

# INTRODUCTION

Since times immemorial, microorganisms have been playing useful roles in human life and this has led to the need of studying these microscopic biological agents extensively. Milk being a nutritious food for human being also provides an ideal environment for microbial growth. Thus, the microorganisms which may gain entry into milk or its product can multiply and bring about spoilage of these products or render them unsafe due to potential health hazards (Yadav *et.al.*, 1993).

Control of food borne pathogens and prevention of related diseases are major tasks that public health will face in the 22nd century. More than 200 known diseases are transmitted through food by a variety of agents that include bacteria, fungi, viruses, and parasites. According to public health and food safety experts, each year millions of illnesses in the USA and throughout the world can be traced to food borne pathogens. While the food supply in the United States is one of the safest in the world, the Center for Disease Control and Prevention (CDC, 2003; CDC, 2004) estimates that 76 million people get sick, more than 3,00,000 are hospitalized, and 5,000 Americans die each year from food borne illness. The risk of food borne illness has increased markedly over the last 20 years, with nearly a quarter of the population at higher risk for illness today.

Consequently, preventing illness and death associated with food borne pathogens remains a major public health challenge. It is not known when man started utilizing milk of others animals for his benefit but the importance of milk in our diet has been recognized since Vedic times. Today, the major portion (91%) of world milk supply comes from cattle, buffalo, goat and sheep. They are the principal milk animals (Yadav *et. al.*, 1993).

Should the dairy industry be concerned about food safety? The answer is yes, and there are several good reasons why such as: Bulk tank milk contains several food borne pathogens that cause human disease, outbreaks of disease in humans have been traced to the consumption of raw unpasteurized milk and have also been traced back to pasteurized milk. Raw unpasteurized milk is consumed directly by dairy producers and their families, farm employees and their families, neighbors, etc. Raw unpasteurized milk is consumed directly by a much larger segment of the population via consumption of several types of cheeses including ethnic cheeses manufactured from unpasteurized raw milk. Entry of food borne pathogens via contaminated raw milk into dairy food processing plants can lead to persistence of these pathogens in biofilms and subsequent contamination of processed food products, pasteurization may not destroy all food borne pathogens in milk and faulty pasteurization will not destroy all food borne pathogens. A logical approach to

control food borne pathogens in dairy farms should be to define areas in the dairy farm that serve as food borne pathogens reservoirs and management practices that contribute to the persistence and spread of pathogens from these reservoirs. Food borne diseases are a common and widespread global problem. Several outbreaks have been reported as a result of eating contaminated dairy food that may look, taste and smell perfectly normal but as in fact contaminated with large number of harmful bacteria (CDC, 2009). The list of pathogens found in dairy shed wastewater is long, detailed descriptions are given by Pell (1997) and Sobsey *et. al.*, (2006) Pell (1997) nominated the pathogens of most concern to the US dairy industry as *Salmonella* spp., *Escherichia coli* and *Staphylococcus aureus*. **Table1** summarises the characteristics of these pathogens.

**Table1: Pathogens relevant to the dairy industry (Sobsey *et. al.*, 2006):**

| Sr. no. | Pathogen                     | Disease in dairy cattle | Disease in humans                  | Transmission              |
|---------|------------------------------|-------------------------|------------------------------------|---------------------------|
| 1       | <i>Salmonella</i> spp.       | May be asymptomatic     | Yes                                | Food, water, and clothing |
| 2       | <i>Escherichia coli</i>      | No                      | Yes<br>(pathogenic strain O157:H7) | Food and water            |
| 3       | <i>Staphylococcus aureus</i> | yes                     | Yes                                | Food, water and clothing. |

Generally, pathogen numbers are reduced by sunlight (UV radiation), drying, high temperatures, and high or low pH, Pathogen viability, more importantly die-off, depends on climate and is therefore difficult to pinpoint. In addition, under given conditions, different pathogens have varying levels of resistance to environmental stresses. Guan and Holley (2003), suggested that the time required for pathogen numbers to return to background levels under dark incubation conditions ranged from 3 days (*Campylobacter*) to 56 days (*Escherichia coli*) at warm conditions (20–37°C) and longer at cold conditions. Coliforms particularly *Escherichia coli* are frequently used in the microbiological analysis of food as an indicator of poor hygienic condition (Chatterjee *et. al.*, 2006). Insufficient lethality of pasteurization treatments and post-pasteurization contamination by pathogens are believed to be the primary contributing factors in outbreaks associated with consumption of dairy products manufactured with Pasteurized milk (Inghama *et. al.*, 2000). Coliform bacteria count in milk and dairy products is an indicator of the sanitary conditions or practices during production, processing and storage of the product, Recovery and counting of *Escherichia coli* is used as an index of recent faecal contamination and suggests that other microorganisms of faecal origin including pathogens might be present (Silva *et.al.*, 2001). In addition, raw or pasteurized milk and cheese are frequently

implicated as vehicle of transmission of pathogenic bacteria and with outbreaks reported all over the world (Araujo, *et. al.*, 2002).

### **1. Milk borne pathogens:**

Milk cannot be preserved in its fresh condition for a long times and soon it is fermented as result of the life activities of the different type of microorganisms that gain entrance to milk from various sources. *Salmonella* infection is the cause of food borne illness in most developing countries. Meat, poultry and dairy products are frequently implicated in outbreaks. The bacterium *Salmonella typhi* causes typhoid fever (Doughari *et. al.*, 2007). The bacterium is a motile, non-sporing, non-capsulated bacillus that can be contracted through contaminated water, milk, food or fruits and vegetables or via convalescent or chronic carriers (Doughari, 2005). Mastitis is the most common infectious disease affecting the dairy buffaloes and remains the most economically important disease of dairy industries around the world. The major cause of bovine mastitis is the infection of the udder by pathogenic bacteria. A wide variety of bacteria can be involved, but the most common mastitis pathogen is *Staphylococcus aureus* (Chiang, 2008). *Staphylococcus aureus* produces a broad spectrum of surface components (proteins and capsular polysaccharide) and exotoxins. They are virulence factors involved in the pathogenesis of bovine mastitis as these toxins and

products are injurious to milk producing cells of the mammary gland, impair glands and immune defense mechanisms. They are capable to reside as an intracellular contributes with the ability to establish a chronic infection that can persist for the life of the animal (Taverna, *et. al.*, 2007).

Milk is supposed to constitute a complex ecosystem for various microorganisms including bacteria. Milk products like cheese and curd are widely consumed and marketed and have enlisted in many parts of the world. There is an increasing demand by the consumer for high quality natural food free from artificial preservatives and contaminating microorganisms. Contaminations of milk and milk products with pathogenic bacteria are largely due to processing, handling and unhygienic conditions (Priyanka singh *et. al.*, 2000). Milk and milk products constitute important nutritional components for all age groups. Good quality milk meets the nutritional needs of the body better than any single food as it contains all the essential food constituents (Sharma and Joshi, 1992). As a result of the presence of these nutrients, milk is an excellent culture medium for many kinds of microorganisms (Henry and Newlander, 1997). A broad spectrum of microbial pathogens contaminates 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* spp. and *Salmonella* spp. were reported as the most common food borne pathogens that are present in many foods and able to survive in milk and fermented milk products (Tekinsons and Ozdemir, 2006).

In order to extend the shelf-life of milk for human consumption by preventing the growth of spoilage organisms as well as preventing the transmission of disease via milk, this highly nutritious, versatile food is usually pasteurized for a short time. The International Dairy Federation defines pasteurization as a process applied to a product with the aim of avoiding public health hazards arising from pathogenic microorganisms associated with such products by heat treatment which is consistent with Minimal chemical, physical and organoleptic changes in the product. Milk is pasteurized by heating at a temperature of about 63° C (145° F) for 30 min, rapidly cooling it, and then storing it at a temperature below 10°C (50°F). Pasteurization kills most, but not all bacteria in milk. The combination of time and temperature used for heat treatment of milk are however, designed to kill all pathogenic microorganisms (ICMSF, 1998; Edema and Akingbade, 2007). Acute diarrhea is a common cause of death in developing countries and the second most common cause of infant deaths worldwide (Victora *et. al.*, 2008).

In Nigeria it is encountered both in urban and rural areas. It is estimated that 1.3 billion episodes and 4 million deaths occur each year in children under five. About 80% of deaths due to diarrhea occur in the first two years of life (Rukunga *et. al.*, 2002). Diarrhea is an important cause of under nutrition because patients eat less during diarrhea and their ability to absorb nutrients is reduced. Moreover, nutrient requirement is increased as results of infection (Sinclair *et. al.*, 2003). Risk factors that predispose children to diarrhea include poor sanitation, poor social and economic status and malnutrition (Andu *et. al.*, 2002). The most important cause of acute watery diarrhea in young children in Nigeria include *rotavirus*, enterotoxigenic *Escherichia coli* *Shigella*, *Campylobacter Jejuni* and *Cryptosporidia*, *Vibrio cholerae*, *Salmonella* and enteropathogenic *Escherichia coli* (Bahal *et. al.*, 2001; Sule *et. al.*, 2011).

The extent of contamination and subsequent microbial multiplication directly determine the microbiological quality of the product hence the major emphasis for combating the harmful effect of microorganisms in dairy products includes the following measures, avoiding or minimizing contamination of milk, refrigerated storage of raw milk before processing, avoiding prolonged storage of processed milk and milk products under favorable ambient conditions to control microbial multiplication.



The milk when secreted in the healthy udder is almost sterile. It is only during subsequent milking and post milking operations that raw milk gets various microorganisms due to contaminations from the surrounding fresh milk obtained from healthy udder under sanitary conditions. This milk, contaminated by man, his practices and the environment (International dairy federation, 1994). Unpasteurized milk contains a wide range of bacteria, principally those causing mastitis and also the fecal flora arising from udder contamination (Ministry of Agriculture Fisheries and Food, 1998).

Heating of milk destroys a majority of the micro flora during processing of milk depending on the severity of the heat treatment. The common method of heating milk include pasteurization method kill all pathogen, microorganisms in the product there are some microorganisms which can either survive under these conditions and multiply. Besides microorganisms there are certain enzymes which survive at this commercial heat treatment. The latter may even include pathogenic microorganisms coming mainly through the personnel's involved in processing of marketing.

Pasteurization remains as essential stage impossible to reduce the microbiological risk food and to prolong the preservability. Pasteurization does not impair the nutritional quality or milk fat, calcium and phosphorus. Independently of the situation of milk

production in any area milk should not be consumable or used in dairy products without pasteurization. Pasteurization cannot guarantee the absence of pathogenic microorganisms when they are present in large numbers in raw milk or due to post-pasteurization contamination (Salmeron *et. al.*, 2002; Karmen torker and Godic torker, 2006).

