

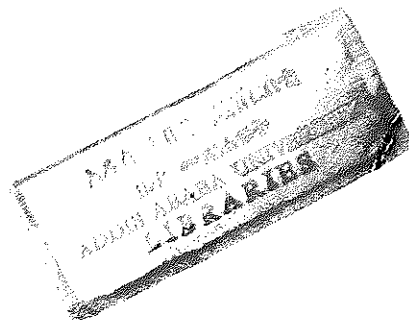
PREVALENCE OF MASTITIC STAPHYLOCOCCI  
STREPTOCOCCI AND COLIFORM (Escherichia coli)  
IN THREE DAIRY FARMS IN AND AROUND  
ADDIS ABABA

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SCHOOL OF GRADUATE STUDIES



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ABSTRACT

Prevalence of mastitic staphylococci, streptococci & coliform species in foremilk samples of apparently healthy lactating cows was studied in 3 dairy farms. One hundred & twenty apparently healthy lactating cows were randomly selected on the basis of lactation numbers. These were 40 from Debre Zeit, 40 from Repi & 40 from Kumbi dairy farms. Pooled foremilk samples were collected from the teats of each lactating cow & totally 120 foremilk samples were collected & analysed from the 3 farms.

The total mesophilic aerobic bacterial, streptococcal & staphylococcal counts & the bacterial counts of Staphylococcus & Streptococcus species were estimated using different kinds of media. The susceptibilities of 55 isolates of mastitic staphylococcal & streptococcal strains to 11 antibiotics was determined using the standard agar disc diffusion technique.

The total mesophilic aerobic bacterial counts was highly variable & ranged from hundreds to millions in all of the dairy farms. The high total mesophilic aerobic bacterial counts greater than or equal to  $1 \times 10^5$  colony forming units / milliliter were mainly associated with high numbers of streptococcal counts. Staphylococci were regularly isolated in all foremilk samples, however, their contribution to increases in the total mesophilic aerobic bacterial count was not as significant as that of streptococci. At the 3<sup>rd</sup> & subsequent lactations, the number of cows that had total mesophilic aerobic bacterial counts greater than or equal to  $2 \times 10^4$  colony forming units/milliliter increased at Kumbi & Repi farms. At the Debre Zeit farm, with increases in lactation numbers the number of cows that had greater than or equal to  $1 \times 10^5$  colony forming units/milliliter was high.

Five hundred & thirty five isolates were biochemically tested from the 120 samples. Of these 312 belonged to Staphylococcus, 215 belonged to Streptococcus, 6 belonged to Micrococcus & 2 belonged to coliform. Five species & 2 subspecies of staphylococci, 7 species of streptococci & 1 coliform species were identified. Among mastitic staphylococci, Staphylococcus epidermidis was the most prevalent and was isolated from 80.8% of the foremilk samples, followed by Staphylococcus aureus (20%), Staphylococcus hyicus subsp. chromogenes (12.5%), Staphylococcus hyicus subsp. hyicus (5.8%). Similarly, other Staphylococcus species were also isolated in different proportions: Staphylococcus simulans (6.7%), Staphylococcus xylosus (4.2%), Staphylococcus intermedius (0.83%) & Staphylococcus hominis/Staphylococcus warneri / Staphylococcus haemolyticus group (6.7%).

Of the mastitic streptococcal strains, Streptococcus bovis was the most prevalent & was isolated from 40.8% of the lactating cows sampled, followed by Streptococcus uberis (15.8%), Streptococcus dysagalactiae (14.2%), Streptococcus agalactiae (9.2%), Streptococcus faecalis (5%), Streptococcus sp. G (5.8%) & Streptococcus infrequens (0.8%). Other streptococcal strains were also isolated. Streptococcus lactis was detected in 0.8% of the samples.

Generally the frequency of isolation for Streptococcus uberis, Streptococcus dysagalactiae, Streptococcus agalactiae & Staphylococcus aureus was high during the latter lactation numbers (3, 4 & 5).

Escherichia coli was not common in the herds & was detected in 1.7% of the foremilk samples from one herd only.

Among 30 isolates of staphylococcal strains tested, 26.7% were resistant to penicillin & tetracycline. All the staphylococcal strains

were sensitive to erythromycin, oxacillin, cephalothin, chloramphenicol, kanamycin, gentamycin, streptomycin, clindamycin & vancomycin.

Of 25 isolates of streptococcal strains, resistance to oxacillin, tetracycline, kanamycin, gentamycin, streptomycin was shown by 8, 28, 8, 16 & 40% of the isolates, respectively. All the streptococcal strains were sensitive to penicillin, erythromycin, cephalothin, clindamycin, vancomycin & chloramphenicol.

## I INTRODUCTION

Milk has been called one of nature's most perfect food (Hodgson, 1979). It is an excellent source of proteins with all the essential amino acids available in high concentration (Tobias, 1974). Calcium and riboflavin are also available in sufficient quantities (Phillips & Briggs, 1975). Because of the nutritional excellence of milk, plans for improving nutrition include plans for developing the role of dairying throughout the world (Hodgson, 1979).

Milk serves as a vehicle for the transmission of several disease causing microorganisms. These pathogens gain access into the milk as a result of infections of organs far removed from the udder or are results of the direct infection of the udder (WHO, 1970). Occasionally milk handlers also contaminate the milk with pathogens (Acha & Sczyfres, 1980). Inflammation of the udder due to microbial infection is generally referred to as mastitis (Hill, 1986).

Mastitis is a multifactor disease that continues to be a problem for the dairy industry. In every herd, there will always be some with full blown or low grade mastitic infections. More than 80 species of microorganisms have been identified as causal agents of mastitis in cattle (Philpot, 1979). However the most common agents include staphylococci, streptococci & coliforms. Other etiological agents less frequently encountered include pseudomonads, nocardia, mycoplasma & yeasts (McDonald, 1979).

Generally acute infections are easily detected by palpation. In addition to the above, another screening method which could be applied is the examination of the foremilk. However, most low grade infections are not detected by palpation or examination of foremilk (Philpot, 1979). Bacteriological findings & cell counts are more

reliable methods to monitor udder health hence, they are used regularly for such purposes (Brolund,1985).

Mastitis is a cause of increased bacterial count in milk, damage to the udder & occasionally death of the cow (Belschner, 1974). As a result of the disease, milk production decreases & considerable change in the characteristics of milk takes place. This makes the milk unsuitable for direct consumption or manufacturing purposes (WHO,1970).

Mastitis is also responsible for milk borne infections and/or intoxications in man (Cousins & Bramley, 1981). Hence, staphylococcal & streptococcal infections are of principal concern to milk hygienists (WHO, 1970). Staphylococcal enterotoxin & some strains of staphylococci are heat resistant and survive pasteurization temperatures (WHO,1970).

Many of the present methods of dairy management seem to be counterproductive in the control of mastitis (Smith,1983). This is because of the possible exposure of teats to pathogens. The chance of acquiring intramammary infections seems to be higher also in commercial dairy management (Bramley & Dodd, 1984). Total confinement housing, increasing cow densities per unit area, use of bedding material that serves as culture medium to the bacteria, & communal milking of cows are known to facilitate the transmission of pathogens between cows (Smith,1983). Milking by hand damages the teat skin causing abrasions. The abrasions then become invaded by pathogens. Moreover, milker's hands, udder cloths or towels & wash water also serve as vehicles for mastitic causing microorganisms (McDonald, 1979).

Susceptibility of mastitic microorganisms to various antibiotics have been observed by researchers (Bishop et al., 1980). The improper & indiscriminate use of antibiotics without evidence of causative organisms results in the failure to reduce the incidence of mastitis (Wright,1977).

Studies dealing with the prevalence of mastitic microorganisms are conducted regularly in most countries. However, there exists no published reports on the prevalence & antibiotic susceptibility of mastitic organisms present in "normal" milking cows in Ethiopia to date.

This study was therefore designed to supply part of the missing information. In its entirety, the objectives of this study are the following.

- 1- To determine the prevalence of mastitic staphylococci, streptococci & coliforms(Escherichia coli) in milking cows at Repi, Debre Zeit & Kumbi dairy farms.

- 2- To compare the prevalence of mastitic microorganisms amongst the farms.

- 3- To estimate what fraction of the total count that these mastitic microorganisms make in raw milk.

- 4- Investigate the antibiotic susceptibility of some of the mastitic microorganisms.

It is believed, that the present study will provide information that could serve as baseline data for implementing control programs as well as initiating trends for future studies in the subject matter.

## II. LITERATURE REVIEW

### The Disease in Cattle

#### Definition & Clinical Symptoms

Mastitis is classically defined as the inflammation of the mammary glands. The term is derived from two Greek words Mastos & itis meaning breast & inflammation of, respectively (Schalm et al., 1971). Injury of any type to the mammary tissue may lead to mastitis. The udder disease of chief concern however, is the one associated with microbial infection (Jain, 1979). The internal environment of a normal mammary gland is basically sterile (Jain, 1979; Volk & Wheeler, 1980). However, mastitis starts due to the entrance of pathogenic bacteria through the teat duct into the interior of the gland (Schalm et al., 1971). As a result of a reaction produced by the host defense system against the invader & the by products of bacterial growth & metabolism, the soft mammary tissue becomes irritated (Jain, 1979). The clinical signs of mastitis differ according to the invading pathogen & the reaction of the host itself which in turn usually affects the severity of the disease (Hill, 1986). Clinical signs include discoloration of milk, often containing fibrin clots, a swollen painful udder & systemic disturbances such as pyrexia, anorexia, if endotoxemia is present shivering, rumen stasis & diarrhoea with associated dehydration (Hill, 1986).

#### The Mammary Gland & Its Mode of Exposure to Infecting Bacteria

The defense systems of the mammary gland plays an important role in protection against infections. The teat is the first barrier against invading pathogens (Adams & Reckard, 1963). Once microorganisms enter the teat canal, they often are exposed to humoral & cellular immune systems (Tarkowski, 1983). Furthermore, non specific bactericidal & bacteriostatic

mechanisms operate in lactating & dry mammary glands, including lactoferrin, the lactoperoxidase system & lysozyme (Skinner & Hugo, 1976; Cousins & Bramely, 1981). In addition, the teat canal is enclosed by a true sphincter of smooth muscle fibres that keep a tight closure of the canal & avoids microbial penetration (Schalm et al., 1971).

To induce mastitis or the development of an intramammary infection, there needs to be penetration of the teat duct by a pathogenic organism & establishment of the organism in the sinuses, ducts or alveolar tissue of the gland (Bramley & Dodd, 1984).

Abnormalities occurring in the mammary gland usually predispose the gland to invasion by microorganisms (Tarkowski, 1983). The wear & tear of milkings, especially when the milking act is performed carelessly, and the stress applied during milking that dilate & lengthen the sphincter muscle, are known to reduce the effectiveness of the teat canal as a barrier to bacteria (McDonald, 1968). As a result of this the Quarters possess patent teat canals (lack tight closure) & hence have a higher incidence of infection (Schalm et al., 1971).

There is no proof that motile bacteria can migrate more readily through the teat canal as compared to non-motile bacteria. However, capillary action within the canal might carry bacteria into the mammary gland (McDonald, 1975). In addition, dilation of the lower one-half of the teat canal between milkings, which is caused by the increased intramammary pressure may also support bacterial migration and invasion (McDonald, 1973).

Many infections occur before the time of first calving, during the dry period, & between milkings during lactation (McDonald, 1975). There is a high rate of infection occurring in the early dry period (Cousins et al., 1980).

