh Ergo must not be underestimated. It was, thus, recommended to inoculate boiled milk with a three day old ergo to ensure the nutritious quality and wholesomeness of ergo (Mogessie Ashenafi, 2006).

The potential of lactic acid fermentation to control the harmful effects of food contamination depends on factors that are difficult to quantify, such as the initial level of contamination, which in turn depends on local conditions, levels of hygiene and sanitation, and the resulting degree of acidity. It should be noted, therefore, that fermentation cannot replace observing the principles of food hygiene/food safety and minimizing the risk of contamination. This is particularly important since some pathogens may be acid resistant and may consequently survive the fermentation process (Motarjemi and Nout, 1996).

There was study done on the milk handling, processing and ways of marketing, as well as milk production, utilization including types of milk products of the urban and prei-urban dairy systems in the milk shade area of Northern Ethiopian highlands showed the use of different milk utensils for collection, storing and processing milk (Yitaye Alemayehu *et al.*, 2009). Another study was carried out by (Asaminew Tassew and Eyassu Seifu, 2011) to evaluate microbial quality of raw cow's milk taken at different sampling points from farmers and dairy cooperatives in Bahir Dar Zuria and Mecha district. But there is no study on microbial quality and safety of *ergo* in the study area. So, this study intended to fill this gap for first time in the study area.

Currently many genera of lactic acid bacteria have been used as a probiotics, enhancing the health of consumer by fighting against the establishment of pathogens in the consumers gastro intestinal tract (GIT), reducing the chance of development of cancer and other disease . Experience has shown that when fermentation has been improperly used, the resulting food products have caused illnesses. Milk and its fermented products have been identified as important means of spreading human pathogenic organisms, which gain access in to milk and milk products from different sources and cause different types of food borne illnesses. On the other hand, with the development that have taken place over the years in food microbiology and the present level of knowledge, it is evident that the micro flora of fermented milks consists of different strains of lactic acid bacteria (LAB) and some pathogens which resist different antibiotics that used for human medication. So, this study was intended to answer the following question:

The general objective of the research was to evaluate the microbial composition of ergo from Bahir Dar town.

The specific objectives of the study were:

- To identify pathogenic microorganisms from ergo sample
- To evaluate the microbial load of ergo.
- To isolate and characterize lactic acid bacteria from ergo sample.
- To determine the predominant microbial groups in "ergo" from study area.

2. LITERATURE REVIEW

2.1 Food fermentation

Fermentation can be defined as the biochemical modification of primary food products brought about by the action of microorganisms and their enzymes. It is intentionally carried out to enhance the taste, aroma, shelf-life, texture, nutritional value, and other properties of food (Motarjemi and Nout, 1996). Fermentation is often part of a sequence of food-processing operations in many parts of the world, with regional differences depending on the availability of raw materials, consumption habits, the laboriousness of the processes involved, and a variety of social factors (Motarjemi and Nout, 1996). Food fermentation is practiced by human cultures all over the world. It is a major component of human survival in places where preserved food is a necessity. Production of fermented food does not require knowledge of the biologically mediated nature of fermentation, because the microbiota that carry out fermentation are present worldwide. These organisms are ambient in the environment, whether humans make use of them or not (Scott and Sullivan, 2008). Traditionally for several centuries man has adopted fermentation as a means of food preservation and to have nutritional and therapeutic advantages from fermented products (Aneja *et al.*, 2002).

Archaeological evidences shows some civilization *e.g.* the Summerians and Babylonians in Mesopotamia, the Pharoes of ancient Egypt and the Indian in Asia, were well advanced in agricultural and animal husbandry and kept cows and buffalo for milk production, which was either consumed or processed in to other products. There are also many sketches that illustrate the milking and milk processing in these areas (Abou-Donia, 1984).

In Ethiopia, a considerable proportion of milk is consumed in fermented form in different areas. The fermentation of these products is carried out naturally without defined starter cultures used to initiate fermentation. In most cases, this is made possible through the proliferation of initial milk flora with microbial succession determined by ambient temperature and chemical changes in the fermenting milk. Especially those people in rural area produce fermented milk by traditional method. Among these *Ergo* (fermented sour milk), *Itity* (fermented milk curd) *Kibe* (traditional butter), *Neterkibe* (Spiced butter), *Ayib* (Cottage Cheese), *Arerra* (Sour defetted

milk) and *Aguat* (whey) are the major milk products that produced by small holder farmers (Mogessie Ashenafi, 2006).

2.2 Some of African traditional fermented milks

Traditional food fermentation is a relatively efficient, low energy preservation process which increases the shelf life and decreases the need for refrigeration or other form of food preservation methods. It is therefore a highly appropriate technique for use in developing countries and remote areas where access to sophisticated equipment is limited. Fermented foods are popular throughout the world and in some regions make a significant contribution to the diet of millions of individuals (Campbell-Platt, 1994). The aim of fermentation was to obtain products with characteristics, aroma and consistency and at the same time could be stored unspoiled for longer time than untreated raw milk. Even though the composition and microbiology of fermented milks were not understood, their beneficial effects over fresh milk were recognized in Europe, Asia and Africa (Lefoka, 2009).

Due to the inexpensive nature of fermentation technology, fermented beverages have made an important contribution to the human diet in many African countries. Several fermented dairy products are known to be produced at the artisan level in different African countries. For example, *Kindirmo*, *Nono* and *warankasi* are common fermented milk products in Nigeria (Belewu *et al*., 2005). *Kindirmo* is prepared by fermenting cow milk with overnight portion of previously prepared *Kindirmo* which is reported to contain strains of lactic acid bacteria. The milk is normally heated, allowed to cool and fermented in a big calabash or any suitable container at ambient temperature for a period of 8 h. The curdled *Kindirmo* is then homogenized by stirring and sweetened to taste (Belewu *et al*., 2005). *Nono* is the fermented skimmed milk that is prepared by the same procedure like *Kindirmo*. *Warankasi* is known among indigenous African consumers as cheese just as *kindirmo* and *nono* are considered as the equivalent of yoghurt. *Warankasi* is a dairy based product that is fermented by the artisans using an overnight portion of *warankasi* which have been reported to contain mainly *Lactococcus, Streptococcus* and *Lactobacillus* strains of lactic acid bacteria which ferment the heated milk within a period of 8-10 h (Belewu *et al.*, 2005).

In Zimbabwe, a traditional fermented milk known as *amasi* or *zifa* is consumed by the people. This product is produced by leaving fresh raw bovin milk to ferment spontaneously at ambient temperature in earthenware pots or plastic containers. The fermentation process occurs within 24-72 h depending on the temperature of the environment. Strains of lactic acid bacteria like *Enterococcus, Lactococcus* and *Lactobacillus* have been implicated to be responsible for the fermentation reaction (Gadaga *et al.*, 1999).

In the lower Egypt, farmers put fresh milk in earthenware pots and leave it undisturbed in a warm place until the cream rises and lower partially skimmed milk coagulates. The cream layer is removed and whipped by hand to butter while the remaining sour milk, often called **'Laban** *Rayeb*' is either consumed as it is or is converted to a soft acid cheese - 'Karish cheese' (El-Gendy, 1983). Amongst the major fermented milk products of Sudan *Rob* is common for its considerable economic and dietary importance to the people (Abdelgadir *et al.*, 1998). It is mainly produced from surplus milk of the rainy season by nomadic tribes. During this season the housewife turns as much milk (about 80 %) into rob each evening.

In Ethiopia, *Ergo*, the most important traditional milk product, which is similar to yogurt; is prepared by ''spontaneous'' fermentation, commonly initiated by either ''back slopping'' or by repeated use of the same utensil (Almaz Gonfa *et al.*, 2001).

2.3. Traditional milk handling and processing practices in Ethiopia

Dairying is practiced almost all over Ethiopia involving a vast number of small or medium or large-sized, subsistence or market-oriented farms. Based on climate, land holdings and integration with crop production as criterion, dairy production systems are categorized as: (i) rural dairy system which is part of the subsistence farming system and includes pastoralists, agro-pastoralists, and mixed crop–livestock producers; (ii) the peri-urban; and (iii) urban dairy systems (Dereje *et al.*, 2005). Among these the rural dairy system (pastoralism, agropastoralism and highland mixed smallholder production system) contributes to 98%, while the peri-urban and urban dairy farms produce only 2% of the total milk production of the country (Ketema, 2000).

