MICROBIAL QUALITY AND SAFETY OF RAW MILK WITH REFERENCE TO SOURCES OF CONTAMINATION

GINI GEORGE

Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

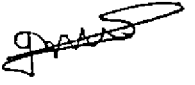
Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2007

Department of Veterinary Public Health
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

DECLARATION

I hereby declare that the thesis entitled **"MICROBIAL QUALITY AND SAFETY OF RAW MILK WITH REFERENCE TO SOURCES OF CONTAMINATION"** is a record of research work done by me during the course of research and this thesis has not previously formed the basis for the award of any degree, diploma, fellowship or associateship or other similar title, of any other University or Society.



Mannuthy
30/05/07.

GINI GEORGE

CERTIFICATE

Certified that the thesis entitled **"MICROBIAL QUALITY AND SAFETY OF RAW MILK WITH REFERENCE TO SOURCES OF CONTAMINATION"** is a record of research work done independently by **GINI GEORGE** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Mannuthy
30/05/07 .

Dr. E. Nanu

(Chairman, Advisory Committee)
Professor and Head,
Department of Veterinary Public Health,
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur-680651.

CERTIFICATE

We, the undersigned members of the Advisory Committee of **GINI GEORGE.,** a candidate for the degree of Master of Veterinary Science in Veterinary Public Health, agree that the thesis entitled **"MICROBIAL QUALITY AND SAFETY OF RAW MILK WITH REFERENCE TO SOURCES OF CONTAMINATION"** may be submitted by Gini George., in partial fulfillment of the requirement for the degree.

Dr. E. Nanu
(Chairman, Advisory Committee),
Professor and Head,
Department of Veterinary Public Health,
College of Veterinary and Animal Sciences, Mannuthy.

Dr. G. Krishnan Nair
Associate Professor and Head,
Department of Veterinary Micrbiology,
College of Veterinary and
Animal Sciences, Mannuthy.
(Member)

Dr. M. Mukundan
Associate Professor and Head,
Department of Dairy Science,
College of Veterinary and
Animal Sciences, Mannuthy.
(Member)

Dr. P.I. Geevarghese
Associate Professor and Head,
K.A.U Dairy Plant,
College of Veterinary and
Animal Sciences, Mannuthy.
(Member)

External Examiner

ACKNOWLEDGEMENT

I hereby convey my profound thanks to ***Dr. E. Nanu,*** *Professor and Head, Department of Veterinary Public Health and Chairman of the Advisory committee who spared no pains in extending his helping hand and for invaluable guidance, constant encouragement and creative suggestions during the entire course of this work. I reckon it a rare privilege to work under his counsel and indomitable spirit.*

I owe my sincere gratitude to ***Dr. M. Mukundan,*** *Associate professor and Head. Deparment of Dairy Science, for his valuable guidance, timely help, moral support and affection rendered during the entire period of research work.*

I remember with great sense of gratitude ***Dr. G. Krishnan Nair,*** *Associate professor and Head, Department of Veterinary microbiology, Mannuthy, for his encouraging advices, whole hearted help, patient guidance and moral support.*

I am cordially obliged to ***Dr. P. I. Geevarghese,*** *Associate Professor and Head, K.A.U Dairy Plant, for the supporting attitude, critical comments, pleasant cooperation and help rendered to me as a member of my advisory committee.*

I am grateful to ***Dean,*** *College of Veterinary and Animal Sciences, Mannuthy, and Kerala Agricultural University for the facilities provided for the conduct of this research work.*

I would like to place on record my heartfelt thanks to ***Dr. Satyanarayana Rao,*** *for the encouraging advices and inimitable help.*

I remember with great sense of gratitude ***Dr. B. Sunil,*** *Associate professor, Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy, for his encouraging advice, whole hearted help, patient guidance and moral support without which the work might have not been completed.*

Bouquets of special thanks are due to ***Dr .C Latha, Dr. M. Mini*** *and* ***Dr. T.V. Aravindakshan*** *for their concern and support.*

I greatfully acknowledge ***Smt. K.S. Sujatha, Smt. K.A. Mercy*** and ***Miss. Jaisy Thomas*** *for the help rendered in statistical analysis.*

I am obliged, thankful and grateful to my beloved friend and colleague ***Dr. Asha. K*** *for her support, encouragement, advice and incessant help at the time of difficulties during various stages of my work.*

I am in short of words to express my deep sense of gratitude to my seniors ***Dr. Prejit, Dr. Praseeda, Dr.Jaiby. K and Dr. Lekha chacko. K.*** *Their immense support and constant encouragement have helped me to successfully complete the research work.*

No words or deeds are sufficient to express my gratitude to ***Dr. Magna Dr. Siji P.C, Dr. Vivek, Dr. Praveena, Dr. Aswathi*** *and* ***Dr. Vinod*** *for all the incessant support, continuous guidance they have showered on me.*

I cherish the spirit of understanding and personal encouragement rendered to me by my friends ***Dr. Siji, Dr. Biju, Dr. Bagya Lekshmi and Dr.Deepa Mary***

I also thankful to ***Mr. Sundaran, Mrs. Suhara, Mrs. Priya, Mrs. Bindu Mr. Prashant, Mr. Dhanesh, Mr.Sandeep, Mr. Sivasankaran, Mrs.Mary*** *and* ***Mr.Achudhanadhan*** *for the co-operation rendered to me during my study.*

Bouquets of special thanks are due to ***Dr. Ambili K.S,*** *Liberararian for the help rendered.*

A special thanks to the Joint Director and Head, National Salmonella and Escherichia Centre, ***Dr. K.A. Mani*** *Central Research Institute, Kasauli for serotyping the E. coli isolates and sending the results on time.*

I greatfully acknowledge ***Mr. Chandrasekharan****, Asociate Professor and Instrumentation engineer and* ***Miss. Annie Thomas*** *and* ***Liji****, ARIS cell for their lots of help rendered.*

A note of special thanks to farmers and members of co-operative societies for their untiring help and cooperation.

No phrase or words in any language can ever express my deep sense of gratitude to my beloved ***Daddy, Mammy, Chechi, Chetan, Ammachi, Appachan, Sister in law*** *and* ***Alan*** *being always with me through thick and thin.*

I am deeply touched by the understanding, love, care, support and constant encouragement by my life partner ***Mr. Vineesh****.*

I gratefully remember all those who directly and indirectly helped in the preparation of thesis.

Above all I bow before ***God, the Almighty*** *for the blessing showered on me.*

GINI GEORGE

CONTENTS

LIST OF TABLES

LIST OF FLOW CHARTS

LIST OF FIGURES

Introduction

1. INTRODUCTION

In India, dairying plays an important role in the national economy and the socioeconomic development of the country by contributing significantly to the employment and income to millions of people in both urban and rural areas but also nutritional security to the people. In 2001, India became the world leader in milk production with a production volume of 84.6 million tonnes. India's milk production increased from 21.2 million tonnes in 1968-69 to 94.5 million tonnes in 2005 to an anticipated 100 million tonnes in 2010, with the annual growth rate of 4 per cent against the world's one per cent level (Gupta 2007).

Despite being the largest milk producer, the per capita availability of liquid milk is woefully low at about 240 g per day in 2005-06, up from 112 g per day in 1968-69, which was still lower than the world average of 285 g per day and 283 g per day as recommended by World health Organization (Gupta 2007). However, the per capita availability of milk in Kerala is only 173 g per day, which is much lower than the national average. In the Indian context of poverty and malnutrition, milk has a special role to play for its many nutritional advantages as well as providing supplementary income to millions of farmers.

Dairying in India is the major source of income to the poor sector of the society and also many people rearing livestock as a source of supplementary income. Initial microbial quality of milk produced has paramount importance and deserves vital consideration because it forecasts the final product quality. The Indian dairy products have good demand globally but the failure to meet the quality standards diminishes its value in world market. About 85 per cent of India's milk production lies in the unorganized sector and many factors like lack of awareness, our climatic conditions, lack of organized milk production and collection, restricted facilities for refrigerated transportation and storage of milk etc. lead to the deterioration of the quality milk produced.

A variety of microorganisms may gain access into milk from different sources *viz.*, unclean animal, milker's hand, utensils, water, air and other conditions prevailing during the production and various stages of handling of milk and causes both milk borne illness and reduction of shelf life of the product. The saprophytic organisms entered by these ways may result in the reduction of shelf life and/or spoilage of milk, which results in economic loss to the farmers and/or milk traders.

Since milk possesses almost all essential nutrients, it can also serves as a potential vehicle for transmission of many diseases by directly transmitting the pathogen or indirectly through its metabolites. Microbiological health hazards' arise from the consumption of contaminated food like milk has grown in recent years and has resulted in national and international intensification of food hygiene programme.

The term "quality" of raw milk is extremely comprehensive and encompasses every trait of importance like quality of content and its physico-chemical condition, hygienic quality which includes bacteriological quality, absence of pathogens and other contaminants, sensory quality, nutritional quality and technological quality (processing ability). To achieve the global perspective of quality assurance, hygiene in all aspects of milk production, handling, transportation and processing by implementing an effective HACCP system are all primary concerns in hygienic milk production.

In view of the immense dietary importance of milk, the need for production of safe, clean and wholesome milk is emphasized. Moreover, in the present days consumers are more health conscious and more concerned about the quality of the product. Emphasis and great care must be excised on clean milk production from healthy animal and should take all precaution from preventing the microbial contamination.

Considering all the above facts, the present study was undertaken with following objectives,

1. To assess the microbial quality of raw milk both from the farmer and society level.

2. Isolation and identification of *Escherichia coli, Staphylococcus aureus* and *Yersinia.*

3. Molecular characterization of *Escherichia coli* using Polymerase Chain Reaction (PCR) technique.

4. To estimate bacterial load of the samples of water, hand wash of the milk handlers and rinsing of utensils from each co-operative society and the samples of water, udder wash, rinsing of utensils and hand wash of the milker collected prior to milking/ milk handling.

Review of literature

2. REVIEW OF LITERATURE

2.1 MICROBIAL COUNTS OF MILK

2.1.1 Total Viable Count

Vijai and Saraswat (1968) examined the bacterial quality of 224 raw market milk samples. The samples collected from rural collection centers (69), city vendors and retailers (70) and mixed herd milk produced at the College of Agriculture Dairy farm (85) in Udaipur. Twenty samples from the later source were produced in controlled and strict clean conditions. The mean standard plate counts of the samples obtained from rural collection centers were 60,00,000 per ml while the count of the samples belonging to city vendors and retailers was 30,00,000 per ml. The samples produced under controlled and strict clean conditions had a mean count of 23,000 per ml and the routine samples had mean count of 18,00,000 per ml.

Davies (1977) analysed the bacterial quality of 2090 milk samples collected from bulk milk supplies in Wales. Frequency distribution of total colony count showed that 19.4 per cent of samples had count less than 10,000/ml, 67 per cent with count less than 50,000/ml, 77.3 per cent with count less than 1,00,000/ml, 97.4 per cent with count less than 10,00,000/ml and 5.4 per cent with counts of 5,00,000/ml or more. The average count of bulk milk samples for the year was just over 33,000/ml.

Garg *et al.* (1977) assessed the bacterial flora of 102 raw milk samples of cows and buffalo obtained from Hissar city. The samples consisted of 57 from cow and 45 from buffalo and were collected during summer and winter months. The standard plate count per ml of cow milk samples collected during summer was in the range of 4 x 10^5/ml to 2 x 10^8/ml and the corresponding count of the samples collected during winter was in the range of 5.4 x 10^5 to 4 x 10^7/ml.

Desai and Natarajan (1981) tested the bacterial quality of raw milk samples procured from three areas *viz.*, A, B and C located around Bangalore city. Pooled milk samples were collected from five, 25 and 13 societies belonging to areas A, B and C, respectively. From each society 5 samples were collected. The average standard plate count of the samples collected from the societies in the areas A, B and C were 205 x 10^5, 441x 10^5 and 92 x 10^5/ml, respectively.

Yadava *et al.* (1983) studied the bacterial quality of 105 milk samples marketed in Ranchi. Of the samples, 42 raw milk samples were collected from Dairy unit, Ranchi Veterinary College (RVC) and 41 from local vendors in Ranchi. The average standard plate count of milk from dairy unit and local vendors during monsoon was 4.08 x 10^5 and 74.20 x 10^5/ml, respectively. The corresponding count of the samples collected from the sources during winter was 0.56 x 10^5 and 12.08 x 10^5/ml.

Reddy *et al.* (1984) studied the bacterial quality of 30 samples of raw milk obtained from Vijayawada milk shed. The standard plate count of the samples ranged from 0.77 to 29.40 million/ml with a mean count of 7099 x 10^3/ml. A high correlation (0.74) was observed between the bacterial count of raw milk and count of dried whole milk made from it.

Yadava *et al.* (1985) analysed the pathogenic bacterial flora of 105 milk samples marketed in Ranchi town. During the study, 42 raw milk samples were collected from organised dairy farm and 41 samples from local vendors. The average total viable count of samples from the former source was 2.23 x 10^5/ml and the count of the samples from the later source was 40.38 x 10^5/ml.

Das and Nag (1986) evaluated the bacterial quality of 162 raw milk samples from vendors in Calcutta. The mean standard plate count of samples collected during

summer was 136 million per ml and the count of samples collected during winter was 61 million per ml.

Misra and Kuila (1989) conducted a study to estimate various groups of bacteria and quality of milk produced and distributed in Calcutta and its suburbs. A total of 125 sample of raw milk, consisting of 15 from organised dairy farm, 60 from city vendors and 50 from sweet meat shops were analysed. The study revealed that the samples from organised dairy farm, city vendors and sweet meat shops had average standard plate counts of 51 x 10^4, 71.73 x10^5 and 72.73 x 10^5 cfu/ml, respectively.

Rajmany *et al.* (1989) evaluated the occurrence of Staphylococci in 20 samples each of raw milk, khoa, curd, ice cream, sweetened condensed milk, milk powder and processed cheese, sold in Udaipur market. The total bacterial count in raw milk samples varied from 11.6 x 10^6 to 98 x 10^6cfu/ml, with an average count of 53.4 x 10^6 cfu/ml.

Rai *et al.* (1990) analysed the quality of milk from four sources, *viz.*, C.S.A. University Dairy farm (A), Milk Board (B), Hawkers (C) and Town Dairies (D) in the month of January to March. Milk from source B was pasteurized. The average Total Plate Counts of samples from source A, C and D were 295.00 x 10^4, 1142.00 x 10^4 and 429.12 x 10^4 cfu/ml, respectively.

Sakkarvarthi *et al.* (1990) evaluated the bacteriological quality of five samples each of raw cow milk and buffalo milk of organised sector and buffalo milk from unorganized sector. The total viable count of cow milk samples varied from 1.38 x 10^6 to 50 x 10^6/ml. The corresponding count for buffalo milk samples from organized sector varied from 1.2 x 10^6 to 66 x 10^6/ml. Buffalo milk samples from unorganized sector had the count in the range of 1.0 x 10^6 to 197 x 10^6/ml.

Rahman *et al.* (1992) investigated the bacterial flora of 83 samples of raw milk obtained from Guwahati city and reported that the Standard Plate Count/ml of milk varied from $1.5x10^4$ to $3.6x10^7$.

Siva *et al.* (1993) collected 32 samples of raw cow milk from individual producers, collection centers and reception dock of dairy plant and evaluated the microbiological status at various stages of collection. Average total plate count of milk samples collected from the individual producers, collection centers and reception dock of dairy plant was $1 \pm 4.15 \times 10^6$, $9.1 \pm 1.6 \times 10^6$ and $17 \pm 0.43 \times 10^6$ cfu/ml, respectively.

Singh *et al.* (1994 b) analysed the sanitary quality of 70 samples of raw milk collected from different cans for distribution to Pantnagar. Standard plate count in the samples ranged from 4.477 to 8.857 $\log_{10}$ cfu/ml with a mean count of 7.30 $\log_{10}$ cfu/ml.

Kapre (1995) studied the microbial quality of 84 milk samples consisting of 28 each from University Livestock Farm, Mannuthy (S_1), Ollukkara Ksheera Vyavasaya Co-operative Society (S_2) and Panancherry Ksheera Udpathaka Sahakarana Sangam (S_3). From each source, 21 individual samples and seven pooled milk samples were collected. The mean standard plate counts of individual samples from S_1, S_2 and S_3 were 7.5×10^4, 1.4×10^5 and 2.0×10^5 cfu/ml, respectively. The corresponding counts of pooled milk samples from S_1, S_2 and S_3 were 4.0×10^4, 1.8×10^6 and 2.1×10^3 cfu/ml, respectively.

Matta and Punj (1996) examined 30 raw milk samples from different local dairy sources, to isolate and identify the presence of aerobic spore forming bacteria and reported that the total viable count (TVC) of 30 raw milk samples ranged from less than 5×10^4 to more than 4×10^6/ml.

Mutukumira *et al.* (1996) evaluated the chemical and microbial quality of 10 samples of bulk raw milk delivered to Nharira/Lancashire milk collection center, Zimbabwe, by 34 dairy producers over six months. The total aerobic counts of the samples ranged between 6.2×10^3 and 7.8×10^7 cfu/ml. Seven out of the ten samples had count less than 10^5 cfu/ml, whereas in three samples the count was more than 5.01×10^5 cfu/ml.

Garg and Mandokhot (1997) analysed the quality of 86 samples of raw milk consisting of 67 samples from local vendors, six from vendors of organised dairy unit and 13 from local milk plant. The standard plate count of the samples ranged between 7×10^4 and 2×10^{10}/ml. High standard plate count (over 5×10^6/ml) in majority of milk sample indicated poor hygienic practice followed at dairy farms in the region.

Jolly *et al.* (2000) evaluated the bacteriological quality of 60 raw milk samples obtained from three sources, *viz.* A, B and C located in and around Mannuthy. From each society, 10 each of pooled and individual samples were collected. The mean total viable counts of pooled milk samples from sources A, B and C was 6.06 ± 0.11, 6.78 ± 0.26 and 6.04 ± 0.10 $\log_{10}$ cfu/ml, respectively with an overall mean count of 6.30 ± 0.20 $\log_{10}$ cfu/ml. The corresponding counts for individual samples from the sources was 5.93 ± 0.05, 6.12 ± 0.23 and 6.2 ± 0.12 $\log_{10}$ cfu/ml and the samples had an overall mean count of 6.08 ± 0.02 $\log_{10}$ cfu/ml. The mean of total viable count of both pooled and individual samples varied significantly between sources.

Oliveira *et al.* (2000) evaluated 16 lots of raw milk to assess the relationship between microbial characteristics of raw milk and the quality of high-heat whole milk powder made from it. The mesophilic count of raw milk samples ranged from 8.2×10^4 to 6.9×10^7 cfu/g, with an average count of 8.1×10^6 cfu/g. Significant positive correlation was observed between the mesophilic count of raw milk and milk powder.

Gopi *et al.* (2001) examined the microbial quality of 12 brands of commercial pasteurized and homoginised milk sold by private vendors in Chennai city. The average standard plate count of these brands varied from 5.5 to 175.17 x 10^4 cfu/ml. The study revealed that more than 94 per cent of samples were of poor quality, compared to BIS standards.

Homhual and Jindal (2001) assessed the microbial quality of 95 raw milk samples and reported that the standard plate count of the samples was in the range of 6.5 x 10^4 to 1.2 x 10^8 cfu/ml.

Khalilur *et al.* (2002) evaluated the microbiological quality of 36 samples comprising six samples of raw milk, nine samples of pasteurized milk, nine samples of vegetables and 12 samples of fruit juices collected from local markets in Aligarh city. The total viable count of raw milk samples ranged from 15,900 x 10^6 to 2,59,000 x 10^6 cfu/ 100ml with mean count of 98,500 x 10^6 cfu/ 100ml ($\log_{10}$ count = 10.99).

Lues *et al.* (2003) assessed microbial quality of milk samples obtained from 60 randomly selected households in the Botshabelo Township, South Africa. The study revealed that the samples had a mean total aerobic mesophilic count of 8.6 x 10^8 cfu/ml.

Raj *et al.* (2003) studied the microbial quality of 40 raw milk samples consisting of 10 samples each from two milk marketing societies (A and B) and also milk from hand milked (C) and machine milked (D) animals of live stock farm, Kerala Agricultural University. Samples of the sources A and B were collected from farmers who brought milk to the society, whereas samples of C and D were collected from individual animals. The mean total viable counts of samples of sources A, B, C and D were 4.96 ± 0.14, 5.09 ± 0.24, 4.13 ± 0.13 and 4.76 ± 0.17 $\log_{10}$ cfu/ml, respectively. Significant difference was observed between the counts of samples from different sources.

Aaku *et al.* (2004) analysed the microbiological quality of 129 milk samples consisting of 43 samples of pooled raw milk and 86 commercial pasteurized milk samples from two processing plants (A and B) in Gaborone, Botswana. The mean total mesophilic counts of pooled raw milk from A and B sources were 3 x 10^7 and 1 x 10^6 cfu/ml, respectively and the counts of pasteurized sample were 7 x 10^3 and 1x 10^4 cfu/ml, respectively.

Chye *et al.* (2004) investigated the microbial quality of 930 raw cow milk samples collected from 360 farmers belonging to southern, central, eastern and northern regions of Peninsular Malaysia. The average total plate counts in samples from the above regions were 14.0x10^6, 8.2 x10^6, 18.0 x10^6 and 8.6 x10^6 cfu ml^{-1}, respectively.

Kessel *et al.* (2004) examined 861 bulk milk samples obtained from the United States to determine the prevalence of *Salmonella*, *Listeria monocytogenes* and faecal coliforms in bulk tank milk. The standard plate count of 419 samples was estimated. The count ranged from 102 to more than 1x10^5cfu/ml. The count of 48 per cent of the samples fell with in 1000-4999 cfu/ml and 40 per cent samples had the count above 5000 cfu/ml. Approximately, 12 per cent samples had count less than 1000 cfu/ml. The standard plate count limit of Grade A milk in the US is 1,00,000 cfu/ml and 29 (6.9 per cent) samples exceeded the limit.

Prejit (2005) investigated the bacteriological quality of 296 milk samples consisting of raw milk (60), milk at various stages of pasteurization (60), pasteurized milk during storage (120) and retail milk samples (56). Among raw milk samples, 20 each were collected from individual animals and pooled raw milk from Livestock Farm, Kerala Agriculture University. Another 20 chilled raw milk samples were collected from Kerala Agriculture University Dairy plant. The average total viable count of raw milk from individual animals, pooled milk and chilled milk was 5.14 ± 0.13, 5.58 ± 0.14 and 5.70 ± 0.13 $\log_{10}$ cfu/ml, respectively.

Chacko (2006) analysed the microbiological quality of 108 raw milk samples consisting of 6 individual milk samples each from six farmers belonging to three societies *viz.*, S_1, S_2 and S_3 of Thrissur district. The mean total viable count of the samples of the farmers belonging to three societies was 6.27 ± 0.14, 6.57 ± 0.13 and 5.59 ± 0.16 log $_{10}$ cfu/ml, respectively with an overall mean count of 6.14 ± 0.09 log $_{10}$ cfu/ml.

Nanu *et al* (2007) investigated microbial quality of 240 milk samples obtained from the farmers belonging three societies *viz*; FS_1, FS_2 and FS_3 and reported the mean total viable count of 6.10 ± 0.17, 6.57 ± 0.18 and 6.40 ± 0.16 $\log_{10}$ cfu/ml, respectively.

2.1.2 Coliform Count

Vijai and Saraswat (1968) evaluated the bacterial quality of 224 raw market milk samples, collected from rural collection centers (A), city vendors and retailers (B) and mixed herd milk produced at the College of Agriculture Dairy farm in Udaipur where the samples had been produced under controlled and strict clean conditions (C). The study revealed that the samples of the sources A, B and C had the mean coliform count of 18,000 per ml, 2200 per ml and 410 per ml, respectively.

Davies (1977) analysed the bacterial quality of 4672 milk samples, which consisted of bulk milk (2090) and churn milk supplies (2582) in Wales. Coliforms were present in the samples at the level of 10^{-2} ml in 57.1 and 80.6 per cent samples from bulk supplies and churn supplies, respectively. The count in 29.1 per cent bulk milk and 56.5 per cent of churn milk supplies had contaminated with coliforms at the level of 10^{-3}ml.

Singh and Ranganathan (1978) analysed the incidence and distribution of *Escherichia coli* in milk and dairy products supplied in Karnal. The samples consisted

of 50 raw cow milk, 78 raw buffalo milk, 30 pasteurized cow milk, 27 pasteurized buffalo milk and 100 milk products. All 128 raw milk samples were positive for coliforms. The count in the raw cow milk ranged between 500 and 50,000 per ml and the count in the raw buffalo milk ranged from 50,000 to 1,960,000 per ml.

Desai and Natarajan (1981) assessed the bacterial quality of raw milk collected from societies in three areas *viz.* A, B and C located in and around Bangalore city and reported that the samples belonging to the areas A, B and C had an average coliform count of 1040 x 10^3, 80 x 10^3 and 282 x 10^3/ml, respectively.

Yadava *et al.* (1983) examined the bacterial flora of raw milk collected from Dairy unit, Ranchi Veterinary College (42) and local vendors of Ranchi (41) during monsoon and winter. The average coliform counts of samples obtained from the two sources during monsoon were 0.778 x 10^5 and 0.32 x 10^5/ml, respectively. The corresponding count of samples collected during winter was 0.33 x 10^5 and 0.343 x 10^5/ml, respectively.

Reddy *et al.* (1984) reported that the coliform count of 30 raw milk samples obtained from Vijayawada milk shed was ranged between 4,280 and 1,32,000/ml with an average count of 28,600/ml.

Das and Nag (1986) examined the bacterial quality of 162 raw market milk samples obtained from vendors of Calcutta and reported that the maximum coliform count of the sample was at the level of 1270/ml.

Raju and Nambudripad (1987) assessed the incidence and growth of heat resistant coliforms bacteria in 78 raw milk and 75 pasteurized milk samples obtained from Banglore city. Raw milk samples were obtained from organized dairy (22), private dairies (30) and village pooled milk at the reception dock (26) and reported that the mean coliform count of samples from the three sources were136 x 10^3, 196 x 10^3 and 1560 x 10^3 cfu/ml, respectively.

Palanniswami *et al.* (1988) conducted a study on the coliforms of farm milk and its environment. The samples were obtained from three sources, *viz*, A - where sanitary conditions were given second preference, B - where sanitary practices were moderate and C - where sanitary practices were experimentally imposed while sampling. Samples consisted of 68 individual and 28 pooled milk samples. The mean coliform count of the individual samples from the 3 sources based on the Most Probable Number method was 10, 10 and 4 per ml and that of pooled samples were 39,000, 27,000 and 30/ml, respectively.

Misra and Kuila (1989) examined bacterial quality of 125 raw milk samples collected from organized dairy farm (15), city vendors (60) and sweet meat shops (50) in Calcutta and reported that the samples had an average coliform count of 3.96×10^3, 6.54×10^3 and 6.74×10^3 cfu/ml, respectively.

Rai *et al.* (1990) analysed the quality of raw and pasteurized milk supplied in the Kanpur city and the samples were collected from four sources, *viz.*, C.S.A. University Dairy farm (A), Milk Board (B), Hawkers (C) and Town Dairies (D) in the month of January to March. Milk from the source B was pasteurized. The average coliform count of raw milk samples from the sources A, C and D were 24.375×10^2, 213.375×10^2 and 64.125×10^2 /ml, respectively.

Patel *et al.* (1993) conducted a study to assess the sources of contamination of raw milk and collected 21 samples each of foremilk and middle milk from buffaloe maintained in the Veterinary College, Anand. The study revealed that the average coliform count of the foremilk samples was $5.1 \pm 1.2 \times 1000$ and that of middle milk was $1.9 \pm 0.57 \times 1000$ cfu/ml.

Siva *et al.* (1993) evaluated microbiological status of 32 samples each of raw cow milk and buffalo milk collected from individual producers, collection centers and dairy plant and 10 pasteurized milk samples from the Dairy science college, Anand.

Average coliform counts of raw cow milk samples collected from the above three sources were 0.63 ± 0.31 x 10^4, 66 ± 38 x 10^4 and 150 ± 0.82 x 10^4 cfu/ml, respectively.

Singh *et al.* (1994b) analysed 70 raw milk samples collected from different distribution cans from Pantnagar and reported that the coliform count ranged between 2.477 and 5.869 $\log_{10}$ cfu/ml, with a mean count of 4.477 $\log_{10}$ cfu/ml.

Kapre (1995) analysed the bacterial quality of 84 raw milk samples from three societies, *viz.* S_1, S_2 and S_3. The samples collected from each society consisted of 21 individual and 7 pooled milk samples. The mean coliform count of individual milk samples from the societies S_1, S_2 and S_3 were 2.4 x 10^1, 4.8 x 10^4 and 3.8 x 10^3 cfu/ml, respectively and the corresponding count of pooled milk samples belonging to the societies were 5.5 x 10^1, 2.0 x 10^5 and 6.4 x 10^3 cfu/ml, respectively.

Mutukumira *et al.* (1996) evaluated the chemical and microbiological quality of ten raw milk samples collected over six months from Nharira milk collection centre, Zimbabwe. The coliform counts of these samples ranged from 3.2 x 10^2 to 2.3 x 10^5 cfu/ml.

Jolly *et al.* (2000) evaluated the bacterial quality of individual and pooled raw milk samples obtained from three sources, *viz.*, A, B and C located in and around Mannuthy. From each source, 10 each of pooled and individual samples were collected. The mean coliform count of pooled milk samples of the sources A, B and C was 4.74 ± 0.54, 6.02 ± 0.19 and 5.31 ± 0.12 $\log_{10}$ cfu/ml, respectively and the samples had an overall mean count of 5.31 ± 0.09 $\log_{10}$ cfu/ml. The corresponding count of the individual milk samples were 5.14 ± 0.15, 5.03 ± 0.58 and 5.34 ± 0.18 $\log_{10}$ cfu/ml, and the overall mean count of the samples was 5.17 ± 0.03 $\log_{10}$ cfu/ml. Coliforms were present in 96.67 per cent of the total milk samples.

Khalilur *et al.* (2002) evaluated the microbiological quality of six raw and nine pasteurized milk samples obtained from local markets in Aligarh city. The raw milk samples had mean coliform count of more than or equal to 2.4×10^3 MPN/100 ml and pasteurized samples showed a mean count of 2.13×10^3 MPN/100 ml.