This could be as a result of the milk load that create pressure & open the teat duct & thereby facilitating bacterial penetration into the duct. The increase in infection during the early dry period is temporary. This is because of the development of an effective keratin seal after the 10-14 days of the early dry period (Cousins et al., 1980).

Pathogens infused into the teat sinus immediately before milking may be less likely to infect the gland than those infused into it after milking. This is because of the greater chance of removal of invading pathogens during milking before they become established (Bramley & Dodd, 1984). However, Frost & Phillips (1970), reported that as few as 5 colony forming units of Staphylococcus aureus which are introduced before milking can infect the exposed quarters. Possibly, adherence to the surface epithelium of the streak canal by Staph. aureus may prevent their removal during milking (Frost, 1975).

Most transmission of Staph. aureus & Streptococcus agalactiae takes place during the milking process via milkers' hands, udder cloths, & teat cups (Philpot, 1979). Some transmissions occur during the intervals between milkings. Possible sources include contaminated bedding, cross licking of teats & udder, & contact of teats with rear legs, tail switch, & flies. Pathogens that are usually transmitted during the intermilking period are Streptococcus uberis & coliforms (Philpot, 1979).

#### Staphylococcal Mastitis

Staphylococcal mastitis is mostly associated with udder infection by Staph. aureus (Neave et al., 1969; Brolund, 1985). Intramammary infections with Staphylococcus epidermidis are also known. Since most infections are eliminated spontaneously, it is still not considered as a serious mammary pathogen (Brown, 1973; McDonald, 1977). Hence Staph. aureus is the only one considered as the major pathogen amongst the species belonging to the genus Staphylococcus (Brolund, 1985).

Staphylococcus hyicus which is found on the skin of pigs & cattle has also been reported to exist in dairy cows with subclinical mastitic infections (Devriese, 1977; Devriese & Derijcke 1979; Devriese & Hajek, 1980).

Staphylococcus species other than Staph. aureus have been also isolated from the extramammary sources of lactating cows. Devriese & Dekyser (1980), stated that Staphylococcus xylosus, Staphylococcus scuri & Staphylococcus haemolyticus predominated in the samples taken from teat skin & apex of lactating cows. Strains belonging to Staphylococcus cohnii, Staphylococcus capti & Staphylococcus hyicus subsp. hyicus & a heterogeneous collection of micrococci & novobiocin resistant staphylococci were more often obtained from teat swab samples.

The important source of infection of Staph. aureus are infected mammary glands, colonized teat ducts, infected teat lesion & the milk from infected lactating cows (Davidson, 1961; Forbes, 1968). There are also extramammary sources like the vagina & tonsils (Daleel & Frost, 1967) that play minor yet important roles in infection.

Infection rate of Staph. aureus increases with age of the cow (Schalm & Woods, 1953). The organism can penetrate deep into tissues & also establish itself in teat glands. This somehow protects the pathogen & helps it make its way into the tissue (Neave et al., 1952; Schalm & Woods, 1953).

Staphylococcus aureus mainly produces subclinical (Wilson & Richards, 1980) & chronic mastitis (Jain, 1979).

Bacterial toxins and toxic products of Staph. aureus are thought to be involved in creating mastitic gangrenous condition (Schalm et al., 1971). Since it causes vasoconstriction leading to ischemic necrosis of infected tissues & gangrene, the  $\alpha$  toxin is potentially the most damaging (Brown & Scherer,

1958). Combinations of  $\beta$  - &  $\delta$ - toxins are also reported to be more irritating than either of the toxins (Slanetz & Bartley, 1953). Furthermore coagulase & other bacterial products originating from it are known to facilitate infection (Anderson, 1976).

It has been confirmed that the phage type 80/81 produced interstitial mastitis in cows. This phage was found among farm hands who tended the animals, indicating that this organism is transmissible from man to animals & that reinfection of man can occur (Acha & Sczyfers, 1980).

#### Streptococcal Mastitis

The major Streptococcal species frequently reported to cause mastitis are Streptococcus agalactiae, Streptococcus dysagalactiae, & Streptococcus uberis (Philpot, 1979). The incidence of infection differ from herd to herd & may surpass staphylococcal mastitis (WHO, 1970; Jain, 1979).

The only notable reservoirs of Strep. agalactiae are infected udders & cows which already have intramammary infections. These serve as the main source of new infections (Hill, 1986).

Streptococcus dysagalactiae & Strep. uberis are the two most important organism that invade from extramammary sources. Streptococcus dysagalactiae to some extent is also the cause of intramammary infections (Hill, 1986). All of them are not, however, obligate udder pathogens. They persist for long periods in the environment of the cow & could be isolated from the skin & other parts of the cow regularly (Jain, 1979). Streptococcus dysagalactiae could be isolated from the tonsils & skin lesions of animals. These serve as the main source for intramammary infection in herds free from mastitis. Organisms causing new cases of mastitis are thought to originate from the mouth of cows (Neave, 1971).

Frequency of isolation of Strep. uberis from udder & teat skin was higher than from within the mammary gland. Skin infections were sometimes independent of intramammary infection (Sharma & Packer, 1976). The belly or lips were also invaded most most heavily with Strep. uberis (Cullen, 1969).

Streptococcus agalactiae is not an active tissue invader. It rather develops in milk & on the wall of cisterns & large ducts producing an irritant which results in an inflammatory reaction that is mostly subclinical. At times this develops into acute mastitis. The affected tissue eventually is destroyed resulting in reduced milk production or agalactia. (Schalm et al., 1971).

Streptococcus agalactiae, of Lancefield group B, is the main agent of chronic catarrhal mastitis of dairy cattle. Streptococcus dysagalactiae (group c) and Strep. uberis (group E) cause sporadic cases of acute mastitis in cattle (Acha & Sczyfres, 1980).

#### Coliform Mastitis

The coliform species most commonly responsible for bovine mastitis is Escherichia coli, although Klebsiella pneumoniae & Enterobacter aerogenes have been found to cause mastitis too (Bramely & Dodd, 1984).

The primary sources of coliform bacteria are the bedding material, damp walk ways, manure covered exercise yards, & heavily contaminated water (Howell, 1972; Brander, 1973). Unlike infections caused by staphylococci & streptococci that of coliform is not reduced by post milking teat dipping. This suggests that coliform bacteria commonly reach the teat end at other time other than the milking time (Eberhart et al., 1979). Most infections result from the penetration by the pathogen through the teat duct into the teat cistern (Eberhart et al., 1979).

The quick growth of coliform (E. coli) bacteria increases the production & dissemination of endotoxin that results in the sudden manifestation of the disease (Schalm et al., 1964). The severity of the disease (Mastitis) may range from peracute to subclinical infections. The subclinical infection is uncommon. The clinical mastitis is common among housed cattle (Eberhart et al., 1979; Jackson & Bramley, 1983; Bramley & Dodd, 1984). Some of the animals affected by the peracute disease die of endotoxemia unless the necessary antibiotic & supportive therapy are immediately given (Buchnel, 1974; Jackson & Bramley, 1983).

#### Pathogenicity of Mastitic Bacteria to Man

##### Staphylococcal Food Poisoning & Intoxication

The staphylococcal food poisoning syndrome was first mentioned in 1894 by J. Denys & later in 1914 by Barber, who produced in himself the signs & symptoms of the disease by consuming milk that had been contaminated with a culture of Staph. aureus (James, 1986).

Staphylococcal gastroenteritis is commonly caused by some strains of Staph. aureus that liberate coagulase. Some coagulase negative strains cause gastroenteritis, however coagulase positive strains are the most frequently encountered (James, 1986). The large majority of the outbreaks is due to human strains (phage group III, types 6, 7, 47, & 53), although a few are due to cattle strains (phage group IV, type 42 C) (Acha & Sczyfres, 1980).

Outbreaks of Staph. aureus poisoning due to the consumption of products of animal origin such as milk, cheese, cream & ice cream are common (Acha & Sczyfers, 1980). Cheese though acidic in nature has been found to serve as a carrier of Staph. aureus (McCoy, 1966).

The presence of Staph. aureus in dairy products could result in a high amount of enterotoxin production if the level of contamination is high and the temperature & the period of holding are favourable to growth (Sheikh & Luedecke, 1974). Consumption of food containing enterotoxin leads to illness, usually of 24 hours duration, characterized by nausea, diarrhoea & abdominal pain (James, 1986). All strains of Staph. aureus do not produce enterotoxin (Olson et al., 1970).

The resistance of staphylococcal enterotoxin & some strains of staphylococci to heat inactivation have raised difficulties in pasteurized milk (WHO, 1970). Jackson & Woodbine (1963), exposed 24 hours cultures of enterotoxigenic strains of Staph. aureus to 60°C for 20 to 40 min & isolated survivors. Dabbah et al., (1971), using an inoculum of about  $10^9$ /ml Staph. aureus found that small numbers of them resist heating at 62.5°C for 30 to 120 minutes.

#### Streptococcal Infections

Milk & milk products are among the most important of the foods involved in the transmission of streptococci. Most milk-borne infections have resulted from the consumption of milk from cows having udder or teat infected with streptococci (WHO, 1970, Groschel et al., 1985). Streptococci of group B, which include the agent of bovine mastitis (Strep. agalactiae) are pathogenic to man (Kaplan & et al., 1962, Cousins & Bramely, 1981). Strep. agalactiae is now known to cause disease in immuno-compromised patients & especially in new borns, causing infections with a very high death rate (Groschel et al., 1985). In milk, streptococci are readily destroyed by heat treatment. Infections are almost always due to the consumption of raw or imperfectly pasteurized or to milk contaminated after pasteurization (Kaplan et al., 1962).

Coliform Infection & Food poisoning

The coliforms group produce endotoxin which is toxic to all types of tissue cells (Schalm et al., 1971). High numbers of Escherichia coli may be present in milk as a consequence of mastitis. Certain strains of this species are responsible for several diseases of man of varying severity. A wide range of E. coli serotypes have been isolated from bovine milk, & it is probable that some of these are pathogenic to man, too (Cousins & Bramley, 1981). The classical enteropathogenic serotypes of E. coli have been found in pasteurized dairy products (Jones et al., 1967), imported & domestic cheeses (Pintasia et al., 1975) & market milk & dairy products (Bonita & Brundvig, 1980).

Escherichia coli has been shown to cause diarrheal disease in humans of all age groups, with symptoms of severe cholera-like diarrhea to a relatively mild food poisoning syndrome (Sack, 1975). Escherichia coli causes diarrheal disease by producing an enterotoxin, & because enterotoxin production does not correlate much with serotypes, it is essential to screen food isolates for enterotoxin synthesis & not just for classical enteropathogenic serotypes (Bonita & Brundvig, 1980).

Although sporadic reports of E. coli associated with gastroenteritis of food origin were observed prior to the 1970's it was the 1971 documented outbreak in the United States that drew the attention of investigators to this organism (Marrier et al., 1973; James, 1986).

The common occurrence of coliform organisms in dairy products & the implication of gastroenteritis caused by E. coli suggest that enteropathogenic E. coli may be the notable food borne pathogen & that dairy products may be the most important medium for their transmission (Bonita & Brundvig, 1980).