Although milk is produced in almost every production system of Ethiopia, a minor portion of this milk enter the commercial sectors. Farmers close to the main road of Addis Ababa do not have market problems. They can sell their milk directly to consumers or to traders, as well as to private dairy plant such as Mamma Dairy Private limited Company. Elsewhere in Ethiopia, farmers near towns generally have ready outlets for their milk. However, most people live far away from major roads and have no nearby markets and for the fact that milk is relatively perishable food and a high percentage is consumed in relatively natural state, handling of milk and its products to preserve its natural and desired characteristics is very important (Duane and Cunnignam, 1991).

Dairy processing in Ethiopia is generally based on *Ergo* (fermented milk in Ethiopia), without any defined starter culture, with natural starter culture. Raw milk is either kept at ambient temperature or kept in a warm place to ferment prior to processing (Mogessie Ashenafi, 2002). The fermented product may also be processed into traditional butter (*qibe*) and butter milk (*arrera*), while the butter milk may further be processed into traditional cottage cheese (*ayib*) and whey (*aguat*) (Mogessie Ashenafi, 2006).

In southern Ethiopia a concentrated fermented milk Called *ititu* is prepared and consumed by the Borana tribes. *Ititu* prepared during the rainy season when milk is available in abundance for later consumption during the drier seasons when fresh milk supply is markedly scanty (Almaz Gonfa *et al*., 2001). *Itittu* has good keeping quality and remains acceptable for about two months at ambient temperature (25^0C-30^0C) and can be stored from about two months to three months (Almaz Gonfa *et al*., 2001). The people consume *ititu* in defferent forms, for example as side dish with traditional porridge or thin-baked cereal chips. It can also be consumed as food or drink alone and considered as one of the special foods and served to respected guests as well as to weaning-age children and the elderly (Almaz Gonfa *et al*., 2001).

2.3.1. Ergo fermentation

Ergo has different vernacular name in different area such as, *Ergo* (among Amhara people) Ititu,(Among Oromo people), *Geinto* or *meomat*a (among Sidama or Wolayta people). The fermentation is usually natural, with no defined starter cultures used to initiate it. In most cases, this is made possible through the proliferation of the initial milk flora, with microbial succession determined by ambient temperatures and chemical changes in the fermenting milk. In most urban homes, no attempt was made to control the fermentation rather; raw milk is left either at ambient temperatures or kept in a warm place to ferment. In rural areas, particularly among the pastoralists, raw milk is usually kept in a well-smoked container and milk from a previous fermentation serves as source of inoculums and lactic acid bacteria also become established on the inner walls of the container and serve as starter culture. Incubation temperature does not usually vary significantly, particularly in the lowlands, and the taste of the fermented product may, in general, be more or less uniform (Mogessie Ashenafi, 2006). Precipitation of the casein is usually the sign of completion of fermentation. Consistency and flavor of *Ergo* was varying among households. Even in experimental controlled fermentations, variability in flavor components occur with different strains of the same species (Mogessie Ashenafi, 2006).

2.3.2 Effect of container smoking and temperature on the microbiological and biochemical qualities of ergo

In the study conducted by (Mogessie Ashenafi, 1996) to study the effect of container smoking on the microbiological and biochemical qualities of fermenting ergo, raw milk was allowed to sour naturally at ambient temperatures (25-30^{0}C) in smoked or non-smoked containers. The result from his study shows that, milk in smoked containers had a lower rate of pH drop and the fermented product had good flavor for a longer time after coagulation. The total count of non-lactic acid bacteria in milk in non-smoked containers reached a high count (>10^8 cfu/ml) within 12 hours, whereas milk in smoked containers required more than 24 hours to reach this level. Similarly, the growth of coliforms and lactic acid bacteria is slow in milk of smoked containers, thus assuring good and slow development of flavor components, safety of finished product and better keeping quality. *Lactobacilli* dominate the flora of fermented product in non-smoked containers while, *lactococci* were equally dominant in fermented milk in smoked containers (Mogessie Ashenafi, 1996).

The study conducted by Mogessie Ashenafi, 1996; showed that, the rate of acid formation was lower at low temperature. In addition to that milk incubated at lower temperatures had a better ergo flavor. But, as the temperature of incubation raised, the rate of pH drop was faster, and the time of coagulation became shorter. On the other hand, the rate of growth of the various groups of bacteria increased with an increase in incubation temperature. At low incubation temperature, *Lactococci* dominated the lactic flora, while *Lactobacilli* dominated at higher incubation temperatures. Finally he concludes from this study the effectiveness of smoking containers to produce a safer and tastier *ergo* with better keeping quality at household level. In addition, lower incubation temperatures (around 20^0C) may favor a gradual proliferation and succession of lactic acid bacteria and thus guarantee a desirable fermentation (Mogessie Ashenafi, 1996).

2.4. Composition and microbiology of fermented milks

The type of bacterial flora developed in fermented food depends on intrinsic factors such as water activity, pH, salt concentration, availability of oxygen, composition of the food matrix, and extrinsic factors such as temperature, relative humidity and other parameters. Most fermented foods are dependent on lactic acid bacteria (LAB) to mediate the fermentation process, although yeasts are also involved in fermentations (Mogessie Ashenafi, 2006).

The microbial load of milk is a major factor in determining the quality of milk and indicates the hygienic level exercised during milking, cleanliness of the milking utensils, condition of storage, manner of transport as well as the cleanliness of the udder of the individual animal (Asaminew Tassew and Eyassu Seifu, 2011). Most of the time naturally fermented milk (NFM) relies on the growth of mesophilic LAB species, which lower the pH and produce the most typical sensorial compounds of the products. Fermented milk classified into two classes of, inoculated and noninoculated based on organisms that initiate fermentation. From these noninoculated NFMs are made by leaving the raw milk at room temperature until it becomes sufficiently acidic for the coagulum to appear. On the other hand inoculated naturally fermented milks (NFMs) are manufactured by adding a portion of a previous NFM batch to a new milk substrate (backslopping). In either case, *Lactobacilus lactis* strains are among the dominant microbiota (Patrignani *et al.*, 2006).

In traditional products manufactured from raw milk it is also common to find species of mesophilic *lactobacilli* such as *Lactobacilus plantarum and Lactobacillus casei /Lactobacillus paracasei*, as well as *Leuconostoc* spp, *Enterococcus* spp and *Pediococcus* spp (Almaz Gonfa *et al.* 2001; Patrignani *et al.,* 2006). In warm climates, other *lactobacilli* such as *Lactobacillus helveticus, Lactobacillus fermentum* and /or *Lactobacillus acidophilus* may also develop. Moderate to high (up to 10^8 cfu / g) numbers of yeast species are also usually present in NFMs (Gadaga *et al.* 2000). *Saccharomyces cerevisiae, Kluyveromyces marxianus* and *Candida lusitaniae* are among dominant yeast species which may cause spoilage or enhance the flavor of naturally fermented milk (Gadaga *et al.*, 2000). *Micrococci*, coliforms and pathogens (*Staphylococcus aureus, Bacillus cereus*) are occasionally found in NFMs, stressing the need for improving the microbial safety of these products (Almaz Gonfa *et al.* 2001). Naturaly fermented milks can also be manufactured from pasteurized milk (at both the artisanal and industrial scales), which renders products safer. Industrial NFMs are inoculated with acidifying and aromatic starter cultures, while artisanal products are usually inoculated via backslopping techniques. Such transfers impose conditions that select strongly for strains that grow rapidly in milk and that show strong resistance to high levels of lactic acid (Alegria *et al.,* 2010).

2.4.1. Lactic acid bacteria

The term LAB is used to describe a broad group of anaerobic, non motile, non spore forming gram positive curved rods, fastidious and acid-tolerant, which produce lactic acid and acetic acid upon fermentation of the milk and able to reduce the pH of the intestine, which in turn limits the growth of pathogens and other putrefactive bacteria (Naidu *et al.* 1999). They have a long history of safe use in fermented foods and they still play an essential role in the majority of food fermentations and one of the most important contributions of these microorganisms is the extended shelf life of fermented products, they also have beneficial influence on nutritional and sensory characteristics as well as on the standardization of end products (De vuyst and Leroy, 2007).

2.4.1.1 Classification of Lactic acid bacteria

The classification of lactic acid bacteria into different genera is largely based on morphology, mode of glucose fermentation, growth at different temperatures, and configuration of the lactic acid produced, ability to grow at high salt concentrations, and acid or alkaline tolerance. Even some of the newly described genera of lactic acid bacteria, additional characteristics such as fatty acid composition and motility are used as the basis of classification (Desai, 2008). Depending on similarities in physiology, metabolism and nutritional requirements they can be grouped as genera of *Streptococcus, Entrococcus,Lactococcus, Leuconostoc, Lactobacillus and Pediococcus* (Collins *et al*., 1987).