The conditions of heat treatment used for pasteurization depend on the final product, lower temperatures are used for refrigerated products and higher heat treatments are used for products stored at room temperature (USCFR, 2006). The main objective of milk heat treatment is to eliminate in born pathogenic organisms or reduce them to a level safe for human consumption during extended-shelf life. Milk must be produced and be maintained in hygienic good conditions. This essential rule is very difficult to respect in developing countries due to unfavorable climatic conditions. The inadequate feeding the lack suitable installations and ignorance the uptake of unhealthy animals which are obstacles reflecting on the quantity and quality of produced milk. However, some agent responsible for zoonoses can be transmitted to the human through even in pasteurized milk reflecting the great importance of hygienic (Aggad *et. al.*, 2010).

Milk is a natural food that has no protection from external contamination and can be contaminated easily when it is separated from the cow (Rosenthal, 1991). Raw milk normally has a varied micro flora arising from a several sources such as the exterior surfaces of the animal and the surface of milk handling equipment such as milking machines, pipeline, and containers (Burton, 1980). Therefore, milk is susceptible to contamination by many pathogenic microorganisms which results in infection and threat to consumer's health additionally; there is the potential that disease of cows such as tuberculosis, brucellosis, typhoid and listeriosis can be transmitted.

An estimated 76 million cases of food borne illness occur each year in the United States costing between \$ 6.5 billion and \$34.9 billion in medical care and lost productivity (Buzby and Roberts, 1997; Mead *et. al.*, 1999) of the estimated 13.8 billion cases of food borne illness due to known agents roughly 30% are due to bacteria. The remaining cases of known etiology are due to parasites in 30% of the cases and viruses in 67% of the cases, Bacteria are the causative agents of food borne illness in 60% of cases enquiring hospitalization (Mead *et. al.*, 1999). The survival of microorganisms in different milk products depend on the processing treatments rendered to milk during the manufacture of these products like heating, freezing, ripening, addition of chemicals like salt, sugar, preservatives etc.

All these process have adverse effect on the viability of commonly associated dairy microorganisms and thus the final effect depends on the nature of the process of its severity. Although there are a number of secondary factors like concentration of solids, pH, moisture etc. which further determine the killing effect of these microbicidal processes, the most common of these processed employed in dairy processing is heating. The common chemical substances (salt, sugar etc) used in certain products like butter, cheese, condensed milk etc. inhibit growth of microorganisms by providing unfavorable ionic concentration and osmotic changes. A variety of microorganisms has been isolated from milk and different milk products. These microorganisms may be bacteria, yeasts, molds and viruses, bacteria have the ability to utilize various milk constituents to grow and multiply their number by binary fission. The Composite micro flora finally affects the keeping quality of the product as most of these microorganisms have been known to cause various spoilages if the product is stored under favorable conditions of their growth.

The dairy products of Indian origin referred to as indigenous dairy products which include different categories, viz, fat rich products (Makkhan or desi butter) Frozen products (Kulfi, Malaika burf etc.) Khoa, pera, kalakand, dahi, lassi, shrikhand most of these products are still produced and sold by unorganized sector. In India

where unhygienic practices are still produced and still followed as compared to commercial organized sector.

### **1.1. Microorganisms associated with milk and milk products:**

A variety of microbes, both saprophytic and pathogenic are associated with milk and milk products because these foods provide all the nutrients required for microbial growth and multiplication. The Microorganisms may range from a few hundred to a few thousand per ml of milk, factors like the animal the milker milking utensils, equipments and the environment may contribute to the micro flora of raw milk. Besides these the unhealthy animal may directly secret the disease causing organisms into raw milk while it is still in the udder hence there is a need for "clean milk production" to obtain milk with a better microbiological quality. The potential sources of microbial contaminants in different dairy products like raw milk include the utensils, equipments, water supply, air, dairy personnel packaging material etc. The adequate cleaning and sanitization of dairy utensils and plant equipment involvement of healthy and clean personal disinfection of dairy environment and water supplies and proper quality as well as care of packaging materials, contamination of raw milk within milking process are originated for the udder, the exterior of the udder, the milking

equipment used, the contamination may further increase for some reasons including the cooling and storage temperature plus holding time (Murphy and Boor, 2000). The bacterial count is a useful method to measure milk quality high bacteria count in milk originates from milking wet dirty udders plus inadequate cleaned and sanitized inflations, milking houses, pipelines and bulk tanks (Shojaei and Yaddollahi, 2008). In general bacteria grow well in milk and hence endanger its keeping quality when they are cleaned and sanitized properly, utensils and milk contact surfaces add few bacteria per milliliter of milk but under very poor conditions these sources may increase the bacterial content of milk by millions per milliliter. Application of quaternary ammonium compounds as sanitizing agents tends to increase the percentage of gram negative rods on the utensils where as hypochlorite favor gram positive bacteria.

The detection of coliforms bacteria and pathogens in milk indicates a possible contamination of bacteria either from the udder, milk utensils or water supply used (Olson and Mocquot, 1980; Bonfoh *et. al.*, 2003; Muhammad Rizwan *et. al.*, 2011). Fresh milk drawn from a healthy cow normally contains a low microbial load less than 1000 ml<sup>-1</sup> but the loads may increase up to 100 fold or more once it is stored for some time at normal temperatures (Richter *et. al.*, 1992). However, keeping milk in clean containers at refrigerated

temperatures immediately after milking process may delay the increase of initial microbial load and prevent the multiplication of microorganisms in milk between milking at the farm and transportation to the processing plant (Adesiyum, 1994; Bonfoh *et al.*, 2003). Contamination of mastitis milk with fresh milk may be one of the reasons for the high microbial load of bulk milk (Jeffery and Wilson, 1987). *Staphylococci* are expected to be among the organisms that contaminate dairy products from handlers. Under favorable conditions, *Staphylococci* can grow and secrete heat resistance enterotoxin which cause vomiting and diarrhea within 30 min to 8 h after ingestion of incriminated foods. A wide variety of bacteria can be involved, but the economically important mastitis pathogen is *Staphylococcus aureus*. *S. aureus* is a major pathogen of bovine mastitis worldwide. Despite implementing intensive control measures, it is difficult to eradicate the intramammary infections caused by this pathogen and it remains a substantial economic problem (Salmon, S.A., 2002). It has been estimated that the major contagious mastitis pathogens are responsible for less than a third of all mastitis cases, compared with more than 75% of all cases 20 years ago (Petzer *et al.*, 2009).

*Enterobacteriaceae spp.* has been implicated in many cases of food poisoning outbreaks (Koneman *et al.*, 1994). *Escherichia coli* is an important organism in the microbiology of the food, besides being

involved in food borne gastroenteritis, it is considered a good indicator of possible fecal contamination of dairy products (El-Bagoury and Mosaad,2002). *Salmonella* is considered among the most important enteric foodborne pathogen that present in the food constitutes a severe health hazard. Many outbreaks of human illness have been associated with the consumption of raw or inadequately heat treated milk or their dairy products (Ellis *et. al.*, 1998; Fadel, 2009). The number and types of microorganisms present in milk and dairy products depends on the microbial quality of milk used, heat treatment of milk, the conditions in which the products are manufactured, the temperatures and duration of storage, feeding of the animals, season, area, general sanitation in the plant, quality of starter cultures, occurrence of phages, quality of rinsing water, etc. (Bramley and Mckinnon 1990; Anonymous, 1994). Examination for the presence and number of specific micro-organisms is, therefore, an integral part of any quality control or quality assurance plan and it may be applied to a number of areas, raw materials, intermediate samples, finished products, or environmental equipment sites. The most common spoilage microorganisms in milk and dairy products are *Pseudomonas*, *Coliforms*, *Bacillus spp*, *Clostridium spp*, lactic-acid producing bacteria, yeasts and moulds, enterococci, etc. On the other hand, milk-borne and milk product borne outbreaks, caused mostly by cheeses, represent 2–6% of bacterial food-borne outbreaks



reported by surveillance systems from several countries. The cheeses represent a large risk of bacterial food-borne outbreaks because of pathogen microflora, divided into pathogens of current concern (*Salmonella* spp., *Campylobacter* spp., Coagulase positive *Staphylococci*, *Listeria monocytogenes* etc.) and those which cause disease only occasionally *Escherichia coli*, *Bacillus cereus*, *Clostridium perfringens* etc. (De Buyser *et. al.*, 2001).

The contamination of raw milk with *Salmonella* usually occurs as a result of transfer of faces from an animal to the milk via unclean teats and udders. Such contamination can pass into milk during milking and, once present on milking parlor equipment, it can then readily proliferate and spread if such equipment is not adequately cleaned and sanitized. Its growth in milk should be limited by effective refrigeration (<8°C). Effective milking parlor hygiene (cleaning and disinfection of udders and teats), cleaning and sanitization of milking equipment and subsequent milk storage systems are essential elements in preventing the spread of this organism (McManus and Lanier, 1987; Karmen Godic Torker and Slavica Golc Teger, 2006).

**Table: A summary of microorganisms associated with milk and milk products:**

| Section according to 9 <sup>th</sup> edition of Bergey's Manual | Family  | Genus   |
|---|---|---|
| <i>Bacteria</i>   | <i>Spirillaceae</i>   | <i>Camphylobacter</i>   |
|   | <i>Pseudomonadaceae</i>   | <i>Pseudomonas, Brucella</i>  |
|   | <i>Neisseriaceae</i>  | <i>Actinobacter, Moraxella like organisms</i>   |
|   | <i>Enterobacteriaceae</i>   | <i>Escherichia, Enterobacter, Salmonella, Yersinia</i>  |
|   | <i>Vibrionaceae</i>   | <i>Aeromonas, Chromobacterium, Flavobacterium, Vibrio</i>   |
|   | <i>Rickettsiaceae</i>   | <i>Coxiella</i>   |
|   | <i>Streptococcaceae</i>   | <i>Micrococcus, Staphylococcus</i>  |
|   | <i>Bacillaceae</i>  | <i>Streptococcus, Leuconostoc</i>   |
|   | <i>Lactobacillaceae</i>   | <i>Bacillus, Clostridium</i>  |
|   | <i>Propionibacteriaceae</i>   | <i>Lactobacillus, Listeria</i>  |
|   | <i>Corynebacteriaceae</i>   | <i>Propionibacterium</i>  |
|   | <i>Actinomycetaceae</i>   | <i>Corynebacterium, Arthobacter, Microbacterium</i>   |
|   | <i>Mycobacteriaceae</i>   | <i>Actinomyces</i>  |
|   | <i>Nocardiaceae</i>   | <i>Mycobacterium</i><br><i>Nocardia</i>   |
|   | <i>Yeasts</i>   | <i>Saccharomyces cerevisiae, Kluyveromyces fragilis, K. lactis, Debryomyces hansennii, Candida lipolytica var lipolytica, C. kefir, Torulopsis lactis condens</i> |
| <i>Mold</i>   | <i>Geotrichum candidum, Penicillium roqueforti, P. casei, P. camemberti, Rhizopus stolonifer, Mucor, Aspergillus.</i> |   |

### **Family *Enterobacteriaceae*:**

This family comprises of twelve genera like *Escherichia*, *Salmonella*, *Shigella*, *Enterobacter*, *Proteus* etc. all of which may be associated with milk and milk products.