Changes in Composition & Properties of Milk  
due to Mastitis or Mastitic organisms

It is known that bacterial infection of a quarter usually causes a decrease in production and a change in composition of milk (Wheelock et al., 1966). Infected quarters show decreases in the concentration of lactose, potassium, fat, casein & total solids. There occurs an increase in those of sodium, chloride ions and serum protein. It also reduce the curd tension of milk (Wheelock et al., 1966; Ashworth, et al., 1967; Tallamy et al., 1969b). In mastitic milk, proteinases levels may be so high that substantial proteolysis of the caseins can occur in the udder. Proteolytic activity in mastitic skim-milk is often 5-10 fold higher than in normal milk. The increase in proteinases activity depends upon the severity of the infection & the extent of mammary tissue damage (Barry & Donnely, 1981; DeRham & Andrews, 1982).

It seems likely that the activity of proteinases, is a primary cause of the losses in yield observed when cheese is prepared from mastitic milk as opposed to normal milk (Erwin et al., 1972; Ali et al., 1980). Much of the proteinase action will persist cheese making conditions & continue to act during cheese maturation, almost certainly with harmful results on cheese quality in terms of texture & flavour (DeRham & Andrews, 1982).

The udder health status of the cow may also affect the susceptibility of the milk fat to development of hydrolytic rancidity. Milk classified as mastitis positive based upon the Wisconsin Mastitis Test values has higher lipase activity & higher concentrations of free fatty acids than mastitis negative milk (Tallamy & Randolph, 1969a; Randolph & Erwin, 1974). Lipolysis of milk fat results in flavor deterioration (Downey, 1980).

Gudding (1982), also stated that milk from cows with subclinical

mastitis has a higher concentration of free fatty acids than milk from non mastitic cows. Milk from a mastitic cow is thus more subject to flavor deterioration. Furthermore, milk samples from Staph. aureus infected cows show a higher degree of lipolysis than milk samples from cows with similar high somatic cell counts but not infected with Staph. aureus. This is due to the release of extracellular enzymes by Staph. aureus during growth in the mammary gland (Gudding, 1982).

As mastitic milk has an increased level of free fatty acids, prevention of mastitis should also be justified by the positive effect in milk quality (Gudding 1982).

Growth of coliforms also influence the flavor of milk. Milk samples containing coliforms have higher standard plate counts & psychrotrophic counts than coliform - negative samples. Pyruvate contents of milk, lipolytic & proteolytic activities are greater in samples positive for coliforms (Ledford et al., 1983).

#### Control of Mastitis

Mastitis has been studied for over 100 years, but advance in its control has been slow. The reasons for this are numerous. Mastitis is caused due to several types of infection, each with a different etiologic agent. Possible methods of control include eradication of the aetiologic agent, chemotherapy, breeding for resistance or improving the management factors (Dodd, 1983).

Prevention of the spread of pathogens from cow to cow implies that the teats must be kept free of pathogens. To this end methods have been investigated including the use of disinfectants, paper towels, or boiled cloths for washing each individual udder, the wearing of rubber gloves that

may be readily disinfected between milking of each of the cows, & wearing of washable coats or overalls & hats (Neave et al., 1969; Sanisbury & Sanisbury, 1982). However, these have not effectively brought about full control of environmental pathogens such as Strep. uberis and coliforms.

The use of teat dipping is found to be less effective in controlling coliforms (Natzke et al., 1972; Schultze & Smith, 1972) & Strep. uberis infections (Neave et al., 1969). However, teat dipping is highly effective in the control of staphylococcal & streptococcal mastitis (Natzke et al., 1972, Schultze & Smith 1972). Most teat dips are not effective for the duration of the interval between milking & thus the pathogens (Coliform & Strep. uberis) & host may come in contact with each other when the germicidal activity of the dip is reduced or lost (Eberhart et al., 1979).

Field experiments were conducted on commercial farms to analyse practical hygiene routines, one where no disinfection was practised during milking & the other where the fullest hygiene system was practised on farms. Results have shown that the new infection rate on commercial farms could be lowered by more than 50% with improved hygiene (Neave et al., 1969; Dodd, 1983). The success varies with the type of pathogen.

A combination of teat dipping after every milking during lactation concomitantly with medication of the udder during the early dry period within a herd, is known to limit udder diseases (Natzke, 1977).

In many respects further investigation of the genetic basis of resistance to mastitic microorganisms seems to be a more promising approach to prevention of infection, although, it will necessarily be slow to take within the cattle population (Bramley & Dodd, 1984). When comparing the udder health condition of different breeds, Holstein cows seem to be more susceptible to mastitis than Ayrshires (Batra & McAllister, 1983). The

Swedish Red & White (SRB) breed have generally a lower incidence of clinical mastitis than the Swedish Friesian breed (SLB). Moreover, study made on subclinical mastitis has shown that the SLB breed had worse udder infections than the SRB breed (Brolund, 1985).

Host specific resistance can be supplemented through immunization. This increases the response of cellular & humoral defense systems in the mammary gland to entering mastitic pathogens. Nevertheless there exist no vaccine of proven efficacy, so far (Dodd, 1983).

Culling of cows with mastitis could be employed to advantage in herds affected with an atypical pathogen which is resistant to therapy. Or it could also be employed as an initial step to reduce very high levels of staphylococcal infection in herds where antibiotic therapy has been ineffective (Bramley & Dodd, 1984).

In general, a control program for any mastitic pathogen must either reduce the rate at which new infection occurs &/or shorten the duration of infection (Eberhart et al., 1979).

### III. MATERIALS AND METHODS

#### Study Areas

Three separate farms, possessing a total of 455 lactating cows: 170 at Debre Zeit, 215 at Repi, 70 at Kumbi were selected for the study. Selection of the study site, were mainly dictated by farm proximity to the microbiology laboratory and availability of reliable transportation. Kumbi & Repi dairy farms are situated on the Jimma road, about 15 to 18 kilometers away from the Addis Ababa University. Debre Zeit dairy farm is situated in Debre Zeit town, about 50 kilometers away to the south east.

Forty Holstein cows were randomly selected from each herd on the basis of lactation numbers. Only lactating cows which were considered to be healthy & have healthy udders by the milkers employed on each farm were included for the study. Cows were considered unhealthy by the milkers, if they showed discomfort during palpation or had bloody, purulent, discolored milk or had swollen and/or bruised teats. Those cows which were under antibiotic treatment or had had treatment a week or two in the past were not included in the study.

#### Sampling

Milk samples were collected from November 1986 to December 1987 from the three dairy farms.

Milk samples were removed in the afternoon when all the cows were in their stalls. Before milking, the udder was washed with water thoroughly & subsequently dried with a towel that was treated with 70% ethanol. There after the teat area was disinfected with pieces of cotton soaked with 70% ethanol. During sampling the first 2 streams of the foremilk from each quarter were discarded. Of the remaining fraction of the foremilk, about 10 ml was collected from each quarter following aseptic techniques. The milk from each quarter of a cow was then pooled to give a 40 ml sample.

This pooled sample was then used for bacteriological analysis.

The milk samples collected from the cows were transported to the laboratory in an ice bath. Analysis of the samples was carried out within 5 hours after collection.

#### Bacteriological Examination of Milk Samples.

Duplicate 0.1 ml amounts of decimal dilutions in quarter strength Ringer's solution were surface plated on each of the following media: Mannitol Salt Agar (Oxoid) for the detection & enumeration of staphylococci; Azide Blood Agar Base (Oxoid) + 5% sheep blood for the detection & enumeration of streptococci, Nutrient Agar [Nutrient Broth Number 2 (Oxoid) + Agar (Oxoid)] for the total mesophilic aerobic bacterial count & Violet Red Bile Agar (Oxoid) for the detection of coliform (Escherichia coli)

Azide Blood Agar Base + 5% sheep blood & Mannitol Salt Agar media were incubated at 37°C for 48 hours. Violet Red Bile Agar medium was incubated at 44-45°C for 48 hours and Nutrient Agar medium at 32°C for 3 days.

Furthermore Edwards Medium (Oxoid) & 7% sheep blood was used for identification of mastitic streptococci & the Hotis test for differentiating & isolating Streptococcus agalactiae. The procedure for the Hotis test involves the followings: Nine & a half (9.5) ml. of each milk sample obtained from individual cows were added in sterile test tubes. Half (0.5) ml of sterile 1:200 aqueous bromocresol purple solution was then added to each of these samples, mixed thoroughly & incubated at 37°C for 24 to 48 hours. Samples that produced small flakes or yellow colonies adherent to the side of the tube after 15-24 hours of incubation were selected. The adherent colony from the test tube was removed with a sterile loop & surface streaked on Azide Blood Agar Base (Oxoid) + 5% sheep blood. The plate was then incubated for 24-48 hours. Pure colonies were isolated & further analysed biochemically for the presence of Strep. agalactiae.

### Biochemical Identification

Representative streptococcal colonies were isolated based on colony morphology, size, color and the type of haemolysis. Similarly representative staphylococcal colonies were isolated based on colony morphology, color and whether the isolates were mannitol fermenters or non fermenters.

Representative isolates obtained during the study period were identified to the species level using methods previously described by Harvey & Gilmour (1985), for staphylococci, Buxton & Fraser (1977), & Carter (1984), for streptococci, Eberhart et al., (1979), Edwards & Ewing (1972) & Carter (1984), for Enterobacteriaceae, Buchanan & Gibbons (1974), (Bergey's Manual of Determinative Bacteriology) for staphylococci, streptococci & Enterobacteriaceae.

### Antibiotic Susceptibility Testing

Fifty five staphylococcal & streptococcal isolates were subjected to disc assay sensitivity tests. Eleven antibiotics were used for the test (Bauer et al., 1966). The antibiotics used were penicillin, erythromycin, oxacillin, cephalothin, tetracycline, chloramphenicol, kanamycin, gentamycin, streptomycin, clindamycin & vancomycin.

The diameters of the zone of inhibition were measured in millimeters & were interpreted as sensitive, intermediate or resistant based on Bauer et al., (1966), recommendations.

Staphylococcus aureus (ATCC 25923) sensitive to all antibiotics used in this study was used as control strain.

#### IV. RESULTS

The total mesophilic aerobic bacterial count of the foremilk collected from the individual cows was found to run from as low as hundreds to the millions per milliliter of sample analyzed. Hence the total mesophilic aerobic bacterial & streptococcal counts were grouped into sub groups containing a) less than  $2 \times 10^4$  colony forming units (cfu)/ml, b) between  $2 \times 10^4$  to  $1 \times 10^5$  cfu/ml & c) counts exceeding  $1 \times 10^5$  cfu/ml, as used by Bramely et al., (1984). Similarly the staphylococcal counts, were also divided into sub groups with a) counts less than  $1 \times 10^3$  cfu/ml, b) between  $1 \times 10^3$  to  $1 \times 10^4$  cfu/ml & c) counts greater than  $1 \times 10^4$  cfu/ml.

During this study period, a total of 535 isolates obtained from the 3 farms were biochemically tested. Of the total number of isolates, 195, 171 & 169 were foremilk samples of cows obtained, from Debre Zeit, Kumbi & Repi dairy farms, respectively. Of the 535 isolates, staphylococci streptococci, coliform (Escherichia coli) & Micrococcus represented 312, 215, 2 & 6, respectively.

#### Total Mesophilic Aerobic, Streptococcal & Staphylococcal

##### Counts of the Foremilk Samples

##### Debre Zeit Dairy Farm

The total mesophilic aerobic bacterial counts (TMABC) varied from about  $1 \times 10^2$  up to  $5 \times 10^6$  cfu/ml. As much as 55% (22 out of 40 cows in all lactation numbers) of the foremilk samples collected from the cows had counts less than  $2 \times 10^4$  cfu/ml while 20% had counts between  $2 \times 10^4$  to  $1 \times 10^5$  cfu/ml & 25% of the samples contained bacteria whose population was greater than  $1 \times 10^5$  cfu/ml (Table, 1). With increases in the lactation number, the number of cows which had bacterial counts greater than

$1 \times 10^5$  cfu/ml also increased. During the 5<sup>th</sup> lactation number, as many as 50% of the cows had counts greater than  $1 \times 10^5$  cfu/ml in their foremilk.