Lues *et al.* (2003) obtained raw milk samples from 60 randomly selected households in the Botshabelo Township, South Africa and evaluated the potential microbiological hazards in milk. The study revealed that the samples had a mean coliform count of 6.7×10^7 cfu/ml.

Raj *et al.* (2003) examined 40 raw milk samples consisted of 10 samples each from the farmers who brought milk into two milk marketing societies (A and B) and milk from hand milked (C) and machine milked (D) animals of live stock farm, Kerala Agricultural University. The average coliform counts of samples from sources A, B, C and D were 3.34 ± 0.14, 2.48 ± 0.13, 0.45 ± 0.24 and 0.63 ± 0.28 $\log_{10}$ cfu/ml, respectively.

Chye *et al.* (2004) investigated the microbial quality of 930 raw cow milk samples collected from 360 farmers belonging to southern, central, eastern and northern regions of Peninsular Malaysia. The average coliform count in the samples from the four regions were 28.0×10^4, 23.0×10^4, 11.0×10^4 and 7.5×10^4 cfu ml^{-1}, respectively with an overall mean count of 17.0×10^4 cfu ml^{-1}. The organisms were present in 89.9 per cent of samples.

Prejit (2005) investigated the bacteriological quality of 296 milk samples consisting of raw milk (60), milk at various stages of pasteurization (60), pasteurized milk during storage (120) and retail milk samples (56). Twenty samples each of raw milk were taken from individual animal and pooled milk from farm and chilled milk from dairy plant. The average coliform counts of raw milk from individual animals,

pooled milk and chilled milk was 1.83 ± 0.23, 3.24 ± 0.19 and 3.10 ± 0.17 $\log_{10}$ cfu/ml, respectively.

Chacko (2006) analysed the bacterial quality of milk samples with special emphasis on the quality assurance programme. A total of 108 milk samples were collected from 18 farmers at the point of production belonging to three societies in and around Mannuthy. The mean coliform count of the samples from the farmers of the three societies, S_1, S_2 and S_3, was 3.70 ± 0.10, 3.89 ± 0.08 and 3.39 ± 0.09 $\log_{10}$ cfu/ml, respectively with an overall mean count of 3.66 ± 0.05 $\log_{10}$ cfu/ml.

Nanu *et al* (2007) investigated microbial quality of 240 milk samples obtained from the farmers belonging three societies *viz*; FS_1, FS_2 and FS_3. The mean coliform count of the samples from the three societies was 2.97 ± 0.05, 3.20 ± 0.06 and 3.13 ±0.06 $\log_{10}$ cfu/ml, respectively.

2.1.3 *Escherichia coli* Count

Kapre (1995) evaluated the microbial quality of 84 milk samples consisted of 28 each from three sources, namely, S_1, S_2 and S_3. From each source, 21 individual samples and seven pooled milk samples were collected. The mean *Escherichia coli* count of individual samples from S_1, S_2 and S_3 was 2.0×10^2, 1.2×10^4 and 1.5×10^3 cfu/ml, respectively. The corresponding count of pooled milk samples from S_1, S_2 and S_3 was 2.7×10^2, 8.9×10^4 and 1.9×10^3 cfu /ml, respectively.

Jolly *et al.* (2000) evaluated the bacterial quality characteristics of individual and pooled raw milk samples obtained from three sources, *viz.* A, B and C located in and around Mannuthy. From each source, 10 each of pooled and individual samples were collected. The mean *Escherichia coli* count of pooled milk from A, B and C sources were 4.02 ± 0.47, 4.97 ± 0.18 and 4.33 ± 0.14 $\log_{10}$ cfu/ml, respectively with an overall mean count of 4.44 ± 0.61 $\log_{10}$ cfu/ml. The mean count of individual milk

samples was 4.11 ± 0.20, 3.13 ± 0.7 and 4.08 ± 0.48 log_{10} cfu/ml, respectively and the samples had an overall mean count of 3.77 ± 0.31 log_{10} cfu/ml.

Gran *et al.* (2003) conducted a study on the occurrence of pathogenic bacteria in 12 milk samples obtained from three small-scale societies in Zimbabwe and reported the samples had a mean *Escherichia coli* count of 4.5 log_{10} cfu ml^{-1}

Lues *et al.* (2003) evaluated the potential microbiological hazards in raw milk samples obtained from 60 randomly selected households in the Botshabelo township, South Africa and reported that the mean *Escherichia coli* count of the samples as 1.2 x 10^4 cfu/ml.

Chye *et al.* (2004) investigated the microbial quality of 930 raw cow milk samples collected from 360 farmers belonging to southern, central, eastern and northern regions of Peninsular Malaysia. The average *Escherichia coli* counts in samples from the above four regions were 15.0 x 10^3, 5.4 x 10^3, 4.8 x 10^3 and 1.9 x 10^3 cfu ml^{-1}, respectively, with an overall mean count of 6.8 x 10^3 cfu ml^{-1}.

Prejit (2005) investigated the bacteriological quality of 296 milk samples consisting of raw milk (60), milk at various stages of pasteurization (60), pasteurized milk during storage (120) and retail milk samples (56). Twenty samples each of raw milk were taken from individual animal, and pooled milk from farm and chilled milk from dairy plant. The average *Escherichia coli* counts of raw milk from individual animals, pooled milk and chilled milk were 1.01 ± 0.21, 0.63 ± 0.31 and 1.05 ± 0.29 log_{10} cfu/ml, respectively.

Chacko (2006) analysed the bacterial quality of milk samples with special emphasis on the quality assurance programme. A total of 108 milk samples were collected from 18 farmers at the point of production belonging to three societies, *viz*, S_1, S_2 and S_3 in and around Mannuthy. The mean *Escherichia coli* count of the

samples from the sources S_1, S_2 and S_3 was 0.90 ± 0.17, 1.11 ± 0.19 and 0.57 ± 0.15 $\log_{10}$ cfu/ml, respectively, with an overall mean count of 0.86 ± 0.10 $\log_{10}$ cfu/ml.

2.1.4 Faecal Streptococcal Count

Davies (1977) analysed the bacterial quality of 4672 milk samples, which includes both bulk milk samples (2090) and churn milk supplies (2582) obtained from Wales. The faecal streptococcal count of the samples from both the supplies ranged from less than 10,00 to greater than 5000000/ml. The count in 39.8 percent of the samples belonging to bulk tank supplies and 31.4 per cent of the samples belonging to churn supplies were between more than 1000 to less than 5000/ml.

Yadava *et al.* (1983) examined the bacterial flora of 83 raw milk samples collected from Dairy unit, Ranchi Veterinary College (42) and local vendors of Ranchi (41) during monsoon and winter. The average faecal streptococcal count of samples from the dairy unit and local vendors during monsoon was 0.183 x 10 and 1.76 x 10/ml, respectively. The corresponding count of the samples from the sources during winter was 0.003 x 10 and 0.102 x 10/ml.

Kapre (1995) evaluated the microbial quality of 84 milk samples consisting of 28 each from three sources, *viz.*, S_1, S_2 and S_3. From each source, 21 individual samples and seven pooled milk samples were collected. The mean faecal streptococcal count of individual samples from S_1, S_2 and S_3 was 1.5×10^2, 2.1×10^3 and 1.7×10^3 cfu/ml, respectively. The corresponding count of pooled milk samples from the sources S_1, S_2 and S_3 was 2.0×10^2, 4.8×10^3 and 2.9×10^3 cfu/ml.

Jolly *et al.* (2000) investigated bacterial quality of 30 each pooled and individual raw milk samples collected from sources A, B and C located in and around Mannuthy. From each source, 10 individual and 10 pooled milk samples were collected. The overall mean faecal streptococcal count of the sample was 3.06 ± 0.05 $\log_{10}$ cfu/ml and the organism was detected in 83.33 per cent of the samples. The

mean faecal streptococcal count of pooled milk samples from the sources A, B and C was 2.90 ± 0.38, 2.00 ± 0.49 and 2.56 ± 0.32 log_{10} cfu/ml, respectively with an overall mean count of 2.49 ± 0.04 log_{10} cfu/ml and the corresponding count of individual milk samples were 2.55 ± 0.13, 1.44 ± 0.49 and 2.46 ± 0.34 log_{10} cfu/ml, with an overall mean of 2.15 ± 0.12 log_{10} cfu/ml.

Raj *et al.* (2003) analysed bacterial quality of a total of 40 raw milk samples consisting of 10 samples each from two milk marketing societies (A and B) and hand milked (C) and machine milked (D) animals of live stock farm, Kerala Agricultural University. Samples from A and B were collected from farmers who brought milk to the society. Samples of C and D were collected from individual animals. The overall mean Faecal streptococcal counts of samples from the sources A, B, C and D was 1.7 ± 0.32, 1.30 ± 0.45, 0.53 ± 0.22 and 0.94 ± 0.28 log_{10} cfu/ml, respectively.

Prejit (2005) investigated the bacteriological quality of 296 milk samples consisting of raw milk (60), milk at various stages of pasteurization (60), pasteurized milk during storage (120) and retail milk samples (56). Twenty samples each of raw milk were taken from individual animal and pooled milk from farm and chilled milk from dairy plant. The average faecal streptococcal counts of raw milk from individual animals, pooled milk and chilled milk were 1.89 ± 0.08, 2.59 ± 0.11 and 2.57 ± 0.12 log_{10} cfu/ml, respectively.

Chacko (2006) analysed the bacterial quality of milk samples with special emphasis on the quality assurance programme. A total of 108 milk samples were collected from 18 farmers at the point of production belonging to three societies, *viz.*, S_1, S_2 and S_3 in and around Mannuthy. The mean faecal streptococcal counts of the samples from the farmers of the three societies S_1, S_2 and S_3 was 3.68 ± 0.09, 3.86 ± 0.08 and 3.31 ± 0.07 log_{10} cfu/ml, respectively with an overall mean count of 3.62 ± 0.05 log_{10} cfu/ml.

Nanu *et al.* (2007) evaluated the microbial quality of 240 raw milk samples collected from farmers belonging to three societies of Kerala *viz.*, FS_1, FS_2 and FS_3. The mean faecal streptococcal counts obtained from three societies were 1.76 ± 0.13, 2.66 ± 0.27 and 2.80 ± 0.28 $\log_{10}$ cfu/ml, respectively.

2.1.5 Yeast and Mould Count

Mutukumira *et al.* (1996) carried out quality analyses of 10 samples of raw milk obtained from the milk collection center, Zimbabwe. The yeast and mould count were less than 100 cfu/ml in 7 of the 10 samples.

Lues *et al.* (2003) enumerated microbial quality of raw milk samples obtained from 60 randomly selected households in the Botshabelo township, South Africa and that the samples had reported an average yeast count of 2.3×10^6 cfu ml^{-1} ($\pm 9.7 \times 10^6$ cfu ml^{-1}) and the average mould count of 1.1×10^3 cfu ml^{-1} ($\pm 3.8 \times 10^3$cfu ml^{-1}).

Prejit (2005) investigated the bacteriological quality of 296 milk samples consisting of raw milk (60), milk at various stages of pasteurization (60), pasteurized milk during storage (120) and retail milk samples (56). Twenty samples each of raw milk were taken from individual animal and pooled milk from farm and chilled milk from dairy plant. The average yeast and mould count of raw milk from individual animals, pooled milk and chilled milk were 1.58 ± 0.27, 1.86 ± 0.19 and 1.84 ± 0.24 $\log_{10}$ cfu/ml, respectively.

Chacko (2006) analysed the bacterial quality of milk samples with special emphasis on the quality assurance programme. A total of 108 milk samples were collected from 18 farmers at the point of production belonging to three societies, *viz.*, S_1, S_2 and S_3 in and around Mannuthy. The average yeast and mould count of the samples from the farmers of the three societies S_1, S_2 and S_3 was 4.08 ± 0.07, 3.50 ± 0.11 and 3.65 ± 0.09 $\log_{10}$ cfu/ml, respectively with an overall mean of 3.75 ± 0.06 $\log_{10}$ cfu/ml.

2.2 ISOLATION AND IDENTIFICATION OF BACTERIA FROM MILK

2.2.1 *Escherichia coli*

Singh and Ranganathan (1978) analysed 128 milk samples consisted of 50 raw cow milk and 78 raw buffalo milk samples obtained from National Dairy Research Institute, Karnal, and reported the isolation of *Escherichia coli* from 33 out of 50 raw cow milk and 59 out of 78 raw buffalo milk.

Yadava *et al.* (1985) analysed the bacterial flora of 105 milk samples obtained from Ranchi town and isolated *Escherichia coli* from 78.09 (82) per cent of the samples. The isolates belonged to serogroups, *viz*, O1, O17, O22, O11, O84, O55, O125, O86, O36, O45, O18, O2, O76, O9, O58, O30, O82, O34 and O59.

Yadava *et al.* (1987) reported the isolation of 31, 30 and 21 *Escherichia coli* from 42 raw milk samples obtained from organised dairy farm, 41 raw milk samples received from local vendors and 22 pasteurized milk samples collected from centralized milk supply organizations, Ranchi town, respectively. The 82 isolates obtained from 105 samples belongs to serogroups O1, O17, O22, O11, O84, O55, O125, O86, O36, O2, O9, O18, O30, O34, O45, O58, O69, O76 and O82.

Rahman *et al.* (1992) investigated the bacterial flora in 83 raw milk samples collected from Guwahati city and reported the isolation of six strains of *Escherichia coli*. The isolates belong to serogroups O15 (2), O9 (1), O60 (1) and O186 (1). One of the isolates was untypable.

Gill *et al.* (1994) analysed bacteriological quality of the milk samples of cow (36) and buffalo (40) and also 215 samples of milk products were collected at random from retail shops of Ludhiana city. During the investigation *Escherichia coli* was isolated from five out of 36 cow milk and four out of 40 buffalo milk samples.

Singh *et al.* (1994c) made a study on bacteriological quality of milk and its products and reported the isolation 49 strains of *Escherichia coli* from 70 raw milk samples collected from milk cans intended for distribution to Pantnagar.

Kapre (1995) evaluated the microbial quality of 21 individual milk samples and seven pooled milk samples collected from each of three sources, *viz.*, S_1, S_2 and S_3. *Escherichia coli* were isolated from 16 (76.19 per cent) individual samples obtained from S_1. Cent percent of the individual samples from S_2 and S_3 and all pooled samples revealed the presence of the organism.

Steel *et al.* (1997) isolated 15 (0.87 per cent) Verocytotoxigenic *Escherichia coli* from 1720 random bulk tank milk samples obtained from Ontario, Canada.

Heuvelink *et al.* (1998) examined 1011 raw milk samples obtained from farm bulk tanks in Netherlands and reported that none of the samples yielded O157 VTEC strain of *Escherichia coli*.

Jayarao and Henning (2001) studied the prevalence of Foodborne pathogens in bulk tank milk from 131 dairy herds in Dakota and Minnesota and isolated shiga-toxin producing *Escherichia coli* from five (3.8 per cent) samples.

Kapoor *et al.* (2002) examined 88 milk samples and reported the isolation of 32 (36.36 per cent) *Escherichia coli* and 14 were serogrouped. The serogroups consisted of O148 (3), O142 (2), O127 (2), O 125(1), O119 (1), O53 (2), O18 (2) and O11 (1).

Dontorou *et al.* (2003) evaluated various foods of animal origin obtained from North Western Greece and reported the isolation of one *Escherichia coli* O157: H7 strain from100 cow milk samples.

Gran *et al.* (2003) conducted a study on the occurrence of pathogenic bacteria in milk obtained from three small-scale societies in Zimbabwe and isolated six strains

of *Escherichia coli* from 12 samples of raw milk. During the investigation it was observed that two of these isolates produced Enterotoxigenic *E.coli* producing the heat stable enterotoxin.

Raj *et al.* (2003) studied the microbial quality of 40 raw milk samples consisting of 10 samples each from two milk marketing societies (A and B) and milk from hand milked (C) and machine milked (D) animals of live stock farm, Kerala Agricultural University. During the investigation *Escherichia coli* were isolated from five (12.5 per cent) samples from the above four sources. Two samples (20 per cent) from source A and one sample (10 per cent) each from sources B, C and D revealed the presence of the organism.

Aaku *et al.* (2004) isolated 10 (23 per cent) *Escherichia coli* from the 43 pooled raw milk samples collected from two processing plants *viz.* A and B in Gaborone, Botswana.

Chye *et al.* (2004) investigated the microbial quality of 930 raw cow milk samples collected from 360 farmers belonging to southern, central, eastern and northern regions of Peninsular Malaysia. During the investigation, *Escherichia coli* was isolated from 68.5, 57.2, 72.2 and 59.9 per cent of the samples from the above four regions, respectively. *E.coli* O157: H7 was isolated from 312 (33.5 per cent) of the 930 samples tested.

Oksuz *et al.* (2004) reported the isolation of *Escherichia coli* O157 from one of the 100 raw milk samples randomly collected from 10 villages in Tekirday, Turkey.

Prejit (2005) isolated four strains of *Escherichia coli* from the 20 pooled raw milk samples obtained from Livestock Farm, Kerala Agricultural University.

Chacko (2006) isolated *Escherichia coli* from 45 (41.67 per cent) samples out of the 108 raw milk samples collected from societies S_1, S_2 and S_3 located in and

around Mannuthy. Only 22 (48.89 per cent) out of the 45 isolates were serotyped. The isolates fell in to 10 serotypes belonged to O116 (4), O84 (3), O24 (3), 0172(3), 0145(3), O125 (2), O79 (1), O87 (1), O103 (1) and O157 (1).

Manna *et al.* (2006) detected *Escherichia coli* O157 from one each of the 32 raw milk sample and 49 pasteurized milk sample obtained from 3 districts of West Bengal.

Nanu *et al.* (2007) assessed the microbial quality of 240 raw milk samples collected from farmers belonging to three societies of Kerala *viz.*, FS_1, FS_2 and FS_3 and isolated 76 (31.60 per cent) *Escherichia coli*. The isolates serogrouped into the different serotypes and they were 0172 (8), O24 (9), O157 (3), O103 (3), O25 (2), O125 (2), O145 (2), O5 (1), O68 (1), O84 (7), O87 (3) and O116 (9).

2.2.2 *Staphylococcus aureus*

Mohan and Misra (1967) examined a total of 200 milk samples collected from producer, agent, collection center, can samples and bulk milk samples from dairy and raw cow milk samples supplied to Patna milk supply scheme and isolated 71 strains of Staphylococci. Among the isolates 33 were coagulase positive and 38 were coagulase negative.

Garg *et al.* (1977) assessed the bacterial flora of raw market milk of cows (57) and buffalo (45) from Hissar city during summer and winter and reported the isolation of 54 *Staphylococcus aureus* strains from the cow milk samples.

Shah *et al.* (1984) isolated *Staphylococcus aureus* from 20 per cent of the milk samples obtained from 134 healthy cows belonging to the University farm, Anand.

Yadava *et al.* (1985) analysed bacterial flora of 105 milk samples consisted of raw milk samples from organised dairy farm (42) and local vendors (41) and pasteurized milk from milk supply scheme (22) of Ranchi town. *Staphylococcus*

aureus was isolated from 7 (16.66 per cent), 10 (24.39 per cent) and 3 (13.63 per cent) samples from the above sources, respectively. A total of 20 *Staphylococcus aureus* was isolated from105 samples.

Rajmany *et al.* (1989) examined 20 samples each of raw milk, khoa, curd, ice cream, sweetened condensed milk, milk powder and processed cheese, obtained from local markets of Udaipur city to find out the incidence of Staphylococci in milk and milk products. The staphylococcal count in raw milk samples ranged from 20.5 x 10^3 to 104 x 10^3 cfu/ml with an average of 63.5 x 10^3 cfu/ml. Coagulase positive staphylococcal count in the samples was ranged between 11.2 x 10^3 and 57 x 10^3 cfu/ml with an average count of 31.5 x 10^3 cfu/ml. Cent per cent of the raw milk samples were positive for the staphylococci and coagulase positive staphylococci.

Sen *et al.* (1989) analysed total of 178 cow milk samples belonging to an organised dairy farm, West Bengal and reported the isolation of *Staphylococcus aureus* from 24.1 per cent (43) samples.

Rahman *et al.* (1992) examined 83 raw milk samples obtained from Greater Guwahati city, Assam and reported that 56.13 per cent samples yielded *Staphylococcus aureus*. Of the 21 strains of *Staphylococcus aureus* subjected to phage typing, 16 (76.2 per cent) were typable with international set of phages.

Gill *et al.* (1994) analysed 76 samples of market milk of cow (36) and buffalo (40) and 215 milk products were collected at random from retail shops of Ludhiana city. During the investigation, *Staphylococcus aureus* was isolated from 6 (16.67 per cent) cow milk, 8 (20 per cent) buffalo milk samples and 39 (18.13 per cent) milk products.

Singh *et al.* (1994c) evaluated 70 raw milk samples collected from the distribution cans of Livestock Research Center, Nagla and reported that cent per cent of the samples had *Staphylococcus aureus*.

Kapre (1995) examined 21 individual samples and seven pooled milk samples each obtained from three sources *viz.* S_1, S_2 and S_3. *Staphylococcus aureus* was isolated from 14 (66.66 per cent), 13 (61.91 per cent) and 10 (47.61 per cent) of individual samples from S_1, S_2 and S_3 sources, respectively. The organism was also isolated from four (57.1 per cent), six (85.71 per cent) and six (85.71 per cent) pooled milk samples from sources, S_1, S_2 and S_3, respectively.

Adesiyun *et al.* (1998) conducted a study to find out the prevalence and characteristics of *Staphylococcus aureus* from samples of milking centers in Trinidad and reported that all 175 bulk milk samples and 280 (97.6 per cent) out of 287 composite milk samples had *Staphylococcus aureus.*

Jolly *et al.* (2000) evaluated the bacteriological quality of 60 raw milk samples obtained from three societies, *viz.*, A, B and C located in and around Mannuthy. From each source, 10 individual and 10 pooled milk samples were collected. *Staphylococcus aureus* was present in 50 per cent of pooled and 36.67 per cent of individual milk samples and the overall mean count of *Staphylococcus aureus* in pooled and individual milk sample was 1.48 ± 0.13 and 1.24 ± 0.34 $\log_{10}$ cfu/ml, respectively.

Carmo *et al.* (2002) analysed cheese and raw milk samples collected during two food poisoning outbreaks in Brazil. *Staphylococcus aureus* was present at the level of 2.4×10^3 and 2.0×10^8 cfu/g in cheese and raw milk samples, respectively, in the samples collected during the first outbreak. Milk samples collected during the second outbreak had coagulase negative Staphylococci at levels exceeding 2.0×10^8 cfu g^{-1}.

Gran *et al.* (2003) isolated *Staphylococcus aureus* from seven out of 12, 15 out of 27 and 20 out of 21 raw milk, cultured pasteurized milk and natural soured raw milk samples, respectively, obtained from three small scale societies in Zimbabwe.

Raj *et al.* (2003) conducted a study on the bacterial quality of 40 raw milk samples consisting of 10 samples each from two milk marketing societies (A and B) and hand milked (C) and machine milked (D) animals of live stock farm, Kerala Agricultural University. *Staphylococcus aureus* was isolated from 15 (37.5 per cent) samples. The samples from source B was found free from the organism and the organism was isolated from 40, 50 and 60 per cent of samples belonged to the sources A, C and D, respectively.

Aaku *et al.* (2004) analysed microbiological quality of 129 milk samples consisted of pooled raw milk (43) and bottled commercial pasteurized milk (86) from two processing plants, Gaborone, Botswana. The study revealed that none of the samples of raw milk and 10 (11.6 percent) of the pasteurized milk samples had *Staphylococcus spp.*

Chye *et al.* (2004) investigated the microbial quality of 930 raw cow milk samples collected from 360 farmers belonging to southern, central, eastern and northern regions of Peninsular Malaysia. *Staphylococcus aureus* was isolated from 565 (61 per cent) samples, with a frequency of detection of 63.3, 55.7, 60.3, and 61.3 per cent of the samples from the above four regions, respectively.

Prejit (2005) isolated *Staphylococcus aureus* from 12 (60 per cent) out of 20 raw pooled milk samples collected from Livestock Farm, Kerala Agricultural University.

Chacko (2006) isolated 33 *Staphylococus aureus* from 33(30.56 per cent) out of the 108 raw milk samples collected from societies, *viz.*, S_1, S_2 and S_3 located in and around Mannuthy. The highest number of the organism was isolated from the samples of the source S_2 (14), followed from the sources, S_1 (12) and S_3 (7).

Nanu *et al.* (2007) assessed the microbial quality of 240 raw milk samples collected from farmers belonging to three societies of Kerala *viz.*, FS_1, FS_2 and FS_3 and isolated 84 (35 per cent) *Staphylococus aureus.*

2.2.3 *Yersinia*

Schiemann and Toma (1978) reported the isolation of *Yersinia enterocolitica* from 19 (31.1 per cent) pooled raw milk samples (61) obtained from Southern Ontario. The organism was also isolated from 10 (14.3 per cent) samples collected from individual producers.

Schiemann (1978) isolated *Yersinia enterocolitica* from 10 (18.2 per cent) out of the 55 raw milk samples collected from cheese manufacturing plants in Ontario.

Hughes and Jensen (1981) isolated *Yersinia enterocolitica* from 35 (12.8 per cent) out of 274 samples of raw goat milk produced in New South Wales, Australia.

Vidon and Delmas (1981) analysed a total of 75 raw milk samples consisted of 56 pooled and 19 processed samples obtained from Central Dairy and retailers in Alsace, France. *Yersinia enterocolitica* was isolated from 61(81.4 per cent) out of the 75 raw milk samples. The occurrence of *Yersinia enterocolitica* was slightly higher in processed milk than in bulk raw milk.

Rohrbach *et al.* (1992) analysed the prevalence of *Yersinia enterocolitica* in 292 farm bulk tank milk samples obtained from East Tennesse and South West Virginia and reported that the organism was isolated from 44 (15.1 per cent) samples.

Jayarao and Henning (2001) examined the prevalence of Foodborne pathogens in bulk tank milk obtained from 131 dairy herds in Dakota and Minnesota and reported the isolation of *Yersinia enterocolitica* from eight (6.1 per cent) samples.

Kushal and Anand (2001a) isolated 36 *Yersinia enterocolitica* strains from 80 raw milk samples collected from the villages of Haryana.

Gran *et al.* (2003) reported that none of the 12 raw milk, 27 cultured pasteurized and 21 naturally soured raw milk samples obtained from small scale dairies in Zimbabwe, had *Yersinia.*

Nihal and Huriye (2006) examined 100 milk samples obtained from Ankara and reported that 55 per cent of the sample were contaminated with *Yersinia.* The per cent of isolation of *Yersinia enterocolitica, Y. frederiksenii, Y. kristensenii, Y. intermedia* and atypical *Yersinia* spp. was 47.3, 31.0, 12.7, 7.2 and 1.8 per cent, respectively.

Chacko (2006) analysed the bacterial quality of milk samples with special emphasis on the quality assurance programme. None of the sample was positive for *Yersinia enterocolitica* among the108 milk samples were tested.

Nanu *et al.* (2007) isolated 118 (49 per cent) *Yersina* spp from the 240 raw milk samples collected from farmers belonging to three societies of Kerala *viz.*, FS_1, FS_2 and FS_3.

2.3 BACTERIAL STANDARDS OF MILK

The Bureau of Indian Standards prescribed the microbial quality of milk. Milk contracts bacterial contamination mainly from animals, human beings, environment and utensils at various stages of production, processing, transport and distribution, which leads to spoilage of milk and causes milk borne infection and intoxication.

2.3.1 Raw Milk

The Indian Standards (1977) prescribed the following criteria as a guideline for grading of milk. Raw milk with a standard plate count not exceeding two lakhs

per milliliter is graded as very good, the counts between two and 10 lakhs/ml is graded as good, the counts between 10 and 50 lakhs /ml is graded as fair and the counts over 50 lakhs/ml is graded as poor. The standard also prescribed that coliforms should be absent in 1:100 dilution of satisfactory grade raw milk.

The standard for individual producer samples is 1,00,000/ml and for commingled samples is 3,00,000/ml according to Food And Drug Administration for Grade A raw milk for pasteurization (Yadava *et al.*, 1993).

2.4 GRADING OF SAMPLES BASED ON TOTAL VIABLE COUNT

Yadava *et al.* (1983) studied the bacterial quality of 105 raw milk samples marketed in Ranchi. Of the samples, 42 raw milk samples were collected from Dairy unit, Ranchi Veterinary College (RVC) and 41 from local vendors of Ranchi. Based on Standard Plate Count, most of the samples of RVC and local vendors were classified as good or very good quality. One of the samples belonging to dairy farm, RVC and ten samples from local vendors were classified as poor quality.

Misra and Kuila (1989) conducted a study to estimate various groups of bacteria and quality of milk produced and distributed in Calcutta and its suburbs. A total of 125 sample of raw milk, consisting of 15 from organized dairy farm, 60 from city vendors and 50 from sweet meat shops were analyzed. On the basis of Standard Plate Count, 60 per cent of samples from organized dairy farms were graded as good, 33.3 per cent as fair and 6.75 per cent as poor. None of the samples from vendors and

sweet meat shops was graded as good. However, 45 per cent of samples from vendors were graded as poor and 48 per cent of samples from sweet meat shops were graded as fair.

Singh *et al.* (1994b) analysed the sanitary quality of 70 samples of raw milk collected from different cans for distribution to Pantnagar and reported that 28.75 per cent samples were graded as very good, 14.28 per cent as good, 21.43 per cent as fair and 37.73 per cent as poor.

Kapre (1995) studied the microbial quality of 84 milk samples consisting of 28 each from University Livestock Farm, Mannuthy (S_1), Ollukkara Ksheera Vyavasaya Co-operative Society (S_2) and Panancherry Ksheera Udpathaka Sahakarana Sangam (S_3). From each source, 21 individual samples and seven pooled milk samples were collected. Only 28.56 per cent pooled milk samples from S_2 were below good quality, as per BIS standards. The study revealed that 95.24 per cent of individual samples from S_1 were of very good quality and 4.76 per cent were of good quality. All pooled milk samples were very good. Out of individual samples from S_2, 76.20 and 23.80 per cent were graded as very good and good, respectively. Among pooled samples 42.84, 28.60, 14.28 and 14.28 per cent samples were graded as very good, good, fair and poor, respectively. Quality of 80.95 and 19.05 per cent individual samples from S_3 were very good and good, respectively, whereas 57.14 and 42.86 per cent samples of pooled milk were graded as very good and good, respectively.