### **Family *Pseudomonadaceae*:**

The family comprises of four genera viz. *Pseudomonas*, *Xanthomonas*, *Zooglea* and *Gluconobacter* the only genus important to dairy industry.

### **Family *Vibrionaceae*:**

This family includes some potential spoilage organisms which can produce undesirable changes in milk and milk products.

### **Family *Brucellaceae*:**

This family includes a very important member *Brucella* which is capable of introducing illness in both man and animals.

### **Family *Micrococcaceae*:**

The important of this milk and milk products is mainly due to two of its members namely *Staphylococcus* and *Micrococcus* which can invariably gain access into milk and milk products from different sources and may cause milk borne intoxication as well as spoilage.

### **Family *Streptococcaceae*:**

Some of these organisms may reach milk and milk products from a variety of sources and hence may take part in food borne illness both in man and animal.

### **Family *Lactobacillaceae*:**

Lactobacillaceae includes some very important organisms because of their tremendous nutritional, therapeutic and preservative potentials in foods and feed.

### **Family *Bacillaceae*:**

The two genera important to family Bacillaceae are *Bacillus* and *Clostridium*.

### **Family *Propionibacteriaceae*:**

The important genus, which is also associated with dairy products is *propionibacterium*.

### **Family *Spirillaceae*:**

The genus of importance here is *Camphylobacter* which has been recorded in several outbreak of food poisoning.

### **Family *Rickettsiaceae*:**

Rickettsia, the active member of this family is known for its ability to produce Q fever in man and animals. The organisms are secreted in milk by the infected animal and hence may reach human consumers if not destroyed by heat treatment.

### **Family *Nocardia*:**

The important genus is Nocardia which may be encountered in milk and milk products like Butter. However, its exact role in food spoilage is not known.

### **Family *Actinomycetaceae*:**

Although the role of this family in foods is not exactly known a few members appear to be pathogenic to man as well as dairy animals. (Yadav, 1993)

## **1.2. Microbiological spoilage of dairy products:**

Milk and milk products are popular because of their unique flavor and texture so much, so that if these attributes are not attractive the product is rejected by the consumers. Yeast and mold are common contaminants in food. While yeast does not result in

food poisoning, it does cause food to spoil (Deak, 2008). A large number of molds produce toxic substances designated as mycotoxin. Some are mutagenic and carcinogenic, some display specific organ toxicity and some are toxic by other mechanisms (Fulya Tasci, 2011).

Markets and consumers for raw milk and their products have existed in many part of the world. Being a highly nutritious medium many bacteria including spoilage and pathogenic bacteria can grow and propagate in it. Generally, bacteria in the milk can occur through colonization of the teat canal of an infected udder (clinical and sub clinical mastitis) or contaminated at various stages be it from the animal, milker (Manual as well automates) extraneous diet or unclean process water (Gruetzmacher and Bradley, 1999; Hayes *et .al.*, 2001).

Several studies have identified milk borne pathogens including shigatoxin producing *Escherichia coli* in form bulk tank milk (Moustafa *et .al.*, 1983; Lovett *et. al.*, 1987; McManus and Lanier, 1987). Apart from the gross manifestations of spoilage such as lactic acid production and souring milk and milk products also undergo a number of other changes like proteolysis sweet curdling etc. with the current high consumers price of certain dairy products coupled with dietary considerations it becomes highly desirable to prevent the microbial spoilage of milk and milk products by taking appropriate

precautions. Food spoilage is an enormous economic problem worldwide. Through microbial activity alone, approximately one fourth of the world food supply is lost. Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms. The microbiological quality of milk and dairy products is influenced by the initial flora of raw milk the processing conditions and post heat treatment contamination undesirable microbes that can cause spoilage of dairy products include Gram negative psychrotrophs, coliforms and lactic acid bacteria. In addition, various bacteria of public health concern such as *salmonella spp.* *Listeria Monocytogenes*, pathogenic strains of *Escherichia coli* and enterotoxigenic strains of *staphylococcus aureus* may also be found in milk and dairy products (Laszlo varga *et. al.*, 2007).

There are many constraints or problems that milk traders face in marketing their milk. Many of these constraints or problems cause milk spoilage or are the result of it. These may include the following factors (Kenya dairy board, Nairobi, May 2004). Long distance or time between collection and resale point, Type of containers used, how the containers are washed, Method of preservation, Low profits Lack of training, all these factors related to milk spoilage.

Most foods contain viable bacteria unless thoroughly heated or made sterile, otherwise, it serves as an important medium for transmission of pathogenic organisms may influence considerably their harmlessness, endanger the health of consumers and decrease shelf quality resulting in food born infections intoxications and economic losses from food spoilage (Le Loir Yves *et. al.*, 2003).

### **1.3. Mechanism of microbial spoilage:**

The microbiological changes in milk and milk products are produced as a result of fermentation of one or more of the milk constituents by the causative microorganisms. This milk fermentation may be normal or abnormal type.

Since microorganisms in dairy products are present as a mixed flora another phenomenon called associative action among different microorganisms may also be responsible for causing microbial changes in milk and milk products. Associative action among microorganisms is a combined action of two or more organisms in producing a change which is not possible due to either of them alone such interactions among causative organisms may be of the following three type's synergism, metabiosis and antibiosis.

1) Synergism:



In this type of association the two organisms bring about a change which neither of them could carry out alone. e.g. In mixed starter containing *Streptococcus lactis* and *Leuconostoc* spp. like *Leucodextranicum*, characteristic flavor due to conversion of citrate to volatile compounds by *Leuconostoc*s is possible only at low pH. The lowering pH due to lactic acid production is brought about by *Streptococcus lactis*.

ii) The production of blue discoloration in milk by *Pseudomonas syncyanea* is possible only in association with *Streptococcus lactis* which produces acidic conditions.

## 2) Metabiosis:

In this type of association among microorganisms a "food chain" is formed which means the metabolic end products of one are utilized as food staff by the other for producing the final change. e.g. In Swiss cheese making the lactic acid bacteria convert lactose to lactic acid, which in turn is utilized by propioni bacteria to produce propionic acid. (The compound responsible for characteristic flavor production in Swiss cheeses)

## 3) Antibiosis:

In this phenomenon, the growth of one organism inhibits or suppresses the growth of the second organism. e.g. The growth of

lactic acid bacteria causes the inhibition of proteolytic organisms (mainly spore formers) due to production of lactic acid by the former group. The various type of microbiological spoilage occurs with regard to their causative organisms (Yadav, *et. al.*, 1993).

#### **1.4. Milk borne Diseases:**

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 is a compulsory part of daily diet for the expectant mother as well as growing children. Milk can also serves as potential vehicle for transmission of some diseases under certain circumstances. Moreover, by virtue of processing almost all the essential nutritional factors milk can also serve as an excellent culture and protective medium for certain microorganisms which may include potential pathogens capable of causing serious health problem to the consumers. In this way, milk may serve not only as vehicle of transmission of disease causing organisms but it can also allow this pathogen to grow multiply and produce certain toxic metabolites. Milk and milk products derive from dairy cow's milk can harbour a variety of microorganisms and can be important sources of food borne pathogens (Oliver *et. al.*, 2005). Milk being nutritional food for human beings also serves as a good medium for the growth of many microorganisms, especially *Lactobacillus*, *Streptococcus*,

*Staphylococcus* and *Micrococcus* spp. Bacterial contamination of raw milk can originate from different sources such as air, milking equipment, feed, soil, faces and grass. The number and type of microorganisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health. It is hypothesized that different feeding and housing strategies of cows may influence the microbial quality of milk (Torker and Teger, 2008).

The numerous food borne diseases outbreaks in humans is related to milk, were mainly caused by pathogens such as *Campylobacter* spp. *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* General infections such as typhoid fever, diphtheria, scarlet fever and mastitis related enterotoxaemia are also often transmitted in milk, whilst the most severe zoonoses transmitted from animals to humans via milk are tuberculosis and brucellosis (Foster, 1990; Ruegg, 1991; Orr *et. al.*, 1994; Heuvelink *et. al.*, 1998). The safety of dairy products with respect to food borne disease is of great concern around the world. This is especially true in developing countries where production of milk and various milk products takes place under unsanitary conditions and poor production practices (Mogessic Ashnafi, 1990).

A variety of pathogenic organisms may gain access into milk and milk product from different sources and cause different types of food borne illnesses. Milk and milk products carry organisms as such or their toxic metabolites called toxin to the sensitive consumers. Ingestion of toxins already synthesized in the food, bring about poisoning in the consumers. The ingestion of food leads to their lodgment and establishment in consumer's organs; this is termed as food infection. A broad spectrum of microbial pathogens can contaminate human food and water supplies and causes illness after they or their toxins are consumed. This includes a variety of enteric bacteria, aerobes and anaerobes, viral pathogens and yeasts (Tauxe, 2002).

Food borne diseases affect people's health and well being as well as have an economics impact on individuals and nations. Diarrheal disease has been a major public health problem causing high morbidity and mortality among children in Thailand form many years. Approximately one million cases of acute diarrhea and more than 20,000 cases of food poisoning are reported in Thailand each year (Bureau of Epidemiology, 2004). Food is considered the main source of microorganisms causing diarrheal disease (Rabbani and Greenough, 1999; Jay, 2000; Fang, *et. al.*, 2003). Food borne disease outbreaks from enteropathogenic bacteria such as *Salmonella*, *Vibrio cholera* and *Staphylococcus aureus* are common causes of food borne

infection throughout the world including Thailand (Chomvarain *et al.*, 1993; Adams and moss, 2000; Bangtrakulnonth *et. al.*, 2004; Meldrum *et. al.*, 2006).