The streptococcal count was also as variable as the TMABC (none to  $2 \times 10^6$  cfu/ml). As many as 12.5 to 37.5 % of the cows which were in their first 4 lactation numbers had no streptococci detected in their foremilk (Table, 2). However, amongst those which harboured streptococci in their foremilk 57.5% had counts less than  $2 \times 10^4$ , 7.5% between  $2 \times 10^4$  &  $1 \times 10^5$  & 12.5% had greater than  $1 \times 10^5$  cfu/ml. The high total mesophilic aerobic bacterial count was mostly associated with large numbers of the streptococcal population. Generally total streptococcal count increased in most of the cows as the lactation number increased. During the 5<sup>th</sup> lactation number as many as 37.5% of the cows had streptococcal counts exceeding  $1 \times 10^5$  cfu/ml.

The staphylococcal count ranged from 60 to  $3 \times 10^4$  cfu/ml (Table 3). Thirty two & a half percent (32.5%) of the staphylococci count fell less than  $1 \times 10^3$  cfu/ml while 60% between  $1 \times 10^3$  to  $1 \times 10^4$  cfu/ml & 7.5% of the staphylococci count fell greater than  $1 \times 10^4$  cfu/ml. During the first two lactations the staphylococcal population was found to be less than  $1 \times 10^4$  cfu/ml in the foremilk. However, as the lactation number increased thereafter the foremilk of some of the cows appeared to harbour more staphylococcal populations (exceeding  $1 \times 10^4$  cfu/ml). This was particularly more noticeable in the 3<sup>rd</sup> & 4<sup>th</sup> lactation numbers.

#### Kumbi Dairy Farm

As can be seen in table 4, the total mesophilic aerobic bacterial count of the foremilk collected fell between  $2 \times 10^3$  to  $1 \times 10^7$  cfu/ml. As much as 50% (20 out of 40) of the samples analysed contained counts less than  $2 \times 10^4$  cfu/ml. Those falling between  $2 \times 10^4$  to  $1 \times 10^5$  cfu/ml

Table -1- Distribution of the total mesophilic aerobic bacterial counts of foremilk of cows from Debre Zeit dairy farm.

Lactation number	Number of cows sampled	Colony forming units/ml of foremilks		
		$< 2 \times 10^4$	$2 \times 10^4 - 1 \times 10^5$	$> 1 \times 10^5$
1	8	$9 \times 10^2$ $4 \times 10^3$ $5 \times 10^3$ $7 \times 10^3$	$3 \times 10^4$ $7 \times 10^4$ $7 \times 10^4$	$2 \times 10^6$
2	8	$1 \times 10^2$ $3 \times 10^2$ $4 \times 10^2$ $1 \times 10^3$ $4 \times 10^3$ $4 \times 10^3$	$2 \times 10^4$	$5 \times 10^5$
3	8	$2 \times 10^3$ $5 \times 10^3$ $7 \times 10^3$ $9 \times 10^3$	$2 \times 10^4$ $2 \times 10^4$ $6 \times 10^4$	$6 \times 10^5$
4	8	$6 \times 10^3$ $7 \times 10^3$ $8 \times 10^3$ $1 \times 10^4$	$4 \times 10^4$	$1 \times 10^5$ $2 \times 10^5$ $3 \times 10^5$
5	8	$2 \times 10^2$ $5 \times 10^3$ $5 \times 10^3$ $5 \times 10^3$	-	$2 \times 10^5$ $2 \times 10^5$ $3 \times 10^6$ $5 \times 10^6$

Table -2- Distribution of streptococci in the foremilk of cows from Debre Zeit dairy farm.

Lactation number	Number of cows sampled	Colony forming units/ml of foremilk		
		$< 2 \times 10^4$	$2 \times 10^4 - 1 \times 10^5$	$> 1 \times 10^5$
1	8	0 0 $4 \times 10^2$ $4 \times 10^2$ $1 \times 10^3$ $1 \times 10^3$ $4 \times 10^3$	-	$1 \times 10^6$
2	8	0 0 0 $2 \times 10^2$ $5 \times 10^2$ $7 \times 10^3$ $8 \times 10^3$	-	$3 \times 10^5$
3	8	0 0 0 90 $3 \times 10^3$ $5 \times 10^3$ $1 \times 10^4$	$9 \times 10^4$	-
4	8	0 $3 \times 10^2$ $6 \times 10^2$ $3 \times 10^3$ $3 \times 10^3$ $5 \times 10^3$ $1 \times 10^4$	$6 \times 10^4$	-
5	8	$1 \times 10^3$ $1 \times 10^3$ $7 \times 10^3$ $7 \times 10^3$	$3 \times 10^4$	$2 \times 10^5$ $1 \times 10^6$ $2 \times 10^6$

Table -3- Distribution of staphylococci in the foremilk of cows from Debre Zeit dairy farm.

Lactation number	Number of cows sampled	Colony forming units/ml of foremilk		
		$< 1 \times 10^3$	$1 \times 10^3 - 1 \times 10^4$	$> 1 \times 10^4$
1	8	$4 \times 10^2$ $7 \times 10^2$	$1 \times 10^3$ $2 \times 10^3$ $2 \times 10^3$ $5 \times 10^3$ $5 \times 10^3$ $8 \times 10^3$	
2	8	60 70 $2 \times 10^1$ $3 \times 10^2$ $7 \times 10^2$	$1 \times 10^3$ $4 \times 10^3$ $4 \times 10^3$	
3	8	-	$1 \times 10^3$ $1 \times 10^3$ $1 \times 10^3$ $2 \times 10^3$ $3 \times 10^3$ $5 \times 10^3$ $5 \times 10^3$	$2 \times 10^4$
4	8	$2 \times 10^2$ $2 \times 10^2$ $9 \times 10^2$	$3 \times 10^3$ $4 \times 10^3$ $9 \times 10^3$	$1 \times 10^4$ $3 \times 10^4$
5	8	$3 \times 10^2$ $4 \times 10^2$ $7 \times 10^2$	$1 \times 10^3$ $1 \times 10^3$ $3 \times 10^3$ $4 \times 10^3$ $1 \times 10^3$	

represented 27.5% of the cows while those with counts greater than  $1 \times 10^5$  cfu/ml were 22.5% of the cows.

Most counts during the first & second lactation period of the cows, were below  $2 \times 10^4$  cfu/ml. However, cows with bacterial counts equal to or greater than  $2 \times 10^4$  cfu/ml in their foremilk increased during lactations 3, 4 & 5. At the 5<sup>th</sup> lactation number, 62.5% of the cows had counts equal to or in excess of  $2 \times 10^4$  cfu/ml.

The streptococcal count ranged from none to  $1 \times 10^7$  cfu/ml (Table 5). Most of the foremilk samples (75%) had less than  $2 \times 10^4$  cfu/ml. Only 12.5% of the samples had counts greater than  $1 \times 10^5$  cfu/ml. Those with  $2 \times 10^4$  to  $1 \times 10^5$  cfu/ml represented 12.5%. Seventy five, 25, 37.5, 25 & 12.5% of the cows during the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> & 5<sup>th</sup> lactation numbers did not have any streptococci in their foremilks (Table, 5). Generally, streptococcal counts increased in number as the lactation number increased. However, there was only a cow that had a count of  $2 \times 10^4$  cfu/ml during the first lactation. On the other hand as many as 37.5% of the cows had counts equal to or greater than  $2 \times 10^4$  cfu/ml in lactation 5.

Staphylococci were detected from all samples examined. However, their contribution to the total aerobic mesophilic bacterial count was low. Their count ranged from 60 to  $2 \times 10^4$  cfu/ml (Table, 6). Twenty two & a half percent (22.5%) of the staphylococcal count fell less than  $1 \times 10^3$  cfu/ml while 57.5% between  $1 \times 10^3$  to  $1 \times 10^4$  cfu/ml & 20% had counts greater than  $1 \times 10^4$  cfu/ml. There were 2 samples with high staphylococcal count greater than  $1 \times 10^4$  cfu/ml in lactations 1, 3 & 4 & a single sample each in lactation 2 & 5. Increases in staphylococcal population with increases in lactation number was not apparent on this farm.

Table -4- Distribution of the total mesophilic aerobic bacterial counts of foremilk of cows from Kumbi dairy farm

Lactation number	Number of cows sampled	Colony forming units/ml of foremilk		
		$< 2 \times 10^4$	$2 \times 10^4 - 1 \times 10^5$	$> 1 \times 10^5$
1	8	$4 \times 10^3$ $4 \times 10^3$ $5 \times 10^3$ $8 \times 10^3$ $1 \times 10^4$	$2 \times 10^4$ $2 \times 10^4$	$3 \times 10^5$
2	8	$4 \times 10^3$ $6 \times 10^3$ $7 \times 10^3$ $8 \times 10^3$ $1 \times 10^4$	$4 \times 10^4$	$2 \times 10^5$ $2 \times 10^5$
3	8	$3 \times 10^3$ $3 \times 10^3$ $1 \times 10^4$	$5 \times 10^4$ $6 \times 10^4$ $8 \times 10^4$	$1 \times 10^5$ $1 \times 10^5$
4	8	$3 \times 10^3$ $3 \times 10^3$ $4 \times 10^3$ $7 \times 10^3$	$3 \times 10^4$ $6 \times 10^4$	$1 \times 10^6$ $1 \times 10^7$
5	8	$2 \times 10^3$ $4 \times 10^3$ $8 \times 10^3$	$3 \times 10^4$ $3 \times 10^4$ $4 \times 10^4$	$3 \times 10^5$ $2 \times 10^6$

Table -5- Distribution of streptococci in the foremilk of cows from Kumbi dairy farm

Lactation number	Number of cows sampled	Colony forming units/ml of foremilk		
		$< 2 \times 10^4$	$2 \times 10^4 - 1 \times 10^5$	$> 1 \times 10^5$
1		0 0 0 0 0 0 $7 \times 10^3$	$2 \times 10^4$	-
2	8	0 0 80 $4 \times 10^2$ $8 \times 10^2$ $7 \times 10^3$	$4 \times 10^4$	$5 \times 10^4$
3	8	0 0 0 $7 \times 10^2$ $2 \times 10^3$ $3 \times 10^3$	$2 \times 10^4$ $4 \times 10^4$	-
4	8	0 0 $1 \times 10^3$ $2 \times 10^3$ $2 \times 10^3$ $6 \times 10^3$	-	$1 \times 10^6$ $1 \times 10^7$
5	8	0 $2 \times 10^2$ $4 \times 10^3$ $4 \times 10^3$ $5 \times 10^3$	$2 \times 10^4$	$2 \times 10^5$ $1 \times 10^6$

Table -6- Distribution of staphylococci in the foremilk  
of cows from Kumbi dairy farm