Garg and Mandokhot (1997) analysed the quality of 86 samples of raw milk consisting of 67 from local vendors, six from vendors of organised dairy unit and 13 from local milk plant. Out of the samples, 64 (74.4 per cent), eight (9.3 per cent) and 12 (13.95 per cent) were graded as poor, fair and good, respectively. Only two (2.33 per cent) samples were graded as very good. High standard plate count (over 5 x 10^6/ml) in majority of milk sample indicated poor hygienic practice followed at dairy farm in the region.

Jolly *et al.* (2000) evaluated the bacteriological quality of 60 raw milk samples obtained from three sources, namely, A, B and C located in and around Mannuthy, which received milk from farmers. The samples consist of 10 each of pooled and individual samples from each source. Based on TVC, the per cent of pooled samples graded as good, fair and poor were 40, 50 and 10 from source A; 10, 50, and 40 from source B and 40, 40 and 20 from source C, respectively. None of the pooled samples was graded as very good. The percentage of individual samples graded as very good, good, fair and poor were nil, 60.00, 40.00 and nil in source A; 20.00, 20.00,50.00 and 10.00 in source B; and nil, 50.00, 40.00 and 10.00 in source C, respectively.

Raj *et al.* (2003) studied the microbial quality of 40 raw milk samples consisting of 10 samples each from two milk marketing societies (A and B) and hand milked (C) and machine milked (D) animals of live stock farm, Kerala Agricultural University. According to BIS, percentage of samples from sources A, B, C and D, graded as very good were 90.00, 60.00, 100.00 and 80.00, respectively. Of the samples from the sources A, B and D 10.00, 30.00 and 20.00 per cent was graded as good quality, respectively and 10 per cent samples from source B was graded as fair. Based on total viable count, none of the samples was graded as poor.

Chacko (2006) analysed the bacterial quality of 108 individual raw milk samples with special emphasis on the quality assurance programme. Based on TVC, the per cent of samples graded as very good, good, fair and poor were 16.67, 29.63, 20.37 and 33.33, respectively.

Jaibi (2006) analysed the bacterial quality of 36 pooled raw milk samples and reported that 16.67, 58.33 and 25.00 per cent samples fell under the grade good, fair and poor. None of the pooled samples was graded as very good.

2.5 SOURCES OF MILK CONTAMINATION

Palanniswami *et al.* (1988) studied the contribution of different farm environmental niches to the coliform contamination of farm milk in three groups of farms, namely group A, B and C. In group A, sanitary conditions were given second preference, in group B, sanitary practices were moderate and in Group C, sanitary practices were experimentally imposed while sampling. Coliform counts of tap water in farms A, B and C were 450, 72 and 40 MPN /100ml respectively. Coliform counts of milk pail in farms A, B and C were 17.5 x 10^4, 450 and zero per litre capacity, respectively.

Patel et al. (1993) assessed the contribution of milk cans to the microbial load of raw buffalo milk by comparing 10 rinse samples obtained from washed milk cans with another 10 samples obtained from unwashed milk cans at the collection point. The Total Plate Count of washed cans ranged from 40,000 to 45,00,000, with an average of 1.1 ± 0.51 x 10,00,000 per can. The corresponding count for unwashed cans ranged from 5.6 x 1,00,000 to more than 3,00,00,000, with an average of 1.7 ± 0.44 x 10 million per can.

Jaibi (2006) assessed the critical control points at the society level of three societies, Thrissur District. The mean total viable count, coliform count, *Escherichia coli* count and faecal streptococcal count of the water samples collected were 176.61 ± 16.25, 1.41 ± 0.31, 0.92 ± 0.28 and 0.70 ± 0.27 log $_{10}$ cfu/ml. The corresponding counts of hand wash and utensil wash were 2.98 ± 0.10, 2.23 ± 0.30, 1.45 ± 0.32 and 1.57 ± 0.31 $\log_{10}$ cfu/ml and 2.75 ± 0.04, 1.56 ± 0.30, 1.09 ± 0.29, 1.40 ± 0.30 $\log_{10}$ cfu/ml, respectively.

2.6 DETECTION OF ADULTERANTS AND PRESERVATIVES IN MILK

Garg and Mandokhot (1997) examined 80 milk samples and reported that 41 samples were adulterated with carbonates/bicarbonates.

Jolly *et al.* (2000) investigated the bacterial quality of 30 each of the pooled and individual raw milk samples collected from sources A, B and C located in and around Mannuthy. From each source, 10 individual and 10 pooled milk samples were collected. All milk samples were found to be free of preservatives like carbonates, formalin, salicylic acid and benzoic acid and adulterants like cane sugar, starch and nitrates.

Rao *et al.* (2002) analysed chemical quality of 20 samples each of full cream milk obtained from three sources *viz.*, Andra Pradesh Dairy Development Cooperative Federation (APDDCF), private dairies and local vendors. None of the samples from APDDCF contained any added adulterant, Neutralizer or preservative. Water and bicarbonate were detected in 30 and 40 per cent samples, respectively from private dairies, but free from added sugar, formalin and hydrogen peroxide. Of the samples from local venders 60, 95 and 10 per cent samples were positive for added bicarbonates, water and sugar, respectively, but free from formalin and hydrogen peroxide.

Arora *et al.* (2004) collected 996 milk samples from different States of North India to detect the presence of adulterants and found that 91, 58, 25, 10 and 120 samples were adulterated out of 337, 254, 64, 20 and 321 samples collected from Punjab, Utter Pradesh, Delhi, Rajasthan and Haryana, respectively. The most common adulterant encountered was water (11.7 per cent) followed by neutralizers (9.2 per cent), sugar (2.3 per cent), urea (0.9 per cent), starch (0.8 per cent), glucose (0.7 per cent), salt (0.6 per cent) and formalin (0.4 per cent).

Saxena and Agrawal (2004) assessed quality of 81 milk samples collected from 3 sources, *viz.*, Government dairy (8), private dairies (37) and vendors (36). The percentage of sugar, salt, neutralizer and formaldehyde from the private dairies and vendors was 51.35 and 33.30, 32.40 and 8.30, 62.10 and 56.60 and 35.10 and 27.80,

respectively. Adulteration of any kind was not observed in Government dairy milk samples. None of the samples from 3 sources showed the presence of starch.

Mankar *et al.* (2005) surveyed on adulteration of 100 raw milk samples, 25 samples each from four sources *viz.*, individual producers, co-operative societies, milk chilling centers and milk receiving platform of Government Milk Scheme, Nagpur. The percentage of water was more (28 per cent) followed by sugar (16 per cent), sodium bicarbonate (14 per cent) and urea (8 per cent). Not a single sample was adulterated with starch.

2.7 POLYMERASE CHAIN REACTION.

Allmann *et al.* (1995) studied the sensitiveness of PCR for detection of Escherichia coli from dairy products over the conventional microbiological methods. A total of 90 milk samples were analysed and reported the detection of 41 (46 per cent) isolates by PCR method whereas culture method revealed the presence of only 21 (23 per cent) isolates.

Desmarchelier *et al.* (1998) developed a PCR for the detection of *Escherichia coli* O157 based on the *rfbE* O-antigen synthesis genes. A 479-bp product was amplified specifically from *Escherichia coli* O157 in cell lysates containing 2 cfu following crude DNA extraction. The PCR detected <1 cfu of the organism per ml in raw milk following enrichment.

Reid *et al.* (1999) designed a multiplex PCR to detect the *eae* gene and simultaneously identify specific alleles in pathogenic *Escherichia coli*. The method was tested on 87 strains representing the diarrhoeagenic *Escherichia coli* clones. The results showed that the PCR assay accurately detected *eae* gene and as this gene is

lineage specific, this multiplex PCR method provides a rapid way to classify suspected pathogens into the major clonal groups of EPEC and EHEC.

Kumar *et al.* (2001) standardized a PCR based assay targeted against 'uidR' gene specific for all *Escherichia coli* biotypes using primers URL-301 and UAR-432 and template DNA from *Escherichia coli.* The assay was fairly sensitive as it could detect as low as 10 cells of the organism in broth cultures and milk spiked with *Escherichia coli* ATCC 25922 after 4h enrichment. The total time for detection of the organism by this method was less than 10h.

Daly *et al.* (2002) used PCR-ELISA for detection of *Escherichia coli* in raw and pasteurized milk using unique *alr* primers (Yokoigawa *et al.,* 1999) and reported the detection of 5 *Escherichia coli* colony forming units (cfu) equating to a sensitivity of detection of 100 *Escherichia coli* cfu/ml of pasteurized milk.

Jothikumar and Griffiths (2002) studied the sensitiveness of a SYBR Green Light Cycler PCR assay using a single primer pair, which allowed simultaneous detection of *stx1* and/or *stx2* of *Escherichia coli* O157:H7. A distinct sequence of the Shiga-like toxin genes was amplified to yield products of 227 and/or 224 bp, respectively.

Materials and methods

3. MATERIALS AND METHODS

In the present investigation, a total of 180 raw milk samples consisted of 108 individual milk samples obtained at the point of production from farmers belonging to three co operative societies *viz.* S_1, S_2 and S_3 and 72 pooled milk samples from these societies were collected. From each society, samples were collected from six farmers and collection from each farmer was repeated six times. The samples were tested to determine the Total Viable Count (TVC), Coliform Count (CC), *Escherichia coli* Count (ECC), Faecal Streptococcal Count (FSC) and Yeast and Mould Count (YMC) per ml of the sample. The samples were also subjected for isolation and identification of *Escherichia coli*, *Staphylococcus aureus* and *Yersinia*.

In order to assess the critical control points of bacterial contamination of milk, during milking, samples of air, water used in milking barn, rinsing of utensils, milker's hand wash and udder wash of the animal were collected and subjected to estimation of various bacterial load. In order to identify the critical control points of microbial contamination at the society level, samples of air, water, hand wash of the milk handlers and rinsing of utensils were collected from each co-operative society. Collection of these samples was repeated six times. These samples were evaluated for bacterial load as discussed above.

The pooled milk samples obtained from the co operative societies were tested to detect the adulterants (starch and cane sugar) and preservatives (boric acid, formaldehyde and neutralizers) added in the milk. *Escherichia coli* isolates obtained from the milk samples were confirmed by Polymerase Chain Reaction (PCR) technique.

3.1 MICROBIAL QUALITY OF MILK

In order to get an insight on the microbial quality and the presence of bacterial pathogens in milk, produced at the point of production (farmer's level)

and at the society level, the milk samples were collected from three selected co-operative societies, *viz.* S_1, S_2 and S_3, located in Thrissur district and examined for their microbial count and bacterial pathogens.

3.1.1 Collection of milk samples

The samples included a total of 180 raw milk samples, consisted of 108 individual and 72 pooled milk samples from three societies.

3.1.1.1 Raw milk from individual farmers

A total of 108 raw milk samples were collected from 18 farmers belonging to three co-operative societies at the point of production in the farmer's premises. On each day, the samples were collected from six randomly selected farmers of a society and the collection was repeated six times. Each sample consisted of 500 ml of milk collected in a clean and sterile conical flask and brought to laboratory in an insulated container. Sampling plan of individual and pooled milk samples and also the critical control point samples are shown in the flow chart 1.

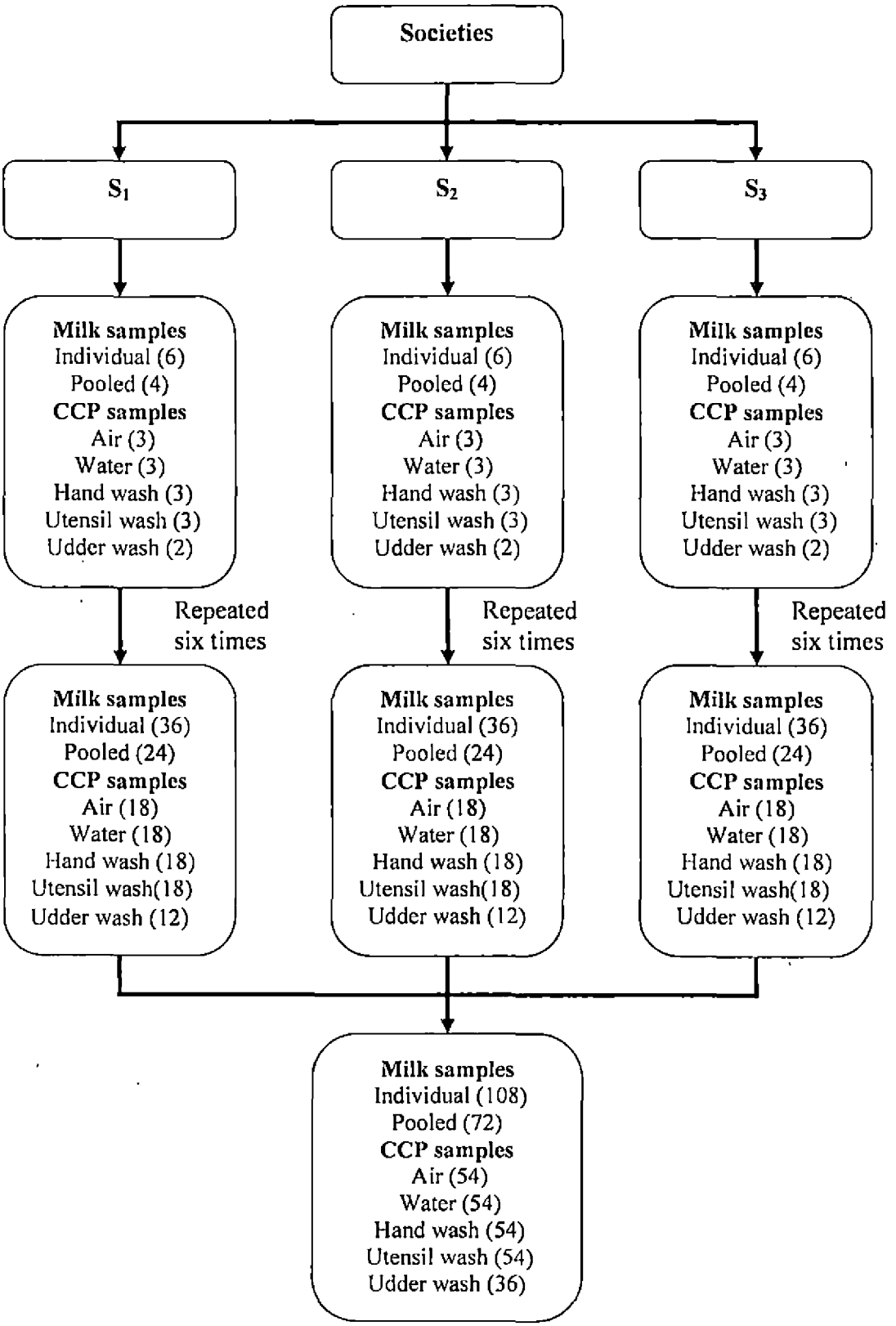
3.1.1.2 *Pooled raw milk*

Pooled raw milk samples were collected after thorough mixing of milk in the cans using a plunger and transferring 500 ml of milk into a sterile conical flask. At a time four pooled samples each were collected from a society and the collection was repeated on six days. Thus, a total of 24 pooled milk samples were collected from each society. All samples collected from the societies were brought to the laboratory in an insulated container. The details of samples collected are shown in the flow chart 1.

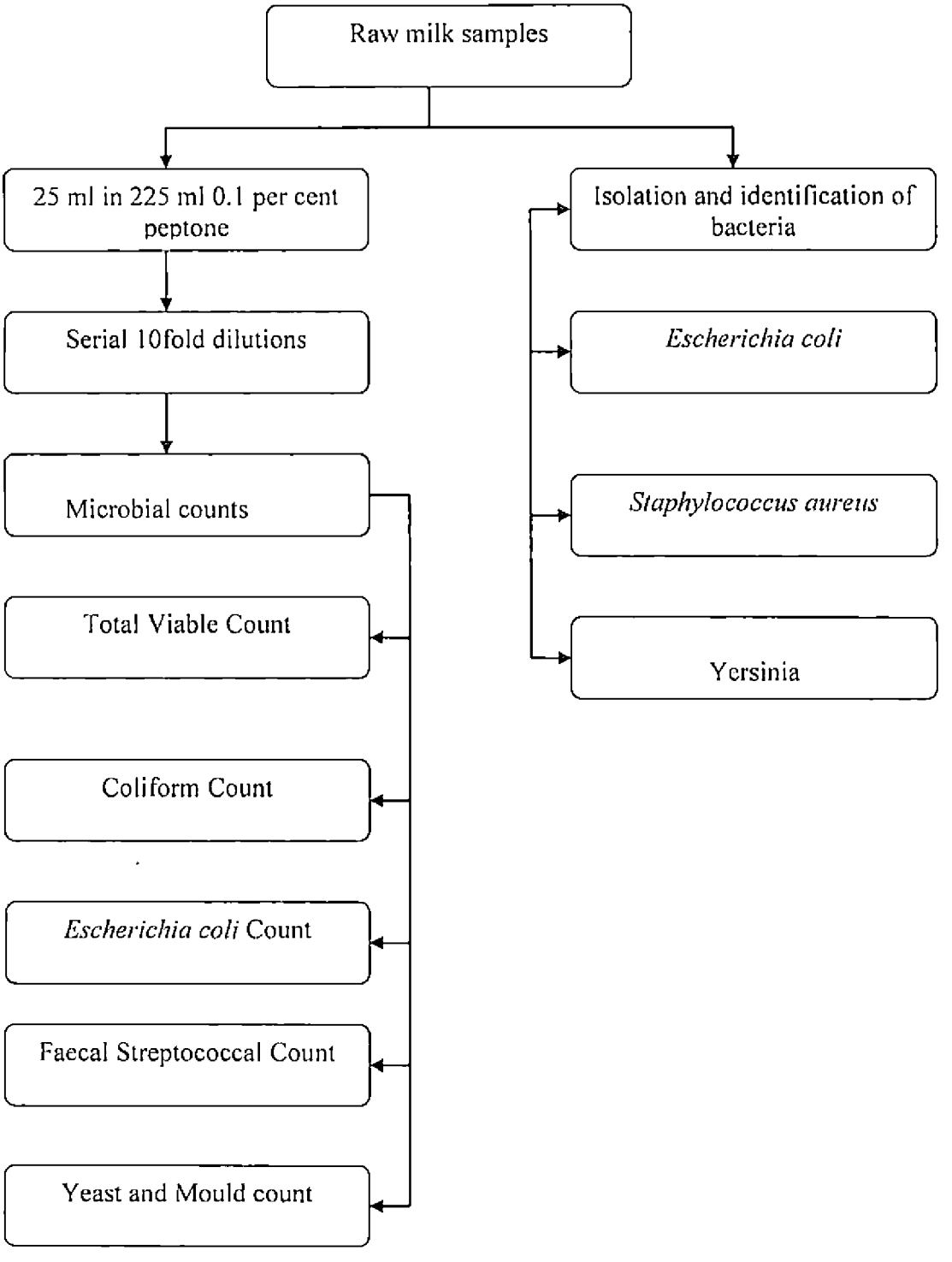
3.1.2 Processing of milk samples

In order to estimate the microbial load per ml of milk, the sample was agitated thoroughly and 25 ml was transferred into 225 ml of 0.1 per cent peptone water (diluent) so as to form one in 10 dilution. Further 10 fold serial dilutions were made by transferring one ml of inoculum into nine ml of diluent. Dilutions

Flow chart 1. Sampling plan



Flow chart 2. Microbial Analysis



were made up to 10^{-8} and selected dilutions were used for the estimation of various microbial loads per ml of the sample. All aseptic precautions were taken during collection and processing of milk samples. Collection and processing of samples are described in flow chart 2.

3.1.3 Microbial counts

The selected serial dilutions of each sample were used to estimate the Total Viable Count (TVC), Coliform Count (CC), *Escherichia coli* Count (ECC), Faecal Streptococcal Count (FSC) and Yeast and Mould Count (YMC). The count is expressed as $\log_{10}$ cfu/ml.

3.1.3.1 *Total Viable Count*

Total viable count (TVC) of each sample was estimated by pour plate technique, as described by Mortan (2001). From the selected ten fold dilution of each sample, one ml of the inoculum was transferred on to duplicate Petri- plates of uniform size. To each of the inoculated plates about 15-20 ml sterile molten standard plate count agar (Hi-media) maintained at 45°C was poured and mixed with the inoculum, by gentle rotatory movement i.e., clock wise, anticlock wise, forward and backward directions. The inoculated plates were left at room temperature and allowed to solidify, and incubated at 37°C for 24 h. At the end of incubation, plates showing 30 and 300 colonies were selected and counts were taken with the help of a colony counter. The number of colony forming units (cfu) per ml of sample was calculated by multiplying the mean colony count in the duplicate plates with the dilution factor and expressed as $\log_{10}$ cfu/ml.

3.1.3.2 *Coliform Count*

Coliform count (CC) per ml of sample was estimated according to the procedure described by Kornacki and Johnson (2001). From the selected dilution, 0.1 ml of the inoculum was inoculated onto duplicate plates of violet red bile agar (VRBA) (Hi-media) and was uniformly distributed with a sterile 'L' shaped glass rod. The plates were incubated at 37°C for 24 h. At the end of incubation, purplish

red colonies with diameter of at least 0.5 mm, surrounded by a reddish zone of precipitate were counted as coliforms. The number of organisms per ml of the sample was estimated by multiplying the mean count of duplicate plate samples with dilution factor and expressed as $\log_{10}$ cfu/ml.

3.1.3.3 *Escherichia coli Count*

The number of *Escherichia coli* (ECC) per ml of sample was estimated as prescribed by Indian standards (1980). To estimate the organism, 0.1 ml of inoculum from the selected dilution was transferred onto duplicate plates of Eosin Methylene Blue (EMB) Agar (Hi-media) and was evenly distributed over the medium with a sterile 'L' shaped glass rod. The plates were incubated at 37°C for 24 h. After the incubation period, colonies with a greenish black metallic sheen on deflected light were counted as *Escherichia coli*. The number of organism per ml of sample were estimated as described in coliform count and expressed as $\log_{10}$ cfu/ml.

3.1.3.4 *Faecal Streptococcal Count*

The standard procedure prescribed by Nordic Committee (1968) was followed to estimate the number of faecal streptococci per ml of sample. Accordingly, 0.1 ml of the inoculum from the selected dilution was transferred onto duplicate plates of Karl Friedrich (KF) streptococcal agar (Hi-media). The inoculum was uniformly distributed onto the plates using a 'L' shaped glass rod. The plates were incubated at 37°C for 48 h. Pink to dark red colonies with a diameter between 0.5 and three mm and surrounded with a narrow whitish zone were counted as faecal streptococci. The number of organisms per ml of the sample was estimated as described in coliform count and expressed as $\log_{10}$ cfu/ml.

3.1.3.5 *Yeast and Mould Count*

Method described by Beuchat and Cousin (2001) was followed for estimation of yeast and mould count per ml of milk sample. Potato dextrose agar (Hi-media) was used for the estimation of yeast and mould count by spread plate technique.

From the selected dilution of each sample, 0.1 ml of inoculum was transferred onto duplicate plates containing the media and the inoculum was evenly distributed on the media with a sterile 'L' shaped glass rod. The plates were incubated at 25°C for 3 to 5 days. After the period of incubation the colonies in duplicate plates were counted with the help of a colony counter and the mean count was multiplied with the dilution factor and expressed as $\log_{10}$ cfu/ml.

3.1.4 Isolation and identification of bacteria

All raw milk samples were subjected for the isolation and identification of *Escherichia coli*, *Staphylococcus aureus* and *Yersinia*.

3.1.4.1 *Escherichia coli*

In order to isolate *Escherichia coli*, a loopful of inoculum from each sample was inoculated on to duplicate plates of Eosin methylene blue agar and incubated at 37°C for 24 h. (Indian Standards, 1980). At the end of incubation period, three or four colonies with a dark center and a distinct indelible-ink greenish black metallic sheen on deflected light were selected and transferred on to nutrient agar slants and incubated at 37°C for overnight. These isolates were subjected to further characterization and identification by cultural, morphological and biochemical reactions as described by Barrow and Feltham (1993) and are shown in flow chart 3. The isolates were serotyped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh.

3.1.4.2 *Staphylococcus aureus*

For the isolation of *Staphylococcus aureus*, a loopful of the sample was inoculated onto Baird-Parker (BP) agar medium (Hi-media) and was incubated at 37°C for 48 h (Lancette and Bennett, 2001) (Fig.1). At the end of incubation, colonies showing characteristics appearance (circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, gray black to jet black, frequently with light coloured margin, surrounded by opaque zone and frequently with outer clear zone) on BP agar medium were selected and transferred to nutrient agar slants and incubated at 37°C for overnight. The isolates were stored at refrigeration

Flow chart 3. Isolation and identification of *Escherichia coli*

	Characteristics/Reactions
Milk sample	
↓	
EMB agar	
↓	colonies with a dark center and a distinct indelible-ink greenish black metallic sheen on deflected light
Suspected colonies	
↓	
Nutrient agar	
↓	
Grams' staining reaction and cell morphology	Gram negative small rods
↓	
Motility test	+
↓	
Growth aerobically	+
↓	
Catalase	+
↓	
Oxidase	-
↓	
Glucose (acid)	+
↓	
OF test	F
↓	
Urease	-
↓	
ONPG	+
↓	
Indole	+
↓	
MR	+
↓	
VP	-
↓	
Citrate Utilization test	-
↓	
Carbohydrate utilization	
Lactose	+
Gluose	+
Mannitol	+
Inositol	-
Maltose	+

Flow chart 4. Isolation and identification of *Staphylococcus aureus*

	Characteristics/Reactions
Milk sample	
↓	
Inoculated on to BP agar	
↓	
Suspected colonies on to Nutrient agar slant	Gray black to jet black, frequently with light coloured margin, surrounded by opaque zone
↓	
Gram's staining reaction and cell morphology	Gram positive cocci in singles, pairs, cluster or bunch of grapes appearance
↓	
Motility test	-
↓	
Growth aerobically	+
↓	
Growth anaerobically	+
↓	
Catalase	+
↓	
Oxidase	-
↓	
Glucose (acid)	+
↓	
OF test	F
↓	
VP	+
↓	
Arginine hydrolysis	+
↓	
Phosphatase	+
↓	
Gelatin liquefaction	+
↓	
Urease	+
↓	
Coagulase test	+
↓	
Carbohydrate utilization	
Glucose	+
Lactose	+
Mannitol	+
Aerobic	+
Anaerobic	+

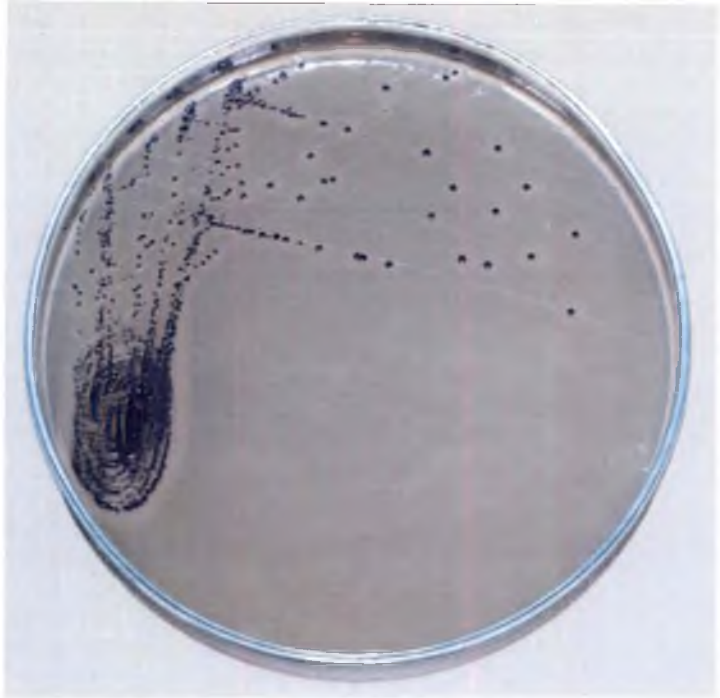


Fig. 1 *Staphylococcus aureus* in Baird - Parker agar

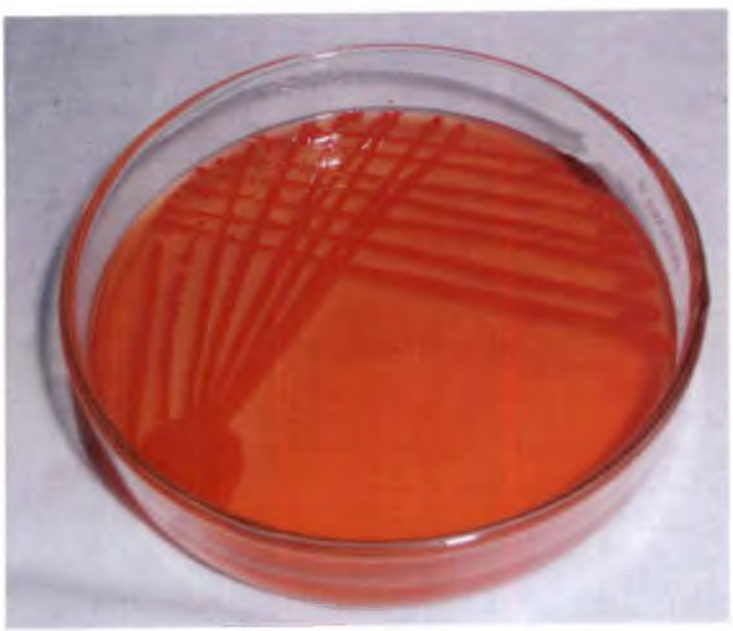


Fig. 2 Congo Red binding property of *Escherichia coli*

temperature. Characterization and identification of the isolates were done following the procedure described by Barrow and Feltham (1993) and are shown in the flow chart 4. The isolates were identified based on the cultural, morphological and biochemical characteristics.