Foods borne disease have a major public health impact. In the United States each year foodborne illnesses affect 6-80 million persons, cause 9000 death and cost an estimated 5 billion U.S.dollars (Council for agriculture science and technology food borne pathogens, 1994). The epidemiology of food borne diseases is rapidly changing as newly recognized pathogens emerge well recognized pathogens increase in prevalence or become associated with new food vehicles. In addition to acute gastroenteritis many emerging food borne diseases may cause chronic disability listeriosis for ex. cause miscarriages (Schuchat *et.al.*, 1991) or result in meningitis in patients with chronic disease. Toxoplasmosis is an important cause of congenital malformation (Kapperud *et. al.*, 1996) and *Escherichia coli* O157; H7 infection is leading cause of hemolytic uremic syndrome, the most common cause of acute kidney failure in children in the United States (Boyce *et. al.*, 1995) congenital malformation (Kapperud *et. al.*, 1996).

Contaminated milk and milk products have been associated with food borne illnesses caused by *Salmonellas* (Tacket *et. al.*, 1985; D'Aoust *et. al.*, 1985; Lecos, 1986) *Listeria Monocytogenes* (Fleming

*et. al.*, 1985; James *et. al.*, 1985) *Staphylococcus aureus* (Zehren and Zehren, 1968) *Yersinia enterocolitica* (Barrett, 1986; Greenwood *et. al.*, 1990). Milk borne infection should be made notifiable and reported by national authorities according to the standard classification commonly followed. A great variety of bacteria can produce specific types of milk borne illnesses and may subsequently lead to food poisoning outbreaks. Among the bacterial pathogens which can cause a variety of food infection through the ingestion of milk and milk products the following types are more prevalent.

#### **1.5.1. Salmonella poisoning (Salmonellosis):**

Salmonellosis is a disease resulting from the ingestion of *Salmonella* along with contaminated food. The frequency of both small and large scale illness attributed to milk borne. *Salmonella* and other microorganisms underscore the importance of milk and milk products as vehicle of human infection. For these reasons, the presences of *Salmonella* and other human pathogens in unpasteurized milk remain a public health hazard. It is known that high parentage of clinically normal animals on dairy farms can be infected with *Salmonella* spp. (McEwes *et.al.*, 1988). *Salmonella* caused disease ranging from diarrhea to septicemia Salmonellosis from contaminated food generally causes diarrhea (Jay, 2000; Murray, 2002; Montville and Matthews, 2005).

When a person ingests contaminated food the *Salmonella* bacteria take up residence in the small intestine. The onset of symptoms typically occurs from 8h and 72h after ingestion. The most common symptoms are nausea, vomiting, abdominal cramps, diarrhea, fever and headache. Treatment is not usually necessary. Unless the infection spreads outside of the intestines or the patient becomes dehydrated. However, Salmonellosis can still be life threatening to infants the elderly one immune compromised individual. Resistance to antibiotic therapy for the *Salmonella* infection has been reported and is thought to be a result of the large scale use of antibiotics to promote the growth of feed animals (Kai Lin Hwang *et. al.*, 2010).

To become ill from *Salmonella* a person needs to consume food that contains the viable bacteria. The pathogen then enters the digestive tracts, grows in the small intestine and causes inflammation resulting in enterocolitis (Gravani, 1984). Pathogenicity of food borne *Salmonella* has been reviewed recently by D'Aoust. Although salmonellosis commonly results from consumption of foods contaminated with the pathogen. *Salmonella heidelberg* was transmitted by direct contacts from ill calves to a pregnant woman (Holmberg *et. al.*, 1984). The woman subsequently transmitted the infection at birth to her infant and then further transmission occurred to other infants in a hospital nursery.

Almost all of the 2200 salmonella species can infect humans, the most common infectious *Salmonella species* are *Salmonella enteritidis* and *Salmonella typhimurium* which together accounts for three quarters of all salmonellosis cases each year. *Salmonella* particularly *Salmonella typhimurium* and *Salmonella dublin* are commonly found in cattle and are excreted in the faeces. This provides an easy route of contamination during milking and milk processing. The consumption of contaminated unpasteurized milk was a common cause of infection in England and Wales during the 1950 and in Scotland in the 1980. Products in which unpasteurized milk is used as an ingredient may also become contaminated (Maguire *et. al.*, 1992; Papadopoulou *et. al.*, 1993). Schimann *et. al.*, (1989) described *Salmonella typhimurium* strains with an unusual plasmid profile isolated from pasteurized milk and from asymptomatic patient during 1985 Marth reviewed literature through 1968 on salmonellae and salmonellosis associated with milk and milk products.

Contamination of raw milk by *Salmonella*, usually takes place from external sources and the reduction in salmonella number due to freezing temperature is limited, since it can survive a prolonged time in frozen foods (Asperger *et. al.*, 1994). Similarly the survival of *Salmonella* for at least 63 days in cultured buttermilk stored at 8°C. However, *Salmonella* are readily destroyed during milk pasteurization



(Asperger *et.al.*, 1994; Wigdan Abdalla and Ibtisam and E.M.El Zubair, 2010).

### **1.5.2. Staphylococcal poisoning:**

Among the milk borne illnesses intoxications have been extensively studied and are of serious public health concern as they lead to frequent food poisoning outbreaks. Some very common types of food intoxications through milk and milk products are discussed below. Infection of milk by some strains of *Staphylococcus aureus* leads to the elaboration of enterotoxins by them that cause gastroenteritidis in human beings. Information gained from several out breaks of *Staphylococcal* foods including milk has been summarized by many workers. Malik (1968) reported out breaks of food poisoning. The incidence of Staphylococcal enterotoxin in milk and milk products sampled from different sources in India has been recorded by NDRI Karnal (Batish *et. al.*, 1978).

Contamination of dairy products with pathogenic bacteria can serve as a source of spread of certain harmful human bacterial diseases like tuberculosis, gastroenteritidis, brucellosis, Salmonellosis and staphylococcal food poisoning, besides this enterotoxin producing *Staphylococcus aureus* is most dangerous and harmful for the human health. About 50% strains of these organisms are able to produce enterotoxin associated with food poisoning (Payne and wood,

1974). The incidence of Staphylococcal food poisoning due to the consumption of dairy products is not uncommon in our country (Masud *et. al.*, 1989).

The most common food that may cause food poisoning is dairy products (Bijker and Vincentic, 1977). There is evidence that *Staphylococcus aureus* was isolated from khoa samples (Masud *et. al.*, 1989), Rasmalai (Grewal and Tiwari, 1996) and Moroccan traditional milk product samples contaminated with enterotoxin and producing *Staphylococcus aureus* strains (Hamama and Tatini, 1991). The most important source of contamination is probably the human. The contaminants reached the products either during cooking or handling after cooking (Ghosh and Laximinarayan, 1976).

Illness through *Staphylococcus aureus* range from minor skin infection such as pimples, boils, cellulites toxic shock syndrome, impetigo and abscesses to life threatening disease such as pneumonia, meningitis, endocarditic, septicemia (Soomro *et. al.*, 2003). *Staphylococcus aureus* can also cause food poisoning even with very small amounts (100-200ng) of its heat stable enterotoxin (Evenson *et. al.*, 1988). *Staphylococcal* food poisoning is one of the most prevalent causes of gastro enteritidis world wide (Jablonski *et. al.*, 1997). Symptoms of *Staphylococcal* food poisoning have a rapid onset of abdominal cramps, nausea and vomiting, sometimes

followed by diarrhea (Jorgensen *et. al.*, 2005). Milk and milk products are common vehicles for *staphylococcal* food poisoning (Wieneke *et. al.*, 1993).

Hobbs and Roberts (1993) reported that in England and Wales there are yearly outbreaks of food poisoning from *Salmonella* and *Campylobacter jejuni* in milk not receiving heat treatment or imperfectly pasteurized. *Staphylococcal aureus* can be isolated from most samples of raw milk and may be found in unheated or lightly heated dairy products (Tahiri, 2005).

Food borne disease are of major concern worldwide, to date around 250 different food borne diseases have been described and bacteria are the causative agents of two thirds of food borne disease outbreaks. Among the predominant bacteria involved in these diseases, *Staphylococcal aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food (Le Loir Yves, 2003). Studies have shown that white soft cheese and dairy products have been involved in much *Staphylococcal* food poisoning outbreaks in Brazil and other countries (Carmo and Bergdoll, 1990; Adesiyum, *et. al.*, 1998; Almeida Filho and Nader-Filho, 2000). Some Coagulase negative *Staphylococcal* are also able to produce enterotoxin (Anunciacao *et. al.*, 1994). The main carriers of

antimicrobial resistance in milk are the *Staphylococcal*, some of this resistance is transferable (Muhammad *et. al.*, 1993).

Staphylococcal food poisoning (SFP) is one of the most common foodborne diseases (FBD) worldwide with high occurrence second to Salmonellosis (Aycicek *et. al.*, 2005). *Staphylococcal* food poisoning is often associated with the ingestion of manually handled foods that contain one or more highly heat stable *Staphylococcal* enterotoxins (Smith, 2007; Mekonnen Addis, 2011).

### **1.5.3. *Escherichia coli*:**

*Escherichia coli* are known to be associated with enteritis in infants and adults with traveller's diarrhea and food poisoning. *Escherichia coli* is responsible for several out breaks of diarrhea in children and adults after ingestion of contaminated milk and dairy ingestion of contaminated milk and dairy products different stories showed that 1-5 % of food borne infection were related to consumption of milk and dairy products that 53% of cases of food borne infections caused by contaminated cheese and that enteropathogenic *Escherichia coli* (EPEC) is the causative agent of 18.33% of these cases. The relation between pathogenicity and different serotypes of *Escherichia coli* has been suggested and proved (Crossy *et. al.*, 1995). Cattle manure is considered the primary source

of *Escherichia coli* contamination of foods and the environment (Lejeune, 2005).

*Escherichia coli* located in the tropics can cause bladder infections, bronchopneumonia, common cold in children and lower respiratory infections. In many clinical studies, it had been showed that the mortality was forty percent when some strains of *Escherichia coli* were present in the blood stream instead of infecting the surface of human skin. In the other example, influenza alone was rarely lethal, but secondary bacterial infections with simple *Escherichia coli* made situations worst and could cause death (Smith, 1982; Mihn Than Nguyen, 2006). Recovery and counting of *Escherichia coli* is used as an index of recent faecal contamination and indicate that other micro organisms of fecal origin may be present (Portaria *et. al.*, 1977).