Lactation numbers	Number of cows sampled	Colony forming units/ml of foremilk		
		$< 1 \times 10^3$	$1 \times 10^3 - 1 \times 10^4$	$> 1 \times 10^4$
1	8	$2 \times 10^2$	$2 \times 10^3$ $4 \times 10^3$ $4 \times 10^3$ $5 \times 10^3$ $6 \times 10^3$	$1 \times 10^4$ $1 \times 10^4$
2	8	$7 \times 10^2$	$2 \times 10^3$ $3 \times 10^3$ $3 \times 10^3$ $4 \times 10^3$ $6 \times 10^3$ $7 \times 10^3$	$2 \times 10^4$
3	8	$1 \times 10^2$ $4 \times 10^2$	$1 \times 10^3$ $2 \times 10^3$ $9 \times 10^3$ $9 \times 10^3$	$2 \times 10^4$ $2 \times 10^4$
4	8	60 $3 \times 10^2$ $4 \times 10^2$	$4 \times 10^3$ $7 \times 10^3$ $8 \cdot 10^3$	$1 \times 10^4$ $2 \times 10^4$
5	8	$3 \times 10^2$ $7 \times 10^2$	$2 \times 10^3$ $3 \times 10^3$ $3 \times 10^3$ $3 \times 10^3$ $1 \times 10^4$	$2 \times 10^4$

### Repi Dairy Farm

The aerobic plate counts of foremilk samples collected from cows at Repi dairy farm are shown in (Table, 7). The range in counts was between  $2 \times 10^2$  to  $9 \times 10^6$  cfu/ml. As many as 37.5% of those sampled during lactation 1 to 5 had counts less than  $2 \times 10^4$  cfu/ml. Those with counts between  $2 \times 10^4$  to  $1 \times 10^5$  cfu/ml represented 35% of the total, while, 27.5% represented samples where counts were greater than  $1 \times 10^5$  cfu/ml. Fifty percent of the cows in 1<sup>st</sup>, 2<sup>nd</sup> & 3<sup>rd</sup> lactations had counts less than  $2 \times 10^4$  cfu/ml. However, after lactation 3, with increases in lactation number, the bacterial count also increased. As can be seen from Table 7, those cows with counts of  $2 \times 10^4$  cfu/ml or greater constituted 75% & 87.5% at lactations 4 & 5, respectively.

As shown in Table 8 the streptococcal counts ranged from none up to  $9 \times 10^6$  cfu/ml. Seventy five percent of the samples represented counts less than  $2 \times 10^4$  cfu/ml. Those with counts between  $2 \times 10^4$  to  $1 \times 10^5$  cfu/ml & greater than  $1 \times 10^5$  cfu/ml represented 17.5% & 7.5% of the total respectively. The contribution of the streptococcal count to the total bacterial population appeared to be significant. Some cows did not have streptococci in their foremilk & it was not related to lactation number one way or the other. Twenty five percent, 62.5, 37.5, 50% and 37.5% of the foremilk samples from the cows in lactations 1,2,3,4 & 5, respectively did not contain streptococci. Increases in streptococcal population were not apparent in this farm as lactation number increased.

The staphylococcal count varied from 80 to  $6 \times 10^4$  cfu/ml (Table 9). As many as 55% of the samples contained counts between  $1 \times 10^3$  to  $1 \times 10^4$  cfu/ml while 25% had counts less than  $1 \times 10^3$  cfu/ml & 20% of the samples had staphylococci whose population was greater than  $1 \times 10^4$  cfu/ml. At

Table - 7 - Distribution of the total mesophilic aerobic bacterial counts of foremilk of cow from Repi dairy farm

Lactation number	Number of cows sampled	Colony forming units/ml of foremilk		
		$< 2 \times 10^4$	$2 \times 10^4 - 1 \times 10^5$	$> 1 \times 10^5$
1	8	$5 \times 10^2$ $6 \times 10^2$ $9 \times 10^3$ $1 \times 10^4$	$2 \times 10^4$	$1 \times 10^5$ $2 \times 10^5$ $9 \times 10^6$
2	8	$4 \times 10^2$ $1 \times 10^3$ $6 \times 10^3$ $1 \times 10^4$	$2 \times 10^4$ $4 \times 10^4$ $7 \times 10^4$	$4 \times 10^5$
3	8	$8 \times 10^3$ $9 \times 10^3$ $1 \times 10^4$ $1 \times 10^4$	$3 \times 10^4$ $3 \times 10^4$	$2 \times 10^5$ $6 \times 10^5$
4	8	$2 \times 10^2$ $6 \times 10^3$	$3 \times 10^4$ $6 \times 10^4$ $8 \times 10^4$	$2 \times 10^5$ $7 \times 10^5$ $1 \times 10^6$
5	8	$1 \times 10^4$	$2 \times 10^4$ $3 \times 10^4$ $3 \times 10^4$ $5 \times 10^4$ $5 \times 10^4$	$1 \times 10^5$ $3 \times 10^5$

Table -8- Distribution of streptococci in the foremilk  
of cows from Repi dairy farm

Lactation number	Number of cows sampled	Colony forming units/ml of foremilk		
		$< 2 \times 10^4$	$2 \times 10^4 - 1 \times 10^5$	$> 1 \times 10^5$
1	8	0 0 $4 \times 10^2$ $2 \times 10^3$ $4 \times 10^3$	$2 \times 10^4$ $7 \times 10^4$	$9 \times 10^6$
2	8	0 0 0 0 0 $4 \times 10^2$ $3 \times 10^3$	$2 \times 10^4$	
3	8	0 0 0 $3 \times 10^2$ $4 \times 10^3$ $8 \times 10^3$ $9 \times 10^3$	-	$6 \times 10^5$
4	8	0 0 0 0	$3 \times 10^4$ $4 \times 10^4$ $8 \times 10^4$	$1 \times 10^6$
5	8	0 0 0 $2 \times 10^2$ $5 \times 10^2$ $9 \times 10^3$ $2 \times 10^4$	$8 \times 10^4$	-

latter lactation numbers the number of cows that had staphylococcal counts greater than  $1 \times 10^3$  cfu/ml increased. As many as 87.5% of the cows had staphylococcal count greater than  $1 \times 10^3$  cfu/ml during the 4<sup>th</sup> & 5<sup>th</sup> lactations. It was not unusual to detect foremilk samples with staphylococcal counts exceeding  $1 \times 10^4$  cfu/ml at any one of the lactation numbers.

Prevalence of Species belonging to Genus Staphylococcus  
and Streptococcus in the Foremilk of Cows

Debre Zeit Dairy Farm

Out of the 195 isolates subcultured from foremilk samples obtained from Debre Zeit, 110 were species belonging to genus Staphylococcus while 82 belonged to genus Streptococcus (Table 10 & 11). Staphylococcus epidermidis was the most common isolate making up 64 of the total staphylococcal isolates. This was followed by Staphylococcus aureus (16). Other Staphylococcus species which were also isolated were Staphylococcus hyicus subsp. chromogenes (8), Staphylococcus xylosus (6), Staphylococcus simulans (4), Staphylococcus hyicus subsp. hyicus (1) & the Staphylococcus homini/ Staphylococcus warneri/ Staphylococcus haemolyticus group (7). Four other Staphylococcus isolates could not be identified to the species level.

During each lactation number Staph. epidermidis was found to be the most prevalent (85%), while Staph. aureus, Staph. hyicus subsp. chromogenes, Staph. hominis / Staph. Warneri / Staph. haemolyticus group, Staph. xylosus, Staph. simulans, Escherichia coli, Staph. hyicus subsp. hyicus & Micrococcus sp. were found to be 20, 17.5, 17.5, 7.5, 5, 5, 2.5 & 2.5 % respectively (Table,10).

Staphylococcus epidermidis was isolated from most of the samples in all

Table --9-- Distribution of staphylococci in the foremilk of cows from Repi dairy farm.

Lactation number	Number of cows sampled	Colony forming units/ml of foremilk		
		$< 1 \times 10^3$	$1 \times 10^3 - 1 \times 10^4$	$> 1 \times 10^4$
1	8	$2 \times 10^2$ $4 \times 10^2$ $5 \times 10^2$	$3 \times 10^3$ $4 \times 10^3$ $5 \times 10^3$ $7 \times 10^3$	$1 \times 10^4$
2	8	$1 \times 10^2$ $3 \times 10^2$	$1 \times 10^3$ $5 \times 10^3$ $5 \times 10^3$ $6 \times 10^3$	$1 \times 10^4$ $6 \times 10^4$
3	8	80 $1 \times 10^2$ $8 \times 10^2$	$5 \times 10^3$ $6 \times 10^3$ $8 \times 10^3$ $9 \times 10^3$	$2 \times 10^4$
4	8	90	$2 \times 10^3$ $5 \times 10^3$ $6 \times 10^3$ $6 \times 10^3$ $7 \times 10^3$ $9 \times 10^3$	$3 \times 10^4$
5	8	$2 \times 10^2$	$2 \times 10^3$ $2 \times 10^3$ $2 \times 10^3$ $3 \times 10^3$	$1 \times 10^4$ $6 \times 10^4$ $2 \times 10^4$

lactation numbers. Staphylococcus aureus was also detected in each of the lactation numbers. The rate of isolation was high in lactation 2, 3 & 5. Staphylococcus hyicus subsp. hyicus was only detected in lactation 5 whereas Staph. hyicus subsp. chromogenes was isolated in all lactation period except in lactation 3. The rate of isolation was high in lactation 5 & this was followed by lactation 1. Staphylococcus hominis / Staph. warneri / Staph. haemolyticus group was isolated in all lactation numbers except lactation 4. The rate of isolation of the group was equal in all lactation numbers except lactation 1. Staphylococcus simulans was isolated in lactation 1 & 4, Staphylococcus xylosus in lactation 2 & 3 & E. coli in lactation 2 & 5.

The streptococcus species isolated during the study were mostly Streptococcus bovis (38), Streptococcus dysagalactiae (15) and Streptococcus uberis (12). Other streptococcal species isolated were Streptococcus agalactiae (3), Streptococcus faecalis (5) Streptococcus sp. G(4) & Streptococcus infrequens(2). Three of the streptococcal isolates were n't identified to the species level.

Among the streptococcal strains (Table 11), Strep. bovis was the most prevalent (50%) followed by Strep. dysagalactiae (20%), Strep. uberis(15%), Strep. faecalis (7.5%), Strep. agalactiae (5%) Strep. sp. G (5%) & Strep. infrequens (2.5%).

Except Strep. bovis and Strep. dysagalactiae, all the other streptococcal strains were only detected in lactation numbers 3, 4 & 5. Streptococcus bovis was isolated in all lactation numbers & the rate of isolations varied from 37.5% to 62.5% in the different lactation numbers. Streptococcus dysagalactiae was also isolated in all lactation numbers. The rate of isolation of Strep. dysagalactiae & Strep. uberis was the highest in lactation 4. The highest rate of isolation for Strep. faecalis was in lactation 5,

Table -10- Prevalence of staphylococci, coliform (E. coli) & Micrococcus species in foremilk samples from individual cows in Debre Zeit dairy farm.

Species (strains)	Lactation number					Percent prevalence
	1	2	3	4	5	
<u>Staph. aureus</u>	1	2	2	1	2	20
<u>Staph. epidermidis</u>	7	6	7	8	6	85
<u>Staph. hyicus</u> subsp. <u>hyicus</u>	-	-	-	-	1	2.5
<u>Staph. hyicus</u> subsp. <u>chromogenes</u>	2	1	-	1	3	17.5
<u>Staph. simulans</u>	1	-	-	1	-	5
<u>Staph. xylosus</u>	-	2	1	-	-	7.5
<u>Staph. hominis</u> / <u>Staph. warneri</u> / <u>Staph. haemolyticus</u>	1	2	2	-	2	17.5
<u>E. coli</u>	-	1	-	-	1	5
<u>Micrococcus</u>	-	-	-	1	-	2.5

Table 11. Prevalence of streptococci species in foremilk samples from individual cows in Debre Zeit dairy farm.