Based on the mode of glucose fermentation under standard conditions, *i.e.,* nonlimiting concentrations of glucose and growth factors (amino acids, vitamins and nucleic acid precursors) and limited oxygen availability, lactic acid bacteria can be classified as; homofermentative, which convert glucose almost quantitatively to lactic acid, and heterofermentative, which ferment glucose to lactic acid, ethanol/acetic acid, and CO2 . In practice, a test for gas production from glucose can separate between the homofermentative and heterofermentative. So, *Leuconostocs* and a subgroup of *Lactobacillus* are heterofermentative while, *Streptococcus* , *Entrococcus* ,*Lactococcus Pediococcus* homofermentative genera of lactic acid bacteria (Sharpe, 1979) as sited in Desai; 2008).

2.4.1.2. Lactic acid bacteria in milk fermentation

Variety of microorganisms including yeasts, molds and bacteria are present in raw milk. However, among these organisms, only the lactic acid bacteria (LAB) have the property of producing lactic acid from milk sugars by the process of fermentation and thus LAB constitute the predominant microflora of milk. These bacteria are responsible for most of the physiochemical and aromatic transformations intrinsic to fermented dairy products (Ogier *et al*, 2002). The LAB consumes natural milk sugars and release lactic acid that increases acidity, rendering milk proteins, especially casein, to denature and tangle into a solid mass or curd. They also cause the release of bioactive compounds which contribute to important physical, chemical and therapeutic properties of fermented milk products (Otles and Cagindi, 2003). Interest in these microbes, their various species, biotypes and strains is primarily because of their

biotechnological potential of efficient biotransformation essential for dairy product preparation (Leisner *et al,* 1999).

The lactic acid bacteria used in dairy fermentation can roughly be divided into two groups on the bases of their growth optimum. The lactic acid bacteria which have an optimum growth temperature between 20^{oC} and 30^{oC} grouped under mesophilic lactic acid bacteria while the other group called thermophilic have their optimum between 30°C and 40°C. It is not surprising to discover that traditional fermented products from sub-tropical countries harbor mainly themophilic lactic acid bacteria, whereas the products with mesophilic bacteria originated from Western and Northern European countries (Wouters *et al*., 2002).

2.5. **Contamination and spoilage of fermented milk**

Food can be contaminated from deferent sources, including polluted water (e.g. wastewater and household water), dirty hands, flies, pests, domestic animals, dirty pots and cooking utensils, and human and animal excreta in the environment (Ogwaro *et al*., 2002). Food themselves are also frequently the source of contaminants, as some foodstuffs naturally harbor pathogens or may have been obtained from infected animals. Moreover, during food handling there is a risk of cross contamination of foods which leads to food borne disease (Motarjemi and Nout, 1996).

Since milk is an excellent medium for growth for a variety of bacteria; spoilage bacteria can inter from different sources. They may originate on the farm from the environment or milking equipment or in processing plants from equipment, handler, or from the air. LAB is usually the predominant microbes in raw milk and proliferates if milk is not cooled adequately. When populations reach about10^6 cfu/ml, off-flavors develop in milk due to production of lactic acid and other compounds. Refrigeration suppresses growth of LAB and within one day psychrophilic bacteria (*Pseudomonas, Enterobacter, Alcaligenes* and some spore-formers) grow and can eventually produce rancid odors through the action of lipases and bitter peptides from protease action (Dogan, and Boor, 2003). Though Pasteurization kills the psychrophiles and mesophilic bacteria, heat-tolerant species (*Alcaligenes*, *Microbacterium,* and the sporeformers *Bacillus* and *Clostridium*) survive and may later cause spoilage in milk or other dairy products (Dogan and Boor, 2003).

2.5.1. Pathogenic organisms in indigenous fermented milk products

For several centuries man has adopted fermentation as a means of food preservation and also found them to have nutritional and therapeutic advantages (Soomro *et al.*, 2002). The significance of milk in human nutrition is now well established as it is considered as the best, ideal and complete food for all age groups. Milk and dairy products are an excellent source of calcium, phosphorus and magnesium. These minerals in optimum ratio are present in milk and are required for optimum growth and maintenance of bones (Aneja *et al.*, 2002). However, in spite of this, milk can also serve not only as a potential vehicle for transmission of some pathogens but also allows these organisms to grow, multiply and produce toxins. A variety of pathogenic organisms may gain access in to milk and milk products from different sources and cause different types of food borne illnesses. Milk and milk products may carry toxic metabolites of different organisms growing in it. Ingestion of such products, contaminated with these metabolites, cause food poisoning. On the other hand the ingestion of viable pathogenic bacteria along with the food product leads to food borne infection. Some time these organisms undergo lysis in the gastrointestinal tract and liberate toxic substances from inside the cells which are detrimental to the health of the consumers (Aneja *et al.*, 2002).

The results of microbiological analysis from the study conducted by Karagözlü, 2007; revealed that, *Salmonella typhimurium*, *E. coli* 0157:H7 and *S. aureus* can survive and multiply during fermentation of kefir. Since these pathogens are resistant to acidic conditions, in the case of the contamination of milk used for kefir, they can presumably survive and cause foodborne illnesses.

In Ethiopia the study conducted by, Mogessie Ashenafi, 1992 to determine the possibility of contamination of milk by food-borne pathogens from various sources and the fate of pathogenic microorganism such as, *Salmonella* spp, *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes* during the souring of milk into *Ergo* showed that, all the test pathogens could grow to levels as high as 10^7- 10^8 cfu/ml within 12 hours in fermenting milk. The duration of inhibition of pathogenic microorganism by lactic acid bacteria is vary from one the organism to the other. For example, *Salmonella typhimurium* and *Salmonella enteritidis* were inhibited between 48 and 60 hours of fermentation of milk in non-smoked and smoked containers while, *Staphylococcus aureus* and *Bacillus cereus* were inhibited within 24 to 38 hours of fermentation

in non-smoked containers and within 24 hours in smoked containers. On the other hand, *Listeria monocytogenes* in fermenting milk in non-smoked containers was inhibited after 48-60 hours, whereas inhibition was observed at 36 hours in smoked containers. It was suggested that the synergistic effect of pH, acids and container smoking were important in the complete inhibition of the test organisms (Mogessie Ashenafi, 1992).

E. coli O157:H7 inoculated in souring milk at initial levels of 10^3cfu/ml grew to over 10^6cfu/ml within 24 hours and counts at 72 hours were still at the level of 10^3cfu/ml. Post-souring inoculation of the pathogen in ergo, however, resulted in complete elimination of the pathogen within 6 hours at ambient temperature storage, but it was recovered until 72 hours at refrigeration storage (Mekonnen Tsegaye and Mogessie Ashenafi, 2005).

3. MATERIALS AND METHODS

3.1 Study Area

This study was conducted between December 2010 and February 2011 in Bahir Dar town, which is the capital city of Amhara National Regional State (ANRS). The town is located at 11° 36' 0'' North and 37° 23'0'' East .The latitude of the town is about 1801m above sea level and covers an area of 16000 hectares (Mogose, 2008). Bahir Dar town is located in northwestern part of Ethiopia at a distance of 565 km from Addis Ababa. According to the central statistical Agency of the Federal Democratic Republic of Ethiopia 2006 census report , the population of Bahir Dar town is estimated to be around 175185 (Abel Teferi, 2010). Bahir Dar is one of the leading tourist destinations in Ethiopia with a variety of attractions in the nearby Lake Tana and Blue Nile River. The town has tropical type of climate with an average temperature of 18.5°C. The high ambient temperature in most seasons of the year and the location of the town leads to a great spoilage rate of the food by microorganisms.

3.2. Study design

To assess microbial quality and safety of *ergo* in cafeterias and restaurants of Bahir Dar town, a cross-sectional study was conducted from December 2010 - April 2011.

3.3. Sampling

Thirty samples of fermented milk (*Ergo*) were randomly collected from different restaurants and cafeterias found in Bahir Dar town. Samples were collected under aseptic conditions in sterile airtight sampling jars and brought to the laboratory and stored under refrigeration at 4°C for 1-2 hour until they were used in experiments. Microbiological analysis was conducted.