3.1.4.3 *Yersinia*

The procedure used for the isolation and identification of *Yersinia enterocolitica* was described by Weagant and Feng (2001). Milk sample (10ml) was mixed with 90 ml of the enrichment medium (Peptone Sorbitol Bile Salt broth of pH 7.6 ± 0.2) (Hi-media) and incubated at 10^0C for 10 days. On 10^{th} day of incubation the sample was mixed well and a loopful of the enriched inoculum was streaked on to *Yersinia* Selective Agar (Hi-media) supplemented with CIN (Celfsulodin, Irgasan - Novobiocin). The plates were incubated at 25^0C for 24 h. Typical colonies of *Yersinia* of 1-2 mm diameter and with dark red centre and sharp border surrounded by clear transparent zone were transferred to nutrient agar slants. The isolates were characterized by the cultural, morphological and biochemical characters as described by Barrow and Feltham (1993) and are shown in flow charts 5 to 10.

3.1.5 Characterisation and identification of isolates

The suspected colonies selected as *Escherichia coli*, *Staphylococcus aureus* and *Yersinia* were subjected to various tests and identified based on the cultural, morphological and biochemical characteristics described by Barrow and Feltham (1993) except for the triple sugar iron agar test (Edwards and Ewing, 1972).

3.1.5.1 *Primary identification test*

I. Catalase test

a) Slide test : A small quantity of colony was transferred onto a clear, grease free, glass slide and mixed well with a drop of three per cent hydrogen peroxide. Evaluation of effervescence within a few seconds indicated a positive reaction.

b) Tube test : One ml of three percent hydrogen peroxide solution was poured over the slope of a nutrient agar slant on which the isolates was grown. A positive reaction was indicated by the development of effervescence immediately.

2. Gram staining

The procedure for gram staining was as follows:

a. A thin smear of each isolate was made on a clean, grease free glass slide. Air-dried the smear and then heat fixed by passing over a flame.

b. The smear was then flooded with 0.5 per cent crystal violet in water and allowed to act for 30 seconds.

c. Poured off the stain and washed with water.

d. Flooded the smear with Grams' iodine solution (one per cent iodine and two per cent potassium iodide in water) for 30 sec.

e. Poured off the solution and the smear was decolourised with a few drops of acetone and allowed to act for two to three seconds.

f. Washed the smear and counter stained with dilute carbol fucshin for 30 seconds.

g. Poured off the stain from the slide, washed, dried and examined under oil immersion objective of the microscope.

3. Motility test

Motility of the organism was assessed by stabbing the isolate into the Hugh Leifson's medium with a straight wire up to a depth of 5 mm. Motility was indicated by a spreading growth into the medium from the line of inoculations and growth of non-motile organisms is confirmed to the stab.

4. Oxidase test

A filter paper strip was moistened with a few drops of an aqueous solution

Flow chart 5. Isolation and identification of *Yersinia*

(Y. enterocolitica)

	Characteristics/Reactions
Milk sample	
↓	
Enrichment in peptone sorbitol bile salt broth (10^0C for 10 days)	
↓	
Streak on Yersinia selective agar	Colonies with dark red centre, sharp border and having one to two mm diameter surrounded by clear transparent zone
↓	
Nutrient agar	
↓	
Gram's reaction and cell morphology	Gram negative rods
↓	
Motility (25° C)	+
↓	
Catalase	+
↓	
OF	F
VP	+
Citrate utilisation	-
↓	
Arginine dihydrolase	-
↓	
Urease	+
↓	
Lysine decarboxylase	-
↓	
Ornithine decarboxylase	+
↓	
Carbohydrate utilization	
Sucrose	+
Melibiose	-
Rhamnose	-
Raffinose	-
Maltose	+

Flow chart 6. Isolation and identification of *Yersinia*

(Y. pseudotuberculosis)

	Characteristics/Reactions
Milk sample	
↓	
Enrichment in peptone sorbitol bile salt broth (10^0C for 10 days)	
↓	
Streak on Yersinia selective agar	Colonies with dark red centre, sharp border and having one to two mm diameter surrounded by clear transparent zone
↓	
Nutrient agar	
↓	
Gram's reaction and cell morphology	Gram negative rods
↓	
Motility (25° C)	+
↓	
Catalase	+
↓	
OF	F
↓	
Citrate utilisation	-
↓	
Arginine dihydrolase	-
↓	
Urease	+
↓	
Lysine decarboxylase	-
↓	
Ornithine decarboxylase	+
↓	
Carbohydrate utilization	
Sucrose	-
Cellobiose	-
Rhamnose	+
Inositol	-
Maltose	+

Flow chart 7. Isolation and identification of *Yersinia*

(Y. frederiksenii)

	Characteristics/Reactions
Milk sample	
↓	
Enrichment in peptone sorbitol bile salt broth (10^0C for 10 days)	
↓	
Streak on Yersinia selective agar	Colonies with dark red centre, sharp border and having one to two mm diameter surrounded by clear transparent zone
↓	
Nutrient agar	
↓	
Gram's reaction and cell morphology	Gram negative rods
↓	
Motility (25° C)	+
↓	
Catalase	+
↓	
OF	F
↓	
VP	+
↓	
Arginine dihydrolase	-
↓	
Urease	+
↓	
Lysine decarboxylase	-
↓	
Ornithine decarboxylase	+
↓	
Carbohydrate utilization	
Sucrose	+
Melibiose	-
Rhamnose	+
Raffinose	-
Maltose	+

Flow chart 8. Isolation and identification of *Yersinia*

(Y. kristensenii)

	Characteristics/Reactions
Milk sample	
↓	
Enrichment in peptone sorbitol bile salt broth (10^0C for 10 days)	
↓	
Streak on Yersinia selective agar	Colonies with dark red centre, sharp border and having one to two mm diameter surrounded by clear transparent zone
↓	
Nutrient agar	
↓	
Gram's reaction and cell morphology	Gram negative rods
↓	
Motility (25° C)	+
↓	
Catalase	+
↓	
OF	F
VP	-
Citrate utilisation	-
↓	
Arginine dihydrolase	-
↓	
Urease	+
↓	
Lysine decarboxylase	-
↓	
Ornithine decarboxylase	+
↓	
Carbohydrate utilization	
Sucrose	-
Melibiose	-
Rhamnose	-
Raffinose	-
Maltose	+

Flow chart 9. Isolation and identification of *Yersinia*

(Y. aldovae)

	Characteristics/Reactions
Milk sample	
↓	
Enrichment in peptone sorbitol bile salt broth (10^0C for 10 days)	
↓	
Streak on Yersinia selective agar	Colonies with dark red centre, sharp border and having one to two mm diameter surrounded by clear transparent zone
↓	
Nutrient agar	
↓	
Gram's reaction and cell morphology	Gram negative rods
↓	
Motility (25° C)	+
↓	
Catalase	+
↓	
OF	F
↓	
Citrate utilisation	-
↓	
Arginine dihydrolase	-
↓	
Urease	+
↓	
Lysine decarboxylase	-
↓	
Ornithine decarboxylase	+
↓	
Carbohydrate utilization	
Sucrose	+
Melibiose	-
Cellobiose	+
Rhamnose	-
Inositol	+
Maltose	+

Flow chart 10. Isolation and identification of *Yersinia*

(Y. intermedia)

Milk sample	
↓	Characteristics/Reactions
Enrichment in peptone sorbitol bile salt broth (10^0C for 10 days)	
↓	
Streak on Yersinia selective agar	Colonies with dark red centre, sharp border and having one to two mm diameter surrounded by clear transparent zone
↓	
Nutrient agar	
↓	
Gram's reaction and cell morphology	Gram negative rods
↓	
Motility (25° C)	+
↓	
Catalase	+
↓	
OF	F
VP	+
Citrate utilisation	+
↓	
Arginine dihydrolase	-
↓	
Urease	+
↓	
Lysine decarboxylase	-
↓	
Ornithine decarboxylase	+
↓	
Carbohydrate utilization	
Sucrose	+
Melibiose	+
Rhamnose	+
Raffinose	+
Maltose	+

of 1 per cent tetramethyl paraphenyline diamine dihydrochloride. Each isolate was then smeared across the paper strip with a platinum loop. The appearance of a dark purple colour on the paper strip within 30 second indicated a positive reaction.

5. ***Oxidation – Fermentation test***

Each isolate was inoculated into duplicate tubes of Hugh Liefson's media by stabbing with a straight wire. One of the tubes was sealed with a layer of melted soft paraffin to a depth of about 3 cm above the medium. The tubes were incubated at 37°C for up to 14 days. A change in colour of the medium from green to yellow in the open tubes alone was taken as oxidation whereas a change in colour from green to yellow in both the tubes was regarded as fermentation. Absence of colour change in both tubes indicated no action on carbohydrates.

3.1.5.2 *Secondary tests*

1) Aesculin hydrolysis

The organism was inoculated into aesculin broth and was incubated at 37°C and examined daily for five days. Blackening of the broth due to hydrolysis of aesculin indicated a positive reaction.

2) Arginine hydrolysis

The organism was inoculated into five ml of arginine broth and was incubated at 37°C for 24 h. At the end of incubation period, added 0.25 ml of Nessler's reagent. Arginine hydrolysis is indicated by the development of brown colour.

3) Carbohydrate utilization test

Each isolate was inoculated into two test tubes containing peptone water with Andrade's indicator and one per cent of the appropriate sugar. One of the tubes contained an inverted Durham's tube. The inoculated tubes were incubated at 37°C and examined daily for seven days to detect the production of acid and/or gas. A change in colour of the medium to pink indicated acid production and the production of gas was indicated by the appearance of air bubbles in the inverted

Durham's tube. Anaerobic condition of the medium was provided by adding a layer of sterile molten soft paraffin to a depth of about one centimeter above the media.

4) Citrate utilisation test

A light suspension of the organism was made in normal saline and was inoculated with a straight wire onto the slope of Simmon's citrate agar. The inoculated medium was incubated at 37°C and examined daily up to seven days. The ability of the organism to utilize citrate as the sole source of carbon was indicated by a change in colour of the medium from green to blue and growth of the organism along the streak line.

5) Coagulase test

a) Slide test : A small quantity of the culture was emulsified in a drop of saline on a microscope slide to produce a thick suspension. The suspension was stirred with a straight wire dipped in rabbit plasma. Macroscopic clumping with in few seconds indicated a positive result and delayed clumping is considered as a negative reaction.

b) Tube test : Mixed 0.5 ml undiluted rabbit plasma with an equal volume of an 18 to 24 h broth culture of the test organism and incubated at 37°C and examined after one and four h for coagulation. Negative tubes were left at room temperature overnight and re-examined.

6) Decarboxylase reaction

Each isolate was heavily inoculated with straight wire into three test tubes containing decarboxylase media. One of the tube contained lysine and other contained ornithine. The third tube taken as the control. The organism was inoculated through the paraffin layer and incubated at 37°C for five days. In a positive reaction, the medium first turns yellow and then becomes purple and the control tubes remain yellow.

7) Eijkman test

Each test organism was inoculated into tubes containing MacConkey broth

with inverted Durham's' tube, warmed to 37°C and incubated at 44 ± 0.1°C in a water bath for 48 h. Production of both acid and gas indicated a positive reaction.

8) Gelatin hydrolysis/liquefaction

Each isolate was inoculated into nutrient gelatin and incubated at 37°C up to 14 days. An uninoculated control tube was also set. The tubes were cooled every two to three days in a refrigerator for 2 h and then examined for liquefaction. A positive result was indicated by liquefactions of gelatin.

9) Hippurate hydrolysis

The slope of hippurate agar was lightly inoculated with the test organism and examined daily for seven days. Hydrolysis of hippurate was indicated by growth and the development of a pink colour due to alkali production.

10) Indole production

The isolate was inoculated into peptone water and incubated at 37°C for 48 h. At the end of incubation added 0.5 ml of Kovac's reagent mixed well and examined. A red colour in the reagent layer indicated a positive reaction.

11) Methyl red (MR) reaction

The MR-VP medium was inoculated with the isolate and incubated at 37°C for two days. Added two drops of methyl red solution at the end of incubation period and examined. Development of a red colour indicated positive reaction.

12) ONPG (O-nitrophenyl-P-D-glactopyranocide) test

Each isolate was inoculated into ONPG broth and incubated at 37°C for 48 h. The p-galactosidase activity of the organism was indicated by the development of a yellow colour due to the production of O-nitrophenol.

13) Phenylalanine deamination

The phenylalanine agar slope was heavily inoculated with the test organism and incubated at 37°C for overnight. At the end of incubation, 0.2 ml of 10 per cent aqueous solution of ferric chloride was poured over the slope. A positive result was

indicated by the development of a green colour on the slope and in the free liquid at the base.

14) Phosphatase test

The phenolphthalene phosphate agar was lightly inoculated with the test organism to obtain discrete colonies and incubated at 37°C for 18 h. At the end of incubation, 0.1 ml of ammonia solution (specific gravity –0.880) was placed in the lid of the Petri-dish and the medium was inverted above it. Free phenolphthalein liberated by phosphatase react with the ammonia and phosphatase positive colonies became bright pink.

15) *Triple sugar iron agar test*

Each isolate was stab inoculated into the butt of triple sugar iron agar with straight wire and the slope of the agar was streaked with the wire. The inoculated tubes were incubated at 37°C for 24 h. The tubes were examined at the end of incubation for the development of an alkaline slant and an acid butt, with or without the production of hydrogen sulphide (Edwards and Ewing, 1972).

16) Urease activity

Slopes of Christensens' urea agar was heavily inoculated with the test organism and incubated at 37°C. The tubes were examined after 4 h of incubation and daily for 5 days. Development of a red colour in the medium indicated a positive reaction.

17) Voges-Proskauer reaction

The MR-VP medium inoculated with the isolate was subjected to methyl-red test. After completion of the test, added 0.6 ml of 5 per cent ά- naphthol solution and 0.2 ml of 40 per cent aqueous potassium hydroxide into the tube. After thorough mixing of the contents, the tube was kept in a slanting position and examined after 15 min and one h. A positive reaction is indicated by the development of a strong red colour.

3.1.6 In-vitro pathogenicity studies for *Escherichia coli*

Congo red binding assay

Congo red binding assay of the *Escherichia coli* isolates were carried out by the method given by Rajil *et al.* (2003). Tryptone Soya Agar was supplemented with 0.03 per cent congo red dye (Nesslers) and 0.15 per cent bile salts (Loba Chemie) was used for assay. *Escherichia coli* isolates were cultured on duplicate plates of the congo red medium and incubated at 37°C for 24 h. After incubation, the cultures were left at room temperature for 48 h to facilitate annotation of results. Invasive *Escherichia coli* were identified by their ability to take up congo red dye and production of characteristic brick red colonies (Fig. 2)

3.2 GRADING OF MILK BASED ON TOTAL VIABLE COUNT

Milk was graded based on total viable count according to standard prescribed by Bureau of Indian Standards (IS, 1977) and the criteria for grading of milk is given in table 1.

Table 1. The criteria for grading of milk based on total viable count

Grade	Bacterial count (Lakh/ml)
Very good	Less than two
Good	Between two and 10
Fair	Between 10 and 50
Poor	Greater than 50

3.3 CRITICAL CONTROL POINTS OF BACTERIAL CONTAMINATION OF MILK

In order to assess the critical control points of bacterial contamination of milk during various stages of production, the samples of air, water, washing of utensils, milker's/ milk handler's hand wash and udder washes of animal were collected and their bacterial load were determined. The details of major and minor

sources of contamination at the farmer level and society level are given in the flow chart 11 and 12, respectively.

3.3.1. Collection of samples

3.3.1.1 *Water sample*

The samples of water used in milking shed were collected following the procedures described by Indian Standards (1978). Allowed the water from the tap to run to waste for about two min in order to flush the interior of the nozzle and discharge the stagnant water. A sterile bottle of 250 ml capacity was used to collect the water. The bottle was held near the base with one hand and filled from a gentle stream of water from the tap, avoiding splashing and brought to the laboratory in an insulated container.

3.3.1.2 *Milking utensils*

Rinse method (Evancho *et al.*, 2001) was followed for the collection of samples from utensil. One hundred ml of sterile 0.1 per cent peptone water was poured into the utensil and mixed thoroughly by agitating. The sample was transferred into a sterile conical flask and brought to the laboratory in an insulated container.

3.3.1.3 *Milker's or milk handlers' hand wash*

On each visit the hand washings of individual milker involved in milking operation was collected. The individual's hand was washed in 100 ml of 0.1 per cent sterile peptone water and washing was collected in sterile conical flask and brought to the laboratory in an insulated container.

3.3.1.4 *Udder washes of the animal*

On each visit udder wash from animals were collected. The udder and teat of the animal was washed with 100 ml of 0.1 per cent sterile peptone water and

washes were collected in sterile conical flask and brought to the laboratory in an insulated container.

3.3.2 Processing of samples

Samples brought to the laboratory were agitated vigorously for about 25 times. In order to estimate the bacterial load per ml of water sample, 25 ml was transferred to 225 ml of 0.1 per cent peptone water so as to form one in 10 dilution of the sample. Further 10 fold serial dilutions were prepared by transferring one ml of inoculum to nine ml of the diluent. Dilutions were made up to 10^{-4}.

3.3.3 Bacterial counts

The selected serial dilutions of each sample were used to estimate the Total Viable Count (TVC), Coliform Count (CC), *Escherichia coli* Count (ECC) and Faecal Streptococcal Count (FSC) as described earlier.

3.3.4 Collection and estimation of microbial load in air

Air sample was collected from milking barn during milking process.

3.3.4.1 *Total Viable Count*

Direct exposure method described by Evancho *et al.* (2001) was employed for the estimation of total viable count in the air samples of milking barn. In order to estimate the count, duplicate Petri-dishes (90 mm diameter) containing sterile triple soya agar medium were exposed in the rooms for 15 min. The plates were brought to the laboratory in thermocool container and incubated at 37°C for 24 h. The number of colonies developed in the duplicate plates was counted and the mean count was expressed as cfu/ft^2/min.

3.4 ADULTERANTS AND PRESERVATIVES

All pooled milk samples were tested to detect the presence of adulterants *viz.*, starch and cane sugar and preservatives *viz.*, formaldehyde, neutralizer and boric acid as prescribed by Indian standards (1981).

1) ***Starch***

About 3 ml of well mixed sample was taken in a clean test tube and brought it to boil by holding the tube over a flame. Allow to cool the sample to room temperature. Add a drop of one per cent iodine solution. Presence of starch is indicated by the appearance of a blue colour, which disappears when the sample is boiled and reappears on cooling.

2) ***Cane sugar***

To about 15 ml of milk in a test tube, add 1 ml of concentrated hydrochloric acid and 0.1 g of resorcinol and mix. Place the tube in boiling water bath for 5 minutes. In the presence of cane sugar, a red colour is produced.

3) ***Boric acid***

Immerse a strip of turmeric paper in a sample of milk previously acidified with hydrochloric acid in the proportion of seven ml of concentrated hydrochloric acid to each 100 ml of milk. Allow the paper to dry spontaneously. If boric acid or borax is present, the paper will acquire a characteristic red colour. The addition of ammonium hydroxide will change the colour of the paper to a dark green, but the red colour may be restored by hydrochloric acid.

4) ***Formaldehyde***

To about 10 ml of milk in a wide mouthed test tube add about half the volume of concentrated sulphuric acid pouring the acid carefully down the side of the tube so that it forms a layer at the bottom without mixing with the milk. A violet or blue colour at the junction of the two liquid indicates the presence of formaldehyde. The result is sensitive to one part in 10000.

5) *Neutralizers (Carbonates)*

To about 5 ml of milk in a test tube, add 5 ml of alcohol, a few drops of 1 per cent (*w/v*) alcoholic solution of rosalic acid, and mix. If a carbonate is present, a rose-red colour appears whereas pure milk shows only a brownish colouration.

3.5 POLYMERASE CHAIN REACTION

3.5.1 Materials

- PCR reaction buffer (10X)

  This includes 500mM KCl, 100mM Tris-HCl (pH 9.0) and 15 mM $MgCl_2$.

- Taq DNA polymerase

  Taq DNA polymerase enzyme with a concentration of 3U/µl.

- Deoxy ribo Nucleotide Triphosphate (dNTP) mix 10mM (2.5 mM of each dGTP, dCTP, dATP and dTTP in equal volume)

All the above reagents were obtained from Bangalore Genei, India Limited.

Primers for genus specific PCR

Specific primers to detect *Escherichia coli* (Genus specific) designed by Daly *et al.* (2002) were used. The sequences of the primers were as follows:

5'-CTG GAA GAG GCT AGC CTG GAC GAG-3'

5'-AAA ATC GGC ACC GGT GGA GCG ATC-3'

3.5.2 Reconstitution and dilution of primers

Primers obtained in lyophilized form were reconstituted in 100µl of sterile triple distilled water to a concentration of 200 picomoles. The tubes were kept at room temperature with occasional shaking for one hour. They were spun briefly to pellet down the insoluble particles if any and the stock solution was distributed into 10µl aliquots and stored at -7°C. At the time of use the aliquots were thawed and further diluted ten fold to obtain a concentration of 20 picomoles/µl. before using for PCR.

3.5.3 Method

Polymerase Chain Reaction was conducted for the detection of *Escherichia coli* by the method as described by Daly *et al.* (2002).PCR technique was employed using template DNA prepared from the following method.

3.5.3.1 *Preparation of template DNA*

Overnight culture of isolates of *Escherichia coli* (37°C for 18h in Trypticase Soya broth or Soybean Casein digest broth) obtained from raw milk is taken in an eppendorf tube (1.5ml) and centrifuged at 3000g for 10 minutes. The supernatant is discarded and the pellet obtained at the bottom of the tube is washed twice with sterile PBS and finally the pellet is resuspended in 100µl of triple distilled water. The mixture is boiled for 10 minutes and then immediately chilled on ice for 30 minutes. The samples were thawed and centrifuged at 3000g for 5 minutes and the supernatant is stored at -20°C for further use as template for PCR.

3.5.3.2 *Setting up of PCR*

PCR was performed in a total volume of 25µl reaction mixture. A master mix prepared before setting up the PCR reaction by combining the following reagents in a 20µl volume. The reaction mixture consisted of

Primers	20 picomole of each primer
10X PCR buffer	50mMKCl, 10mM Tris-HCl and 1.5 mM $MgCl_2$
Taq DNA polymerase	1.0 unit
dNTP mix	200µl of each dNTP

Preparation of 200µl master mix for 10 reactions

PCR reaction buffers	25µl
Forward primer	10µl
Reverse primer	10µl
dNTPmix	20µl

Taq DNA polymerase	3.3µl
Triple distilled water	200µl

To each PCR tube 20µl of master mix and 5µl of template DNA were added. One negative control without DNA was also added. The PCR amplification was carried out in an automated thermal cycler (Eppendorf Master Cycler, Germany) according to the following programme.

Initial denaturation at 95°C for one minute followed by 35 cycles of denaturation at 95°C for 20 seconds, annealing at 72°C for 90 seconds and extension at 72°C for 5 minutes and a final extension at 72°C for 6 minutes. The whole reaction was conducted under the heated lid.

The product was analyzed by submarine agarose gel electrophoresis.

3.5.4 Submarine Agarose Gel Electrophoresis

3.5.4.1 *Materials*

A. (0.5 M) EDTA (pH 8.0)

Dissolved 18.61g of EDTA (disodium, dihydrate) in 70ml of triple distilled water. The pH was adjusted to 8.0 with 1N NaOH. The volume was made upto 100ml, filtered, autoclaved at 121°C for 15 minutes at 15 lbs pressure and stored at room temperature.

B. TAE (Tris-Acetate EDTA) buffer (50X) pH 8.0

Tris base	48.40 g
Glacial acetic acid	11.42 ml
0.5 M EDTA pH 8.0	20.00 ml
Distilled water to	1000 ml

Autoclaved at 121°C for 15 minutes at 15 lbs pressure and stored at room temperature.

C. TAE (1X)

TAE 50X	2 ml
Distilled water	98 ml

D. Agarose Gel (1.5 per cent)

Agarose low EEO (Genei)	1.5 g
TAE buffer (1X)	100 ml

E. Gel loading buffer (6X)

Bromophenol blue	0.25 g
Xylene cyanol	0.25 g
Sucrose	40.00 g
Distilled water to	100 ml

Stored at 4°C.

F. Ethidium bromide

Ethidium bromide	100 mg
Distilled water	10 ml

Stored at 4°C in amber coloured bottles.

G. DNA molecular size marker

pUC19DNA/*Msp*I digest with fragments 501, 489, 404, 331, 242, 190, 147, 111, 110, 67, 34 and 26 bp.

The molecular size markers were obtained from Bangalore Genei (India).

3.5.4.2 *Method*

The PCR product was detected by electrophoresis in 1.5 per cent agarose gel in TAE buffer (1X). Agarose was dissolved in TAE buffer (1X) by heating. When the mixture was cooled to around 50oC, Ethidium bromide was added to a final concentration of 0.5 µg/ml. Melted agarose was then poured into clean, dry, gel platform, the edges of which were sealed with adhesive tape and the comb was kept in proper position. Once the gel was set, the comb and adhesive tape were removed gently and the tray containing the gel was completely covered

Amplified PCR product (5 µl) was mixed with one µl of 6X gel loading buffer and the samples were loaded in the wells. The pUC19DNA/*Msp*I DNA molecular size digest was used as marker. Electrophoresis was carried at 5V/cm for one hour (or) until the bromophenol blue dye migrated to more than two-third of the length of the gel.

The gel was visualized under UV transilluminator (Hoefer, USA) and the images were documented in a gel documentation system (Bio-Rad Laboratories, USA).

3.6 STATISTICAL ANALYSIS

The data obtained from the various studies were subjected to statistical analysis following procedure described by Rangaswamy (1995).

Results

4. RESULT

In the present investigation a total of 180 raw milk samples, consisted of 108 individual milk samples from farmers belonging to three co- operative societies (S_1, S_2 and S_3) and 72 pooled milk samples from the three societies were collected and evaluated the microbial quality. The samples were tested to detect the presence of *Escherichia coli, Staphylococcus aureus* and *Yersinia.* During the investigation the factors contributing the bacterial contamination of milk from various sources were also evaluated to identify the critical control points. The pooled milk samples obtained from the societies were also tested to detect the adulterants (starch and cane sugar) and preservatives (boric acid, formaldehyde and neutralizers) added in the milk. Polymerase Chain Reaction (PCR) technique was employed to confirm the isolated *Escherichia coli* cultures from milk.

4.1 MICROBIAL QUALITY OF MILK

All raw milk samples were tested to determine the microbial quality by estimating Total Viable Count (TVC), Coliform Count (CC), *Escherichia coli* Count (ECC), Faecal Streptococcal Count (FSC) and Yeast and Mould Count (YMC).

4.1.1 Microbial counts of individual raw milk samples of S_1, S_2 and S_3

The microbial load of 36 individual milk samples each obtained from the farmer belonging to three societies was evaluated and was illustrated in fig. 3.

4.1.1.1 *Total Viable Count*

The mean total viable count of individual raw milk samples obtained from the farmers of three societies and the overall mean count are given in table 2. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between mean counts of the samples from the three societies. The analysis of the data by Least Significant Difference (LSD) test showed significant ($P<0.05$) difference between the mean count of samples of S_1 and S_2 and S_2 and S_3. The

overall mean total viable count of the samples was 6.01 ± 0.07 $\log_{10}$ cfu/ml. The highest mean count, 6.37 ± 0.13 $\log_{10}$ cfu/ml, was observed in the samples of S_2 and the lowest count was in the samples of S_3 (5.67 ± 0.13 $\log_{10}$ cfu/ml).

Table 2. Mean total viable count of individual raw milk samples from S_1, S_2 and S_3

Sources of milk samples	Total viable count
	Mean ± SE ($\log_{10}$ cfu/ml)
S_1	$5.99^b \pm 0.92$
S_2	$6.37^a \pm 0.13$
S_3	$5.67^b \pm 0.13$
Overall	6.01 ± 0.07

Figures bearing the same superscript in the same column do not differ significantly., N = 36 from each source

Table 3. Frequency distribution of milk samples of S_1, S_2 and S_3 based on total viable count

Sources of milk samples	Total viable count (cfu/ml)			
	10^4	10^5	10^6	10^7
S_1	1 (2.78)	14 (38.89)	21 (58.33)	0
S_2	1 (2.78)	9 (25.00)	17 (47.22)	9 (25.00)
S_3	8 (22.22)	19 (52.78)	6 (16.67)	3 (8.33)
Overall	10 (9.26)	42 (38.89)	44 (40.74)	12 (11.11)

Figures in parenthesis indicate per cent., N = 36 from each source

The distribution of individual milk samples, from the three societies, based on total viable count is given in table 3. The highest count at the level of 10^7 cfu/ml was seen in 12 (11.11 per cent) out of 108 samples, while 44 (40.74 per cent) samples had the count at the level of 10^6 cfu/ml. Forty two (38.89 per cent) of the 108 samples showed count at the level of 10^5 cfu/ml, whereas 10 (9.26 per cent) samples had count at the level of 10^4 cfu/ml.

4.1.1.2 *Coliform Count*

The mean coliform count of raw milk samples from individual farmers belonging to the three societies and the overall mean count are given in table 4. The overall mean coliform count of the samples was 4.44 ± 0.07 $\log_{10}$ cfu/ml. The highest mean coliform count of 4.63 ± 0.11 $\log_{10}$ cfu/ml was observed in samples of S_2, whereas the lowest count was seen in the samples of S_3 (4.31 ± 0.10 $\log_{10}$ cfu/ml).

Table 4. Mean coliform count of individual raw milk samples from S_1, S_2 and S_3

Sources of milk samples	Coliform count
	Mean ± SE ($\log_{10}$ cfu/ml)
S_1	4.41 ± 0.16
S_2	4.63 ± 0.11
S_3	4.31 ± 0.10
Overall	4.44 ± 0.07

N = 36 from each source

Table 5. Frequency distribution of milk samples based on coliform count from S_1, S_2 and S_3

Sources of milk samples	Coliform count (cfu/ml)		
	10^3	10^4	10^5
S_1	9 (25.00)	19 (52.78)	8 (22.22)
S_2	6 (16.67)	18 (50.00)	12 (33.33)
S_3	12 (33.33)	20 (55.56)	4 (11.11)
Overall	27 (25.00)	57 (52.78)	24 (22.22)

Figures in parenthesis indicate per cent., N = 36 from each source

The distribution of individual milk samples, from the three societies, based on coliform count is given in table 5. Of the 108 samples, count at the level of 10^3 cfu/ml was observed in 27 (25.00 per cent) samples whereas the count at the level

of 10^4 and 10^5 cfu/ml was observed in 57 (52.78 per cent) and 24 (22.22 per cent) of the samples, respectively.