Species (strains)	Lactation number					Percent prevalence
	1	2	3	4	5	
<u>Strep. agalactiae</u>	-	-	-	-	2	5
<u>Strep. dysagalactiae</u>	2	1	1	3	1	20
<u>Strep. uberis</u>	-	-	1	3	2	15
<u>Strep. bovis</u>	5	4	3	3	5	50
<u>Strep. faecalis</u>	-	-	-	1	2	7.5
<u>Strep. infrequens</u>	-	-	1	-	-	2.5
<u>Strep. spp. G</u>	-	-	-	2	-	5

Kumbi Dairy Farm

Amongst the 171 isolates obtained during the study period, 100 & 68 were Staphylococcus & Streptococcus species respectively. The rest of the isolates were species belonging to genus Micrococcus. Several Staphylococcal strains were identified. Of these Staph. epidermidis constituted 60 of the 100 isolates while Staph. aureus, Staph. simulans, Staph. hyicus subsp. chromogenes, Staph. xylosus & Staph. hominis/ Staph. warneri / Staph. haemolyticus group made up 12, 10, 5, 4 & 2 respectively. Seven of the staphylococcal strains isolated could not be identified.

As shown in Table 12, the most prevalent staphylococcal strain in the herd was Staph. epidermidis (77.5%) followed by Staph. aureus (15%) Staph. simulans (12.5%) Staph. hyicus subsp. chromogenes (7.5%), Staph. xylosus (5%) & Staph. hominis/ Staph. warneri / Staph. haemolyticus group (2.5%).

Staphylococcus epidermidis was detected in 6 of the samples in each of the lactation numbers except lactation 1 where 7 of the samples had the bacterium. Staphylococcus aureus was isolated in all lactation numbers except lactation 1 where it was not detected. The rate of isolation of Staph. aureus increased in lactation 3 & 5. Staphylococcus hyicus subsp. chromogenes was isolated in the first 3 lactation numbers (1, 2, & 3). Staphylococcus simulans was isolated in the early lactations (1 & 2) as well as latter lactation number (5). The rate of isolation of Staph. simulans was the highest in lactation 5. Staphylococcus xylosus was isolated from samples in lactation 1 & 4. Staphylococcus hominis / Staph. warneri / Staph. haemolyticus group was isolated in lactation 2.

Among the 6 streptococcal strains identified, Strep. bovis made up 25 of the 68 isolates while Strep. uberis, Strep. agalactiae,

Strep. sp. G, Strep. faecalis & Strep. dysagalactiae constituted 23, 6, 5, 4, & 3 respectively. There were also 2 streptococcal isolates which were not identified to the species level (Table 13).

As can be seen from Table 13, Strep. bovis was the most prevalent (40%) followed by Strep. uberis (27.5%), Strep. faecalis (7.5%) each of Strep. agalactiae (7.5%), Strep. sp. G. (7.5%) & Strep. dysagalactiae (5%).

Streptococcus bovis & Strep. uberis were isolated from each of the lactation numbers. The rate of isolations in the different lactation number varied from 12.5 to 37.5% for Strep. uberis & 25 to 62.5% for Strep. bovis. Streptococcus dysagalactiae was only detected in lactation 4 whereas Strep. agalactiae was detected in lactation 2, 3 & 4. Streptococcus faecalis was isolated from cows in lactation 3 & 4 while Strep. sp. G was isolated in lactation 2 & 5.

#### Repi Dairy Farm.

Of 169 total number of isolates subcultured from foremilk sampled from this farm, 102 were Staphylococcus species, 65 Streptococcus, & 2 Micrococcus. Some strains of Streptococcus (5) & Staphylococcus (8) were not identified to the species level.

Six species of Staphylococcus were identified. Among these species Staph. epidermidis was the most common isolate & constituted 59 of the total staphylococcal isolates; Staph. aureus, Staph. simulans, Staph. hyicus subsp. chromogenes, Staph. hyicus subsp. hyicus & Staph. intermedius constituted 20, 2, 5, 6 & 2 of the isolates respectively.

Of the staphylococcal strains Staph. epidermidis was the most prevalent (80%) followed by Staph. aureus (25%), Staph. hyicus subsp. hyicus (15%), Staph. hyicus subsp. chromogenes (12.5%), each of Staph. simulans (2.5%) & Staph. intermedius (2.5%) (Table 14).

The rate of isolation of Staph. epidermidis varied between 62.5% to

Table -12- Prevalence of staphylococci & Micrococcus strains in foremilk samples from individual cows in Kumbi dairy farm

Species(strain)	Lactation numbers					Percent prevalence
	1	2	3	4	5	
<u>Staph. aureus</u>	-	1	2	1	2	15
<u>Staph. epidermidis</u>	7	6	6	6	6	77.5
<u>Staph. hyicus</u> subsp. <u>chromogenes</u>	1	1	1	-	-	7.5
<u>Staph. xylosus</u>	1	-	-	1	-	5
<u>Staph. simulans</u>	1	1	-	-	3	12.5
<u>Staph. hominis</u> / <u>Staph. warneri</u> / <u>Staph. haemolyticus</u>	-	1	-	-	-	2.5
<u>Micrococcus</u>	2	-	-	1	-	7.5

Table -13- Prevalence of streptococci strains in foremilk samples from individual cows in Kumbi dairy farm.

Species(strains)	Lactation number					Percent prevalence
	1	2	3	4	5	
<u>Strep. agalactiae</u>	-	1	1	-	1	7.5
<u>Strep. dysagalactiae</u>	-	-	-	2	-	5
<u>Strep. uberis</u>	1	2	3	2	3	27.5
<u>Strep. bovis</u>	2	5	3	3	3	40.0
<u>Strep. faecalis</u>	-	-	1	2	-	7.5
<u>Strep. spp. G</u>	-	1	-	-	2	7.5

100% in the different lactation numbers. Staphylococcus aureus was isolated in all lactation numbers & the rate of isolation increased in lactations 4 & 5 . Staphylococcus hyicus subsp. chromogenes was also isolated in all lactation numbers except lactation 4 where it was not detected. Staphylococcus hyicus subsp. hyicus was isolated in lactation numbers 3, 4, & 5 & its rate of isolation increased in lactation 3 & 5 Staphylococcus simulans & Staph. intermedius were detected only in lactation 1 & 3 respectively.

Of the indentified streptococcal strains Strep. bovis constituted 24 of the total streptococcal isolates while Strep. uberis, Strep. agalactiae, Strep. dysagalactiae, Strep. lactis & Strep. sp. G made up 4, 12, 14, 2 & 4 respectively.

As shown in Table 15 the most prevalent streptococcal strain was Strep. bovis (32.5%) followed by Strep. dysagalactiae (17.5%) Strep. agalactiae (15%), Strep. uberis (5%), Strep sp. G (5%) & Strep. lactis (2.5%).

Streptococcus bovis was isolated in all lactation numbers & the rate of isolations varied from 25 to 37.5% in the different lactation numbers. Streptococcus agalactiae was isolated in all lactation numbers except lactation 4 where it was not detected. The rate of isolation was the highest in lactation 3. Streptococcus dysagalactiae was isolated from 3 lactation numbers & the rate of isolation increased in lactation 5 followed by lactation 1 & 4. Streptococcus uberis was only isolated from lactation 1 & 3. Similarly Strep. sp. G & Strep. lactis were only isolated in lactation 5 & 3 respectively.

#### Microbial Load and Relative Distribution of Mastitic

##### Bacteria in the Farms under Study

The relative differences in microbial load & distribution of mastitic bacteria in the three farms with especial emphasis on total mesophilic

Table 14 Prevalence of staphylococci & Microcococcus strains in foremilk samples from individual cows in Repi dairy farm

Species (strains)	Lactation number					Percent prevalence
	1	2	3	4	5	
<u>Staph. aureus</u>	1	1	1	3	4	25.0
<u>Staph. epidermidis</u>	7	8	6	6	5	80.0
<u>Staph. hyicus</u> subsp. <u>hyicus</u>	-	-	2	1	3	15.0
<u>Staph. hyicus</u> subsp. <u>chromogenes</u>	1	2	1	-	1	12.5
<u>Staph. simulans</u>	1	-	-	-	-	2.5
<u>Staph. intermedius</u>	-	-	1	-	-	2.5
<u>Microcococcus</u>	-	2	-	-	-	5.0

Table 15- Prevalence of streptococci strains in foremilk samples from individual cows in Repi dairy farm

Species (strains)	Lactation number					Percent prevalence
	1	2	3	4	5	
<u>Strep. agalactiae</u>	1	1	3	-	1	15.0
<u>Strep. dysagalactiae</u>	2	-	-	1	4	17.5
<u>Strep. uberis</u>	1	-	1	-	-	5.0
<u>Strep. bovis</u>	3	2	3	3	2	32.5
<u>Strep. spp. G</u>	-	-	-	-	2	5
<u>Strep. lactis</u>	-	-	1	-	-	2.5

aerobic counts and the different Streptococcus & Staphylococcus species were compared. As can be seen from Table 16 there appeared to be no significant difference in the total aerobic mesophilic counts between the three farms.

Staphylococcus hyicus subsp. hyicus was found to be extremely high at Repi, but was present in low numbers at Debre Zeit & absent in Kumbi (Table 17). In addition Staph. intermedius though absent, at Debre Zeit & Kumbi, was present at the Repi dairy farm. Staphylococcus epidermidis was found in high numbers in all the three farms.

Streptococcus bovis & Strep. uberis were found in high numbers followed by Strep. agalactiae in all the three farms. Streptococcus infrequens & Strep. lactis were found in low numbers and they were isolated only from Debre Zeit & Repi farms, respectively.

#### Antimicrobial Sensitivity Testing

The sensitivity of 55 staphylococcal & streptococcal strains isolated during the study period were tested (Table 19). All the isolates were found to be sensitive to erythromycin, cephalothin, chloramphenicol, clindamycin & vancomycin. The staphylococcal strains were resistant only to penicillin & tetracycline. Forty percent of the Staph. hyicus subsp. chromogenes & 40% of Staph. aureus were resistant to penicillin, while 20% of Staph. epidermidis & 40% of Staph. aureus were resistant to tetracycline.

All the streptococci strains were sensitive to penicillin, erythromycin cephalothin, chloramphenicol, clindamycin & vancomycin (Table, 19). Ten percent of the Strep. uberis & 20% of Strep. dysagalactiae were resistant to oxacillin. Forty percent of Strep. agalactiae were resistant to Kanamycin. The resistance of the streptococcal strains to tetracycline varied from 20 to 40%. Resistance to gentamycin was shown by 40% of

Table - 16- Arithmetic mean of the total mesophilic aerobic count on the three farms.

Lactation Number	Colony forming units/ml.		
	Debre Zeit	Kumbi	Repi
1	$2 \times 10^5$ ( $9 \times 10^2 - 2 \times 10^6$ )*	$1 \times 10^5$ ( $4 \times 10^3 - 8 \times 10^5$ )	$1 \times 10^6$ ( $5 \times 10^2 - 9 \times 10^6$ )
2	$6 \times 10^4$ ( $1 \times 10^2 - 5 \times 10^5$ )	$6 \times 10^4$ ( $4 \times 10^3 - 2 \times 10^5$ )	$7 \times 10^4$ ( $4 \times 10^2 - 4 \times 10^5$ )
3	$9 \times 10^4$ ( $2 \times 10^3 - 6 \times 10^5$ )	$5 \times 10^4$ ( $3 \times 10^3 - 1 \times 10^5$ )	$1 \times 10^5$ ( $8 \times 10^3 - 6 \times 10^5$ )
4	$9 \times 10^4$ ( $6 \times 10^3 - 3 \times 10^5$ )	$1 \times 10^6$ ( $3 \times 10^3 - 1 \times 10^7$ )	$3 \times 10^5$ ( $2 \times 10^2 - 1 \times 10^6$ )
5	$1 \times 10^6$ ( $2 \times 10^2 - 5 \times 10^6$ )	$3 \times 10^5$ ( $2 \times 10^3 - 2 \times 10^6$ )	$8 \times 10^4$ ( $1 \times 10^4 - 3 \times 10^5$ )

\* values in parenthesis indicate the minimum & maximum total mesophilic aerobic count.