3.4. Enumeration and Isolation of Microorganisms

3.4.1. Enumeration of aerobic mesophilic bacteria

Twenty five ml of "*ergo*" was aseptically removed from 500 ml and added into 225 ml of sterile 0.85 % NaCl solution and mixed thoroughly. Serial dilutions of (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) were made using 9 ml sterile physiological saline solution. Then from each dilution 1ml was pour plated on to sterile plate count agar (PCA) (H.P.O., Secunderabad-5000003 A.P., India) and

incubated at 37° C for 48 hours. The total aerobic mesophilic count of bacteria was determined using the procedure described by Akabanda (2010). The numbers of colonies were counted with colony counter (Stuart Scientific Colony Counter, UK). Finally the results were reported as a logarithm of cfu/ml of the sample analyzed.

3.4.2 Enumeration yeasts and molds

To determine the number of yeasts and molds in *ergo* samples, 25 ml f ergo were aseptically added into 225 ml of sterile 0.85 % NaCl solution and mixed thoroughly. Serial dilutions of (10^{-1}, 10^{-2} 10^{-3}, 10^{-4} and 10^{-5}) were made using 9 ml sterile physiological saline solution. Then from each dilution 1ml was pour plated on to Sabouraud Dextrose Agar (SDA) (Blulux, 121001,India) and incubated at 25°C for 2-3 days then the colonies were enumerated from the surface of SDA (Akabanda, 2010). The numbers of colonies were counted with colony counter (Stuart Scientific Colony Counter, UK). Finally the results were reported as a log10 of cfu/ml of the sample analyzed.

3.4.3. Enumeration and isolation of *Salmonella*

From 500 ml collected *ergo* samples, 25 ml were aseptically added into 225 ml of sterile 0.85 % NaCl solution and mixed thoroughly. Serial dilutions of (10^{-1}, 10^{-2} and 10^{-3}) were made using 9 ml sterile physiological saline solution. From each dilution 1ml was pour plated on to sterile salmonella shigella Agar (SSA) (Blulux, 121001, India) and incubated at 37°C for 48 hours. The numbers of colonies were counted with colony counter (Stuart Scientific Colony Counter, UK). Two or three of typical colonies (colorless with black center on SS) were selected and transferred to nutrient broth and incubated at 37°C for 24 hrs. The culture from nutrient broth were streaked onto nutrient agar slope and incubated at 37°C for 24 hour and stored at 4°C for further biochemical identification (Abd El-Atty and Meshref, 2007). All isolates were characterized using triple sugar iron agar (TSIA) for sugar fermentation, Motility test by Sulphare Indole motility, lysine utilization, gas production and citrate utilization. Finally the results were reported as a log 10 cfu/ml of the sample analyzed.

3.4.4. *Staphylococcus aureas* count

Enumeration of *Staphylococcus aureus* were conducted by transferring, 25 ml of *ergo* sample into 225 ml of sterile 0.85 % NaCl solution and mixed thoroughly. Serial dilutions of (10^{-1}, 10^{-2} and 10^{-3}) were made using 9 ml sterile physiological saline solution. From each dilution 1ml was pour plated on to sterile Manitol Salt Agar (MSA) (Blulux, 121001, India) and incubated at 37°C for 48 hours. The numbers of colonies were counted with colony counter (Stuart Scientific Colony Counter, UK). Two or three of typical colonies were selected and transferred to nutrient broth and incubated at 37°C for 24 hours. The culture from nutrient broth were streaked onto nutrient agar slope and incubated at 37 °C for 24 hour and stored at 4°C for further biochemical identification (da Silva *et al.*, 2000). Finally the results were reported as a log10 of cfu/ml of the sample analyzed.

3.4.5. Enumeration and Isolation of lactic acid bacteria

After sample collection, 25 ml of ergo were aseptically removed and added into 225 ml of sterile 0.85 % NaCl solution and mixed thoroughly. Serial dilutions of (10^{-1}, 10^{-2} 10^{-3}, 10^{-4} and 10^{-5}) were made using 9 ml sterile physiological saline solution. From each dilution 1ml was pour plated on to sterile de Mann Rogosa Sharp agar (MRS) (H.P.O., Secunderabad-5000003 A.P., India) (De Man *et al.*, 1960). The plates were incubated anaerobically for 48 hrs at 37°C under carbon dioxide atmospheric conditions using anaerobic jars with gas generating kits (Oxoid BR38, Ltd, Basingstoke, Hampshire, England). After incubation, the colonies that grew on the surface of MRS were counted as lactic acid bacteria. Representative colonies were randomly picked from countable MRS agar plates for further identification. Colonies of LAB were transferred into about 5ml MRS broth (H.P.O., Secunderabad-5000003 A.P., India) and purified by repeated streaking on MRS agar. The pure cultures were streaked on slants of MRS agar and were stored at 4°C for further biochemical characterization. All isolates were characterized with gram reaction, catalase test, gas production from glucose and microscopically examined for cell morphology and cellular arrangement (Akabanda, 2010).

3.5 Data analysis

All microbial counts were converted to the base 10 logarithm of the number of colony forming units per ml of *ergo* samples (log cfu/ml) and from the means microbial load was determined.

4. RESULTS AND DISCUSSION

From a total of 30 samples of *ergo* five types of microorganisms which include, Aerobic mesophilic count, *Salmonella* ,*Staphylococcus aureus,* yeasts and molds and lactic acid bacteria where analyzed.

4.1 Aerobic mesophilic bacteria count

Dairy products prepared under unhygienic conditions pose a great threat to the health of consumers. Aerobic mesophilic bacteria (AMB) are often considered as an important microbiological parameter for milk and dairy products quality and when present at high levels (higher than 10^5cfu/ml) indicate serious deficiencies in production hygiene, whereas values lower than 20,000 cfu/ml reflect good sanitary practices (Chambers, 2002).

The result of this study showed that 26 (86.66%) of AMB counted from *ergo* sample exceed the typical guide line of aerobic mesophilic count set at $<10^5$ cfu/ml for ready to eat food products. This high level of aerobic mesophilic bacteria may be due to contamination of the milk either from the milking cows milking equipments or from milk handler. In contrary, 4 (13.33%) (Table 1) of AMB was below the recommended value. This may be as a result of safe handling practices and the health of the milking cows. Similar to this idea Al-Khatib and Al-Mitwalli, (2009) reported that AMB is a quality control test that is a basic measure of the bacteria in dairy products. It reveals general sanitation and herd health conditions. So the high proportion of samples with unacceptable AMB in this study (86.66%) may be a result of poor sanitation and bad herd health conditions.

The aerobic mesophilic bacteria count ranged from 5.38 log10 cfu/ml to 4.64 log10 cfu/ml (Table1). These range is higher than high counts of aerobic mesophilic bacteria) obtained by Mourad and Nour-Eddine (2006) which ranges from 2.76 to 3.88 log10 cfu/ml. In contrary high range of aerobic mesophilic bacteria (5.9 log10 cfu/ml to 8.556 log10 cfu/ml) was reported by (Savadogo, 2004). The mean counts of AMB in this study also showed that 5.18 log10cfu/ml Table1.

Table 1 Aerobic mesophilic counts of *ergo* collected from Café and Restaurants in Bahir Dar (December 2010 – April 2011)

Sample No	Aerobic mesophilic bacteria (log10 cfu/ml
1	5.25
2	5.43
3	5.04
4	4.77
5	5.04
6	5.32
7	5.17
8	5.19
9	4.92
10	5.19
11	4.90
12	5.17
13	5.31
14	5.18
15	5.32
16	5.33
17	5.33
18	5.33
19	5.19
20	5.14
21	5.22
22	5.20
23	5.21
24	5.33
25	5.31
26	5.32
27	5.20
28	5.13
29	4.64
30	5.38
Mean±0.33	5.18

4.2. Detection of *Salmonella*

The result of this study indicates that the mean count of *Salmonella* (2.7 log10 cfu/ml) from the ergo sample analyzed. The result also showed the range between zero to 4.05 log10 cfu/ml Table2. This value is beyond the acceptable guide lines set for *Salmonella* which is zero tolerance. So the result indicates that the ergo sample collected were highly contaminated by this

organism. From the total 30 samples collected only 7(23.33%) samples are negative for *salmonella* while, 23 (76.66%) of total samples highly contaminated by *salmonella.*

Table 2 Counts of *Salmonella* from *ergo* collected from Café and Restaurants in Bahir Dar (December 2010 – April 2011).