4.1.1.3 *Escherichia coli* Count

The mean *Escherichia coli* count of the 108 milk samples obtained from three societies and the overall mean *Escherichia coli* count of the samples are given in the table 6. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between mean counts of the samples from the three societies. The analysis of the data by Least Significant Difference (LSD) test showed significant ($P<0.05$) difference between the mean count of samples of S_2 and S_3. The overall mean *Escherichia coli* count from the samples of three societies was 0.86 ± 0.11 $\log_{10}$ cfu/ml. The highest mean *Escherichia coli* count was observed in samples of S_2 (1.25 ± 0.22 $\log_{10}$ cfu/ml) and the lowest count was observed in the samples of S_3 (0.54 ± 0.16 $\log_{10}$ cfu/ml).

Table 6. Mean *Escherichia coli* count of individual milk samples of S_1, S_2 and S_3

Sources of milk samples	*Escherichia coli* Count
	Mean ± SE ($\log_{10}$ cfu/ml)
S_1	$0.78^{ab} \pm 0.19$
S_2	$1.25^{a} \pm 0.22$
S_3	$0.54^{b} \pm 0.16$
Overall	0.86 ± 0.11

Figures bearing the same superscript in the same column do not differ significantly., N = 36 from each source

The distribution of milk samples, from three societies based on *Escherichia coli* count is given in table 7. The organism was not detected in 69 (63.89 per cent) out of 108 milk samples at 1 in 100 and above dilutions. The organism was not detected in 24 (66.67 per cent) samples from S_1, 18 (50.00 per cent) samples from S_2 and 27 (75.00 per cent) samples from S_3. In 36 (33.33 per cent) out of 108

samples had the count at the level of 10^2 cfu/ml and only 3 (2.78 per cent) samples had count at the level of 10^3 cfu/ml.

Table 7. Frequency distribution of milk samples of S_1, S_2 and S_3 based on *Escherichia coli* count

Sources of milk samples	*Escherichia coli* Count (cfu/ml)		
	ND	10^2	10^3
S_1	24 (66.67)	11 (30.56)	1 (2.78)
S_2	18 (50.00)	16 (44.44)	2 (5.56)
S_3	27 (75.00)	9 (25.00)	0
Overall	69 (63.89)	36 (33.33)	3 (2.78)

Figures in parenthesis indicate per cent, ND - Not Detected ., N = 36 from each source

4.1.1.4 *Faecal Streptococcal Count*

The mean faecal streptococcal count of raw milk samples from individual farmers belonging to the three societies and overall mean count are given in table 8. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between mean counts of the samples from the three societies. Least Significant Difference test of the data showed significant ($P<0.05$) difference

Table 8. Mean faecal streptococcal count of individual raw milk samples from S_1, S_2 and S_3

Sources of milk samples	Faecal streptococcal count
	Mean ± SE (log_{10} cfu/ml)
S_1	$2.86^b \pm 0.20$
S_2	$3.66^a \pm 0.10$
S_3	$2.92^b \pm 0.17$
Overall	3.14 ± 0.10

Figures bearing the same superscript in the same column do not differ significantly., N = 36 from each source

between the mean counts of samples between S_2 and S_1 and the mean count of the samples between S_2 and S_3. The overall mean fecal streptococcal count of samples was 3.14 ± 0.10 $\log_{10}$ cfu/ml. The highest mean count of 3.66 ± 0.10 $\log_{10}$ cfu /ml was observed in the samples of S_2 and lowest mean count was seen in samples of S_1 (2.86 ± 0.20 $\log_{10}$ cfu/ml).

The distribution of individual milk samples from the three societies based on faecal streptococcal count is given in table 9. Faecal streptococci were not found in 7 (6.48 per cent) of the 108 samples. Of the 108 samples examined 58 (53.70 per cent) samples had count at the level of 10^3 cfu/ml, 25 (23.15 per cent) samples showed the count at the level of 10^2 cfu/ml and 18 (16.67 percent) samples had count at the level of 10^4 cfu/ml.

Table 9. Frequency distribution of milk samples based on faecal streptococcal count from S_1, S_2 and S_3

Sources of milk samples	Faecal streptococcal count (cfu/ml)			
	ND	10^2	10^3	10^4
S_1	4 (11.11)	11 (30.56)	17(47.22)	4 (11.11)
S_2	0	3 (8.33)	21 (58.33)	12 (33.33)
S_3	3 (8.33)	11 (30.56)	20 (55.56)	2 (5.56)
Overall	7 (6.48)	25 (23.15)	58 (53.70)	18 (16.67)

Figures in parenthesis indicate per cent, ND - Not Detected ., N = 36 from each source

4.1.1.5 *Yeast and Mould Count*

The mean yeast and mould count of raw milk from individual farmers belonging to the three societies and overall mean count are given in table 10. The overall mean yeast and mould count of samples was 2.09 ± 0.12 $\log_{10}$ cfu/ml. The highest mean count, 2.21 ± 0.20 $\log_{10}$ cfu/ml, was observed in the samples of S_3 and the lowest count was in the samples of S_2 (2.00 ± 0.21 $\log_{10}$ cfu/ml).

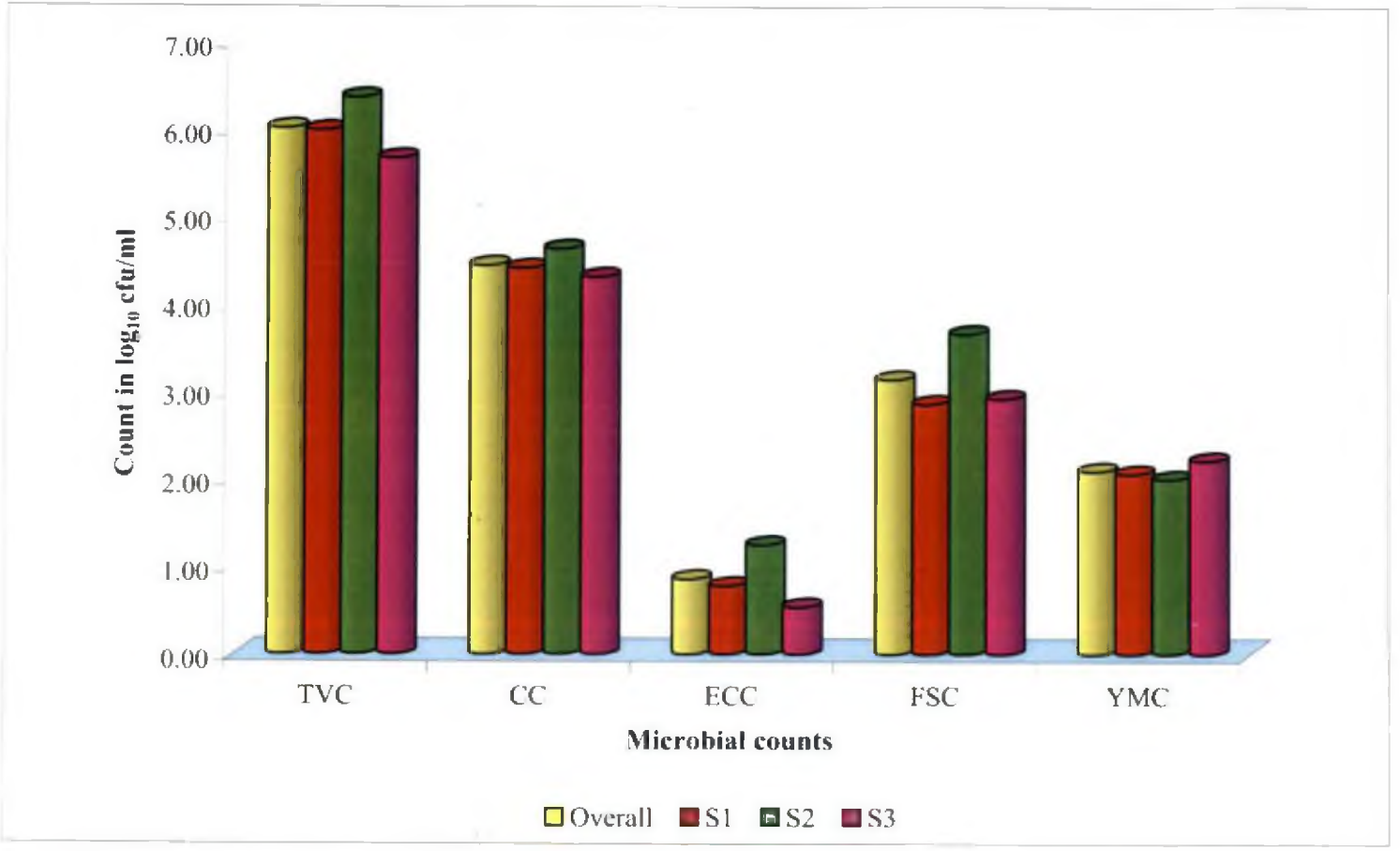


Fig. 3 Comparison of microbial quality of individual milk samples from S_1, S_2 and S_3

Table 10. Mean yeast and mould count of individual raw milk samples from S_1, S_2 and S_3

Sources of milk samples	Yeast and mould count
	Mean ± SE (log_{10} cfu/ml)
S_1	2.06 ± 0.21
S_2	2.00 ± 0.21
S_3	2.21 ± 0.20
Overall	2.09 ± 0.12

N = 36 from each source

The distribution of individual milk samples from the three societies based on yeast and mould count is given in table 11. Yeast and mould was not detected in 26 (24.07 per cent) of the 108 samples at 1 in100 and above dilutions. Of the 108 samples, 61 (56.48 per cent) samples had count at the level of 10^2 cfu/ml but the count in 21 (19.44 per cent) samples was at the level of 10^3 cfu/ml.

Table 11. Frequency distribution of milk samples based on yeast and mould count from S_1, S_2 and S_3

Sources of milk samples	Yeast and mould count (cfu/ml)		
	ND	10^2	10^3
S_1	9 (25.00)	21 (58.33)	6 (16.66)
S_2	9 (25.00)	24 (66.66)	3 (8.33)
S_3	8 (22.22)	16 (44.44)	12 (33.33)
Overall	26 (24.07)	61 (56.48)	21 (19.44)

Figures in parenthesis indicate per cent, ND - Not Detected., N = 36 from each source

4.1.1.6 *Correlation coefficient between bacterial counts of individual raw milk samples obtained from S_1, S_2 and S_3*

The correlation coefficient between various bacterial counts of raw milk samples belonging to three societies is shown in table 12. A significant ($P<0.05$) and positive association was observed between various bacterial counts. Analysis

of the data revealed significant ($P<0.05$) and positive correlation between total viable count and faecal streptococcal count and also between total viable count and coliform count. A similar correlation was observed between coliform count with faecal streptococcal count.

Table 12. Correlation coefficient between bacterial counts of individual raw milk samples of S_1, S_2 and S_3

Counts	CC	ECC	FSC	YMC
TVC	0.289*	0.060^{NS}	0.353*	0.016^{NS}
CC		0.100^{NS}	0.231*	0.063^{NS}
ECC			-0.018^{NS}	-0.141^{NS}
FSC				-0.031^{NS}

*Significant at 5 per cent ($P<0.05$)., NS- Non significant

4.1.2 Microbial counts of individual raw milk samples from society 1

Microbial quality of the samples collected from farmers belonging to S_1 were assessed and was illustrated in fig. 4.

4.1.2.1 *Total Viable Count*

The mean total viable count of milk samples collected from six farmers of S_1 is given in table 13. The overall mean count of the 36 samples was 5.99 ± 0.09 $\log_{10}$ cfu/ml. The samples obtained from F_1 had the highest mean count of 6.29 ± 0.15 $\log_{10}$ cfu/ml and the samples of F_5 showed the lowest count of 5.58 ± 0.37 $\log_{10}$ cfu/ml.

The distribution of milk samples based on total viable count received from the farmers of the S_1 is depicted in table 14. Of the 36 samples, 21 (58.33 per cent) samples had count at the level of 10^6 cfu/ml and the count at the level of 10^5 cfu/ml was present in 14 (38.89 per cent) samples. Only one of the samples belonging to F_5 had the count at the level of 10^4 cfu/ml.

Table 13. Mean total viable count of individual raw milk samples from S_1

Sources of milk samples	Total viable count
	Mean ± SE (log_{10} cfu/ml)
F_1	6.29 ± 0.15
F_2	5.94 ± 0.18
F_3	6.11 ± 0.16
F_4	5.86 ± 0.17
F_5	5.58 ± 0.37
F_6	6.19 ± 0.21
Overall	5.99 ± 0.09

N = 6 from each farmer

Table 14. Frequency distribution of milk samples based on total viable count from S_1

Sources of milk samples	Total viable count (cfu/ml)		
	10^4	10^5	10^6
F_1	0	1 (16.67)	5 (83.33)
F_2	0	2 (33.33)	4 (66.67)
F_3	0	2 (33.33)	4 (66.67)
F_4	0	3 (50.00)	3 (50.00)
F_5	1(16.67)	3 (50.00)	2 (33.33)
F_6	0	3 (50.00)	3 (50.00)
Overall	1 (2.78)	14 (38.89)	21 (58.33)

Figures in parenthesis indicate per cent., N = 6 from each farmer

4.1.2.2 *Coliform Count*

The mean coliform count of raw milk samples collected from farmers belonging to S_1 is given in table 15. The overall mean count of the samples from S_1 was 4.41 ± 0.16 log_{10} cfu/ml. The highest mean count was observed in the samples of the farmer F_3 (4.77 ± 0.19 log_{10} cfu/ml) and the lowest count of 3.52 ± 0.17 log_{10} cfu/ml was observed in the samples obtained from F_2.

Table 15. Mean coliform count of individual raw milk samples from S_1

Sources of milk samples	Coliform count
	Mean ± SE (log_{10} cfu/ml)
F_1	4.40 ± 0.21
F_2	3.52 ± 0.17
F_3	4.77 ± 0.19
F_4	4.72 ± 0.26
F_5	4.64 ± 0.24
F_6	4.40 ± 0.18
Overall	4.41 ± 0.16

N = 6 from each farmer

Table 16. Frequency distribution of milk samples based on coliform count from S_1

Sources of milk samples	Coliform count (cfu/ml)		
	10^3	10^4	10^5
F_1	1 (16.67)	4 (66.67)	1 (16.67)
F_2	4 (66.67)	1 (16.67)	1 (16.67)
F_3	1 (16.67)	3 (50.00)	2 (33.33)
F_4	1 (16.67)	3 (50.00)	2 (33.33)
F_5	1 (16.67)	3 (50.00)	2 (33.33)
F_6	1 (16.67)	5 (83.33)	0
Overall	9 (25.00)	19 (52.78)	8 (22.22)

Figures in parenthesis indicate per cent., N = 6 from each farmer

The distribution of milk samples based on coliform count received from the farmers of the S_1 is depicted in table 16. Count at 10^3 and 10^5 cfu/ml was present in 9 (25.00 per cent) and 8 (22.22 per cent) samples, respectively. The count in 19 (52.78 per cent) samples was at the level of 10^4 cfu/ml.

4.1.2.3 *Escherichia coli Count*

The mean *Escherichia coli* count of the samples obtained from the farmers of the S_1 is given in table 17. The samples had an overall mean count of 0.78 ± 0.19 log_{10} cfu/ml. The samples obtained from F_6 did not reveal the presence of the organism. The samples of the F_5 had the highest mean count of 1.50 ± 0.49 log_{10} cfu/ml.

Table 17. Mean *Escherichia coli* count of individual raw milk samples from S_1

Sources of milk samples	*Escherichia coli* Count
	Mean ± SE (log_{10} cfu/ml)
F_1	0.67 ± 0.42
F_2	0.96 ± 0.62
F_3	0.80 ± 0.51
F_4	0.72 ± 0.46
F_5	1.50 ± 0.49
F_6	0.00
Overall	0.78 ± 0.19

N = 6 from each farmer

Table 18. Frequency distribution of milk samples based on *Escherichia coli* count from S_1

Sources of milk samples	*Escherichia coli* count (cfu/ml)		
	ND	10^2	10^3
F_1	4 (66.67)	2 (33.33)	0
F_2	4 (66.67)	1 (16.67)	1(16.67)
F_3	4 (66.67)	2 (33.33)	0
F_4	4 (66.67)	2 (33.33)	0
F_5	2 (33.33)	4 (66.67)	0
F_6	6 (100.00)	0	0
Overall	24 (66.67)	11 (30.56)	1 (2.78)

Figures in parenthesis indicate per cent., ND -Not detected., N = 6 from each farmer

Based on *Escherichia coli* count the distribution of the milk samples obtained from farmers of the S_1 is given in table 18. The organism was not detected in 24 (66.67 per cent) out of the 36 of the samples. Of the samples, 11 (30.56 per cent) samples showed the count at the level of 10^2 cfu/ml and 1 (2.78 percent) sample had count at the level of 10^3 cfu/ml. The sample received from F_6 did not reveal the presence of the organism.

4.1.2.4 *Faecal Streptococcal Count*

Mean faecal streptococcal count of raw milk samples from S_1 is given in table 19. Analysis of variance test of the data showed a highly significant ($P<0.01$) difference between the mean count of the samples. The least significant difference test of the data revealed significant ($P<0.05$) difference between the mean count of the samples from F_2 and F_6, F_3 and F_5 and also between F_5 and F_6. The overall mean count of the sample was 2.86 ± 0.20 $\log_{10}$ cfu/ml. The highest mean count was seen in the samples of F_6 (3.97 ± 0.09 $\log_{10}$ cfu/ml), while the samples of F_5 had the lowest mean count (1.80 ± 0.59 $\log_{10}$ cfu/ml).

Table 19. Mean faecal streptococcal count of individual raw milk samples from S_1

Sources of milk samples	Faecal streptococcal count
	Mean ± SE ($\log_{10}$ cfu/ml)
F_1	$2.89^{abc} \pm 0.59$
F_2	$2.22^{bc} \pm 0.47$
F_3	$3.21^{ab} \pm 0.27$
F_4	$3.10^{abc} \pm 0.19$
F_5	$1.80^{c} \pm 0.59$
F_6	$3.97^{a} \pm 0.09$
Overall	2.86 ± 0.20

Figures bearing the same superscript in the same column do not differ significantly., N = 6 from each farmer

Table 20. Frequency distribution of milk samples based on faecal streptococcal count from S_1

Sources of milk samples	Faecal streptococcal count (cfu/ml)			
	ND	10^2	10^3	10^4
F_1	1 (16.67)	0	4 (66.67)	1 (16.67)
F_2	1 (16.67)	5 (83.33)	0	0
F_3	0	2 (33.33)	4 (66.67)	0
F_4	0	2 (33.33)	4 (66.67)	0
F_5	2 (33.33)	2 (33.33)	2 (33.33)	0
F_6	0	0	3 (50.00)	3 (50.00)
Overall	4 (11.11)	11 (30.56)	17 (47.22)	4 (11.11)

Figures in parenthesis indicate per cent., N = 6 from each farmer

Distribution of the samples from S_1 based on faecal streptococcal count is given in table 20. Out of the 36 samples, four samples did not showed the presence of organism at and above 1 in 100 dilution. The count in 30.56, 47.22 and 11.11 per cent of the samples was at the level of 10^2, 10^3 and 10^4 cfu/ml, respectively. The count in 83.33 per cent of the samples belonging to F_2 was at the level of 10^2 cfu/ml, while 66.67 per cent samples obtained from F_1, F_3 and F_4 had the count at the level of 10^3 cfu/ml.

4.1.2.5 *Yeast and Mould Count*

The mean yeast and mould count of raw milk samples is given in table 21. The overall mean count of the sample was 2.06 ± 0.21 $\log_{10}$ cfu/ml. The samples belonging to F_3 had the highest mean count (2.74 ± 0.25 $\log_{10}$ cfu/ml) and the lowest mean count (1.75 ± 0.56 $\log_{10}$ cfu/ml) was observed in the samples of F_5.

Distribution of milk samples belonging to S_1 based on yeast and mould count is given in table 22. Nine (25 per cent) samples did not show the presence of the organism at and above 1 in 100 dilution. The count at the level of 10^2 and 10^3 cfu/ml was seen in 21 (58.33 per cent) and 6 (16.67 per cent) samples, respectively.

None of the samples from F_5 and F_6 had count at the level of 10^3 cfu/ml. The count in 83.33 per cent samples belonging to F_6 and 66.67 per cent samples belonging to F_3 and F_5 was at the level of 10^2 cfu/ml.

Table 21. Mean yeast and mould count of individual raw milk samples from S_1

Sources of milk samples	Yeast and mould count
	Mean ± SE (log_{10} cfu/ml)
F_1	1.89 ± 0.60
F_2	1.76 ± 0.59
F_3	2.74 ± 0.25
F_4	2.01 ± 0.67
F_5	1.75 ± 0.56
F_6	2.22 ± 0.45
Overall	2.06 ± 0.21

N = 6 from each farmer

Table 22. Frequency distribution of milk samples based on yeast and mould count from S_1

Sources of milk samples	Yeast and mould count (cfu/ml)		
	ND	10^2	10^3
F_1	2 (33.33)	3 (50.00)	1 (16.67)
F_2	2 (33.33)	3 (50.00)	1 (16.67)
F_3	0	4 (66.67)	2 (33.33)
F_4	2 (33.33)	2 (33.33)	2 (33.33)
F_5	2 (33.33)	4 (66.67)	0
F_6	1 (16.67)	5 (83.33)	0
Overall	9 (25.00)	21 (58.33)	6 (16.67)

Figures in parenthesis indicate per cent., N = 6 from each farmer

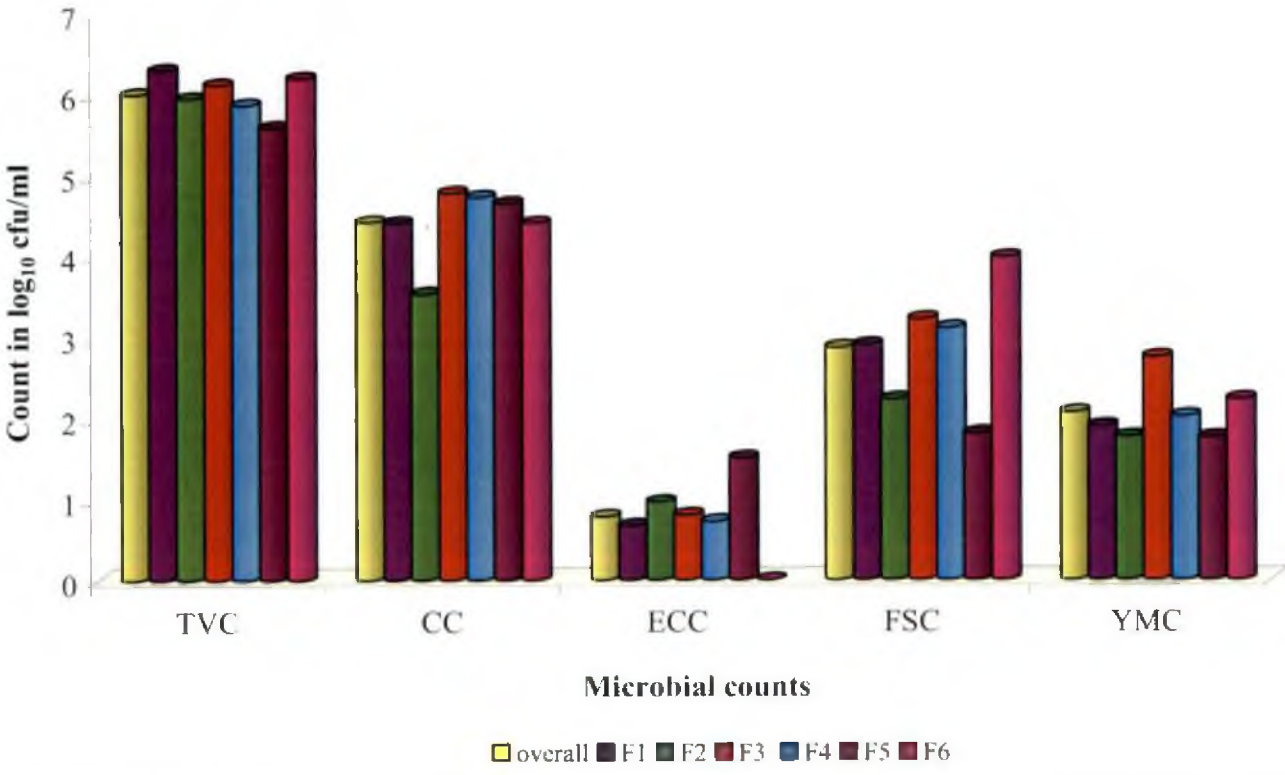


Fig. 4 Comparison of microbial quality of individual milk samples of S_1

4.1.2.6 *Correlation coefficient between bacterial counts of individual raw milk samples from S_1*

Critical difference test of the data revealed that none of the bacterial association was significant in the samples of S_1.

4.1.3 Microbial counts of individual raw milk from society 2

Microbial quality of the samples collected from farmers belonging to S_1 was assessed and was illustrated in fig. 5.

4.1.3.1 *Total Viable Count*

All samples collected from farmers of S_2 were evaluated and the mean total viable count (TVC) and the overall mean count of the samples are shown in table 23. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference in the mean count of samples belonging to the farmers. Least significant difference test of the data revealed significant ($P<0.05$) difference between mean count of samples of F_1 and F_4, F_1 and F_5, F_2 and F_4, F_2 and F_5, F_2 and F_6, F_3 and F_4, and F_3 and F_5 and also between F_5 and F_6. The overall mean count of the samples collected from six farmers was 6.37 ± 0.13 $\log_{10}$ cfu/ml. Samples belonging to F_2

Table 23. Mean total viable count of individual raw milk samples from S_2

Sources of milk samples	Total viable count
	Mean ± SE ($\log_{10}$ cfu/ml)
F_1	$6.72^{ab} \pm 0.24$
F_2	$7.08^{a} \pm 0.20$
F_3	$6.76^{ab} \pm 0.32$
F_4	$5.84^{cd} \pm 0.20$
F_5	$5.47^{d} \pm 0.16$
F_6	$6.36^{bc} \pm 0.10$
Overall	6.37 ± 0.13

Figures bearing the same superscript in the same column do not differ significantly., N = 6 from each farmer

had highest mean count (7.08 ± 0.20 $\log_{10}$ cfu/ml) and the lowest count (5.47 ± 0.16 $\log_{10}$ cfu/ml) was seen in samples collected from F_5.

Table 24. Frequency distribution of milk samples based on total viable count from S_2

Sources of milk samples	Total viable count (cfu/ml)			
	10^4	10^5	10^6	10^7
F_1	0	0	3 (50.00)	3 (50.00)
F_2	0	0	3 (50.00)	3 (50.00)
F_3	0	1(16.67)	2 (33.33)	3 (50.00)
F_4	0	4 (66.67)	2 (33.33)	0
F_5	1(16.67)	4 (66.67)	1 (16.67)	0
F_6	0	0	6 (100.00)	0
Overall	1 (2.78)	9 (25.00)	17 (47.22)	9 (25.00)

Figures in parenthesis indicate per cent., N = 6 from each farmer

Based on total viable count, the distribution of milk samples from the farmers of S_2 is given in table 24. Of the 36 samples, the count at the level of 10^5 and 10^7 cfu/ml was seen in 9 (25.00 per cent) samples each and the count at the level of 10^4 and 10^6 cfu/ml was seen in 1(2.78 per cent) and 17 (47.22 per cent) samples, respectively. One of the samples from the farmer F_5 had the count at the level 10^4 cfu/ml. Samples from F_4, F_5 and F_6 did not show the presence of organism at the level of 10^7 cfu/ml.

4.1.3.2 *Coliform Count*

The mean coliform count of raw milk samples collected from farmers belonging to S_2 is given in table 25. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between the mean coliform count of the samples collected from six farmers. The data were subjected to least significant difference test and the results revealed a significant ($P<0.05$) difference between mean count of samples of F_1 and F_4, F_1 and F_5, F_2 and F_4, F_2 and F_5, F_2 and F_6, F_3 and F_4, F_3 and F_5, F_4 and F_6, and also between F_5 and F_6. The overall mean count of

36 samples from the six farmers of S_2 was 4.63 ± 0.11 $\log_{10}$ cfu/ml. The samples from the F_2 had highest mean count (5.33 ± 0.15 $\log_{10}$ cfu/ml), whereas the samples from F_5 had the lowest mean count (3.89 ± 0.18 $\log_{10}$ cfu/ml).

Table 25. Mean coliform count of individual milk samples from S_2

Sources of milk samples	Coliform count
	Mean ± SE ($\log_{10}$ cfu/ml)
F_1	4.36^{ab} ± 0.29
F_2	5.33^{a} ± 0.15
F_3	5.29^{ab} ± 0.11
F_4	4.12^{c} ± 0.11
F_5	3.89^{c} ± 0.18
F_6	4.36^{b} ± 0.16
Overall	4.63 ± 0.11

Figures bearing the same superscript in the same column do not differ significantly., N = 6 from each farmer

Table 26. Frequency distribution of milk samples based on coliform count from S_2

Sources of milk samples	Coliform count (cfu/ml)		
	10^3	10^4	10^5
F_1	2 (33.33)	2 (33.33)	2 (33.33)
F_2	0	1 (16.67)	5 (83.33)
F_3	0	1 (16.67)	5 (83.33)
F_4	0	6 (100.00)	0
F_5	3 (50.00)	3 (50.00)	0
F_6	1 (16.67)	5 (83.33)	0
Overall	6 (16.67)	18 (50.00)	12 (33.33)

Figures in parenthesis indicate per cent., N = 6 from each farmer

Distribution of milk samples collected from farmers of S_2 based on coliform count is given in table 26. All samples revealed the presence of organisms. Of the

36 samples, the count at the level of 10^3, 10^4 and 10^5 cfu/ml were seen in 6 (16.67 per cent) 18 (50.00 per cent) and 12 (33.33 per cent) samples, respectively. All the samples from F_4 had count at the level of 10^4 cfu/ml. None of the sample from F_4, F_5 and F_6 showed the presence of organism at the level of 10^5 cfu/ml. In 83.33 per cent samples each from F_2 and F_3 had count at the level of 10^5 cfu/ml, while 83.33 per cent samples obtained from F_6 had the count at the level of 10^4 cfu/ml.