Table 17

Microbial load (Range) & the relative distribution of staphylococci species in the 3 farms.

"All counts as colony forming units/ml."

Species	Debre Zeit	Kumbi	Repi
<u>Staph. aureus</u>	70-1x10 <sup>4</sup>	3x10 <sup>2</sup> - 9x10 <sup>3</sup>	1x10 <sup>2</sup> - 3x10 <sup>3</sup>
<u>Staph. epidermidis</u>	60-3x10 <sup>4</sup>	60-2x10 <sup>4</sup>	80-6x10 <sup>4</sup>
<u>Staph. hyicus</u> <del>abbpp.</del> <u>hyicus</u>	5x10 <sup>2</sup>	-	4x10 <sup>3</sup> - 2x10 <sup>4</sup>
<u>Staph. hyicus</u> . subsp. <u>chromogenes</u>	60-3x10 <sup>3</sup>	2x10 <sup>2</sup> - 2x10 <sup>3</sup>	9x10 <sup>2</sup> - 4x10 <sup>4</sup>
<u>Staph. xylosus</u>	40-1x10 <sup>3</sup>	50-1x10 <sup>2</sup>	-
<u>Staph. simulans</u>	80-1x10 <sup>3</sup>	60-2x10 <sup>4</sup>	1x10 <sup>2</sup>
<u>Staph. intermedius</u>	-	-	6x10 <sup>2</sup>

Table-18- Microbial load (Range) & the relative distribution of streptococci species in the 3 farms

"All counts as colony forming units/ml."

Species	Debre Zeit	Kumbi	Repi
<u>Strep. agalactiae</u>	3x10 <sup>3</sup> -2x10 <sup>5</sup>	2x10 <sup>3</sup> - 2x10 <sup>5</sup>	4x10 <sup>2</sup> - 2x10 <sup>4</sup>
<u>Strep. dysagalactiae</u>	80-2x10 <sup>3</sup>	6x10 <sup>2</sup> - 2x10 <sup>3</sup>	3x10 <sup>2</sup> - 5x10 <sup>3</sup>
<u>Strep. uberis</u>	2x10 <sup>2</sup> -2x10 <sup>6</sup>	1x10 <sup>2</sup> - 1x10 <sup>7</sup>	7x10 <sup>2</sup> - 9x10 <sup>6</sup>
<u>Strep. bovis</u>	90-1x10 <sup>6</sup>	80-1x10 <sup>6</sup>	2x10 <sup>2</sup> - 1x10 <sup>6</sup>
<u>Strep. faecalis</u>	3x10 <sup>2</sup> -4x10 <sup>2</sup>	3x10 <sup>2</sup> - 2x10 <sup>3</sup>	-
<u>Strep. sp. G</u>	5x10 <sup>2</sup> -9x10 <sup>2</sup>	2x10 <sup>2</sup> - 5x10 <sup>3</sup>	1x10 <sup>2</sup> - 2x10 <sup>2</sup>
<u>Strep. lactis</u>	-	-	1x10 <sup>2</sup>
<u>Strep. infrequens</u>	1x10 <sup>2</sup>	-	-

the Strep. dysagalactiae and 40 percent of the Strep. agalactiae. Resistance to streptomycin was shown by all strains of streptococci (20 to 50%).

As shown in Table 20, resistance to one drug was exhibited in 30% of the Strep. uberis. Resistance to two drugs in 30%, 40% & 20% of Strep. uberis, Strep. dysagalactiae & Strep. bovis respectively. Similarly resistance to 3 drugs was shown in 20% in each of Strep. dysagalactiae & Strep. agalactiae & to 4 drugs only in 20% of Strep. agalactiae.

Three different patterns of resistance were observed among the staphylococci strains while 8 resistance patterns were observed for the streptococci strains.

Table 19- Sensitivity tests of staphylococci & Streptococci  
to different antibiograms.

Staphylococci & streptococci strains	Number of strains (55)	Percent sensitive (susceptible) to										
		Pen.	Ery.	Oxa.	Cep.	Tet.	Chl.	Kan.	Gen.	Str.	Clin	Van.
<u>Staph. aureus</u>	10	60	100	100	100	60	100	100	100	100	100	100
<u>Staph. epidermidis</u>	10	100	100	100	100	80	100	100	100	100	100	100
<u>Staph. hyicus</u> subsp. <u>hyicus</u>	5	100	100	100	100	100	100	100	100	100	100	100
<u>Staph. hyicus</u> subsp. <u>chromogenes</u>	5	60	100	100	100	100	100	100	100	100	100	100
<u>Strep. uberis</u>	10	100	100	90	100	70	100	100	100	50	100	100
<u>Strep. dysgalactiae</u>	5	100	100	80	100	60	100	100	60	60	100	100
<u>Strep. agalactiae</u>	5	100	100	100	100	80	100	60	60	60	100	100
<u>Strep. bovis</u>	5	100	100	100	100	80	100	100	100	80	100	100

Table -20- Resistance patterns of staphylococci and streptococci strains to different antibiograms

Strains	Resistant pattern	Number of strains	%
<u>Staph. aureus</u> (10 strains)	Pen Tet	4	40
<u>Staph. epidermidis</u> (10 strains)	Tet	2	20
<u>Staph. hyicus</u> subsp. <u>Chromogenes</u> ( 5 strains)	Pen	2	40
<u>Strep. uberis</u> (10 strains)	Oxa	1	10
	Str	2	20
	Tet Str	3	30
<u>Strep. dysgalactiae</u> ( 5 strains)	Oxa Tet	1	20
	Gen Str	1	20
	Tet Gen Str	1	20
<u>Strep. agalactiae</u> ( 5 strains)	Kan Gen Str	1	20
	Tet Kan Gen Str	1	20
<u>Strep. bovis</u> ( 5 strains)	Tet Str	1	20

V. DISCUSSION

The teat duct is the first to be colonized and hence the foremilk drawn always contains organisms (Volk & Wheeler, 1980). The number of bacteria in the foremilk, however is known to fluctuate (Cousins & Bramley, 1981; Bramley et al., 1984). The occurrence of bacteria and there of infection in many instances is related to the state of the health of the host & the sanitary aspects of the environment it lives in (Jain,1979). The limited immunological capability of the mammary gland to prevent invasion is overcome when the teat end is exposed to microorganisms (Smith,1983). Any factor that increase the number of bacteria on apical teat skin will increase the incidence of the probability of gaining entrance of some organisms into the udder while factors that decrease presence of bacteria (pathogen) will reduce the incidence of infection (Jain,1979,Philpot,1979). This variability of exposure (infection) of the udder by microorganisms together with the host factor might be the cause for variability of the bacterial count in the foremilk samples analysed during this study period.

In the 3 farms, on the average, 75% of the apparently healthy cows contained total mesophilic aerobic bacterial count (TMABC) less than  $1 \times 10^5$  colony forming units (cfu)/ml & 25% had greater than  $1 \times 10^5$  cfu/ml in their foremilk. Bacic et al., (1968), on the other hand, reported that 95% & 5% of the healthy cows had TMABC less than  $1 \times 10^5$  cfu/ml & greater than  $1 \times 10^5$  cfu/ml respectively in their foremilk. There were more cows with counts greater than  $1 \times 10^5$  cfu/ml in these farms than those reported by Bacic et al., (1968). The bacterial content (count) of raw milk also increase tremendously when cows are infected with mastitic organisms (Cousins & Bramley, 1981; Bramley et al., 1984). The high bacterial count (greater than  $1 \times 10^5$  cfu/ml) in the 3 farms was mainly associated with large number of

streptococcal species in the foremilk (Table 2, 5 & 8). Cousins & Bramley, (1981) & Bramley et al., (1984) also came to a similar conclusion when they were studying the udder disease & bacterial content of raw milk.

The foremilk when compared with the main (mid) milk, is small in volume & also contains higher bacterial count. The TMABC of the foremilk is 6.5 x greater than the mid (main) milk in healthy cows (Bacic et al., 1968). Bacterial counts greater than  $1 \times 10^7$  cfu/ml in milk from infected udder does increase the bulk milk count significantly (Cousins & Bramley, 1981). In all the farms there were only few cows with counts approaching or equal to  $1 \times 10^7$  cfu/ml, hence their contribution to the total bacterial count in bulk milk collected from all cows is assumed to be low. Mesophilic aerobic bacteria should not be recovered from raw milk used for manufacturing dairy products in a number exceeding  $2 \times 10^6$ /ml (Refai, 1979). This is because of the contribution of high bacterial count to poor keeping quality & inferior product originating from the milk (Law, 1979).

There were more cows (66.7%) in lactation 5 with TMABC equal to or greater than  $2 \times 10^4$  cfu/ml than in lactation 4 (58.3%) in the farms. This decrease in TMABC during the early lactation number were also observed by Bacic (1968). Age and stress of milking lead to loss in the effectiveness of the streak canal as a barrier to bacteria (Schalm, et al., 1971). Hence the increase in the number of cows that had high TMABC count in this study, as lactation number increased could be ascribed to the gradual loss of teat patency with increasing age.

Among the streptococcal species, Streptococcus agalactiae, Strep. dysagalactiae, Strep. uberis, Strep. bovis, Strep. sp. G, Strep. infrequens & Strep. lactis were isolated from the dairy farms. Their presence in the milk of cows is not unusual. Buxton & Fraser (1974) & Newbould (1984), have reported findings similar to this one, previously.

Even though Strep. agalactiae, Strep. dysagalactiae & Strep. uberis, mostly associated with infected udders, Strep. bovis, Strep. faecalis, Strep. sp. G & Strep. infrequens have also been isolated from mastitic cows occasionally (Buxton & Fraser, 1977; McDonald, 1979; Philpot, 1979; Wilson & Richards, 1980; Rangasamy et al., 1983; Newbould, 1984).

Of the streptococcal species Strep. bovis was the most predominant isolate. It was responsible for most of the high bacterial counts (Table 1, 2, 4 & 6). This bacterium was detected in all of the lactation numbers in the 3 farms (Table 2, 5 & 8). Strep. bovis inhabits the gastro intestinal tract of cattle (Buxton & Fraser, 1977; Gillespie & Timoney, 1981). Because of fecal contamination of the udder, this organism is usually detected in milk (Gillespie & Timoney, 1981). That must be why it was found in such greater numbers in all milk samples analysed from the 3 farms. Strep. uberis was also responsible for high bacterial counts in all the farms (Table 18). Bacterial counts greater than  $1.5 \times 10^6$  cfu/ml was reported from the clinical cases of Strep. uberis mastitic infection (Neave, 1975). However, Strep. uberis counts greater than or equal to  $2 \times 10^6$  cfu/ml have been recorded in the 3 farms in milking cows. Nevertheless, these cows did not show overt clinical symptoms of mastitis. This bacterium can survive & multiply in extramammary sources of the cow (Bramely, 1982). It also colonizes the external orifice of the streak canal and multiplies in the udder (Cousins et al., 1980). The degree of infection due to Strep. uberis has been found to be variable. This has been associated with its ability to survive and grow at different sites outside the mammary gland and in the surrounding environment (Bramely & Dodd, 1984).