Sample No	*Salmonella* (log10 cfu/ml
1	2.96
2	2.63
3	ND
4	3.85
5	3.20
6	2.80
7	3.45
8	ND
9	3.29
10	ND
11	ND
12	3.89
13	3.82
14	ND
15	3.83
16	2.56
17	3.97
18	4.05
19	4.00
20	3.48
21	3.84
22	ND
23	3.78
24	3.90
25	ND
26	3.38
27	3.73
28	3.42
29	3.70
30	3.46
Mean±0.29	2.7

ND: Not detected

Although fermented milk products have been considered intrinsically safe due to their high acidity, several investigators have demonstrated that pathogenic microorganisms such as *Salmonella* and *Staphylococcus* can survive in fermented milks over several days and weeks (Hal-Haddad, 2003). These organisms are some of the most important bacterial foodborne pathogens that can lead to foodborne diseases through consumption of contaminated milk and fermented milks (Mead *et al*., 1999). Therefore, the survival of these pathogens for up to several weeks illustrates the potential health risks associated with pre-and-post processing contamination of fermented milks. Similarly, these pathogenic bacteria is detected in *ergo* which could be attributed to the contamination from the milking cows as well as from the milk handler and hygienic quality of the equipments used for storing and selling of *ergo*.

In most cases, preparation of *ergo* requires a one-day incubation at ambient temperatures. Many people consider as spoilage when the *ergo* ferment for a long period of time due to high amount of lactic acid produced by lactic acid bacteria. Similarly Mogessie Ashenafi, (2006) reported that, longer keeping is not desirable because further drop in pH will result in increased wheying off, which, in turn, results in loss of protein as whey. So, fermentation for one day may not sufficient to produce an acid that inhibit the survival of pathogenic and spoilage microorganism because, many species of *Salmonella* survive for a long period in fermented milk. Park and Marth, (1972) confirmed survival of *Salmonella typhimurium* up to 9 days in refrigerated cultured skim milk.

It is clear, that in any hazard analysis of a food production process, due consideration must be given to the potential for acid tolerance to allow *Salmonella* survival. Many finding suggest that acid adaptation of *Salmonella* in fermented milk products. For example, Leyer and Johnson (1992) showed that acid adaptation prolonged the survival of *Salmonella typhimurium* in cheeses and during milk fermentation. It is may be due to this adaptation that allow the high number of *Salmonella* in *ergo* from study area.

4.3 *Staphylococcus aureus* count

Staphylococcus aureus exists in air, dust, sewage, water, milk and food. Although this pathogen is transmitted to food from a human source, equipment and environmental surfaces can also be sources of contamination. Foods that are frequently associated with staphylococcal food poisoning include meat and meat products, bakery products and milk and dairy products (Cliver, 1990).

Table 3 *Staphylococcus aureus* counts of *ergo* collected from Café and Restaurants in Bahir Dar (December 2010 – April 2011)

Sample No	*Staphylococcus aureus* (log10 cfu/ml
1	2.80
2	3.73
3	3.14
4	3.78
5	3.77
6	ND
7	3.77
8	3.81
9	3.20
10	3.93
11	3.75
12	ND
13	ND
14	4.12
15	3.96
16	ND
17	4.14
18	ND
19	ND
20	3.98
21	ND
22	4.10
23	ND
24	3.91
25	ND
26	3.67
27	ND
28	3.78
29	ND
30	3.72
Mean±0.34	3.74

The *Staphylococcus aureus* was detected in 19 (63.33%) of samples collected and the count ranging from zero to 4.14 log10 cfu/ml (Table 3). The presence of *Staphylococcus aureus* is potential health hazard as this high count may indicate the presence of enterotoxigenic strains. Gran *et al*., (2003) reported the presence of *Staphylococcus aureas* in naturally fermented milk in Zimbabwe. The organism was also isolated from samples of traditional fermented milk produced in a plastic container, clay pot and a calabash gourd from individual households in South Africa and Namibia (Beukes *et al*., 2001). Similarly in most of cafeterias and restaurants *ergo* is fermented in plastic container which is difficult to smoke. Since *ergo* come from different cows this pathogenic bacteria may contaminate the milk from the milking cow or from the hand of milk handler or milking equipments. Abdalla and Ahmad, (2010), suggest that, the high *S. aureues* count is indicative of poor hygienic conditions during production/ processing of fermented milk products or contamination after processing. From the total of analyzed *ergo* samples 19 (63.33%) samples were positive for *Satphylococus aureus*, while 11(36.66%) of analyzed ergo samples are negative for *Staphylococcus aureus.* In addition to that these high counts of *Staphylococcus* indicate the extent to which the fermented milk is contaminated. The results of microbiological examination indicate that this product is highly contaminated with microorganisms of public health concern. As being popular fermented milk, ergo is fermented at room temperature. So, high number of *S. aureus* bacteria indicates unhygienic conditions during production of milk and further processing into *ergo* without heat treatment because, *Staphylococcus aureus* is sensitive to high temperature. Therefore if there is no cross contamination it cannot detected in analyzed sample.

4.4. Isolation of lactic acid bacteria

It is well known that lactic acid bacteria are inhibitory to the growth and survival of pathogenic and spoilage microorganisms. Lactic acid bacteria utilize the lactose present in the milk and by the lactic acid fermentation produces organic acids, mainly lactic acid. These organic acids cause a decline in pH and thereby inhibiting the growth of pathogens (Garrote *et al.*, 2000).

Table 4 Counts LAB of *ergo* collected from Café and Restaurants in Bahir Dar (December 2010 –April 2011).

Sample No	Lactic acid bacteria (log10 cfu/ml
1	5.46
2	5.45
3	5.42
4	5.30
5	5.16
6	5.35
7	5.50
8	5.45
9	5.37
10	5.31
11	5.40
12	5.17
13	5.52
14	5.47
15	5.52
16	5.44
17	5.49
18	5.46
19	5.50
20	5.23
21	5.45
22	5.41
23	5.46
24	5.49
25	5.47
26	5.50
27	5.51
28	5.39
29	5.47
30	5.49
Mean±0.018	5.42

The results of lactic acid bacteria count show that fermentation is mainly carried out by lactic acid bacteria in uncontrolled conditions of fermentation because in all samples they dominate the microflora of ergo. Similar results were reported by Abdelgadir *et al.*, (2001) but, the mean count of LAB in this study are below the average mean value of 8.0 log10 cfu/ ml which recorded by Beukes *et al.*, 2001 from South African traditional fermented milk. In a study carried out by Mufandaedza *et al.*, 2006 the lactic acid bacteria in spontaneously fermented raw milk grew to maximum population of about 8.9 log10 cfu /ml over 48 hrs. This increase in LAB resulted in a rapid decrease in pH, thus resulting in a much higher death rate of pathogens.

Mogossie Ashenafi, (2006) reported that growth of lactic acid bacteria in souring milk resulted in inhibition of *Salmonella typhimurium* and *Salmonella enteritidis* between 48 and 60 hours of fermentation of milk in non-smoked and smoked containers. Findings from the various workers indicate that *ergo* is produced by spontaneous fermentation and cannot be defined in terms of its microbiological or biochemical properties. It does not have definite temperature and duration of incubation. Fermentation is carried out at ambient temperatures. This indicates that at high temperature the pathogenic bacteria may proliferate during fermentation of *ergo*.

4.5. Yeast and mould counts

Many species of yeasts are able to grow in milk and fermented milk products. Yeasts' growth in milk products is attributed to their ability to utilize milk constituents such as proteins, fat, lactose, galactose, glucose, citrate and the low pH (Fleet, 1990). The source of yeasts in raw milk and naturally fermented milk has been assumed to be chance contamination from the animals, the milk handler or the contaminated equipment.

Yeasts and moulds were detected in all samples with the range being 4.09 log10 cfu/ml to 5.23 log10 cfu/ml (Table 5). In this study yeasts and mold are the third dominant group next to lactic acid bacteria and aerobic mesophilic bacteria. Although high acidity is considered inhibitory to the vegetative cells of pathogenic microorganisms, it considered promising to growth of yeasts and molds. Nearly similar results were obtained by Rihab, *et al.*, (2006). Isono *et al.* (1994) reported high number of yeasts and molds in traditional fermented milk in Northern Tanzania with mean counts ranging from 6.0 to 8.0 log10 cfu/ml while, Abdelgadir *et* al. (2001) reported

yeast counts of 7 log10 cfu/ml in Sudanese *Rob*. Similarly, Al-Tahiri (2005) and Hassan *et al.*, (2008) reported the result in the same order to this study which has Log10 5.30 from yogurt and Lebnah. But, 7.9 log10 cfu/ml of wild yeast recorded by (Mufandaedza *et al.*, 2006) from naturally fermented raw milk of Zimbabwe was higher than the mean count recorded in this study. These high counts of yeasts and molds might be an indication of poor handling practice from milking up to consumption or from environmental contamination when the product is exposed to air. Abdalla and Ahmed, (2010) indicated that, high count of yeasts and molds reflect unsanitary hygienic conditions during processing and distribution of the product. According to Abdalla and Hussain, (2010) the presence of yeasts might be attributed to contamination by air or to the lack of proper hygiene by the producers, while the presence of molds indicates the contamination of the product by air or by persons who were engaged in the preparation, or transportation. The finding of this study is not far from the idea given by many writers. Because, high counts of yeasts and molds indicate, there might be a contamination either from the air or from milk handler at the farming area or during fermentation in cafeteria and restaurants as well as during serving.