4.1.3.3 *Escherichia coli Count*

The mean *Escherichia coli* count of 36 raw milk samples collected from S_2 is given in table 27. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between the mean count of the samples collected from six farmers. Least significant difference test of the data showed significant ($P<0.05$) difference between the mean count of samples from F_1 and F_2, F_2 and F_3, F_2 and F_6, and also between F_5 and F_6. The overall mean count of the samples of S_2 was 1.25 ± 0.22 $\log_{10}$ cfu/ml. The samples of the F_6 had the highest mean count of 2.23 ± 0.49 $\log_{10}$ cfu/ml and none of the samples from F_2 revealed the presence of organism at and above 1 in 100 dilution.

Table 27. Mean *Escherichia coli* count of individual raw milk samples from S_2

Sources of milk samples	*Escherichia coli* Count
	Mean ± SE ($\log_{10}$ cfu/ml)
F_1	1.66^{ab} ± 0.53
F_2	0.00^{c} ± 0.00
F_3	1.67^{ab} ± 0.53
F_4	1.16^{abc} ± 0.53
F_5	0.72^{bc} ± 0.46
F_6	2.23^{a} ± 0.49
Overall	1.25 ± 0.22

Figures bearing the same superscript in the same column do not differ significantly., N = 6 from each farmer

Based on *Escherichia coli* count, the distribution of milk samples belonging to the farmers of S_2 is given in table 28. The organism was not detected in 18 (50.00 per cent) of the 36 samples from S_2 and the count at the level of 10^2 and 10^3 cfu/ml was seen in 16 (44.44 per cent) and 2 (5.56 per cent) of the 36 samples, respectively. None of the samples from F_2 revealed the presence of organisms even in 1 in 100 dilution. Only 2 (33.33 per cent) out of the 36 samples belonging to F_6 showed the presence of the organism at the level of 10^3 cfu/ml. In 66.67 per cent samples belonging to F_1, F_3 and F_4 had the count at the level of 10^2 cfu/ml.

Table 28. Frequency distribution of milk samples based on *Escherichia coli* count from S_2

Sources of milk samples	*Escherichia coli* Count (cfu/ml)		
	ND	10^2	10^3
F_1	2 (33.33)	4 (66.67)	0
F_2	6 (100.00)	0	0
F_3	2 (33.33)	4 (66.67)	0
F_4	2 (33.33)	4 (66.67)	0
F_5	5 (83.33)	1 (16.67)	0
F_6	1 (16.67)	3(50.00)	2 (33.33)
Overall	18 (50.00)	16 (44.44)	2 (5.56)

Figures in parenthesis indicate per cent., ND- Not detected., N = 6 from each farmer

4.1.3.4 *Faecal Streptococcal Count*

All samples collected from farmers of the S_2 were evaluated and the mean faecal streptococcal count (FSC) of the samples of each farmer and the overall mean count are shown in table 29. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between the mean count of the samples belonging to the farmers of S_2. Least significant difference test of the data revealed that significant ($P<0.05$) difference between the mean count of the samples F_2 and F_4, F_2 and F_5, F_3 and F_4, F_3 and F_5, also between F_5 and F_6. The overall mean count of the samples collected from six farmers was 3.66 ± 0.10 $\log_{10}$ cfu/ml. The highest

mean count (4.10 ± 0.18 $\log_{10}$ cfu/ml) was observed in samples from F_3. The lowest count (3.13 ± 0.21 $\log_{10}$ cfu/ml) was seen in samples collected from F_5.

Table 29. Mean faecal streptococcal count of individual raw milk samples from S_2

Sources of milk samples	Faecal streptococcal count
	Mean ± SE ($\log_{10}$ cfu/ml)
F_1	$3.57^{abc} \pm 0.19$
F_2	$3.99^{a} \pm 0.19$
F_3	$4.10^{a} \pm 0.18$
F_4	$3.31^{bc} \pm 0.13$
F_5	$3.13^{c} \pm 0.21$
F_6	$3.86^{ab} \pm 0.09$
Overall	3.66 ± 0.10

Figures bearing the same superscript in the same column do not differ significantly., N = 6 from each farmer

Table 30. Frequency distribution of milk samples based on faecal streptococcal count from S_2

Sources of milk samples	Faecal streptococcal count (cfu/ml)		
	10^2	10^3	10^4
F_1	1 (16.67)	5 (83.33)	0
F_2	0	1 (16.67)	5 (83.33)
F_3	0	2 (33.33)	4 (66.67)
F_4	1(16.67)	4 (66.67)	1(16.67)
F_5	1 (16.67)	5 (83.33)	0
F_6	0	4 (66.67)	2 (16.67)
Overall	3 (8.33)	21 (58.33)	12 (33.33)

Figures in parenthesis indicate per cent., N = 6 from each farmer

Distribution of milk samples belonging to S_2 based on faecal streptococcal count is given in table 30. All samples belonging to the source showed the presence of the organism. The count at the level of 10^2, 10^3 and 10^4 cfu/ml was seen in 3

(8.33 per cent), 21 (58.33 per cent) and 12 (33.33 per cent) samples, respectively. None of the samples from F_2 and F_3 had count at the level of 10^2 cfu/ml. The count at the level of 10^4 cfu/ml was not observed in samples of F_1 and F_5. In 83.33 per cent samples belonging to F_1 and F_5 had the count at the level of 10^3 cfu/ml, while an equal per cent of sample belonging to F_2 had the count at the level of 10^4 cfu/ml. The count in 66.67 per cent samples belonging to F_3 was at the level of 10^4 cfu/ml. The later per cent of the samples belonging to F_4 and F_6 had count at the level of 10^3 cfu/ml.

4.1.3.5 *Yeast and Mould Count*

The mean yeast and mould count of raw milk samples from S_2 is given in table 31. The overall mean yeast and mould count of all samples collected from S_2 was 2.00 ± 0.20 $\log_{10}$ cfu/ml. The samples belonging to F_3 had the highest mean count (2.99 ± 0.24 $\log_{10}$ cfu/ml). The lowest mean count (0.96 ± 0.61 $\log_{10}$ cfu/ml) was observed in the samples of F_6.

Table 31. Mean yeast and mould count of individual raw milk samples from S_2

Sources of milk samples	Yeast and mould count
	Mean ± SE ($\log_{10}$ cfu/ml)
F_1	1.73 ± 0.55
F_2	2.19 ± 0.44
F_3	2.99 ± 0.24
F_4	2.18 ± 0.45
F_5	1.96 ± 0.42
F_6	0.96 ± 0.61
Overall	2.00 ± 0.20

N = 6 from each farmer

Distribution of milk samples based on yeast and mould count from S_2 is given in table 32. Organism was not detected in 9 (25.00 per cent) of the 36 samples. The

count at the level of 10^2 and 10^3 cfu/ml was observed in 24 (66.67 per cent) and 3 (8.33 per cent) of the 36 samples.

Table 32. Frequency distribution of milk samples based on yeast and mould count from S_2

Sources of milk samples	Yeast and mould count (cfu/ml)		
	ND	10^2	10^3
F_1	2 (33.33)	4 (66.67)	0
F_2	1 (16.67)	5 (83.33)	0
F_3	0	4 (66.67)	2 (33.33)
F_4	1 (16.67)	5 (83.33)	0
F_5	1(16.67)	5 (83.33)	0
F_6	4 (66.67)	1 (16.67)	1(16.67)
Overall	9 (25.00)	24 (66.67)	3 (8.33)

Figures in parenthesis indicate per cent., ND – Not Detected., N = 6 from each farmer

4.1.3.6 *Correlation coefficient between bacterial counts of individual raw milk samples from S_2*

The correlation coefficient between various bacterial counts of raw milk samples collected from S_2 is given in the table 33. A significant ($P<0.05$) and positive association was observed between various bacterial counts. Analysis of the

Table 33. Correlation coefficient between bacterial counts of individual raw milk samples from S_2

Counts	CC	ECC	FSC	YMC
TVC	0.625*	0.048^{NS}	0.567*	0.169^{NS}
CC		-0.054^{NS}	0.568*	0.419*
ECC			0.055^{NS}	-0.087^{NS}
FSC				0.042^{NS}

* Significant at 5 per cent ($P<0.05$)., NS- non significant

data revealed that significant ($P<0.05$) and positive correlation between total viable count and faecal streptococcal count and also between total viable count and

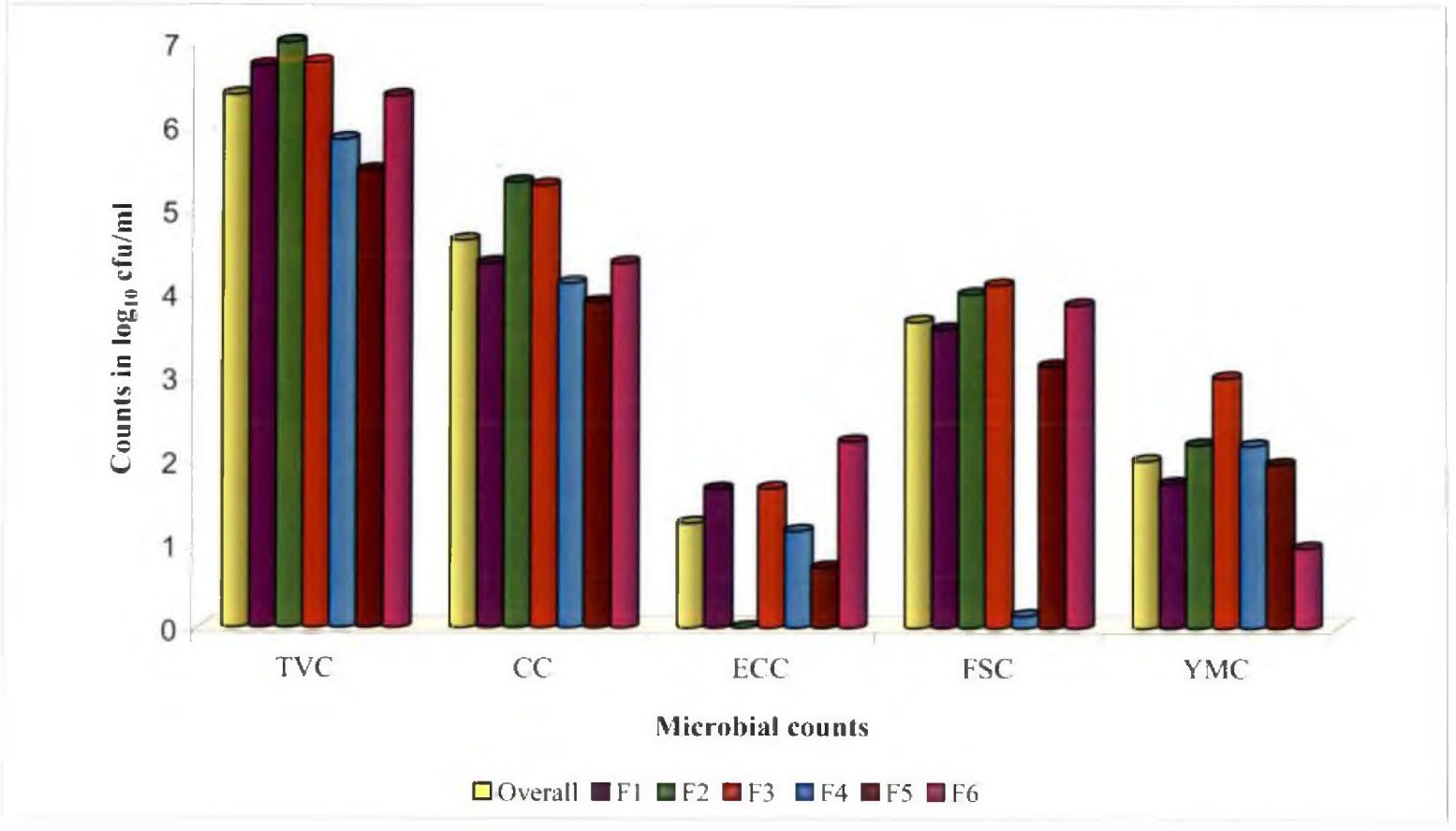


Fig. 5 Comparison of microbial quality of individual milk samples of S_2

coliform count. A similar correlation was observed between coliform count with faecal streptococcal count and yeast and mould count.

4.1.4 Microbial counts of individual raw milk from society 3

Microbial quality of the samples collected from farmers belonging to S_1 was assessed and was illustrated in fig. 6.

4.1.4.1 *Total Viable Count*

The mean total viable count of 36 samples collected from six farmers of S_3 is shown in table 34. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between mean total viable count of the samples collected from six farmers. The data were subjected to least significant difference test and the results revealed significant ($P<0.05$) difference between mean count of samples from F_1 and F_2, F_1 and F_6, F_2 and F_4, F_2 and F_5, F_2 and F_6, F_3 and F_4, F_3 and F_5, F_3 and F_6, F_4 and F_6, and also between F_5 and F_6. The overall mean count of 36 samples from the six farmers of S_3 was 5.67 ± 0.13 $\log_{10}$ cfu/ml.

Table 34. Mean total viable count of individual raw milk samples from S_3

Sources of milk samples	Total viable count
	Mean ± SE ($\log_{10}$ cfu/ml)
F_1	$5.53^{bc} \pm 0.18$
F_2	$4.85^{d} \pm 0.18$
F_3	$4.97^{cd} \pm 0.13$
F_4	$6.00^{b} \pm 0.16$
F_5	$6.00^{b} \pm 0.16$
F_6	$6.66^{a} \pm 0.38$
Overall	5.67 ± 0.13

Figures bearing the same superscript in the same column do not differ significantly., N = 6 from each farmer

The highest mean total viable count (6.66 ± 0.38 $\log_{10}$ cfu/ml) was observed in samples from F_6 whereas the samples from F_2 had the lowest mean count (4.85 ± 0.18 $\log_{10}$ cfu/ml).

Based on total viable count, the distribution of milk samples from S_3 is given in table 35. Of the 36 samples, 8 (22.22 per cent), 19 (52.78 per cent), 6 (16.67 per cent) and 3 (8.33 per cent) samples had count at the level 10^4, 10^5, 10^6 and 10^7 cfu/ml, respectively. Fifty per cent of the samples each from F_6 had count at the level of 10^5 and 10^7 cfu/ml. Count at the level of 10^7 cfu/ml was absent in the samples of F_1, F_2, F_3, F_4 and F_5 and the count at the level of 10^6 cfu/ml was not observed in any of the samples of F_1, F_2, F_3 and F_6.

Table 35. Frequency distribution of milk samples based on total viable count from S_3

Sources of milk samples	Total viable count (cfu/ml)			
	10^4	10^5	10^6	10^7
F_1	1 (16.67)	5 (83.33)	0	0
F_2	3 (50.00)	3 (50.00)	0	0
F_3	4 (66.67)	2 (33.33)	0	0
F_4	0	3 (50.00)	3 (50.00)	0
F_5	0	3 (50.00)	3 (50.00)	0
F_6	0	3 (50.00)	0	3 (50.00)
Overall	8 (22.22)	19 (52.78)	6 (16.67)	3 (8.33)

Figures in parenthesis indicate per cent., N = 6 from each farmer

4.1.4.2 *Coliform Count*

All samples collected from the farmers of S_3 were evaluated and the mean coliform count (CC) of the samples from each farmer and the overall mean count of the samples are given in table 36. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between the mean coliform count of the farmers. The data were subjected to least significant difference test and the result revealed significant ($P<0.05$) difference between the mean count of samples

collected from F_1 and F_4, F_1 and F_5, F_1 and F_6, F_2 and F_4, F_2 and F_5, F_2 and F_6, F_3 and F_4, F_3 and F_5, F_3 and F_6 and also between F_4 and F_6. The overall mean Coliform count of all the samples collected from S_3 was 4.31 ± 0.10 $\log_{10}$ cfu/ml. The samples belonging to F_4 had the highest mean count (4.96 ± 0.17 $\log_{10}$ cfu/ml), while the lowest count was observed in samples of F_1 (3.74 ± 0.13 $\log_{10}$ cfu/ml).

Table 36. Mean coliform count of individual raw milk samples from S_3

Sources of milk samples	Coliform count
	Mean ± SE ($\log_{10}$ cfu/ml)
F_1	$3.74^{d} \pm 0.13$
F_2	$3.80^{d} \pm 0.21$
F_3	$4.22^{cd} \pm 0.20$
F_4	$4.96^{a} \pm 0.17$
F_5	$4.75^{ab} \pm 0.16$
F_6	$4.35^{bc} \pm 0.11$
Overall	4.31 ± 0.10

Figures bearing the same superscript in the same column do not differ significantly., N = 6 from each farmer

Distribution of milk samples from S_3 based on coliform count is given in table 37. Of the 36 samples, the count at the level of 10^3, 10^4 and 10^5 cfu/ml was seen in 12 (33.33 per cent), 20 (55.56 per cent) and 4 (11.11 per cent) samples, respectively. Cent per cent of the samples received from the F_6 had count at the level of 10^4 cfu/ml. None of samples from the F_1, F_2, F_3 and F_6 had count at the level of 10^5 cfu/ml. The count in 66.67 per cent samples belonging to F_4 and F_5 was at the level of 10^4 cfu/ml. The equal per cent of samples from F_2 had count at the level of 10^3 cfu/ml. In 33.33 per cent samples of F_4 and F_5 the count was at the level of 10^5 cfu/ml, while the same per cent samples of F_2 had count at the level of 10^4 cfu/ml. Of the samples of F_1, 83.33 per cent had count at the level of 10^3 cfu/ml.

Table 37. Frequency distribution of milk samples based on coliform count from S_3

Sources of milk samples	Coliform count (cfu/ml)		
	10^3	10^4	10^5
F_1	5 (83.33)	1 (16.67)	0
F_2	4 (66.67)	2 (33.33)	0
F_3	3 (50.00)	3 (50.00)	0
F_4	0	4 (66.67)	2 (33.33)
F_5	0	4 (66.67)	2 (33.33)
F_6	0	6 (100.00)	0
Overall	12 (33.33)	20 (55.56)	4 (11.11)

Figures in parenthesis indicate per cent., N = 6 from each farmer

4.1.4.3 *Escherichia coli Count*

The mean *Escherichia coli* count (ECC) of raw milk samples from S_3 is given in table 38. The overall mean count of all the samples collected from S_3 was 0.54 ± 0.16 $\log_{10}$ cfu/ml. The highest mean count (0.80 ± 0.51 $\log_{10}$ cfu/ml) of the organisms was seen in the samples belonging to F_1 and F_5. The samples belonging to F_2, F_4 and F_6 had the lowest mean count (0.33 ± 0.33 $\log_{10}$ cfu/ml).

Table 38. Mean *Escherichia coli* count of individual raw milk samples from S_3

Sources of milk samples	*Escherichia coli* Count
	Mean ± SE ($\log_{10}$ cfu/ml)
F_1	0.80 ± 0.51
F_2	0.33 ± 0.33
F_3	0.67 ± 0.42
F_4	0.33 ± 0.33
F_5	0.80 ± 0.51
F_6	0.33 ± 0.33
Overall	0.54 ± 0.16

N = 6 from each farmer

Distribution of the samples from S_3 based on *Escherichia coli* count is given in table 39. The organism was not detected in 27 (75.00 per cent) of the 36 samples. Only 9 (25.00 per cent) of the samples showed the presence of the organism and the count was at the level of 10^2 cfu/ml.

Table 39. Frequency distribution of milk samples based on *Escherichia coli* count from S_3

Sources of milk samples	*Escherichia coli* Count (cfu/ml)	
	ND	10^2
F_1	4 (66.67)	2(33.33)
F_2	5 (83.33)	1 (16.67)
F_3	4 (66.67)	2(33.33)
F_4	5 (83.33)	1 (16.67)
F_5	4 (66.67)	2(33.33)
F_6	5 (83.33)	1 (16.67)
Overall	27 (75.00)	9 (25.00)

Figures in parenthesis indicate per cent., ND-Not Detected., N = 6 from each farmer

4.1.4.4 *Faecal Streptococcal Count*

Mean faecal streptococcal count of raw milk samples from S_3 is given in table 40. The overall mean count of the samples collected from S_3 was 2.92 ± 0.17 $\log_{10}$ cfu/ml. The highest mean count (3.66 ± 0.14 $\log_{10}$ cfu/ml) was seen in the samples of F_4. The samples of F_3 had the lowest mean count (2.13 ± 0.68 $\log_{10}$ cfu/ml).

Based on faecal streptococcal count, the distribution of milk samples from S_3 is given in table 41. All samples collected from F_1, F_4, F_5 and F_6 showed the presence of the organism. Of the 36 samples, the count at the level of 10^2 and 10^3 cfu/ml was seen in 11 (30.56 per cent) and 20 (55.56 per cent) samples, respectively. The count at the level of 10^4 cfu/ml was seen only in 2 (5.56 per cent) samples. In 83.33 per cent samples belonging to F_4 and F_5 had count at the level of

10^3 cfu/ml. The count in 50.00 per cent samples belonging to F_1, F_3 and F_6 had the count at the level of 10^3 cfu/ml and an equal per cent of the samples belonging to these societies had count at the level of 10^2 cfu/ml.

Table 40. Mean faecal streptococcal count of individual raw milk samples from S_3

Sources of milk samples	Faecal streptococcal count
	Mean ± SE (log_{10} cfu/ml)
F_1	2.96 ± 0.23
F_2	2.55 ± 0.58
F_3	2.13 ± 0.68
F_4	3.66 ± 0.14
F_5	3.07 ± 0.23
F_6	3.13 ± 0.21
Overall	2.92 ± 0.17

N = 6 from each farmer

Table 41. Frequency distribution of milk samples based on faecal streptococcal count from S_3

Sources of milk samples	Faecal streptococcal count (cfu/ml)			
	ND	10^2	10^3	10^4
F_1	0	3 (50.00)	3 (50.00)	0
F_2	1 (16.67)	3 (50.00)	1 (16.67)	1 (16.67)
F_3	2 (33.33)	1 (16.67)	3 (50.00)	0
F_4	0	0	5 (83.33)	1(16.67)
F_5	0	1 (16.67)	5 (83.33)	0
F_6	0	3 (50.00)	3 (50.00)	0
Overall	3 (8.33)	11 (30.56)	20 (55.56)	2 (5.56)

Figures in parenthesis indicate per cent., N = 6 from each farmer

4.1.4.5 *Yeast and Mould Count*

The mean yeast and mould count of raw milk samples from S_3 is given in table 42. The overall mean count of 36 samples collected from S_3 was 2.21 ± 0.20 $\log_{10}$ cfu/ml. The samples belonging to F_3 had the highest mean count (2.77 ± 0.11 $\log_{10}$ cfu/ml). The lowest mean count was observed (1.41 ± 0.64 $\log_{10}$ cfu/ml) in the samples from F_1.

Table 42. Mean yeast and mould count of individual raw milk samples from S_3

Sources of milk samples	Yeast and mould count
	Mean ± SE ($\log_{10}$ cfu/ml)
F_1	1.41 ± 0.64
F_2	2.53 ± 0.51
F_3	2.77 ± 0.11
F_4	1.88 ± 0.61
F_5	2.34 ± 0.48
F_6	2.31 ± 0.48
Overall	2.21 ± 0.20

N = 6 from each farmer

Distribution of milk samples based on yeast and mould count from S_3 is given in table 43. Of the 36 samples, the count at the level of 10^2 and 10^3 cfu/ml was seen in 16 (44.44per cent) and 12 (33.33 per cent) samples, respectively, while the organism could not detected in 8 (22.22 per cent) of the samples. Cent per cent of the samples from F_3 revealed the presence of the organisms. In 50.00 per cent samples belonging to F_2 was at the level of 10^3 cfu/ml. The count in 66.67 per cent samples belonging to F_3 was at the level of 10^2 cfu/ml and in 50.00 per cent samples each belonging to F_5 and F_6 was at the level of 10^2 cfu/ml.

Table 43. Frequency distribution of milk samples based on yeast and mould count from S_3

Sources of milk samples	Yeast and mould count (cfu/ml)		
	ND	10^2	10^3
F_1	3 (50.00)	2 (33.33)	1 (16.67)
F_2	1 (16.67)	2 (33.33)	3 (50.00)
F_3	0	4 (66.67)	2 (33.33)
F_4	2 (33.33)	2 (33.33)	2 (33.33)
F_5	1 (16.67)	3 (50.00)	2 (33.33)
F_6	1 (16.67)	3 (50.00)	2 (33.33)
Overall	8 (22.22)	16 (44.44)	12 (33.33)

Figures in parenthesis indicate per cent., ND-Not Detected., N = 6 from each farmer

4.1.4.6 *Correlation coefficient between bacterial counts of individual raw milk from S_3*

The correlation coefficient between various bacterial counts of raw milk samples collected from S_3 is given in the table 44. A significant ($P<0.05$) and positive correlation was observed only between the total viable count and coliform count.

Table 44. Correlation coefficient between bacterial counts of individual raw milk samples from S_3

Counts	CC	ECC	FSC	YMC
TVC	0.415*	0.036^{NS}	0.172^{NS}	-0.040^{NS}
CC		-0.005^{NS}	0.032^{NS}	-0.024^{NS}
ECC			0.068^{NS}	-0.177^{NS}
FSC				-0.048^{NS}

*Significant at 5 per cent ($P<0.05$)., NS- non significant

4.1.5 Microbial counts of pooled samples of raw milk from S_1, S_2 and S_3

The bacterial and yeast and mould counts of 72 pooled raw milk samples

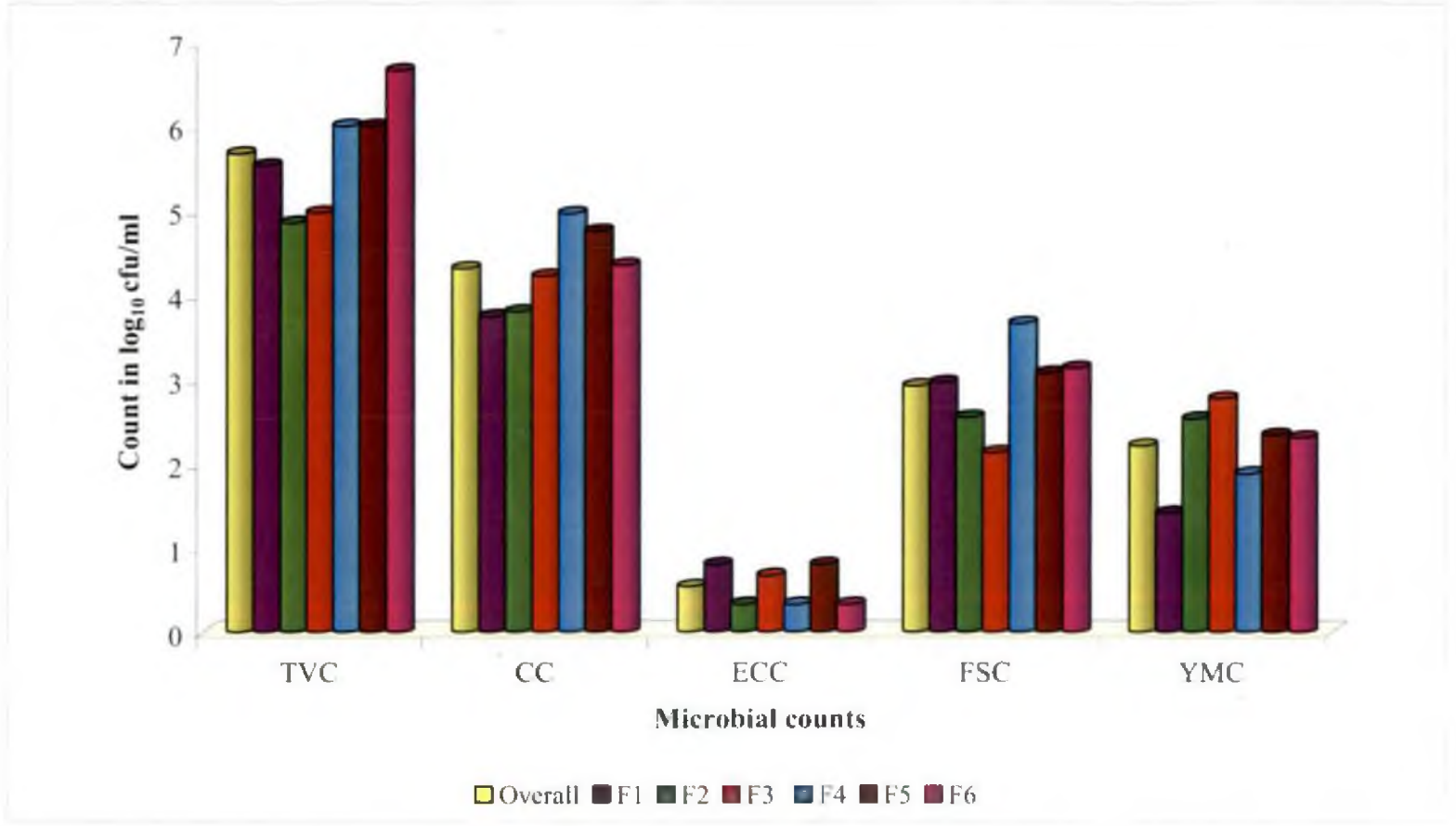


Fig. 6 Comparison of microbial quality of individual milk samples of S_3

consisting of 24 samples each from three societies were evaluated and was illustrated in fig. 7.

4.1.5.1. *Total Viable Count*

The mean total viable count of pooled raw milk samples collected from the three societies and overall mean count are given in table 45. Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between mean counts of the samples from the three societies. Least Significant Difference test of the data showed significant ($P<0.05$) difference between the mean count of the samples from S_1 and S_3 and S_2 and S_3. The overall mean count of the sample was 6.19 ± 0.09 $\log_{10}$ cfu/ml. The highest mean count was observed in the samples of S_2 (6.49 ± 0.11 $\log_{10}$ cfu/ml) and the lowest was seen in the samples of S_3 (5.73 ± 0.08 $\log_{10}$ cfu/ml).