After an outbreak of foodborne disease caused by enteropathogenic *Escherichia coli*. The presence of these micro organisms in cheese acquired additional significance. Toxigenic *Escherichia coli* of bovine origin have been classified into three categories: Enterotoxigenic *Escherichia coli* (ETEC), verotoxigenic *Escherichia coli* (VTED) and necrotoxigenic *Escherichia coli* (NTEC) (Quinto and Cepeda, 1997).

The first group constitutes one of the most important vectors of *Escherichia Coli* diarrhea and is considered the major cause of diarrhea in children in developing countries. It is also the most frequently etiological agent responsible for traveler's diarrhea (Nataro and Kapar, 1998). Entropathogenic *Escherichia coli* (EPEC) strains causes serious diarrhea among infants in developing countries throughout the worlds and have been isolated from a wide range of food and dairy products. Coli form bacteria count in milk and dairy products is an indicator of the sanitary conditions or practices during production, processing and storage of the products (Ministerial da saude, 1997). Recovery and colonies of *Escherichia coli* is used as an index of recent faecal contamination and suggested that other microorganisms of fecal origin including pathogen, might be present (Simango *et. al.*, 1995).

*Escherichia coli* are responsible for several out breaks of diarrhea in children and adults after ingestion of contaminated water and food including milk and dairy products (Feresu *et.al.*, 1990). Entropathogenic *Escherichia coli* (EPEC) have been implicated in food and waterborne human illnesses, especially as an important agent of infantile diarrhea in developing countries (Ramos *et. al.*, 1996). In Brazil, Entropathogenic *Escherichia coli* (EPEC) strains are recovered from 30% or more of the diarrhea causes in infants of low social economic status living in cities (Gomes *et. al.*, 1989). Several studies

have identified milk borne pathogens including shigatoxin producing *Escherichia coli* (STEC) in farm bulk tank milk (Moustafa *et. al.*, 1983; McManus and Lanier, 1987; Rohrbach *et. al.*, 1992; O'Donnell 1995; Rahn *et. al.*, 1997; Steel *et. al.*, 1997). Most *Escherichia coli* are harmless but some known as pathogenic bacteria causing severe intestinal and extra intestinal diseases in man (Kaper *et. al.*, 2004). Studies documented the equation of some *Escherichia coli* isolates from raw milk and its products for virulence markers (Jayarao and Henning, 2001; Holko *et. al.*, 2006; Paneto *et. al.*, 2007).

Selim and cullor (1997) reported that *Escherichia coli* was the most common isolated Gram negative bacteria, in many cases may not only contaminate food but grows in it and reaches high numbers particularly in tropical countries or in the absence of refrigeration.

Antibiotic resistant of *Escherichia coli* was found in 10.6% of milk samples. *Escherichia coli*, frequently contaminate food organism and it is a good indicator of fecal pollution (Dilielo, 1982; Soomro *et. al.*, 2002; Benkerroum *et.al.*, 2004). *Escherichia coli* can cause severe diarrhea and vomiting in infants and young children.

The pathogen *Escherichia coli* has since emergent as a major cause of bloody and non bloody diarrhea, causing as many as 20,000 cases and 250 deaths per year in the United States outbreaks have been reported in Canada, Japan, Africa, United Kingdom and

elsewhere. In addition causing hemolytic uremic syndrome, end stage renal disease, hypertension and neurologic injury (Boyce, 1995). Especially in India, rate of infection is still higher (Bhatia, 2007) because of warm and humid climate. Kagkli *et. al.*, (2006) showed that in addition to fecal contamination, other factors such as milking, wet udders, inadequate cooling of milk and udder infection are the main sources of coli form in bulk milk. Most *Escherichia coli* are harmless but some are known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man.

## **1.6. Milk products:**

Although milk and dairy products are important components of a healthy diet if consumed unpasteurized, they also can present a health hazard due to possible contamination with pathogenic Bacteria. These Bacteria can originate even from clinically healthy animal from which milk is derived or from environmental contamination occurring during collection and storage of milk.

### **1.6.1. Milk:**

Milk is an important food of diet of vast population on earth due to its high nutritional value for human beings. Milk is an excellent growth medium of micro organism when suitable temperature exists. If it is produced unhygienically and handled



carelessly, it gets contaminated very easily leading to its early spoilage. Raw milk is milk in its natural (unpasteurized) state, contaminated raw milk can be a source of harmful bacteria such as those that cause undulant fever, dysentery salmonellosis and tuberculosis "certified milk", obtained from cow's certified as healthy in unpasteurized milk with a bacteria count below a specified standard, but it still can contain significant numbers of disease producing organisms. The use of pasteurized milk and milk products is not a health risk for human. After pasteurization milk product can be contaminated by pathogenic micro organisms (Güven Uraz, 2008). Milk is an excellent medium for bacterial growth. Uncontrolled growth of undesirable microbes negatively impacts dairy product yield. Shelf life and sensory characteristics can lead to significant economic losses for the dairy industry. Identifying sources of contamination and controlling growth of micro organisms in raw milk and in processed products has been an ongoing challenge for the dairy industry (Boor and Fromm, 2006).

The introduction of a few pathogens into milk becomes a much more serious problem because of the ability of these substances to support tremendous increase in bacterial numbers. Many milk borne epidemics of human disease have been spread by contamination of milk by spoiled hands of dairy workers, unsanitary utensils, flies and polluted water supplies. The same thing can be said for improper

handling of foods in the home, restaurants hospitals and other institutions.

Different heat and treatments are given to raw milk in order to remove pathogenic organisms, to increase the shelf life to help subsequent processing e.g. for warming before separation and homogenization or as an essential treatment before cheese making, yoghurt manufacture and production of evaporated and dried milk product (Singh,1993). Pasteurization, sterilization (In bottle) and UHT treatment integrated with aseptic packing, sterilization (in bottle)is the term applied to a heat treatment process which has a bactericidal effect greater than pasteurization. The problem of post treatment contamination in container sterilized products is well known. The contamination can either through poor seal or through pinhole in the containers. Production, collection and handling practices influence the initial micro flora present in raw milk. The predominant micro flora in raw milk typically includes lactic acid bacteria, *Streptococcus* spp., *Pseudomonas* spp. and *Staphylococcus* spp. (Lafarge *et.al*, 2004). Microorganisms found in raw milk can deteriorates finished product quality through various mechanisms. Raw milk storage temperature and duration will greatly influence the predominant micro flora and rate of deterioration. Unpasteurized milk and other dairy products, dairy cows commonly carry the *staphylococcus aureus* on the udder and teats and an infection a form

of bovine mastitis can be set by the organisms. This close association with the udder inevitably means that milk becomes infected, but *staphylococcus aureus* can also be spread from the infected region to milking equipment other utensils and the hands of workers. Contaminations of raw milk within milking process are originated for the udder, the exterior of the udder and the milking equipment used. Further contaminations increase for some reasons including the cooling and storage temperature plus holding time (Murphy and Boor, 2000). Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder. Beyond this stage of milk production microbial contamination can generally occur from three main sources from within the udder, from the exterior of the udder and from the surface of milk handling and storage equipment (Bramley and Mckinnon, 1990).

Milk must be produced and be maintained in hygienic good condition. This essential rule is very difficult to respect in developing countries due to unfavorable climate conditions, the inadequate feeding the lack of suitable installations and ignorance, the upkeep of unhealthy animals, which are obstacles reflecting on the quantity and quality of produced milk. However, some agents responsible for zoonoses can be transmitted to the human through even in pasteurized milk (Fleming *et. al.*, 1985) reflecting the great importance of hygiene. The use of unclean milking and transport

equipment contributed also to the poor hygienic quality (Bonfoh *et. al.*, 2003), the unhygienic condition of preparation of these foodstuffs and water used for washing of utensils has enhanced the bacterial contamination of milk and milk products (Johnson, 1961).

Milk when secreted in udder is free from microorganism i.e, sterile but as it passes through ducts and reservoirs of udder, bacteria are carried into it especially walls of teat canal. A variety of other source during production, handling, transport, and distribution of milk contribute myriad organisms to raw milk before it reaches the consumers or a processing dairy. The micro organism entering in milk may be pathogenic or non pathogenic to human beings. The faces contain an enormous quantity of bacteria and so even a very small amount of dry faces falling in milk would be enough to increase the number if bacteria in milk to a great extent. Milk is one of the most common food sources in the human diet and is also a product that is directly available for consumption (Grimaud *et. al.*, 2009).

### **1.6.2. Marketed milk:**

In advanced countries, the market milk industry has already achieved clean milk production by following good hygienic and sanitary practices during production, transportation, processing and distribution of fluid milk. This is primarily because of introduction of various dairy and milk controls ordinances and control laws besides

setting of high standards by the produces, distributors and dairy equipment manufacture in those countries. In India, the produces distributors and dairy equipment manufacture in these countries. In India, the growth of market milk industry has been greatly retarded due to lack of organized milk production and collection, restricted facilities for refrigerated, transport and shortage of processing and marketing organization. However, recently there is a steady rise in the overall standard of organized marketing of fluid milk in this country. Since it is not possible to produce good quality market milk from bad quality raw milk, there is a need to look at the bacteriological and hygienic quality of milk and find ways and means to improving it right from the point of milk collection in the villages. Modern processing and packaging technique can further improve upon the keeping quality of market milk. In this country especially the aseptically packaged UHT treated milk has picked up and this technology holds promise as it does not require costly low marketing the pasteurized fluid milk.

According to milk legislation, milk and cream products for sale must not have sign of being watery, flaky, stringy, bloody, thick or adulterated milk legislation differs in terms of treatment and delivery of milk and dairy products for ex. in the USA there are two categories of drinking milk in the market including pasteurized, ultra pasteurized and aseptic. It is generally considered that the major

source of contamination of raw milk is the milking machine the bulk tank (Bramley and Mckinnon, 1990), lack of cooling facilities and unsatisfactory transportation means (Oghaiki *et .al.*, 2007).

### **1.6.3. Cheese:**

Many varieties of cheese are known throughout the world. More than 1000 varieties of cheese are produced around the world (Hayaloglu *et. al.*, 2002). Cheese is one of the important a popular products of the dairy world, cheese making has been an age old practice as it served as a means of preserving milk solids through moisture removal; by coagulation and had a longer shelf life besides high nutritive value its ancient origin is reflected by the following historical facts.