Table 5 Yeast and mold counts of *ergo* collected from Café and Restaurants in Bahir Dar (December 2010 – April 2011).

Sample No	Yeasts aand molds (log10 cfu/ml
1	4.26
2	4.28
3	4.14
4	5.15
5	4.91
6	5.02
7	4.09
8	5.11
9	5.07
10	4.07
11	5.05
12	4.90
13	5.11
14	4.18
15	5.15
16	5.11
17	5.23
18	5.08
19	4.19
20	5.13
21	5.12
22	5.15
23	5.12
24	5.00
25	5.07
26	4.11
27	5.10
28	4.98
29	5.06
30	5.11
Mean±0.08	4.84

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. SUMMARY

Fermented foods and beverages constitute a major portion of peoples' diets all over the world. Ergo is the most popular dairy product throughout the country which is consumed by every member of the family and considered as spatial food which serves as a basis for further processing the milk in to different dairy products. Milk and its fermented products have been identified as important means of spreading human pathogenic organisms, which gain access in to milk and milk products from different sources and cause different types of food borne illnesses. So, the aim of this study was to assess the microbiological quality and safety of ergo from Bahir Dar town during consumption in cafeterias and restaurants. From the total 30 analyzed samples 26 (86 .66%) of aerobic mesophilic bacteria (AMB) count exceed the typical guide line of aerobic mesophilic count set at $<10^5$ cfu/mg for ready to eat food products. The count of AMB ranged from 5.38 log10 cfu/ml to 4.64 log10 cfu/ml. From the total samples collected 23 (76.66%) highly contaminated by *salmonella and* 19 (63.33%) samples are positive for *Satphylococus aureus*.

5.2. CONCLUSION

Many fermented milk are considered as safe against pathogenic bacteria but, food-borne pathogens can survive the acidic condition. A variety of pathogenic organisms may gain access in to milk and milk products from different sources and cause different types of food borne illnesses. The high count of *Salmonella* and *Staphylococcus aureus* may be a consequence of the low level of hygiene maintained during the processing and sale of this product. The presence of these pathogenic bacteria, in *ergo* indicate high level of contamination. Because, the counts of these pathogenic bacteria are above the guide line set for *Salmonella* and *staphylococcus aureus*. This study also indicates that ergo which is consumed in Bahir Dar is highly contaminated with aerobic mesophilic bacteria, yeasts and molds, *Staphylococcus aureus* and *Salmonella.* Preparation procedures are still traditional arts and the fermentation is uncontrolled.

5.3. RECOMMENDATIONS

The equipment for milking should be cleaned frequently. Milk must be pasteurized or boiled before the production, and the storage of milk should be realized under refrigeration. Education of producers on basic hygienic principles will equally be crucial to obtain ergo which is pathogen free. The microorganisms isolated from *ergo* were diverse, including aerobic mesophilic bacteria, LAB and Yeasts. These dominant microorganisms should be characterized using modern molecular methods to facilitate selection and development of starter cultures from them for the production of good quality *Ergo* which has defined characteristics thereby enhancing the quality of the product. Generally, the poor bacteriological quality observed in the present study requires further investigation of the status of the animals' health and the significance of the effect of containers to ascertain their contribution on microbial quality. Finally, taking this study as base line the study which includes number of parameters should be conducted to aware the society about health risk associated whit consumption of *ergo*.

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8. APPENDIXES

Table 6 Biochemical and morphological characterization result of LAB isolated from ergo in Bahi Dar from (December 2010 – April 2011)

No	Isolate code	Catalase reaction	Gram reaction	Cell Shape	Gas production from glucose
1	M111	–ve	+ve	Rods	+ve
2	M112	–ve	+ve	Cocci	–ve
3	M113	–ve	+ve	Cocci	+ve
4	M114	–ve	+ve	Rods	–ve
5	M115	–ve	+ve	Cocci	–ve
6	M211	–ve	+ve	Rods	–ve
7	M212	–ve	+ve	Cocci	–ve
8	M213	–ve	+ve	Spherical	–ve
9	M214	–ve	+ve	Rods	–ve
10	M215	–ve	+ve	Cocci	+ve
11	M311	–ve	+ve	Cocci	–ve
12	M312	–ve	+ve	Cocci	–ve
13	M313	–ve	+ve	Rods	–ve
44	M314	–ve	+ve	Cocci	–ve
15	M315	–ve	+ve	Cocci	+ve
16	M411	–ve	+ve	Rods	–ve
17	M412	–ve	+ve	Cocci	–ve
18	M413	–ve	+ve	Rods	+ve
19	M414	–ve	+ve	Cocci	–ve
20	M415	–ve	+ve	Cocci	–ve
21	M511	–ve	+ve	Rods	–ve
22	M512	–ve	+ve	Rods	–ve
23	M513	–ve	+ve	Rods	–ve
24	M514	+ve	+ve	Rods	+ve
25	M111	–ve	+ve	Cocci	–ve
26	M112	–ve	+ve	Cocci	–ve
27	M515	–ve	+ve	Rods	–ve
28	M611	–ve	+ve	Cocci	–ve
29	M612	–ve	+ve	Cocci	+ve
30	M613	–ve	+ve	Rods	+ve
31	M614	–ve	+ve	Rods	–ve
32	M615	–ve	+ve	Spherical	–ve
33	M711	–ve	+ve	Cocci	–ve
34	M712	–ve	+ve	Cocci	–ve
35	M713	–ve	+ve	Cocci	–ve
36	M714	–ve	+ve	Rods	–ve
37	M715	–ve	+ve	Cocci	–ve
38	M811	–ve	+ve	Rods	+ve

Table 6 (continued) Biochemical and morphological characterization result of LAB isolated from ergo in Bahir Dar from (December 2010 – April 2011).

39	M812	–ve	+ve	Cocci	–ve
40	M813	–ve	+ve	Cocci	–ve
41	M814	–ve	+ve	Rods	+ve
42	M912	–ve	+ve	Rods	–ve
43	M913	–ve	+ve	Spherical	–ve
44	M914	–ve	+ve	Cocci	–ve
45	M915	–ve	+ve	Rods	–ve
46	M1011	–ve	+ve	Cocci	+ve
47	M1012	+ve	+ve	Cocci	–ve
48	M1013	–ve	+ve	Cocci	–ve
49	M1014	–ve	+ve	Rods	–ve
50	M1015	–ve	+ve	Cocci	–ve
51	M1111	–ve	+ve	Rods	+ve
52	M1112	–ve	+ve	Cocci	–ve
53	M1113	–ve	+ve	Cocci	+ve
54	M1114	–ve	+ve	Rods	–ve
55	M1115	–ve	+ve	Cocci	–ve
56	M1211	–ve	+ve	Rods	–ve
57	M1212	–ve	+ve	Cocci	–ve
58	M1213	–ve	+ve	Spherical	–ve
59	M1214	–ve	+ve	Rods	–ve
60	M1215	+ve	+ve	Cocci	+ve
61	M1311	–ve	+ve	Cocci	–ve
62	M1312	+ve	+ve	Cocci	–ve
63	M1313	+ve	+ve	Rods	–ve
64	M1314	–ve	+ve	Cocci	–ve
65	M1315	–ve	+ve	Cocci	+ve
66	M1411	+ve	+ve	Rods	–ve
67	M1412	–ve	+ve	Cocci	–ve
68	M1413	+ve	+ve	Rods	+ve
69	M1414	+ve	+ve	Cocci	–ve
70	M1415	–ve	+ve	Cocci	–ve
71	M1511	–ve	+ve	Rods	–ve
72	M1512	–ve	+ve	Rods	–ve
73	M1513	–ve	+ve	Rods	–ve
74	M1514	–ve	+ve	Rods	+ve
75	M1515	–ve	+ve	Cocci	–ve
76	M1611	–ve	+ve	Cocci	–ve

Table 6 (continued) Biochemical and morphological characterization result of LAB isolated from ergo in Bahir Dar from (December 2010 – April 2011).