Table 45. Mean total viable count of pooled milk samples of S_1, S_2 and S_3

Sources of milk samples	Total viable count
	Mean ± SE ($\log_{10}$ cfu/ml)
S_1	$6.33^a \pm 0.21$
S_2	$6.49^a \pm 0.05$
S_3	$5.73^b \pm 0.08$
Overall	6.19 ± 0.09

Figures bearing the same superscript in the same column do not differ significantly; N = 24 from each source

The distribution of pooled raw milk samples from the three societies based on total viable count is given in table 46. Count at the level of 10^4, 10^5, 10^6, 10^7 and 10^8 cfu/ml was observed in 4 (5.56 per cent), 25 (34.72 per cent), 36 (50.00 per cent), 6 (8.33 per cent) and 1 (1.39 per cent) samples, respectively. None of the samples from S_2 and S_3 had count in the level of 10^7 and 10^8 cfu/ml, whereas 6 (25.00 per cent) and 1 (4.17 per cent) samples from S_1 had count at the levels of 10^7 and 10^8 cfu/ml, respectively.

Table 46. Frequency distribution of pooled milk samples of S_1, S_2 and S_3 based on total viable count

Sources of milk samples	Total viable count (cfu/ml)				
	10^4	10^5	10^6	10^7	10^8
S_1	3 (12.5)	8 (33.33)	6 (25.00)	6 (25.00)	1 (4.17)
S_2	0	1 (4.17)	23 (95.83)	0	0
S_3	1 (4.17)	16 (66.67)	7 (29.17)	0	0
Overall	4 (5.56)	25 (34.72)	36 (50.00)	6 (8.33)	1 (1.39)

Figures in parenthesis indicate per cent; N = 24 from each source

4.1.5.2 *Coliform Count*

The mean coliform count of pooled raw milk samples collected from the three societies and overall mean count are given in table 47. The overall mean count of samples was 4.65 ± 0.09 $\log_{10}$ cfu/ml. Samples of S_2 had the highest count (4.75 ± 0.09 $\log_{10}$ cfu/ml). The lowest mean count was observed in samples of S_1 (4.33 ± 0.12 $\log_{10}$ cfu/ml).

Table 47. Mean coliform count of pooled milk samples of S_1, S_2 and S_3

Sources of milk samples	Coliform count
	Mean ± SE ($\log_{10}$ cfu/ml)
S_1	4.33 ± 0.12
S_2	4.75 ± 0.09
S_3	4.37 ± 0.22
Overall	4.65 ± 0.09

N = 24 from each source

The distribution of pooled raw milk samples from the three societies based on coliform count is given in table 48.The organism was not detected in 1 (1.39 per cent) of the 72 samples. Of the 72 samples, 7 (9.72 per cent), 39 (54.17 per cent) and 25 (34.72 per cent) had count at the level of 10^3, 10^4 and 10^5 cfu/ml, respectively. The count in 13 (54.17 per cent) samples obtained from S_1 was at the

level of 10^5 cfu/ml. The count in 16 (66.67 per cent) and 15 (62.50 per cent) samples belonging to S_2 and S_3 was at the level of 10^4 cfu/ml.

Table 48. Frequency distribution of pooled milk samples of S_1, S_2 and S_3 based on coliform count

Sources of milk samples	Coliform count (cfu/ml)			
	ND	10^3	10^4	10^5
S_1	0	3 (12.50)	8 (33.33)	13 (54.17)
S_2	0	1 (4.17)	16 (66.67)	7 (21.17)
S_3	1 (4.17)	3 (12.50)	15 (62.50)	5 (20.83)
Overall	1 (1.39)	7 (9.72)	39 (54.17)	25 (34.72)

Figures in parenthesis indicate per cent; N = 24 from each source

4.1.5.3. *Escherichia coli Count*

The mean *Escherichia coli* count (ECC) of pooled raw milk collected from the three societies and overall mean count are given in table 49. The overall mean *Escherichia coli* count of the samples was 1.27 ± 0.16 $\log_{10}$ cfu/ml. The highest mean count of 1.33 ± 0.43 $\log_{10}$ cfu/ml was observed in the samples of S_3 and the lowest was seen in the samples of S_1 (1.24 ± 0.27 $\log_{10}$ cfu/ml).

Table 49. Mean *Escherichia coli* count of pooled milk samples of S_1, S_2 and S_3

Sources of milk samples	*Escherichia coli* count
	Mean ± SE ($\log_{10}$ cfu/ml)
S_1	1.24 ± 0.27
S_2	1.26 ± 0.29
S_3	1.33 ± 0.43
Overall	1.27 ± 0.16

N = 24 from each source.

The distribution of pooled raw milk samples from the three societies based on *Escherichia coli* count is given in table 50. *Escherichia coli* was not detected in 12 (50.00 per cent) samples from S_1, 13 (54.17 per cent) samples from S_2 and 11

(45.83 per cent) samples from S_3, with a total of 36 (50.00 per cent) samples. Out of the 72 samples count at the level of 10^2 and 10^3 cfu/ml was seen in 29 (40.28 per cent) and 7 (9.72 per cent) samples, respectively. The count in 12 (50.00 per cent) samples of S_3 was at the level of 10^2 cfu/ml.

Table 50. Frequency distribution of pooled milk samples of S_1, S_2 and S_3 based on *Escherichia coli* count

Sources of milk samples	*Escherichia coli* Count (cfu/ml)		
	ND	10^2	10^3
S_1	12 (50.00)	9 (37.50)	3 (12.50)
S_2	13 (54.17)	8 (33.33)	3 (12.50)
S_3	11 (45.83)	12 (50.00)	1 (1.47)
Overall	36 (50.00)	29 (40.28)	7(9.72)

Figures in parenthesis indicate per cent; ND- Not Detected; N = 24 from each source

4.1.5.4. *Faecal Streptococcal Count*

The mean faecal streptococcal count of pooled raw milk samples collected from the three societies and the overall mean count are given in table 51.

Table 51. Mean faecal streptococcal count of pooled milk samples of S_1, S_2 and S_3

Sources of milk samples	Faecal streptococcal count
	Mean ± SE (log_{10} cfu/ml)
S_1	$3.13^b \pm 0.09$
S_2	$3.74^a \pm 0.10$
S_3	$3.63^a \pm 0.09$
Overall	3.50 ± 0.06

Figures bearing the same superscript do not differ significantly., N = 24 from each source

Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between the mean count of the samples from the three societies. The mean count of the samples from the three societies were analyzed by Least

Significant Difference test and the results showed significant ($P<0.05$) difference between the mean count of the samples of S_1 and S_2 and also between S_1 and S_3. The overall mean faecal streptococcal count of the samples was 3.50 ± 0.06 $\log_{10}$ cfu/ml. The samples of S_2 had the highest mean count (3.74 ± 0.10 $\log_{10}$ cfu/ml) followed by samples of S_3 (3.63 ± 0.09 $\log_{10}$ cfu/ml) and S_1 (3.13 ± 0.09 $\log_{10}$ cfu/ml).

The distribution of the pooled raw milk samples from the three societies based on faecal streptococcal count is given in table 52. All the 72 samples showed the presence of organism and count at the level of 10^2, 10^3, 10^4 and 10^5 cfu/ml was present in 10 (13.89 per cent), 47 (65.28 per cent), 14 (19.44 per cent) and 1 (1.39 per cent) samples, respectively. Only one sample from S_2 had the count at the level of 10^5 cfu/ml. In 15 (62.50 per cent) samples of S_1 had count at the level of 10^3 cfu/ml and 66.67 per cent samples each belonging to S_2 and S_3 sources had count at that level.

Table 52. Frequency distribution of pooled milk samples of S_1, S_2 and S_3 based on faecal streptococcal count

Sources of milk samples	Faecal streptococcal count (cfu/ml)			
	10^2	10^3	10^4	10^5
S_1	8 (33.33)	15 (62.50)	1 (4.17)	0
S_2	1 (4.17)	16 (66.67)	6 (25.00)	1 (4.17)
S_3	1 (4.17)	16 (66.67)	7 (29.17)	0
Overall	10 (13.89)	47 (65.28)	14 (19.44)	1 (1.39)

Figures in parenthesis indicate per cent ; N = 24 from each source

4.1.5.5. *Yeast and Mould Count*

The mean yeast and mould count of the samples of thee societies and the overall mean count are shown in table 53. The overall mean yeast and mould count of the samples was 2.37 ± 0.11 $\log_{10}$ cfu/ml. The samples of S_3 had the highest mean count (2.73 ± 0.08 $\log_{10}$ cfu/ml) and the lowest count was in the samples of S_2 (2.16 ± 0.24 $\log_{10}$ cfu/ml).

Table 53. Mean yeast and mould count of pooled milk samples of S_1, S_2 and S_3

Sources of milk samples	Yeast and mould count
	Mean ± SE (log_{10} cfu/ml)
S_1	2.20 ± 0.21
S_2	2.16 ± 0.24
S_3	2.73 ± 0.08
Overall	2.37 ± 0.11

N = 24 from each source

On the basis of yeast and mould count per ml, the distribution of pooled raw milk samples collected from the three societies is shown in table 54. Nine (12.50 per cent) samples did not show the presence of the organism. All samples from S_3 showed the presence of organism. Of the 72 samples, 46 (63.89 per cent) and 17 (23.61 per cent) samples had count at the level of 10^2 and 10^3 cfu/ml, respectively. The count in 17 (70.83 per cent) samples of S_3, 16 (66.67 per cent) samples of S_1 and 13 (54.17 per cent) samples S_2 was at the level of 10^2 cfu/ml.

Table 54. Frequency distribution of pooled milk samples of S_1, S_2 and S_3 based on yeast and mould count

Sources of milk samples	Yeast and mould count (cfu/ml)		
	ND	10^2	10^3
S_1	4 (16.67)	16 (66.67)	4 (16.67)
S_2	5 (20.83)	13 (54.17)	6 (25.00)
S_3	0	17 (70.83)	7 (21.17)
Overall	9 (12.50)	46 (63.89)	17 (23.61)

Figures in parenthesis indicate per cent; ND-Not Detected., N = 24 from each source

4.1.5.6 *Correlation between microbial counts of pooled samples of S_1, S_2 and S_3*

The correlation coefficient between various microbial counts of pooled raw milk samples are given in the table 55. A significant ($P<0.05$) correlation was observed only between total viable count and coliform count.

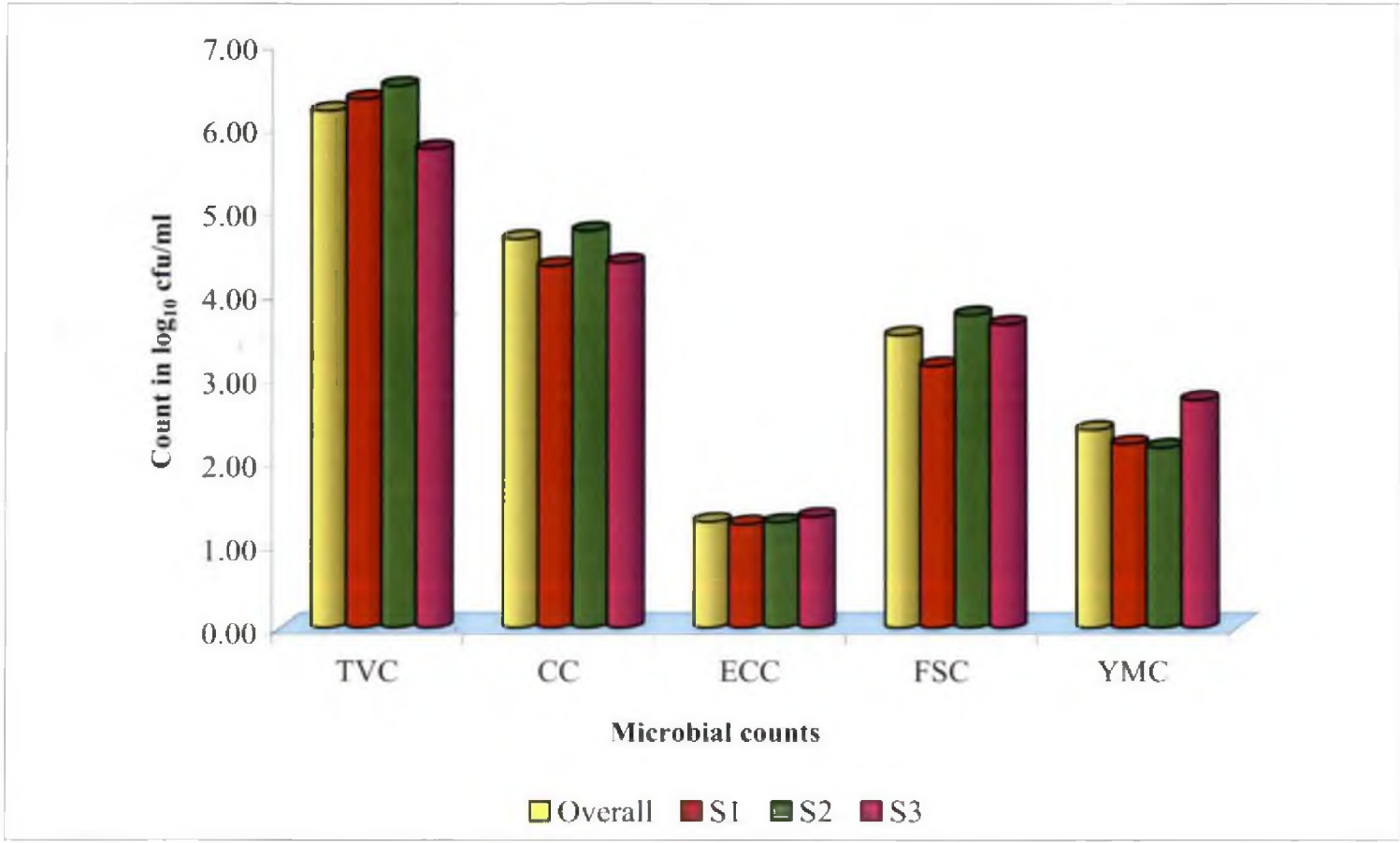


Fig. 7 Comparison of microbial quality of pooled milk samples from S_1, S_2 and S_3

Table 55. Correlation between microbial counts of pooled samples of S_1, S_2 and S_3

Counts	CC	ECC	FSC	YMC
TVC	0.253*	0.081 ^{NS}	0.101 ^{NS}	-0.002 ^{NS}
CC		0.123 ^{NS}	-0.046 ^{NS}	-0.106 ^{NS}
ECC			-0.007 ^{NS}	-0.196 ^{NS}
FSC				0.191 ^{NS}

*Significant at 5 per cent level (P<0.05); NS- Non Significant

4.2 ISOLATION AND IDENTIFICATION OF BACTERIA

In the present investigation, a total of 180 raw milk samples consisting of 108 individual and 72 pooled milk samples, collected from three societies were examined for the isolation and identification of *Escherichia coli, Staphylococcus aureus* and *Yersinia.*

4.2.1 Individual milk samples collected from S_1, S_2 and S_3

The bacteria isolated from individual raw milk samples obtained from S_1, S_2 and S_3 are given in table 56.

Table 56. Bacteria isolated from individual raw milk samples of S_1, S_2 and S_3

Bacteria	Number of samples positive for bacteria			
	S_1	S_2	S_3	Overall
Escherichia coli	12 (33.33)	18 (50.00)	9 (25.00)	39 (36.11)
Staphylococcus aureus	20 (55.56)	13 (36.11)	11 (30.55)	44 (40.74)
Yersinia	10 (27.77)	6 (16.67)	8 (22.22)	24 (22.22)

Figures in parenthesis indicates per cent; N = 36 from each society

4.2.1.1 *Escherichia coli*

Raw milk samples obtained from individual farmers of three societies were tested for the isolation and identification of *Escherichia coli.* The suspected

colonies of the organism were selected from media and transferred to nutrient agar slants and incubated at 37^0 C for overnight. At the end of the incubation period the isolates were stored under refrigeration and were further characterized by cultural, morphological and biochemical reactions. A total of 39 isolates were identified as *Escherichia coli* (Table56). Twelve (30.76 per cent), eighteen (46.15 per cent) and 9 (23.07 per cent) isolates were obtained from the individual milk samples belonging to the societies S_1, S_2 and S_3, respectively.

Table 57. Distribution of *Escherichia coli* serotypes from individual raw milk samples of S_1, S_2 and S_3

Serotypes	Sources			
	S_1	S_2	S_3	Overall
O116	3 (25.00)			3 (7.69)
O12		2 (11.11)		2 (5.13)
O29			2 (22.22)	2 (5.13)
O68		1 (5.56)	1 (11.11)	2 (5.13)
O75	1 (8.33)			1 (2.54)
O79			1 (11.11)	1 (2.54)
O107	1 (8.33)			1 (2.54)
O131	1(8.33)			1 (2.54)
O160			1 (11.11)	1 (2.54)
O172		1 (5.56)		1 (2.54)
UT	4 (33.33)	5 (27.78)	3 (33.33)	12 (30.76)
R	2 (16.67)	9 (50.00)	1 (11.11)	12 (30.76)
Overall	12 (30.76)	18 (46.15)	9 (23.07)	39 (100.00)

UT- Untypable., R- Rough., figures in parenthesis indicate per cent

All isolates obtained from the individual milk samples of S_1, S_2 and S_3 were serotyped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh. Only 15 (38.46 per cent) out of 39 isolates were serotyped. The isolates fell into 10 serotypes and were belonging to O12,

O29, O68, O75, O79, O107, O116, O131, O160 and O172. Out of the 39 isolates 12 each were untypable and rough (Table 57).

Out of the 39 isolates three (7.69 per cent) were belonging to serotypes O116 whereas two (5.13 per cent) of the isolate each were belonging to serotype O12, O29 and O68, respectively. One (2.54 per cent) isolate each belonging to serotypes O75, O79, O107, O131, O160 and O172, were also obtained.

Distribution of *Escherichia coli* serotypes from the individual milk samples of S_1

Distribution of *Escherichia coli* serotypes obtained from individual raw milk samples of S_1 is depicted in table 57. From the 36 samples of S_1, 12 (30.76 per cent) *Escherichia coli* isolates were obtained. Among the isolates six (50.00 per cent) were serotyped, four (33.33 per cent) were untypable and two (16.67 per cent) were rough. The serotype consisted of O116 (3), O75 (1), O107 (1) and O131 (1). The serotype O116 was isolated from the samples obtained from the F_5. The isolates O75, O107 and O131 were obtained from F_1, F_2 and F_4, respectively.

Distribution of *Escherichia coli* serotypes from the individual milk samples of S_2

Different serotypes of *Escherichia coli* obtained from individual raw milk samples of S_2 and their distribution is depicted in table 57. Eighteen (46.15 per cent) *Escherichia coli* isolates were isolated from 36 samples of the S_2 and only four were serotyped. The serotypes were belonging to O12 (2), O68 (1) and O172 (1), whereas 5 (27.78 per cent) were untypable and 9 (50.00 per cent) were rough. Two of the serotype O12 was isolated from the samples of F_3 and the serotypes O68 and O172 were isolated from the samples of F_1 and F_5, respectively.

Distribution of *Escherichia coli* serotypes from the individual milk samples of S_3

The distribution of different serotypes of *Escherichia coli* obtained from individual raw milk samples of S_3 is depicted in table 57. A total of 9 (23.07 per cent) *Escherichia coli* were isolated from the samples of the farmers belonging to

S_3. Of the isolates, five were serotyped and were belonging to serotypes O29 (2), O68 (1), O79 (1) and O160 (1). Three (33.33 per cent) of the isolates were untypable and one (11.11 per cent) was rough. The serotype O29 (2) was isolated from the samples of F_3. The serotypes O68 (1), O79 (1) and O160 (1) were isolated from the samples of F_1, F_4 and F_5, respectively.

Congo red binding test of *Escherichia coli* isolates from the individual milk samples

A total of 15 *Escherichia coli* isolates belonging to 10 serotypes were subjected to congo red binding test and the results are given in table 58. The isolates belonging to serotypes *viz.*, O29, O75, O116, O68 and O172 showed positive congo red binding test, which indicate the property of pathogenisity and the isolates belonging to O12, O79, O107, 0131 and O160 showed negative congo red binding property.

Table 58. Congo red binding test of *Escherichia coli* isolates from individual samples

Serotype	Number	Congo red binding test
O116	3	+
O12	2	-
O29	2	+
O68	2	+
O75	1	+
O79	1	-
O172	1	+
0131	1	-
O107	1	-
O160	1	-

+ Positive, - Negative

4.2.1.2 *Staphylococcus aureus*

All samples from individual farmers of three societies were subjected to the isolation and identification of *Staphylococcus aureus* and the number of samples from each source, which yielded the organism, is given in table 56. The suspected colonies on Baird Parker agar medium were selected and transferred to nutrient agar slants and incubated at 37°C for overnight. The isolates were stored at refrigeration temperature for further characterisation. A total of 44 isolates were identified as coagulase positive Staphylococci. *Staphylococcus aureus* was isolated from 20 (55.56 per cent) samples of S_1. The organism was isolated from 13 (36.11 per cent) and 11 (30.55 per cent) samples of the S_2 and S_3, respectively.

4.2.1.3 *Yersinia*

Raw milk samples obtained from individual farmers of three societies were tested for the isolation and identification of *Yersinia* and the results are shown in table 56.

The characteristic colonies, selected from Yersinia Selective Agar were transferred to nutrient agar slants and incubated at 25^0C for overnight and kept under refrigeration. The isolates were subjected to characterization by cultural, morphological and biochemical tests. The organism was isolated from 10, 6 and 8

Table 59. *Yersinia* isolates from individual milk samples of S_1, S_2 and S_3

Yersinia species	Sources			
	S_1	S_2	S_3	Total
Yersinia enterocolitica	4	1	0	5
Yersinia pseudotuberculosis	0	1	0	1
Yersinia frederiksenii	4	2	4	10
Yersinia aldovae	2	0	0	2
Yersinia intermedia	0	2	3	5
Yersinia kristensenii	0	0	1	1
Total	10	6	8	24

samples of S_1, S_2 and S_3, respectively (Table 59). Ten *Yersinia* isolates were obtained from the samples of S_1. Of the isolates obtained from S_1, four each, were identified as *Yersinia enterocolitica* and *Yersinia frederiksenii*. *Yersinia aldovae* (2) were also isolated from these samples. The six isolates belonging to the samples of S_2 consisted of *Yersinia frederiksenii* (2) *Yersinia intermedia* (2), *Yersinia enterocolitica* (1) and *Yersinia pseudotuberculosis* (1). The eight isolates obtained from the samples of S_3 were identified as *Yersinia frederiksenii* (4), *Yersinia intermedia* (3) and *Yersinia kristensenii* (1).

4.2.2 Pooled milk samples collected from S_1, S_2 and S_3

All pooled raw milk samples obtained from the three societies were tested for the isolation and identification of *Escherichia coli, Staphylococcus aureus* and *Yersinia* and the bacteria isolated from the sources are shown in the table 60.

Table 60. Bacteria isolated from pooled milk samples of S_1, S_2 and S_3

Bacteria	Number of samples positive for bacteria			
	S_1	S_2	S_3	Overall
Escherichia coli	12 (50.00)	11 (45.83)	13 (54.17)	36 (50.00)
Staphylococcus aureus	7 (29.17)	11 (45.83)	7 (29.17)	25 (34.72)
Yersinia	9 (37.50)	4 (16.67)	8 (33.33)	21 (29.17)

Figures in parenthesis indicates per cent., N = 24 from each society

4.2.2.1 *Escherichia coli*

Pooled raw milk samples obtained from three societies were tested for the isolation and identification of *Escherichia coli* and the results are shown in table 60. A total of 36 (50.00 per cent) isolates were identified as *Escherichia coli* by cultural, morphological and biochemical characteristics. *Escherichia coli* was isolated from 12 (50.00 per cent), 11 (45.83 per cent) and 13 (54.17 per cent) samples of S_1, S_2 and S_3, respectively.

Distribution of *Escherichia coli* serotypes obtained from pooled raw milk samples from S_1, S_3 and S_3 is depicted in table 61. Of the 36 isolates 18 (50.00 per cent) were belonging to five different serotypes and they were O116 (10), O68 (3), O75 (2), O60 (2) and O96 (1). Number of rough and untypable isolates was 15 (41.67 per cent) and 3 (8.33 per cent), respectively.

Distribution of *Escherichia coli serotypes* from pooled milk samples of S_1

From the 24 pooled samples of S_1, 12 *Escherichia coli* isolates were obtained. Among the isolates eight (66.67 per cent) were serotyped, one (8.33 per cent) was untypable and three (25.00 per cent) were rough. The serotype consisted of O116 (5), O75 (2) and O96 (1).

Table 61. Distribution of *Escherichia coli* serotypes of pooled raw milk samples from S_1, S_2 and S_3

Serotype	S_1	S_2	S_3	Overall
O116	5 (41.67)	2 (18.18)	3 (23.08)	10 (27.78)
O68		2 (18.18)	1 (7.69)	3 (8.33)
O75	2 (16.67)			2 (5.56)
O60		1 (9.09)	1 (7.69)	2 (5.56)
O96	1 (8.33)			1 (2.78)
UT	1 (8.33)	2 (18.18)		3 (8.33)
R	3 (25.00)	4 (36.36)	8 (61.54)	15 (41.67)
Overall	12 (33.33)	11 (30.56)	13 (36.11)	36 (100.00)

UT- Untypable; R- Rough; figures in parenthesis indicate per cent

Distribution of *Escherichia coli* serotypes from pooled milk samples of S_2

Of the eleven *Escherichia coli* isolates obtained from S_2, two (18.18 per cent) each isolates were belonged to three different serotypes *viz.* O116 and O68 and one belonged to serotype O60. Four of the isolates were rough and two were untypable.

Distribution of *Escherichia coli* serotypes from pooled milk samples of S_3

From the 24 samples of S_3, 13 *Escherichia coli* isolates were obtained. Among the isolates five (38.46 per cent) were serotyped and eight (61.54 per cent) were rough. The isolates were belonging to different serotypes *viz.*, O116 (3), O60 (1) and O68 (1).

Congo red binding test of *Escherichia coli* isolates from pooled milk samples

Escherichia coli isolated from pooled raw milk samples were subjected to Congo red binding test and the results are given in table 62. Of the eighteen serotypes ten isolates belonging to serotype O116, three isolates belonging to serotype O68, two isolates belonging to serotype O75 and one isolates belonging to serotype O96 had Congo red binding characteristics, which indicates the pathogenisity of the isolates whereas the isolates belonging to serotype O60 revealed negative Congo red binding test.

Table 62. Congo red binding test of *Escherichia coli* isolates from pooled milk samples

Serotype	Number of isolates	Congo red binding test
O116	10	+
O68	3	+
O75	2	+
O60	2	-
O96	1	+

+ positive; - negative

4.2.2.2 *Staphylococcus aureus*

Pooled raw milk samples collected from three societies were tested for the isolation and identification of *Staphylococcus aureus* and the results are shown in table 60. The suspected colonies were subjected to identification by the cultural, morphological and biochemical tests and 25 isolates were identified as coagulase positive Staphylococci. The organism was isolated from 25 (34.72 per cent) out of

the 72 samples. Of the 25 *Staphylococcus aureus*, 11 were isolated from the samples of S_2. The samples of S_1 and S_3 yielded seven *Staphylococcus aureus*, each.

4.2.2.3 *Yersinia*

Pooled raw milk samples obtained from three societies were tested for the isolation and identification of *Yersinia* and the results are shown in table 60. The characteristic colonies selected from Yersinia Selective Agar were transferred to nutrient agar slants and incubated at 25^0C for overnight and kept under refrigeration. The isolates were subjected to characterization by cultural, morphological and biochemical tests. The organism was isolated from 9, 4 and 8 samples of S_1, S_2 and S_3, respectively (Table 60). Nine *Yersinia* isolates were obtained from the samples of S_1 and were identified as *Yersinia frederiksenii* (3), *Yersinia kristensenii* (3), *Yersinia intermedia* (2) and *Yersinia enterocolitica* (1). The four isolates belonging to the samples of S_2 consisted of *Yersinia intermedia* (2), *Yersinia frederiksenii* (1) and *Yersinia aldovae* (1). The eight isolates from the samples of S_3 were identified as *Yersinia intermedia* (4), *Yersinia frederiksenii* (2) and *Yersinia aldovae* (2).

Table 63. *Yersinia* isolates from pooled milk samples of S_1, S_2 and S_3

Yersinia species	Sources			
	S_1	S_2	S_3	Total
Yersinia enterocolitica	1	0	0	1
Yersinia frederiksenii	3	1	2	6
Yersinia aldovae	0	1	2	3
Yersinia intermedia	2	2	4	8
Yersinia kristensenii	3	0	0	3
Total	9	4	8	21

4.3 GRADING OF MILK BASED ON TOTAL VIABLE COUNT

Based on total viable count, the milk samples collected from the three societies were graded as very good, good, fair and poor following the criteria prescribed by Indian Standards (1977).

4.3.1 Individual milk samples collected from S_1, S_2 and S_3

The distribution of the samples of different grades was depicted in table 64 and was illustrated in fig. 8. Of the 108 samples, 17 (15.74 per cent) samples were graded as very good. Good, fair and poor grade samples were accounted for 37 (34.26 per cent), 35 (32.41 per cent) and 19 (17.59 per cent) samples, respectively. In the samples of S_3, 33.33 per cent were graded as very good followed by samples of S_2 (11.11 per cent) and S_1 (2.78 per cent). Among the samples of S_3, 15 (41.67 per cent) were graded as good, while 36.11 and 25.00 per cent samples of S_1 and S_2 were also belonging to that grade.