Cheese has found a description in the Old Testament of the Bible and as early as 2,500 years ago. Cheese making process originated as an art several hundred years before Christ exactly the same way as the Indian products like dahi, ghee and khoa. Davies (1975) has defined cheese as a product made from the curd obtained from milk by coagulating the casein with the help of rennet or similar enzymes in the presence of lactic acid produced by added or adventurous micro organisms from which part of the moisture has been removed by cutting, cooking and/or pressing which has been

shaped in a mould and then ripened by holding it for sometimes at suitable temperature and humidity's.

Cheese has been known as an important animal origin product and in most countries domestic and traditional product cheese are in demand. Despite improvements in the cheese making industry in Iran, Domestic Cheese is still very popular. Domestic cheeses are usually produced from raw milk with insufficient hygienic quality. Moreover, production and transportation of these products are carried out in poor hygienic conditions (Najand and Ghanbarpour, 2006). For the years 1973-1992, there were 32 cheeses associated disease out breaks in the United States with 1700 cases and 58 deaths, 52 of the later caused by *Listeria Monocytogens* in the 1985 California outbreaks.

The most common vehicle was soft chesses and improper pasteurization was common. Mina's frescal cheese is one of the most popular cheeses produced in Brazil. This soft, white cheese is made of pasteurized or raw milk. It is characterized by a high water content (43.0%) and low PH (5.1-5.6) (Freitas *et. al.*, 1993). Microbiological standards for soft cheese Inspection are determined by the Brazilian national public health agency (Portaria, 1977). Cheese is a product containing protein, calcium and phosphor at high levels. In Turkey, about 50 different kinds of cheese are produced. But only Beyaz,

Tulum and Kashar cheese are produced in all regions of Turkey (Anonymous, 2000). Other cheese kinds are traditionally made in only some regions. Karin Kay magi cheese one of the types of cheese is widely produced in sarikamis oltu and Kars regions of Turkey. There are a few studies on Karin Kay magi cheese (Cakmakci *et. al.*, 1995). A lot of researches were made on tulum cheese (Diagrak *et. al.*, 1994; Guven and konar, 1995; Keles and Atsever, 1996).

Cheese has been known as an important animal origin product in most countries, domestic and traditional produced Cheeses are in demand. Despite, improvements in the Cheese making industry in Iran, domestic chesses is still very popular. Bacterial contaminations of cheese products may occur during processing and post processing handling where good sanitation practices are not in place, spoilage of microorganism in cheese also may originate from low quality raw milk (Johnson *et. al.*, 1990; Peterson and Marshall, 1990; Hull *et. al.*, 1992).

In tropical countries like India dairy products are responsible for many out breaks of gastrointestinal infection; dairy products prepared under unhygienic conditions pose a great threat to the health of consumers. The Indian cheese is a regular dietary favorite among the north Indians (Vaishnavi *et. al.*, 2001). In some cases milk ingredients used for processed cheese mixture formulation could



also be a source of microbial contamination (Bhowmick *et. al.*, 2006; Kumbhar *et. al.*, 2009).

Productions of processed cheese and factors influencing its characteristic have been described in many publications (Caric and Kalab, 1997; Guinee, 2004). Processed cheese products usually retain their good quality for up to 6-12 months at room temperature (Krumov *et. al.*, 2010). The conventional method for the production of cheese has been discussed extensively by Frazier and Westhoff, (1988). Starter culture in cheese making is a medium of harmless, active microorganisms which by growing in cheese, milk and curd assist the development of natural cheese with desirable characteristic of flavor, aroma, pH, texture and body. Billie *et. al.*, (1992), observed that the rate of acid production is critical in the manufacture of certain product like cheddar cheese (Uaboi *et. al.*, 2010).

#### **1.6.4. Yoghurt:**

The use of yoghurt dates back many centuries although there is no accurate record of the date when it was first made. According to Legend yoghurt was first made by the ancient Turkish people in Asia. Yoghurt is one of the most unique dairy product yet a universal one. The uniqueness of yoghurt is attributable to the symbiotic fermentation involved in its manufacturing. Yoghurt in different forms with appropriate local names is made through about the world.

The manufacture of yoghurt in turkey today is mainly based upon traditional technologies. In Turkey, usually one day old yoghurt is used as a starter culture for production of yoghurt. In principle, worldwide there is not any difference between manufacturing of homemade and factory made yoghurt (Akin *et. al.*, 1994). Yoghurt is being enjoyed everywhere in the world for its beneficial properties. It is easily digestible has high nutritional value (Tamine *et. al.*, 1985; Cakmakci *et. al.*, 1995) and has also therapeutic properties. The chemical composition and microbial quality of yoghurt was reported by several workers. According to yaygin and kilie yoghurt commercially sold in different region of Turkey contained some bacteria and yeast.

#### **1.6.5. Icecream:**

Ice cream represent a congealed dairy product produced by freezing a pasteurized mixture of milk, cream ,milk solid rather than fat, sugars, emulsifier and stabilizers. Product of dairy origin is the main ingredients of ice cream. These include whole milk, skimmed milk, cream, frozen cream, condensed milk products and milk solid other in Ice cream, a milk-based product, is a good media for microbial growth due to high nutrient value, almost neutral pH value (~pH 6–7), and long storage period of ice cream. It is produced by freezing pasteurized mixture of milk, cream, and milk solids other

than fat, sugars, emulsifiers, and stabilizers. Other ingredients include flavoring matters and water. Fruits, nuts, candies and syrups are optionally added into ice cream for flavor enrichment. It is sold in packages or in open containers at retail outlets/ice cream parlors, the open variety being distributed manually in scoops, cones, or sundaes across the counter (Farrag and Marth, 1992; Marshall and Arbuckle, 1996; Warke *et. al.*, 2000). During production, transportation and storage, it may become contaminated with several microorganisms. The microbiological quality of ice cream during retail marketing mainly depends upon the post production handling of the product, in addition to the efficiency and sanitary conditions during frozen storage. Contamination of ice creams by pathogenic microorganisms at some processing steps resulted in several disease outbreaks in numerous countries of Asia, Europe, and North America (Chug, 1996; Djuretic *et. al.*, 1997; Daniels *et. al.*, 2002). Ice cream is one of the major products of the dairy industry and continues to dominate the interest of large segments of the population. As most of the ice cream consumers are children of vulnerable age groups, it is required to be microbiologically safe (Warke *et. al.*, 2000).

In previous studies, high microbial loads in ice cream samples obtained from patisseries in Turkey have been reported (Digrak and Ozcelik, 1991; Kivanc *et. al.*, 1994; Ozcan and Kurdal, 1997; Sezgin *et. al.*, 1997; Erol *et. al.*, 1998; Evrensel and Gunes, 1998; Kanbakan

and Çon, 1999; Digrak *et. al.*, 2000; Korel *et. al.*, 2005; Coskun, 2005; Patir *et. al.*, 2006; Yaman *et. al.*, 2006). Also, it was observed that most of the ice cream samples obtained from patisseries did not confirm to TFC in mentioned studies. Nevertheless, there are only a few studies about industrially produced ice cream sold in retail stores in Turkey (Bostan and Akin, 2002; Korel *et. al.*, 2005).

Ice cream is a nutritionally enriched congealed dairy products consumed by all age groups particularly children, during summer (Sharif *et. al.*, 2005). The ingredients of ice cream may be various combinations of milk, cream, evaporated or condensed milk, dried milk, coloring material, flavors, fruits, nuts, sweetening agents, eggs and eggs product stabilizer. Any of these may account for the various specific species of bacteria (Yaman *et. al.*, 2006). Ice cream is a nutritious food for human and also an excellent medium for the growth of many microorganisms, some of which may cause diseases in human beings e.g. Cholera, Typhoid and Bacillary dysentery.

Contaminated ice cream causes several outbreaks of gastrointestinal diseases in a number of countries in Asia, Europe and North America (Dijuretic and Wall, 1996; Dijuretic *et. al.*, 1997; Chug, 1997; Gulbarn *et. al.*, 2009). In England and Wales two outbreaks of *S. enteritidis* phage type 4 infections were reported in 1990 and 1995 due to consumption of ice cream (Hennessy *et. al.*,

1996). Quality of ice cream depends on extrinsic factors that include manufacture procedure, as well as intrinsic factors that include the proportion of ingredients used. Primary sources of microbial contamination of ice cream include water and raw milk, whereas secondary sources include flavoring agents, utensils and handling. Possible sources of these microorganisms in ice cream have been reported to include raw materials used for the composition of ice cream-mix, such as milk and milk powder, cream, flavoring and coloring substances and sanitizer (Verma, 1972; Bathla and Rao, 1973) and from contaminate air during processing (Gomez, 1969). In Gilgit town ice cream is manufactured on small scale by using the dried and raw milk and sale in the city at retail outlets (Khalil Ahmed, 2009). Ice cream and other frozen dairy desserts have been found to contain enterotoxinogenic strains of *Staphylococcus aureus* (Tamminga *et.al.*, 1980; Batish and Chander, 1987; Massa *et. al.*, 1989) but no outbreaks have been attributed to this kind of dairy product. However, all authors agree that multiplication of *Staphylococcus aureus* with enterotoxin production is always possible in the prefreezing stages of processing and especially during the ripening of the ice cream mixture. In this case enterotoxin would remain active for several months in frozen storage (Gogov *et. al.*, 1984).

Fruit, nuts, candies and syrup are optionally added into ice cream for flavor enrichments. Two types of Ice cream, soft and hard are available in the market (Food and Environmental hygiene Department (HKSAR), 2001). Ice cream is a highly delicious and nutritionally rich frozen milk product occupied an important position amongst the dairy products. According to 1985-86 estimates the production of ice cream in India constituted only about 0.7% of the total milk production.

Ice cream is a popular product consumed particularly in summer as well as throughout all year and continues to be a dominant interest of large segments of the population. The ingredients' of ice cream may be various combination of milk, cream evaporated or condensed milk, dried milk coloring materials, flavors, fruits, nuts sweetening agents, eggs and egg products and stabilizers. Any of these may contribute microorganisms and affect the qualities of the products as judged by its bacterial load or its content of various specific species of bacteria (Frazier and Westhoff, 1988; Ozclik, S. 1998). Time dependent heating during the Ice cream making reduces largely the vegetative forms of the microorganisms. Furthermore, the presence of pathogens in ice cream samples is mostly by means of tools and equipments, water, workers environment, packaging material and contamination during the

transportation and distribution of ice cream (Elliot *et. al.*, 1982; Boston *et. al.*, 2002).