77	M1612	–ve	+ve	Rods	–ve
78	M1613	–ve	+ve	Cocci	–ve
79	M1614	–ve	+ve	Cocci	+ve
80	M1615	–ve	+ve	Rods	+ve
81	M1711	–ve	+ve	Rods	–ve
82	M1712	–ve	+ve	Spherical	–ve
83	M1713	–ve	+ve	Cocci	–ve
84	M1714	+ve	+ve	Cocci	–ve
85	M1715	–ve	+ve	Cocci	–ve
86	M1811	–ve	+ve	Rods	–ve
87	M1812	–ve	+ve	Cocci	–ve
88	M1813	–ve	+ve	Rods	+ve
89	M1814	–ve	+ve	Cocci	–ve
90	M1815	–ve	+ve	Cocci	–ve
91	M1911	–ve	+ve	Rods	+ve
92	M1912	–ve	+ve	Rods	–ve
93	M1913	–ve	+ve	Spherical	–ve
94	M1914	–ve	+ve	Cocci	–ve
95	M1915	–ve	+ve	Rods	–ve
96	M2011	–ve	+ve	Cocci	+ve
97	M2012	–ve	+ve	Cocci	–ve
98	M2013	–ve	+ve	Cocci	–ve
99	M2014	–ve	+ve	Rods	–ve
100	M2015	–ve	+ve	Cocci	–ve
101	M2111	–ve	+ve	Rods	–ve
102	M2112	–ve	+ve	Cocci	+ve
103	M2113	–ve	+ve	Cocci	–ve
104	M2114	–ve	+ve	Rods	–ve
105	M2115	–ve	+ve	Cocci	–ve
106	M2211	–ve	+ve	Rods	+ve
107	M2212	–ve	+ve	Cocci	–ve
108	M2213	–ve	+ve	Spherical	–ve
109	M2214	–ve	+ve	Rods	–ve
110	M2215	–ve	+ve	Cocci	–ve
111	M2311	–ve	+ve	Cocci	–ve
112	M2312	–ve	+ve	Cocci	–ve
113	M2313	–ve	+ve	Rods	–ve
114	M2314	–ve	+ve	Cocci	+ve
115	M2315	–ve	+ve	Cocci	–ve
116	M2411	–ve	+ve	Rods	+ve
117	M2412	–ve	+ve	Cocci	–ve

Table 6 (continued) Biochemical and morphological characterization result of LAB isolated from ergo in Bahir Dar from (December 2010 – February 2011)

118	M2413	–ve	+ve	Rods	–ve
119	M2414	–ve	+ve	Cocci	–ve
120	M2415	–ve	+ve	Cocci	+ve
121	M2511	–ve	+ve	Rods	–ve
122	M2512	–ve	+ve	Rods	–ve
123	M2513	–ve	+ve	Rods	–ve
124	M2514	–ve	+ve	Rods	–ve
125	M2515	–ve	+ve	Cocci	+ve
126	M2611	–ve	+ve	Cocci	–ve
127	M2612	–ve	+ve	Rods	–ve
128	M2613	–ve	+ve	Cocci	–ve
129	M2614	–ve	+ve	Cocci	+ve
130	M2615	–ve	+ve	Rods	–ve
131	M2711	–ve	+ve	Rods	–ve
132	M2712	–ve	+ve	Spherical	–ve
133	M2713	–ve	+ve	Cocci	–ve
134	M2714	–ve	+ve	Cocci	–ve
135	M2715	–ve	+ve	Cocci	–ve
136	M2811	–ve	+ve	Rods	–ve
137	M2812	–ve	+ve	Cocci	+ve
138	M2813	–ve	+ve	Rods	–ve
139	M2814	–ve	+ve	Cocci	–ve
140	M2815	–ve	+ve	Cocci	–ve
141	M2911	–ve	+ve	Rods	–ve
142	M2912	–ve	+ve	Rods	–ve
143	M2913	–ve	+ve	Spherical	–ve
144	M2914	–ve	+ve	Cocci	–ve
145	M2915	–ve	+ve	Rods	–ve
146	M3011	–ve	+ve	Cocci	–ve
147	M3012	–ve	+ve	Cocci	–ve
148	M3013	–ve	+ve	Cocci	–ve
149	M3014	–ve	+ve	Rods	–ve
150	M3015	–ve	+ve	Cocci	–ve

(+ve): Positive, (-ve): Negative

Table 7 Biochemical characteristics of *Salmonella* isolated from ergo in Bahir Dar from (December 2010 – April 2011).

No	Isolate code	Citrate utilization	Motility	Sugar (TSI)Fermentation	Lysine utilization, gas and H_2S formation	Isolates Identity
1	Sa111	+	+	A/Ag	+ + +	*Salmonella*
2	Sa112	-	+	A/Ag	+ - -	*Salmonella*
3	Sa113	-	+	A/A	+ - +	*Salmonella*
4	Sa121	+	+	A/A	+ - -	*Salmonella*
5	Sa122	-	+	A/Ag	++ -	*Salmonella*
6	Sa123	+	+	K/g	+ + -	*Salmonella*
7	Sa211	-	+	A/Ag	+ + +	*Salmonella*
8	Sa212	+	+	A/Ag	+ + -	*Salmonella*
9	Sa213	-	+	A/A	++ -	*Salmonella*
10	Sa221	+	+	K/Ag	+ + +	*Salmonella*
11	Sa222	+	+	A/Ag	+ + +	*Salmonella*
12	Sa223	-	+	A/Ag	+ -+	*Salmonella*
13	Sa311	-	+	K/Ag	+ + -	*Salmonella*
14	Sa312	+	+	A/Ag	+ + +	*Salmonella*
15	Sa313	+	+	A/Ag	+ -+	*Salmonella*
16	Sa321	-	+	A/Ag	+ + -	*Salmonella*
17	Sa322	-	+	A/Ag	+ + -	*Salmonella*
18	Sa323	+	+	A/Ag	+ + +	*Salmonella*
19	Sa411	-	+	A/A	+ -+	*Salmonella*
20	Sa412	+	+	K/Ag	+ + +	*Salmonella*
21	Sa413	+	+	K/Ag	+ + -	*Salmonella*
22	Sa421	-	+	A/A	+ - -	*Salmonella*
23	Sa422	+	+	A/Ag	+ + +	*Salmonella*
24	Sa423	-	+	A/A	+ - +	*Salmonella*
25	Sa511	+	+	A/Ag	+ + +	*Salmonella*
26	Sa512	+	+	A/g	+ + -	*Salmonella*
27	Sa513	-	+	K/g	+ - +	*Salmonella*
28	Sa521	+	+	A/A	- - -	*Salmonella*
29	Sa522	+	+	K/g	+ + +	*Salmonella*
30	Sa523	+	+	A/A	+ + +	*Salmonella*
31	Sa611	-	+	A/A	+ - +	*Salmonella*
32	Sa612	+	+	K/Ag	+ + +	*Salmonella*
33	Sa613	+	+	A/Kg	+ + +	*Salmonella*
34	Sa621	-	+	K/Ag	+ ++	*Salmonella*
35	Sa622	+	+	K/Ag	+ + +	*Salmonella*
36	Sa623	+	+	K/Ag	+ ++	*Salmonella*

Table 7 (continued) Biochemical characteristics of *Salmonella* isolated from ergo in Bahir Dar from (December 2010 – April 2011).