As can be seen in Table 11, 13 & 15, the number of lactating cows that had mastitis causing environmental pathogens (bacteria) (Strep. uberis, Strep. bovis & Strep. faecalis) in their foremilk was higher in Kumbi &

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Debre Zeit than in Repi farm. Escherichia coli was only detected in Debre Zeit farm (Table 10). All these four bacterial commonly inhabit the gastro intestinal tract of cattle (Buxton & Fraser, 1977; Bramley, 1982; Kruze & Bramley, 1982, Jackson & Bramley, 1983). Freshly composed dairy waste solids & straw are potential reservoirs of environmental pathogens including other mastitis pathogens (Bishop et al., 1981). Straw, urine & faecal material are particularly good sources for survival & multiplication of Strept. uberis and E. coli (Sharma & Packer, 1970; Rendos et al., 1975; Kruze & Bramley, 1982; Bramley, 1982). The environmental condition that was observed in Debre Zeit & Kumbi dairy farms appeared to be conducive for the survival & frequent contamination of the udder with urine, fecal material & straw.

At Repi, the urine & faeces are drained into a central drain continuously. The concrete floor in the shed is also washed with water regularly. The floor of the shed is smooth and slopes towards the central drain. As a result the floor is kept clean. Contrary to this, at Debre Zeit & Kumbi farms, straw is being used as bedding material & the faecal material & urine usually fall on their bedding. The urine, faeces together with straw are removed from the bedding using spades. Care is not taken to remove all material, hence it is common to see cows spoilt with faeces urine & straw bedding sticking to them.

The staphylococcal species isolated from the 3 farms during the study period were Staph. aureus, Staph. epidermidis, Staph. hyicus subsp. hyicus, Staph. hyicus subsp. chromogenes, Staph. xylosum, Staph. simulans, Staph. intermedius & Staph. warneri/ Staph. hominis/ Staph. haemolyticus group. Except for Staph. intermedius these bacteria have also been reported by Harvey & Gilmour (1985) from herd bulk milk of cows.

Mastitic staphylococci were more prevalent than mastitic streptococci

(Table 2, 5 & 8). Bishop et al., (1980) studying clinical cases of mastitis also found staphylococci as the most prevalent. Among the staphylococcal species, the population of Staphylococcus epidermidis was high in the foremilk of cows collected from the 3 farms. Similar findings were also reported by (Rangasmy et al., 1983, Devriese & Dekyser, 1984; Brolund, 1985). At any one lactation, one finds Staph. epidermidis as the most prevalent species in the 3 farms (Table 10, 12 & 14). The difference in lactation number with respect to Staph. epidermidis is less accentuated than for major pathogens (Blackburn, 1968).

Staphylococcus epidermidis occurs as a commensal on the skin (udder) of cattle, the skin of man & also in the environment of the cow byre & milk equipment where they remain viable for many days or weeks (Buxton & Fraser, 1977; Nwufoh & Amkori, 1981; Rangasamy et al., 1983). As a result, there could be a greater probability for the udder of a cow to be colonized (infected) by the bacterium. This may be the reason for the frequent detection (isolation) of Staph. epidermidis during the different lactation numbers.

Staphylococcus aureus was the most dominant bacteria next to Staph. epidermidis (Table 10, 12 & 14).

The prevalence of Staph. simulans, Staph. xylosus & Staph. hyicus subsp. chromogenes was found to be much lower than that of Staph. epidermidis. Devriese & Dekyser (1984), studying the prevalence of coagulase negative staphylococcal species in milk found the afore mentioned species to represent only a small fraction of the total staphylococcal population. In the same study (Devriese & Dekyser, 1984) Staph. hyicus subsp. chromogenes was found in cows milk & on teat swab samples whereas Staph. hyicus subsp. hyicus was found only on teat swabs & not in milk. Unlike their findings, Staph. hyicus subsp. hyicus was found in milk at Debre Zeit & Repi dairy farms.

The two major mastitis causing organisms isolated were Staph. aureus and Strep. agalactiae. In general, these bacteria are often responsible for causing mastitic conditions in dairy cows & as a result they are commonly isolated from infected udders (Griffin, 1977; Wilson & Richards, 1980; Rangasamy et al., 1983, Brolund, 1985). Jain (1979), state that Strep. agalactiae cannot survive outside the udder. The usual transmission of Staph. aureus & Strep. agalactiae is through cross contamination from infected to non-infected udders through the radiation of incompletely disinfected hands during the milking process (McDonald, 1979). Hand disinfection and post-milking teat dipping is an effective hygienic method employed for the prevention of new infections by the most common pathogens with the exception of some which survive in the environment for a long time (Neave et al., 1969). Post milking teat dipping usually reduces the rate of intramammary infection by 50% (Dodd, 1983). Hand disinfection & post milking teat dipping was not practiced regularly, though, chemicals were available at the farms. Hence the lack of hygiene in collecting the milk could be one of the reason for the occurrence of Staph. aureus, Strep. agalactiae & other mastitic causing pathogens (bacteria) in all (most) of the lactation numbers on the farm/s .

Treatment (therapy) was only given to clinical cases of mastitis during lactation or dry period. Since regular monitoring of mastitis is not carried out on the farms subclinical cases were not identified. Therefore, they remained as constant sources of infection to other cows. If therapy is to make the desired contribution to the control of mastitis, it is necessary to treat subclinical as well as clinical cases (Philpot, 1979).

These mastitic bacteria are also responsible for food poisoning and/or infections. Raw milk is still consumed in large quantities in Ethiopia. In

addition, since heat treatment is not employed to milk intended for yoghurt, butter etc production, the bacteria can survive and grow in these milk by-products. Hobbs & Christian (1975), recommended that milk intended for human consumption should not contain Strep. agalactiae or Staph. aureus. The bacterial count of Staph. aureus in this study ranged from 70 to  $1 \times 10^4$  cfu/ml. This number can increase to  $1 \times 10^7$  cfu/ml or more within 6 hrs (James, 1986). It has been suggested that  $0.5 - 1 \times 10^6$  staphylococci/gram of food must be present to produce symptoms in man (Kaplan et al., 1962).

Escherichia coli has been detected only at Debre Zeit farm. Eberhart et al., (1979) stated that coliform bacteria appear poorly adapted to survival on skin, & colonization of the skin or teat canal by this bacterium is uncommon.

The major mastitic causing organisms (Staph. aureus, Strep. agalactiae, Strep. dysagalactiae & Strep. uberis) were either present in high numbers or were isolated more frequently during the latter lactation numbers (Table 11, 12, 13, 14, 15). These major mastitis causing organisms were also isolated more frequently at latter lactation numbers by other researchers, (Oliver, et al., 1956; Belackburn, 1968; Brolund 1985). The increase in isolation rate of the major mastitis causing bacteria with increases in lactation number could be due to cumulative infection (Brolund, 1985). The normal streak canal which serves as an effective barrier to bacterial penetration during the first few lactations appears to decrease in efficiency with increasing incidence of udder infection as the lactation number increases.

The resistance of staphylococci to various antibiotics showed that penicillin & tetracycline were relatively ineffective to some strains of Staph. aureus, Staph. epidermidis & Staph. hyicus subsp. chromogenes respectively (Table, 19). The frequency of penicillin resistant Staph. aureus

strains in this study was 40%. Nygard et al., (1968) from Norway, Badenhorst (1977) from Zimbabwe, Rangasamy et al., (1983) from Mauritius reported 7.3, 73 & 71.4% resistance to penicillin respectively. Similarly the frequency of resistant Staph. aureus strains to tetracycline in this study were 40%. Nygard, (1968) from Norway, Badenhorst (1977) from Zimbabwe reported 6 & 36% resistance to tetracycline. The two antibiotics (penicillin & tetracycline) are commonly used to treat clinical cases of mastitis. This might indicate overuse of this antibiotics.

In the case of streptococcal species (Table 19), 30% of the Strep. uberis were resistant to tetracycline in this study. A frequency of resistance (17%) was reported by Badenhorst (1977) from Zimbabwe. Streptomycin was relatively ineffective to some strains of Strep. uberis, Strep. agalactiae, Strep. dysagalactiae & Strep. bovis. Previous reports indicate that mastitis causing streptococcal species are the least sensitive to streptomycin (Wright, 1977, Bishop, et al., 1980).

Nowadays antibiotics have been used on a large scale for treatment of mastitis. The unrestricted use of these drugs has led to the selection of some resistant variants of microorganisms. Among these microbes are certain strains of staphylococci that cause mastitis in cattle (Goda, 1976). The extensive use of antibiotics for treating clinical mastitis has not had a major effect on prevalence of udder infection in most dairy herds. A limiting factor is that only about 40% of the infection can be detected during a 12 month period by abnormalities in milk. Most clinical cases appear to respond to treatment as evidenced by the disappearance of clinical symptoms. Unfortunately many of the infections are not eliminated but continue to persist at subclinical levels (Bishop et al., 1980). Furthermore, the improper & indiscriminate use of

antibiotics for the treatment of mastitis has resulted in antibiotic resistant strains that increase the incidence of mastitis in the herd (WHO,1970). A good management procedure in milk collection, therefore, should not be compromised by the use of antibiotics indiscriminately.

VI. CONCLUDING REMARKS AND  
RECOMMENDATIONS

1. Mastitis causing bacteria (pathogen) have not been eradicated nor will they ever be, but considerable control is now possible by adopting specific management methods. The many vectors for these organisms include the environment bedding, & milkers hands. In the dairy farms the housing methods concentrate the cows & increase exposure to faecal bacteria (pathogens), those that can multiply in bedding material & others.

The following hygienic techniques should be applied regularly at milking time in order to reduce exposure of teats to mastitis causing bacteria.

- a- Keeping stalls clean, & reducing the amount of housing time.
  - b- Segregation of infected animals & also milking them last.
  - c- Teat washing with disinfected water & drying with individual towels.
  - d- Teat disinfection after milking.
  - e- Hand disinfection between milking.
2. The emergence of resistant strains in the farms, is an indication of the **wide** use of antibiotics. Before the use of antibiotics, it is necessary to identify the bacteria (pathogen) and use the appropriate antibiotic(s). The improper & indiscriminate use of antibiotics only increases the spread of resistant strains in the herd.
  3. Generally, a high cell count in milk indicates that the udder is infected. But it is not known whether high cell and bacterial counts are related one way or the other. Therefore studies should be carried out to determine the relationship that might exist between the two in milk samples.
  4. Certain Staph. aureus strains in milk are known for their enterotoxin production. Since the enterotoxin causes illness further study should be

carried out in milk.

5. There are no veterinarians stationed at the dairy farms & hence the mechanism of identifying the infected from the non infected cows on a clinical basis is lacking. Because of this there appears to be an easy transmission of mastitis causing organism in the herd. Hence the presence of a veterinarian who checks the overall health of the cows and specially the udder is essential.
6. Regular medical examination of dairy workers is not carried out in the farms. Workers suffering from bacterial sore throats, nose, aural & skin infections can contaminate the milk with the pathogens, and also inoculate the udder with the organisms. Checking should be carried out in the workers.
7. The dairy barn must be spacious, dry, well ventilated & easy to clean. An easily accessible clean water supply must be present. Flies & rats must be effectively dealt with.

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