#### **1.6.6. Khoa:**

Khoa a traditional Indian milk product is prepared by partial desiccation of milk. In the unorganized sector of the dairy industries Khoa manufacture is a major activity and about 7% of the milk produced in India is converted to Khoa (Rangnathan and Rajorhia, 1993). Khoa is rich in nutrients has a water activity of 0.96 and support bacterial and mold growth.

A number of studies in different part of India have shown that Khoa is contaminated with coli forms (Kumar and Sinha, 1989; Kulshrestha, 1990) *staphylococcus aureus* (Gill *et. al.*, 1994) *Salmonella enteritidis* (Kulshrestha,1978). Many out breaks of food poisoning and gastro enteritis are due to the consumption of khoa based sweets. The main cause of these out breaks is the unhygienic condition during production, handling and preservation of the sweets. Several studies have been reported on the microbiological quality of pedha (Ghodekar *et. al.*, 1974; Kamat and Sulebele, 1974; Singh *et. al.*, 1975; Dwarkanath and Shrikant, 1977; Sharma and Joshi, 1992).

Khoa are partially desiccated milk is a traditional Indian milk product. It is used in preparation of variety of sweets, vegetables,

carry etc. In India, khoa is prepared by condensing milk by regular heating to remove water. During preparation of khoa temperature of milk is raised enough to destroy most of the vegetative cells of bacteria. The keeping quality of the product is adversely affected by thermophilic organisms and organisms acquired during storage. Microbial content of heat dried dairy product depends on the temperature and time of preheating evaporation process contamination and growth during storage, contamination and growth during storage. The important source of contamination of khoa is probably by the human beings (Preeti bhatnagar *et. al.*, 2007). Pal and Gupta (1985) reported that good quality khoa should have uniform white colour, mild cooked flavor and slightly grainy structure (Kulkarni and Hembade, 2009).

Indian system is using various types of milk products including khoa a partially desiccated milk which is traditionally been used in preparation of variety of sweets, vegetable, curry etc. In India, khoa is prepared by condensing milk by regular heating to remove water. It is perishable food products and has short shelf life. During preparation of Khoa temperature of milk is raised enough to destroy most of the vegetative cells of bacteria. Since the product is manufactured by traditional method without any regard to quality of raw material used and hygienic storage so the shelf life of the product is adversely affected by the thermophilic organisms and organisms acquired



during storage. Besides this contamination of products with pathogenic bacteria can results into outbreaks of gastrointestinal infection and thus threat to consumer (Mandokhot *et. al.*, 1986; Gill *et. al.*, 1994).

There is evidence that *Staphylococcus aureus* was isolated from Khoa sample (Navjeevan and Rao, 2005). Landge, (2007) worked on packaging materials to increase the shelf life of indigenous dairy products at room temperature. Misra (1997) stated that most of the Indian milk products are sterile when fresh it is the post manufacture contamination during storage that deteriorates their quality. Hence, it is of almost importance from economic as well as safety point of view to prevent any type of contamination, before during or after the preparation of the products. However, researchers (Kulkarni *et. al.*, 1983; Kumar and Anand, 2003; Patil and Pal, 2005) studied channa, burfi and Khoa indigenous dairy products to increase their shelf life respectively (Gaikwad and Hembade, 2011).

#### **1.6.7. Powdered milk:**

Milk powder is generally considered a product of good microbiological quality. However, several factors may contribute to changes in its physical and chemical properties which reduce shelf-life and thus its commercial value (Cousins *et. al.*, 1987). Different researchers agree that the hygienic conditions under which raw milk

is produced are the main factor affecting powder quality (Muir *et. al.*, 1986; Luck *et. al.*, 1987; Woodhall *et. al.*, 1989). Storage temperature and transportation may also influence the properties of milk powder, especially its insolubility index and acidity (Griffiths *et. al.*, 1988; Carlos *et. al.*, 2000).

*Staphylococcus aureus* may grow and produce *Staphylococcus* enterotoxin during processing of powdered milk (e.g. in storage tanks before spray drying). After processing *Staphylococcus aureus* counts go down. Absence of *Staphylococcus aureus* does not exclude the existence of *Staphylococcus* Enterotoxin since it could have been produced before in the manufacturing process. During storage of powdered milk, *Staphylococcus aureus* cannot grow. However, if powdered milk is used for certain foodstuffs, *Staphylococcus aureus* may grow and produce *Staphylococcus* Enterotoxin. Due to these reasons it is not surprising that powdered milk and foods containing powdered milk have been implicated in a number of staphylococcal food borne intoxication outbreaks (European commission, 2003).

### **1.7. Sources of microbial contamination:**

Microbial contamination occurs mainly during and after milking. Microorganisms in bulk tank milk, milk originate from the interior of teats, the farm environment and surfaces of the milking equipment. Microorganisms are mainly transferred from the farm

environment and to milk via diet e.g. faces, bedding and soil attached to the exterior of teats, In addition, microorganisms attached to the exterior of the teats can enter the teat canal and cause mastitis (Makovec and Reugg, 2003). Finally contamination can originate from insufficiently cleaned milking equipment when during milking microorganisms adhered to surfaces of the milking equipment are released into the milk. Aerial contamination is insignificant under normal production conditions. The concentration of microorganisms in bulk tank milk can further increase due to their growth.

The microbial population in bulk tank milk consists of a variety of bacterial species origin. For ex. the presence of *staphylococcus aureus* in bulk tank milk will generally be traced back to cow suffering from mastitis and silage is the most likely origin of spores of butyric acid bacteria in bulk tank milk (Stadhouders and Jorgensen, 1990; Haven *et. al.*, 1996).

However, there have been a studies amongst others quality of milk in bulk tank and microbial composition of milk and associated milk practices amongst small scale farmers in the informal sector (Prinsloo, 2001; O'Ferrall-Bernelt, 2003; Jansen, 2003) and on milk and food quality in developing urban areas in south Africa (Lues *et. al.*, 2003).

Hayes *et. al.*, (2001) characterized sudden elevations of the total microbial count in bulk tank milk (i.e. spike value). *Staphylococcus urberis* was responsible for 55% and *Escherichia coli* for 20% of the spike values. However, both *staphylococcus urberic* and *Escherichia coli* are environmental pathogens and therefore do not necessarily originate from the interior of infected teat.

### **1.7.1. Microorganisms:**

Besides mastitis pathogens and bacteria from milk contact surfaces bacteria from udder and teat surfaces belongs to the three main causes of microbial contamination of raw milk (Slaghuis, 1996; Sumner, 1996). Clean udder and teats before milking are demanded by directive 89/362/EEc on general conditions of Hygiene on milk production holding to avoid negative influences on holding to avoid negative influences on milk quality (Knappstein, *et. al.*, 2004).

Being a nutritional balanced foodstuff that contains few organisms when it leaves the udder milk gets contaminated at various stages. It may be from the cow, milker (manual as well as automated), extraneous diet or unclean process water (Rohde, 1985; Phillips and Griffiths, 1990; Gruetzmacher and Bradley, 1999; Hayes *et. al.*, 2001). The threat posed by diseases spread through contaminated milk is well known and the epidemiological impact of such diseases is considerable.

Microorganism in raw milk can originate from different sources such as air, milking equipment, feed soil faces and grass (Torker *et. al.*, 2008; Parekh *et. al.*, 2008). The microorganism load and types found in milk shortly after milking are influenced by factors such as animal, equipment cleanliness, season, ambient temperature, storage, personnel health, cleanness and animal health (Zelalem *et. al.*, 2006; Swai *et. al.*, 2011).

Authors agree that the microorganism do play an integral part in the spoilage and contamination of the milk as well as the milk product (Gilmour and Rowe, 1981) it is also eminent that temperature control is critical to prevent milk spoilage due to microbial growth (Frazier and Westhoff, 1988) unfortunately a number of pathogens do grow readily at refrigeration temperatures and a place where milk is normally stored even in the informal sector (Jensen, 2003). Microbes may gain entry into raw milk directly from dairy cows experiencing subclinical or clinical mastitis (Rodojic, Prodova and Necev,1991) from the farm environment particularly the water source and utensils used for storage of milk on farm or during transportation (Freedman,1977).

### **1.7.2. Environment:**

The most common microbial sources in the farm environment are feeds, faces, bedding material and soil. Microorganisms from

these sources are transferred to milk in a number of steps. The consecutive steps from source to milk are referred to as the contamination pathway. A crucial step in the contamination pathway is the transmission of dirt. Composed of faces bedding or soil to milk, microorganisms from transmitted dirt dilate in the milk and pass the flitter of the milking system (Akam *et. al.*, 1989). Dirt is mainly transmitted to milk when it is attached to the exterior of teats and rinses off during the milking operation. Additional dirt and microorganisms can be transmitted from the farm environment to bulk tank milk when the teat cups (that fall on the ground or are kicked off the teats) get contaminated or even such up dirt from the milking parlor floor. The strains and concentration of microorganisms transmitted from the farm environment to milk vide the exterior of teat depends on the composition of the attached dirt and microbial concentration in the dirt when cow's are at pasture, the teat are predominantly contaminated with soil, whereas teats of cows housed in the barn are predominantly contaminated with soil, whereas teats of cows house are mainly contaminated with faces and bedding material (Christianson *et. al.*,1999; Magnusson *et. al.*,2007). Different materials are used for bedding in barns for ex. straw, sawdust, wood, shavings and shredded paper. Fresh bedding contains a large variety of microorganisms. Microbial concentrations in fresh bedding are usually much lower than concentration in used

bedding (Hogan *et. al.*, 1990; Hogan and Smith, 1997; Slaghuis *et. al.*, 1997) however, high coli forms counts have also been measured in unused bedding material (Knapsterin *et. al.*, 2004).

Feeds introduce a large variety of microorganisms to the farm environment and subsequently to milk. The impact of feeds as a hazard of microbial contaminants of raw milk is twofold; feed can be a source of transmission vehicle of pathogens causing infection in cattle and secondly feed is an important source of bacterial spores in raw milk. Basically, the diet of high yielding dairy cows consists of two categories of feedstuffs roughages and concentrate.

The farmer feed proved the animal with dietary fiber, which is essential for the normal functioning of the cow's rumen. The most important roughage crops are grass, Maize, Lucerne (Wilkinson, 2003). Animal pathogens associated with feed included *L. monocytogenes*, *Escherichia coli* O:157 and *Salmonella* enteric outbreaks of listeriosis in cattle herds have been associated with the feeding of poorly conserved silages contaminated with *Listeria monocytogenes* (Fenlon, 1988; Weildmann *et. al.*, 1996).