37	Sa921	-	+	A/A	+ - -	*Salmonella*
38	Sa922	+	+	A/Ag	+ + +	*Salmonella*
39	Sa923	-	-	A/Ag	-+ -	*Salmonella*
40	Sa1211	-	+	K/Ag	+ +-	*Salmonella*
41	Sa1212	+	+	A/Ag	+ + +	*Salmonella*
42	Sa1213	+	-	A/Ag	+ + -	*Salmonella*
43	Sa1221	+	+	K/Ag	+ + +	*Salmonella*
44	Sa1222	-	+	A/Ag	+ - +	*Salmonella*
45	Sa1223	-	+	K/Ag	- + -	*Salmonella*
46	Sa1311	+	+	A/Ag	+++	*Salmonella*
47	Sa1312	-	+	K/Ag	+++	*Salmonella*
48	Sa1313	-	+	A/A	+- -	*Salmonella*
49	Sa1511	+	+	A/Ag	+++	*Salmonella*
50	Sa1512	+	+	A/Ag	+ + +	*Salmonella*
51	Sa1513	+	+	A/Ag	+ + +	*Salmonella*
52	Sa1611	+	+	A/Ag	+++	*Salmonella*
53	Sa1612	+	+	A/Ag	+-+	*Salmonella*
54	Sa1613	+	+	A/Ag	+++	*Salmonella*
55	Sa 1711	+	-	A/A	+-+	*Salmonella*
56	Sa1712	+	-	A/Ag	++-	*Salmonella*
57	Sa1713	+	+	A/Ag	+++	*Salmonella*
58	Sa1811	-	+	A/Ag	+++	*Salmonella*
59	Sa1812	-	+	A/Ag	-++	*Salmonella*
60	Sa1813	-	-	A/Ag	-+-	*Salmonella*
61	Sa1911	+	+	A/A	++-	*Salmonella*
62	Sa1912	+	+	A/Ag	+++	*Salmonella*
63	Sa1913	+	+	A/Ag	+++	*Salmonella*
64	Sa2011	+	+	K/Ag	+++	*Salmonella*
65	Sa2012	-	-	A/A	-+-	*Salmonella*
66	Sa2013	+	-	A/Ag	++-	*Salmonella*
67	Sa2211	+	+	K/A	+++	*Salmonella*
68	Sa2212	+	+	A/Ag	+++	*Salmonella*
69	Sa2213	-	-	A/Ag	-+-	*Salmonella*
70	Sa2311	+	+	K/A	+++	*Salmonella*
71	Sa2312	+	+	K/g	+++	*Salmonella*
72	Sa2313	-	+	K/A	++-	*Salmonella*
73	Sa2411	+	+	A/A	-++	*Salmonella*
74	Sa2412	+	+	K/A	+++	*Salmonella*
75	Sa2413	+	+	A/A	+++	*Salmonella*

Table 7 (continued) Biochemical characteristics of *Salmonella* isolated from ergo in Bahir Dar from (December 2010 – April 2011).

76	Sa2611	+	+	K/A	+-+	*Salmonella*
77	Sa2612	-	+	K/A	+++	*Salmonella*
78	Sa2613	-	+	K/A	-++	*Salmonella*
79	Sa2711	+	+	A/A	+++	*Salmonella*
80	Sa2712	-	+	K/A	+--	*Salmonella*
81	Sa2713	-	-	A/Ag	-+-	*Salmonella*
82	Sa2811	+	+	K/A	+++	*Salmonella*
83	Sa2812	+	-	A/Ag	-++	*Salmonella*
84	Sa2813	+	+	A/A	+++	*Salmonella*
85	Sa2911	-	+	A/Ag	+++	*Salmonella*
86	Sa2912	+	+	A/Ag	+++	*Salmonella*
87	Sa2913	+	+	A/A	+-+	*Salmonella*
88	Sa3011	+	+	K/Ag	+++	*Salmonella*
89	Sa3012	+	+	A/A	---	*Salmonella*
90	Sa3013	-	+	K/Ag	-++	*Salmonella*

A: Acid, **K**: alkaline, **g**: gas, (+): positive, (-): negative

Table 8. Biochemical and morphological characteristics of *Staphylococcus aureus* isolated from Ergo in Bahir Dar tawon (December 2010 – April 2011).

No	Isolates code	Sugar fermentation (TSI)	Cell shape	Cell arrangement	Catalase reaction	Isolates identity
1	St111	A/A	Cocci	pairs	+	*S. aureus*
2	St112	K/A	Cocci	pairs	+	*S. aureus*
3	St113	K/A	Cocci	Clusters	+	*S. aureus*
4	St211	K/A	Cocci	Clusters	+	*S. aureus*
5	St212	K/A	Cocci	Pairs	+	*S. aureus*
6	St213	K/A	Cocci	Pairs	+	*S. aureus*
7	St311	K/A	Cocci	Clusters	+	*S. aureus*
8	St312	K/A	Cocci	Clusters	+	*S. aureus*
9	St313	A/A	Cocci	Singles	+	*S. aureus*
10	St411	K/A	Cocci	Pair	+	*S. aureus*
11	St412	K/A	Cocci	Pairs	+	*S. aureus*
12	St413	K/A	Cocci	Chain	+	*S. aureus*
13	St511	A/A	Cocci	Pairs	+	*S. aureus*
14	St512	K/A	Cocci	Clusters	+	*S. aureus*
15	St513	K/A	Cocci	Clusters	+	*S. aureus*
16	St711	A/A	Cocci	Pairs	+	*S. aureus*
17	St712	A/A	Cocci	pairs	+	*S. aureus*
18	St713	K/A	Cocci	Clusters	+	*S. aureus*
19	St811	A/A	Cocci	Clusters	+	*S. aureus*
20	St812	K/A	Cocci	Clusters	+	*S. aureus*
21	St813	K/A	Cocci	Pairs	+	*S. aureus*
22	St911	K/A	Cocci	Pairs	+	*S. aureus*
23	St912	K/A	Cocci	Tetrated	+	*S. aureus*
24	St913	A/A	Cocci	Chains	+	*S. aureus*
25	St1011	K/A	Cocci	Pairs	+	*S. aureus*
26	St1012	A/A	Cocci	Clusters	+	*S. aureus*
27	St1013	K/A	Cocci	Single	+	*S. aureus*
28	St1111	A/A	Cocci	Clusters	+	*S. aureus*
29	St1112	K/A	Cocci	Chains	+	*S. aureus*
30	St1113	K/A	Cocci	Pairs	+	*S. aureus*
31	St1411	A/A	Cocci	Clusters	+	*S. aureus*
32	St1412	K/A	Cocci	Pairs	+	*S. aureus*
33	St1413	K/A	Cocci	Pairs	+	*S. aureus*
34	St1511	K/A	Cocci	Pairs	+	*S. aureus*

Table 8 (continued) Biochemical and morphological characteristics of *Staphylococcus*

aureus isolated from Ergo in Bahir Dar tawon (December 2010 – April 2011).

35	St1512	K/A	Cocci	Single	+	*S. aureus*
36	St1513	A/A	Cocci	Pairs	+	*S. aureus*
37	St1711	K/A	Cocci	Clusters	+	*S. aureus*
38	St1712	K/A	Cocci	Clusters	+	*S. aureus*
39	St1713	A/A	Cocci	Chains	+	*S. aureus*
40	St2011	K/A	Cocci	Pairs	+	*S. aureus*
41	St2012	K/A	Cocci	Pairs	+	*S. aureus*
42	St2013	K/A	Cocci	Chains	+	*S. aureus*
43	St2111	K/A	Cocci	Pairs	+	*S. aureus*
44	St2112	A/A	Cocci	Clusters	+	*S. aureus*
45	St2113	K/A	Cocci	Single	+	*S. aureus*
46	St2211	K/A	Cocci	Clusters	+	*S. aureus*
47	St2212	K/A	Cocci	Clusters	+	*S. aureus*
48	St2213	KA	Cocci	Clusters	+	*S. aureus*
49	St2411	K/A	Cocci	Pairs	+	*S. aureus*
50	St2412	A/A	Cocci	Clusters	+	*S. aureus*
51	St2413	A/A	Cocci	Chains	+	*S. aureus*
52	St2611	K/A	Cocci	Pairs	+	*S. aureus*
53	St2612	A/A	Cocci	Pairs	+	*S. aureus*
54	St2613	K/A	Cocci	Single	+	*S. aureus*
55	St2811	K/A	Cocci	Clusters	+	*S. aureus*
56	St2812	K/A	Cocci	Clusters	+	*S. aureus*
57	St2813	K/A	Cocci	Single	+	*S. aureus*
58	St3011	A/A	Cocci	Chain	+	*S. aureus*
59	St3012	K/A	Cocci	Pairs	+	*S. aureus*
60	St3013	K/A	Cocci	Pairs	+	*S. aureus*

DECLARATION

The research work described in this thesis was carried out in Bahir Dar city in 2010/11 under the super vision of Dr Mulugeta Kibret.

This study represents original work by author and has not otherwise been submitted in any form for any degree to any university, where use has been made of work of others it has been duly acknowledged in the text.

Name: Abdurkadir Beyan

Signature: .....

Date of submission: .....

This work has been done under my supervision

Name: Dr Mulugeta Kibret

Signature: .....

Date of submission: .....