Furthermore, there is evidence supporting a role of silage in the contamination of raw milk with *Listeria monocytogenes*. In addition, recent studies suggested that cattle feed can be a vehicle for transmission of *Escherichia coli* O157:H7 and *S. enteri* (Fenlon and

Wilson, 2000; Davies *et. al.*, 2003; Dodd *et. al.*, 2003; Dargatz *et. al.*, 2005).

### **1.7.3. Water:**

Water is one of the most important commodities used in the milk processing plants. It is estimated that for processing a liter of milk about 8-10 liters of water has to be used. Water is used in dairy plants for several purposes, namely as an integral part of the products e.g. reconstituted milk for washing of products like butter, for cleaning of dairy equipment and utensils, as a heating and cooling medium and in boilers. The main sources of water used in dairies are wells, river, stream or municipal water supplies. The microbiological quality of water that comes in contact with dairy products or with dairy utensils and equipment is significant for contamination and spoilage of dairy products, transmission of diseases if pathogens are transferred from water to the products.

### **1.7.4. Packaging material:**

Since packaging material is an important aspect in improving the commercial value of processed foods, it merits special consideration in food processing industry, Proper packaging not only protects the nutritional value of the food but also prevent its spoilage apart from attracting the consumers. Packaging of foods is more



relevant to a country like India where unhygienic conditions are extremely prevalent. As with other foods, packaging can play a very important role in dairy industry as well, since are perishable food items. Although the main function of packaging is to protect the food products from physicochemical, microbiological and nutritional deterioration, the packaging material of inferior quality could be possible sources of contamination. A badly packaged food product can nullify the earlier efforts of producing a good quality product. Hence, microbiological quality of packaging material needs special attention.

#### **1.7.5. Animal hygiene and animal health:**

Being a major constituent of the diet, quality control of milk is considered essential to the health and welfare of a community. However, people from low income groups have shown very little interest as to whether food and drink are good or detrimental to their health, their only concern being to buy enough food to keep them from starvation in developed societies; practices generally used to curbe microbial proliferation in milk include pasteurization and refrigeration. These low-income groups however do not utilize such practices mainly due to lack of infrastructure and funds (Rohde, 1985; Collins *et. al.*, 1995). Significant associations between udder and leg hygiene scores were determined by Schreiner and Ruegg

(2003). It can be assumed that with good management of animal health also more attention is to be paid on cow comfort and cow cleanliness (Karin Knappstein, 2004). A lack of knowledge concerning the dangers involved the lack of basic infrastructure as well as poor housekeeping techniques were all thought to be possible causes for this decline in milk quality.

Melin *et. al.*, (2002) used spore for artificial contamination and found a better reduction of carryover of spores into milk by an automated teat cleaning procedure, compared manual cleaning (98.0 verses 66.5%). In contrast, Ten-Hag and Lestie, (2002) could not determine significant differences between effects of automated and manual teat cleaning when a swab method with a simplified determination of bacterial counts was applied. Recent concerns voiced by the environmental health sector of South Africa concerning proper household hygienic have been supported by reports that milk produced by some formal and increasing numbers of informal farmers often fail to meet the national standards.

It is well known that freshly obtained milk contain some bacteria and somatic cells, which constitute the biological constituent of the milk which easily change depending on production conditions such as the health status of the cattle and hygiene practices during

milking as well as keeping and transportation of milk and milk products.

In all dairy operation small or large animal health care represents a major component, next to genetics, feeding and management quality, because healthy animals can produce milk more efficiently and healthy calves can grow in a more optimal way because healthy animals will show a better feed intake pattern throughout lactation as well as a better fertility performance. Because healthy animal will show less welfare problems throughout their life, hence, improving herd longevity (Brand *et. al.*, 1996).

Proper animal health care takes into consideration not only the veterinary technical and zoo technical issues but also the microbiological and epidemiological disease aspects. As an ultimate outcome of such exercise veterinary herd health and production management programs may be designed as well as insecurity, plans, and food quality risk control programs (Noordhuizen, 2003; Lievaart *et. al.*, 2005).

The microbial load of milk is a major factor in determining its quality. It indicates the hygienic level exercised during milking that is cleanliness of the milking utensils, condition of storage, manner of transport as well as the cleanliness of the udder of the individual animal (Spreer, 1998; Asaminew Tassew and Eyassu Seifu, 2011).

Milk from a healthy udder contains few bacterial picks up many bacteria from the time it leaves the teat of cow until it is used for further processing these microorganisms are indicators of both the manner of handling milk from milking till consumption and the quality of the milk, milk produced under hygienic conditions from healthy animals should not contain more than  $5 \times 10^5$  bacteria /ml (O'Connor, 1994). The most evident characteristics of raw milk in the Maghreb cattle systems is its poor hygienic quality, as its microbial load is generally 100 fold more important than international standards. This is due to poor hygiene at farm level (Aggad *et. al.*, 2009; Srairi *et. al.*, 2009; Srairi *et. al.*, 2013).

#### **1.7.6. Milking equipments:**

Modern dairy utensils and milk contact surfaces particularly milking machines pipelines and bulk milk cooler are designed to provide easy access for cleaning, sanitizing and drying farm bulk milk coolers are also equipped with excellent refrigeration capacity and agitation to ensure proper cooling of the milk. Other possible sources of contamination are the hands and arm of the milker or dairy workers. The air of the barn or milking parlor and flies normally these sources would contribute very few bacteria but they might be a source of pathogens or spoilage microorganisms. The quality of the farm water supply used in the milking parlor for cleaning, rinsing etc

will have some effect on the quality of milk. Other sources of contamination after the milk leaves include the tanker, truck, transfer, pipes sampling utensils and the equipment in the market milk plant, cheese factory contamination are the milk contact surfaces, pipelines, vats, tanks, pumps, valves separator, clarifiers, homogenizers, coolers, strainers, stirrer and filters which may serve as possible sources of bacteria. The paper stock used for packaging fluid milk is also an important source of contamination.

Contamination of milk via the milking equipment occurs. The level and tune of contamination of milk via the milking equipment depends largely on the cleaning procedure applied. The milking machine is cleaned after each milking or in case of automatic milking system at regular intervals, to remove residues and prevents contamination during milking. In general microorganisms originating from the farm environment are found on equipment surfaces but also *Staphylococcus aureus* has been recovered from surface of milking equipment, the milking equipment at low temperature or cleaning without sanitizers gives rise to fast growing gram negative rods like coli forms and pseudomonas.

The presence of pathogenic microorganisms in milk and dairy products during different processing indicated the lower standards of hygiene in the selected dairy process. The presence of coli forms

bacteria, furthermore, the presence of *Escherichia coli* and *Staphylococcus aureus* in some of the products emphasized the importance of production hygiene during manufacturing of dairy products in small-scale operations (Beukes, E.M., 1999). Preparation procedures for most products are still traditional arts and the fermentation is uncontrolled starter cultures are not normally used therefore variations in the quality and stability of the products is often observed. The technological and hygienic problems of traditional fermented foods need to be addressed in order to reduce losses due to wasteful and insufficient fermentation pathways, poor quality and unstable shelf-life of products (Odunfa and Oyewelo, 1998; Abeer, 2009).

### **1.8. Hygienic practices and measures for dairy personnel:**

On the basis of surveys conducted at various milk plants, it has been observed that the personnel involved in the manufacture and processing of dairy products observed unhygienic practices like coughing, sneezing, using dirty hands, wearing of dirty clothes and caps, spitting and chewing tobacco, pan etc. All these activities may introduce a variety of organisms like *Staphylococcus aureus*, coli forms etc. in the foods, Hence for the sake of producing clean, wholesome and safe product. Apart from these hygienic practices, the

following measures should be taken to ensure the production of high quality of dairy products.

### **Imparting extensive hygienic training:**

Food handlers should be given training in the hygienic handling of the foods and in the personnel hygiene so as founder stand the importance of hygiene to prevent food contamination.

### **Health supervision:**

Food handlers should be periodically checked for any possible communicable diseases, clinical or subclinical like tuberculosis, typhoid. The diseased person should not be allowed to enter the premises of the dairy plant. Similarly; the carriers should also be identified and kept away from the food processing areas.

### **Wounds and injuries care:**

Any person who happens to have a cut or wounds should not allow handling the food products until injury is protected by water proof covering.

### **Washing of hands:**

All the personnel handling milk and milk products must wash their hands with warm potable water or with a mild antiseptic solution before entering into the processing rooms.

### **Personal cleanliness:**

The persons working in the processing areas must maintain high degree of cleanliness. They should wear clean clothes and caps while on duty. Their facial and head hair should be covered adequately to prevent contamination.

### **Personal behavior:**

Within the premises of the processing unit, all the personnel should avoid such habits like eating, tobacco chewing, talking etc. In addition to these, they should wear gloves, headgear, outer clothings, footwear etc. while performing their duties, the visits of outside personnel should also be discouraged. For rigorous compliance of these hygienic practices, the responsibility should be put on the competent supervisory personnel.

### **Aims and objectives:**

1. The main objective of research is Determination of total microbial count of milk products.



2. Isolation of pathogens from the dairy pack food i.e. milk sample (Plate1), Shrikhand (plate2), Amrakhand (plate3) Chocobar (plate4), Ice cream (Plate5), Curd (Plate6), Milk powder (Plate7), Ghee (Plate 8).
3. Identification of pathogens from milk products.
4. 16s rDNA testing of isolated pathogens.
5. To study the effect of environmental parameter on growth of pathogens.
6. Chemical analysis of milk products.
7. Statistical Analysis of microbial count.

This research is performed to evaluate the hygienic quality and environmental effect on dairy packed food collected from market. The results of this study show that some of the dairy packed food samples were contaminated. Contamination of milk may occur by spoiled hands of dairy workers, unsanitary utensils, flies and polluted water supplies. The problem of post treatment contamination of container sterilized products is well known. The contamination can either through proper seal or through pinhole in the container. The use of unclean milking and transport equipment contributed to the poor hygienic quality, the unhygienic condition of preparation of these foodstuffs and water used for washing of utensils has enhanced the bacterial contamination of milk and milk products. The results obtained were used to study the quality of milk and milk products.

## Milk and milk products



Plate no 1. Milk packet



Plate no 2. Shrikhand



**Plate no 3. Amrakhand**



**Plate no 4. Chocobar**



Plate no 5. Ice cream



Plate no 6. Curd



**Plate no 7. Milk powder**



**Plate no 8. Ghee**