Table 64. Distribution of individual milk samples from S_1, S_2 and S_3 based on total viable count

Sources	Number of samples			
	Very good	Good	Fair	Poor
S_1	1 (2.78)	13 (36.11)	19 (52.78)	3 (8.33)
S_2	4 (11.11)	9 (25.00)	10 (27.78)	13 (36.11)
S_3	12 (33.33)	15 (41.67)	6 (16.67)	3 (8.33)
Overall	17 (15.74)	37 (34.26)	35 (32.41)	19 (17.59)

Figures in parenthesis indicate per cent; N = 36 from each source

4.3.2 Pooled milk samples collected from farmers of S_1, S_2 and S_3

A total of 72 pooled milk samples collected from the three societies were graded as very good, good, fair and poor based on total viable count. The distribution of the samples of different grades was given in table 65 and illustrated in fig. 9. Of the 72 samples, 7 (9.72 per cent) were graded as very good. Good, fair and poor grades were accounted for 23 (31.94 per cent), 29 (40.28 per cent) and 13

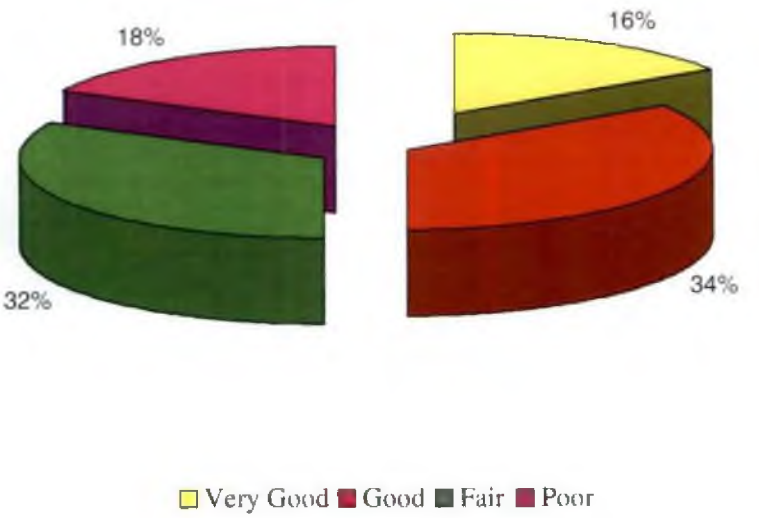


Fig. 8 Distribution of individual milk samples based on total viable count from S_1, S_2 and S_3

Fig. 9 Distribution of pooled milk samples based on total viable count from S_1, S_2 and S_3

(18.06 per cent) samples, respectively. None of the pooled samples from S_2 was graded as very good. Of the samples from S_1, 16.67 per cent was graded as very good and also 12.50 per cent samples from S_3 belonged to that grade. Eight (33.33 per cent), 1 (4.17 per cent) and 14 (58.33 per cent) samples from S_1, S_2 and S_3, respectively were graded as good samples. Samples belonging to fair grade accounted for 16.67, 75.00 and 29.17 per cent of the samples from S_1, S_2 and S_3, respectively. In the samples of S_1 and S_2, 33.33 and 20.83 per cent were graded as poor. But, none of the samples from S_3 was graded as poor.

Table 65. Distribution of pooled milk samples from S_1, S_2 and S_3 based on total viable count

Sources	Number of samples			
	Very good	Good	Fair	Poor
S_1	4 (16.67)	8 (33.33)	4 (16.67)	8 (33.33)
S_2	0	1 (4.17)	18 (75.00)	5 (20.83)
S_3	3 (12.50)	14 (58.33)	7 (29.17)	0
Overall	7 (9.72)	23 (31.94)	29 (40.28)	13 (18.06)

Figures in parenthesis indicate per cent; N = 24 from each source

4.3.3 Individual milk samples collected from farmers of S_1

Based on total viable count, the individual raw milk samples collected from S_1 were graded as very good, good, fair and poor. The distribution of the samples of different grades is given in table 66. Only one out of the 36 samples belonging to F_2 was graded as very good. Thirteen (36.11 per cent), 19 (52.78 per cent) and 3 (8.33 per cent) of the 36 samples were graded as good, fair and poor, respectively. Three (50.00 per cent) samples each from F_4, F_5 and F_6 and two (33.33 per cent) samples from F_3 were fell in the grade good. One sample each from F_1 and F_2 was graded good. One (16.67 per cent) samples each from the F_1, F_2 and F_6 was graded as poor. None of the samples from F_2, F_3 and F_4 were graded as poor.

Table 66. Distribution of individual milk samples from S_1 based on total viable count

Farmers	Number of samples			
	Very good	Good	Fair	Poor
F_1	0	1 (16.67)	4 (66.67)	1 (16.67)
F_2	1 (16.67)	1 (16.67)	4 (66.67)	0
F_3	0	2 (33.33)	4 (66.67)	0
F_4	0	3 (50.00)	3 (50.00)	0
F_5	0	3 (50.00)	2 (33.33)	1 (16.67)
F_6	0	3 (50.00)	2 (33.33)	1 (16.67)
Overall	1 (2.78)	13 (36.11)	19 (52.78)	3 (8.33)

Figures in parenthesis indicate per cent; N = six samples from each farmer

4.3.4 Individual milk samples collected from farmers of S_2

The samples received from the farmers of S_2 were graded based on total viable count and their distribution is given in table 67. The per cent of very good, good, fair and poor samples from S_2 was 11.11, 25.00, 27.78 and 36.11, respectively. None of the individual samples from F_1, F_2 and F_6 were graded as very

Table 67. Distribution of individual milk samples from S_2 based on total viable count

Farmers	Number of samples			
	Very good	Good	Fair	Poor
F_1	0	2 (33.33)	1 (16.67)	3 (50.00)
F_2	0	0	1 (16.67)	5 (83.33)
F_3	1 (16.67)	0	1 (16.67)	4 (66.67)
F_4	1 (16.67)	3 (50.00)	2 (33.33)	0
F_5	2 (33.33)	3 (50.00)	1 (16.67)	0
F_6	0	1 (16.67)	4 (66.67)	1 (16.67)
Overall	4 (11.11)	9 (25.00)	10 (27.78)	13 (36.11)

N = six samples from each farmer; Figures in parenthesis indicate per cent

good. Of the samples, 50.00 per cent each belonging to F_4 and F_5 were graded as good. Among the samples obtained from F_6, 66.67 per cent was graded as fair and an equalent per cent of samples belonging to F_3 was graded as poor. Of the samples belonging to F_2, 83.33 per cent was graded as poor, while 50.00 per cent of samples belonging to F_1 were also belonging to that grade.

4.3.5 Individual milk samples collected from farmers of S_3

The raw milk samples received from the farmers of S_3 were graded according to the total viable count and the distribution of the samples is given in table 68. Among the samples of S_3, 41.67, 33.33, 16.67 and 8.33 per cent were graded as good, very good, fair and poor, respectively. In the samples belonging to F_2, 50.00 per cent each was graded as good and poor. The later per cent of the samples collected from F_4 and F_5 was graded as good and fair, respectively. In the samples of F_2 and F_3, 83.33 per cent were graded as very good and 66.67 per cent samples from F_1 were graded as good.

Table 68. Distribution of individual milk samples from S_3 based on total viable count

Farmers	Number of samples			
	Very good	Good	Fair	Poor
F_1	2 (33.33)	4 (66.67)	0	0
F_2	5 (83.33)	1 (16.67)	0	0
F_3	5 (83.33)	1 (16.67)	0	0
F_4	0	3 (50.00)	3 (50.00)	0
F_5	0	3 (50.00)	3 (50.00)	0
F_6	0	3 (50.00)	0	3 (50.00)
Overall	12 (33.33)	15 (41.67)	6 (16.67)	3 (8.33)

N = six samples from each farmer; Figures in parenthesis indicate per cent

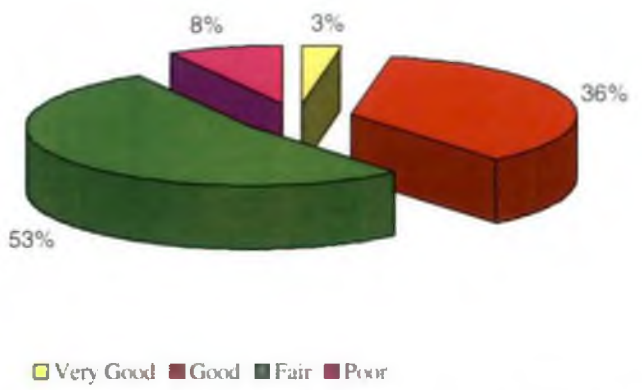


Fig. 10 Distribution of individual milk samples based on total viable count from S_1

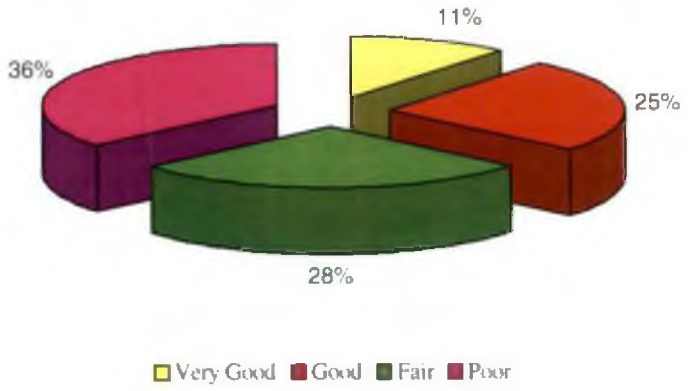


Fig.11 Distribution of individual milk samples based on total viable count from S_2

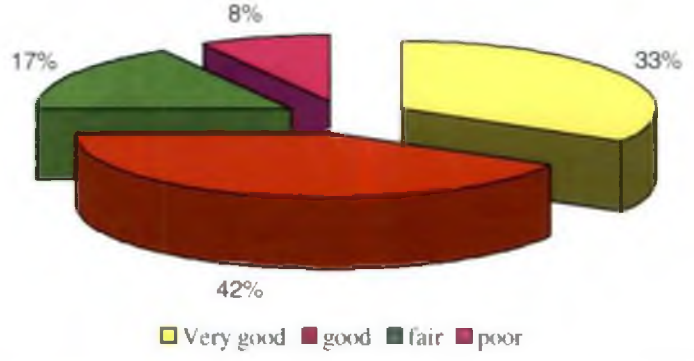


Fig. 12 Distribution of individual milk samples based on total viable count from S_3

4.4 ASSESSMENT OF CRITICAL CONTROL POINTS OF BACTERIAL CONTAMINATION OF MILK AT THE POINT OF PRODUCTION

The samples of air, water, utensil rinsing, hand wash of the milker and udder washes of the animals were tested to evaluate the bacterial load in the samples so as to determine the potential of these samples in the bacterial contamination of milk.

4.4.1 Air

4.4.1.1 *Total Viable Count*

The mean total viable counts of air samples collected from the farmers of three societies are given in table 69. The overall mean total viable count was 149.83 ± 13.21 cfu/ft^2/min. The highest mean count (217.43 ± 21.41 cfu/ft^2/min) was observed in the samples belonging to F_2 of S_1 and the lowest count (119.43 ± 15.22 cfu/ft^2/min) was obtained in the samples of F_1 of S_2. The counts in F_1 of S_1, F_2 of S_2 and F_1 and F_2 of S_3 were 143.67 ± 19.33, 163.34 ± 14.67, 121.87 ± 22.48 and 133.22 ± 15.21 cfu/ft^2/min, respectively.

Table 69. Mean total viable counts of air samples of farmers belonging to S_1, S_2 & S_3

Sources		Mean bacterial count (Mean ± SE cfu/ft^2/min)
S_1	F_1	143.67 ± 19.33
	F_2	217.43 ± 21.41
S_2	F_1	119.43 ± 15.22
	F_2	163.34 ± 14.67
S_3	F_1	121.87 ± 22.48
	F_2	133.22 ± 15.21
Overall		149.83 ± 13.21

N = six from each farmer

4.4.2 Water

4.4.2.1 *Total Viable Count*

Water samples collected from the farmers of three societies were analyzed for total viable count and its mean counts are given in table 70. The samples belonging to the farmers of the three societies had an average mean count of 1.65 ± 0.09 $\log_{10}$ cfu/ml. The samples obtained from F_2 belonging to S_1 had highest mean total viable count (2.44 ± 0.08 $\log_{10}$ cfu/ml) and the lowest count (1.07 ± 0.13 $\log_{10}$ cfu/ml) was observed in the samples of F_1 belonging to S_2. The counts in F_1 of S_1 and F_2 of S_2 were 2.24 ± 0.21 and 1.94 ± 0.10 $\log_{10}$ cfu/ml, respectively. The mean count of the samples of F_1 and F_2 of S_3 were 1.08 ± 0.10 and 1.12 ± 0.12 $\log_{10}$ cfu/ml, respectively.

4.4.2.2 *Coliform Count*

The mean coliform counts of water samples collected from the farmers of three societies are given in table 70. The overall mean count of the samples belonging to the farmers of the societies was at the level of 0.97 ± 0.14 $\log_{10}$ cfu/ml. Water samples obtained from F_2 belonging to S_1 had highest mean coliform count (1.67 ± 0.13 $\log_{10}$ cfu/ml). The lowest count (0.39 ± 0.26 $\log_{10}$ cfu/ml) was observed in the samples of F_2 belonging to S_3. The counts in F_1 of S_1, F_1 and F_2 of S_2 and F_1 of S_3 were 1.49 ± 0.15, 0.60 ± 0.12 and 0.95 ± 0.14 and 0.72 ± 0.17 $\log_{10}$ cfu/ml, respectively.

4.4.2.3 *Escherichia coli Count*

The mean *Escherichia coli* counts of water samples collected from the farmers of three societies are given in table 70. The samples belonging to the farmers of the three societies had an average mean count of 0.10 ± 0.13 $\log_{10}$ cfu/ml. *Escherichia coli* was not detected in the samples from farmers of S_1 and S_3 and the count in the samples belonging to F_1 of S_2 was at the level of 0.50 ± 0.05 $\log_{10}$ cfu/ml and that of F_2 was 0.11 ± 0.1 $\log_{10}$ cfu/ml.

Table 70. Mean bacterial counts of water samples of farmers belonging to S_1, S_2 & S_3

Mean bacterial count (Mean ± SE (log_{10} cfu/ml)							
Bacterial counts	S_1		S_2		S_3		Overall
	F_1	F_2	F_1	F_2	F_1	F_2	
TVC	2.24±0.21	2.44±0.08	1.07±0.13	1.94±0.10	1.08±0.10	1.12±0.12	1.65±0.09
CC	1.49±0.15	1.67±0.13	0.60±0.12	0.95±0.14	0.72±0.17	0.39±0.26	0.97±0.14
EC	ND	ND	0.50±0.05	0.11±0.10	ND	ND	0.10±0.13
FSC	1.19±0.34	1.61±0.24	0.35±0.21	0.83±0.20	0.66±0.20	0.83±0.18	0.91±0.22

N = six from each farmer

4.4.2.4 *Faecal Streptococcal Count*

The mean faecal streptococcal counts of water samples collected from the farmers of three societies are given in table 70. The overall mean count of the samples belonging to the farmers of the societies was at the level of 0.91 ± 0.22 log_{10} cfu/ml. Water samples obtained from F_2 belonging to S_1 had highest mean faecal streptococcal count (1.61 ± 0.24 log_{10} cfu/ml) and the lowest count (0.35 ± 0.21 log_{10} cfu/ml) was in the samples of F_1 belonging to S_2. The counts in F_1 of S_1 and F_2 of S_2 were 1.19 ± 0.34 and 0.83 ± 0.20 log_{10} cfu/ml, respectively. The count in the samples of F_1 and F_2 of S_3 were 0.66 ± 0.20 and 0.83 ± 0.18 log_{10} cfu/ml, respectively.

4.4.3 Hand Wash

4.4.3.1 *Total Viable Count*

The overall mean and mean bacterial counts of hand washings collected from the farmers of three societies are given in table 71. The overall mean total viable count of the samples was 3.38 ± 0.08 log_{10} cfu/ml. The highest mean count (3.88 ± 0.17 log_{10} cfu/ml) was observed in the samples of F_1 belonging to S_2 and the lowest count (2.46 ± 0.11 log_{10} cfu/ml) was observed in the samples of F_2 belonging to society 3. The samples of F_1 and F_2 belonging to S_1 had the count of

3.25 ± 0.22 and 3.84 ± 0.09 log_{10} cfu/ml, respectively. The counts in samples of F_2 of S_2 and F_1 of S_3 were 3.12 ± 0.20 and 3.71 ± 0.21 log_{10} cfu/ml, respectively.

4.4.3.2 *Coliform Count*

The mean coliform counts of samples of hand wash collected from the farmers of the three societies are given in table 71. The samples had an overall mean count of 2.03 ± 0.27 log_{10} cfu/ml. The highest mean count (2.56 ± 0.13 log_{10} cfu/ml) was observed in the samples of F_1 belonging to S_2. The lowest count (1.39 ± 0.31 log_{10} cfu/ml) was seen in the samples of F_2 belonging to society 3. The samples of F_1 and F_2 belonging to S_1 had the count of 1.92 ± 0.27 and 2.22 ± 0.19 log_{10} cfu/ml, respectively. The counts in F_2 of S_2 and F_1 of S_3 were 1.61 ± 0.32 and 2.50 ± 0.12 log_{10} cfu/ml, respectively.

4.4.3.3 *Escherichia coli Count*

The mean *Escherichia coli* counts of hand washings collected from the farmers of three societies are given in table 71. The samples belonging to the farmers of the three societies had an overall mean count of 0.24 ± 0.21 log_{10} cfu/ml. *Escherichia coli* was not detected in the samples of F_1 and F_2 from the society S_1 and F_2 belonging to S_3. The count in the samples of F_1 of S_2 was 0.47 ± 0.29 log_{10} cfu/ml and that of F_2 was 0.72 ± 0.31 log_{10} cfu/ml. The count in the samples of S_3 was 0.22 ± 0.21 log_{10} cfu/ml.

4.4.3.4 *Faecal Streptococcal Count*

The mean faecal streptococcal counts of hand wash samples collected from the farmers of the three societies are given in table 71. The overall mean count of the samples was 2.01 ± 0.18 log_{10} cfu/ml. The highest mean faecal streptococcal count (2.42 ± 0.19 log_{10} cfu/ml) was observed in the samples of F_2 belonging to S_2 and the lowest count (1.39 ± 0.32 log_{10} cfu/ml) was observed in the samples of F_1 belonging to the same society. The counts in F_1 and F_2 of S_1 and F_1 and F_2 of S_3 were 2.37 ± 0.15, 2.25 ± 0.06, 2.06 ± 0.13 and 1.54 ± 0.27 log_{10} cfu/ml, respectively.

Table 71. Mean bacterial counts of hand wash samples of farmers belonging to S_1, S_2 & S_3

Mean bacterial count (Mean ± SE log_{10} cfu/ml)							
Bacterial counts	S_1		S_2		S_3		Overall
	F_1	F_2	F_1	F_2	F_1	F_2	
TVC	3.25±0.22	3.84±0.09	3.88±0.17	3.12±0.20	3.71±0.21	2.46±0.11	3.38±0.08
CC	1.92±0.27	2.22±0.19	2.56±0.13	1.61±0.32	2.50±0.12	1.39±0.31	2.03±0.27
EC	ND	ND	0.47±0.29	0.72±0.31	0.22±0.21	ND	0.24±0.21
FSC	2.37±0.15	2.25±0.06	1.39±0.32	2.42±0.19	2.06±0.13	1.54±0.27	2.01±0.18

N = six from each farmer

4.4.4 Utensil Wash

4.4.4.1 *Total Viable Count*

Utensil wash samples collected from the farmers of three societies were analyzed for total viable count and its mean counts are given in table 72. The samples had an overall mean count of 2.91 ± 0.12 log_{10} cfu/ml. The highest mean count (3.61 ± 0.15 log_{10} cfu/ml) was seen in the samples of F_2 belonging to S_1 and the lowest count (2.16 ± 0.16 log_{10} cfu/ml) was observed in the samples of F_2 belonging to society 3. The counts in F_1 of S_1 and F_1 and F_2 of S_2 were 3.10 ± 0.26, 3.37 ± 0.25 and 2.43 ± 0.14 log_{10} cfu/ml, respectively. The count in the samples of F_1 of S_3 was 2.51 ± 0.19 log_{10} cfu/ml.

4.4.4.2 *Coliform Count*

The mean coliform counts of utensil wash samples collected from the farmers of three societies are given in table 72. The samples had an overall mean count of 1.14 ± 0.16 log_{10} cfu/ml. The highest mean count (1.36 ± 0.19 log_{10} cfu/ml) was observed in the samples of F_2 belonging to S_1 and the lowest count (0.92 ± 0.37 log_{10} cfu/ml) was observed in the samples of F_2 belonging to society 3. The counts in F_1 of S_1 and F_1 and F_2 of S_2 were 1.31 ± 0.28, 0.97 ± 0.26, and 1.17 ± 0.17 log_{10} cfu/ml, respectively. The count in F_1 of S_3 was 1.12 ± 0.02 log_{10} cfu/ml.

4.4.4.3 *Escherichia coli Count*

The mean *Escherichia coli* counts of utensil wash samples collected from the farmers of three societies are given in table 72. The samples had an overall mean count of 0.33 ± 0.24 $\log_{10}$ cfu/ml. The highest mean count (0.61 ± 0.27 $\log_{10}$ cfu/ml) was seen in the samples of F_2 belonging to S_1. The organism was not observed in the samples of F_2 belonging to society 3. The count in F_1 belonging to S_1 was 0.22 ± 0.22 $\log_{10}$ cfu/ml. The samples of F_1 and F_2 belonging to S_2 and F_1 belonging to S_3 had *Escherichia coli* count of 0.27 ± 0.26, 0.56 ± 0.35 and 0.31 ± 0.15 $\log_{10}$ cfu/ml, respectively.

Table 72. Mean bacterial counts of utensil wash samples of farmers belonging to S_1, S_2 & S_3

Bacterial counts	Mean bacterial count (Mean ± SE $\log_{10}$ cfu/ml)						
	S_1		S_2		S_3		Overall
	F_1	F_2	F_1	F_2	F_1	F_2	
TVC	3.10±0.26	3.61±0.15	3.37±0.25	2.43±0.14	2.51±0.19	2.16±0.16	2.91±0.12
CC	1.31±0.28	1.36±0.19	0.97±0.26	1.17±0.17	1.12±0.02	0.92±0.37	1.14±0.16
EC	0.22±0.22	0.61±0.27	0.27±0.26	0.56±0.35	0.31±0.15	ND	0.33±0.24
FSC	2.15±0.19	2.31±0.23	1.42±0.39	2.06±0.41	1.69±0.27	1.21±0.23	1.81±0.35

N = six from each farmer

4.4.4.4 *Faecal Streptococcal Count*

The mean faecal streptococcal counts of utensil wash samples collected from the farmers of three societies are given in table 72. The overall mean count of the samples was 1.81 ± 0.35 $\log_{10}$ cfu/ml. The highest mean count (2.31 ± 0.23 $\log_{10}$ cfu/ml) was observed in the samples of F_2 belonging to S_1 and the lowest count (1.21 ± 0.23 $\log_{10}$ cfu/ml) was seen in the samples of F_2 belonging to society 3. The count in F_1 belonging to S_1 was 2.15 ± 0.19 $\log_{10}$ cfu/ml. The samples of F_1 and F_2 belonging to S_2 and F_1 belonging to S_3 had coliform count of 1.42 ± 0.39, 2.06 ± 0.41 and 1.69 ± 0.27 $\log_{10}$ cfu/ml, respectively.

4.4.5 Udder Wash

4.4.5.1 *Total Viable Count*

The samples of udder washing of the cows belonging to the farmers of the three societies were collected and assessed the bacterial load and the results are given in the table 73. The overall mean count of the samples was 3.06 ± 0.13 $\log_{10}$ cfu/ml. The highest mean count (3.31 ± 0.21 $\log_{10}$ cfu/ml) was seen in the samples of F_1 belonging to S_1. The lowest count (2.54 ± 0.18 $\log_{10}$ cfu/ml) was seen in the samples of F_1 belonging to society 3. The counts in F_1 and F_2 belonging to S_2 were 3.15 ± 0.16 and 3.00 ± 0.11 $\log_{10}$ cfu/ml, respectively and the counts in F_2 of S_1 and F_2 belonging to S_3 were 3.29 ± 0.10 and 3.07 ± 0.12 $\log_{10}$ cfu/ml, respectively.

4.4.5.2 *Coliform Count*

The mean coliform counts of udder washings are given in table 73. The samples had an overall mean count of 1.31 ± 0.20 $\log_{10}$ cfu/ml. The highest mean count (1.68 ± 0.17 $\log_{10}$ cfu/ml) was observed in the samples of F_1 belonging to S_2 and the lowest count (1.02 ± 0.25 $\log_{10}$ cfu/ml) was seen in the samples of F_1 belonging to society 3. The counts in the samples of F_1 and F_2 belonging to S_1 were 1.30 ± 0.13 and 1.05 ± 0.38 $\log_{10}$ cfu/ml, respectively and that of F_2 of S_2 was 1.43 ± 0.24 $\log_{10}$ cfu/ml. The count in the samples of F_2 of S_3 was 1.30 ± 0.22 $\log_{10}$ cfu/ml.

4.4.5.3 *Escherichia coli Count*

The mean *Escherichia coli* counts of udder washing collected from the farmers of three societies are given in table 73. The samples had an overall mean count of 0.43 ± 0.24 $\log_{10}$ cfu/ml. All samples showed the presence of the organism. The highest mean count (0.54 ± 0.22 $\log_{10}$ cfu/ml) was observed in the samples of F_1 belonging to S_3 and the lowest count (0.36 ± 0.14 $\log_{10}$ cfu/ml) was seen in the samples of F_1 belonging to S_1. The counts in the samples of F_2 of S_1, F_1 and F_2 belonging to S_2 were 0.41 ± 0.19, 0.38 ± 0.13 and 0.47 ± 0.14 $\log_{10}$ cfu/ml, respectively and the count in the samples of F_2 of S_3 was 0.41 ± 0.09 $\log_{10}$ cfu/ml.

4.4.5.4 *Faecal Streptococcal Count*

The mean faecal streptococcal count of udder wash samples collected from the farmers of three societies is given in table 73. The samples had an overall mean count of 1.50 ± 0.16 $\log_{10}$ cfu/ml. The samples of F_1 belonging to S_1 had the highest mean count (2.23 ± 0.06 $\log_{10}$ cfu/ml). The lowest count (1.01 ± 0.21 $\log_{10}$ cfu/ml) was observed in the samples of F_1 belonging to society 3. The counts in the samples of F_1 and F_2 belonging to S_2 were 1.78 ± 0.11 and 1.02 ± 0.12 $\log_{10}$ cfu/ml, respectively. The samples of F_2 of S_1 had the count at the level of 1.38 ± 0.18 $\log_{10}$ cfu/ml. The count in the samples F_2 of S_3 was 1.6 ± 0.15 $\log_{10}$ cfu/ml.

Table 73. Mean bacterial counts of udder wash samples of cows belonging to farmers of S_1, S_2 & S_3

Mean bacterial count (Mean ± SE $\log_{10}$ cfu/ml)							
Bacterial Counts	S_1		S_2		S_3		Overall
	F_1	F_2	F_1	F_2	F_1	F_2	
TVC	3.31±0.21	3.29±0.10	3.15±0.16	3.00±0.11	2.54±0.18	3.07±0.12	3.06±0.13
CC	1.30±0.13	1.05±0.38	1.68±0.17	1.43±0.24	1.02±0.25	1.30±0.22	1.31±0.20
EC	0.36±0.14	0.41±0.19	0.38±0.13	0.47±0.14	0.54±0.22	0.41±0.09	0.43±0.24
FSC	2.23±0.06	1.38±0.18	1.78±0.11	1.02±0.12	1.01±0.21	1.6±0.15	1.50±0.16

N = six from each farmer

4.5 ASSESSMENT OF CRITICAL CONTROL POINTS OF BACTERIAL CONTAMINATION OF MILK AT SOCIETY LEVEL

The samples of air, water, utensil rinsing and hand wash of the milk handler of the society were tested to evaluate the bacterial load in the samples so as to determine the potential of these samples in the bacterial contamination of milk.

4.5.1 Air

4.5.1.1 *Total Viable Count*

The mean total viable count of air samples collected from the three societies and overall mean count are given in table 74. The samples from S_1 had the highest

mean count (176.67 ± 23.16 cfu/ft^2/min) and the lowest count was seen in the samples of S_2 (112.34 ± 14.12 cfu/ft^2/min). The count in S_3 was at the level of 164.83 ± 20.17 cfu/ft^2/min. The overall mean count of the samples from the societies was 151.12 ± 19.15 cfu/ft^2/min.

Table 74. Mean total viable counts of air samples from S_1, S_2 and S_3

Sources	Mean bacterial count (Mean ± SE cfu/ft^2/min)
S_1	176.67 ± 23.16
S_2	112.34 ± 14.12
S_3	164.83 ± 20.17
Overall	151.12 ± 19.15

N = six from each society

4.5.2 Water

4.5.2.1 *Total Viable Count*

Water samples collected from the three societies were evaluated for its bacterial count. The mean counts of the samples are given in the table 75. The samples had an overall mean count of 1.82 ± 0.16 $\log_{10}$ cfu/ml. The highest mean count of 2.24 ± 0.13 $\log_{10}$ cfu/ml was observed in the samples collected from S_2. The lowest count was seen in the samples of S_1 (1.41 ± 0.20 $\log_{10}$ cfu/ml) and the count in the samples of S_3 was 1.82 ± 0.18 $\log_{10}$ cfu/ml.

4.5.2.2 *Coliform Count*

The mean coliform counts of water samples collected from the three societies are given in table 75. The overall mean coliform count of the samples was 1.28 ± 0.08 $\log_{10}$ cfu/ml. The samples of S_2 had the highest mean count (1.58 ± 0.14 $\log_{10}$ cfu/ml) followed by S_1 (1.20 ± 0.14 $\log_{10}$ cfu/ml) and the lowest count was seen in the samples of S_3 (1.05 ± 0.09 $\log_{10}$ cfu/ml).

4.5.2.3 *Escherichia coli Count*

The mean *Escherichia coli* counts of water samples obtained from the three societies are given in table 75. The overall mean *Escherichia coli* count of the samples was 0.56 ± 0.06 log_{10} cfu/ml. Samples of the S_2 had the highest mean count (0.68 ± 0.07 log_{10} cfu/ml). The count in the samples of S_3 was at the level of 0.51 ± 0.04 log_{10} cfu/ml and the lowest count was seen in the samples of source S_1 (0.50 ± 0.05 log_{10} cfu/ml).

4.5.2.4 *Faecal Streptococcal Count*

The mean faecal streptococcal counts of water from the three societies are given in table 75. The overall mean faecal streptococcal count of the sample was 0.98 ± 0.11 log_{10} cfu/ml. Samples of the S_2 had the highest mean count (1.67 ± 0.18 log_{10} cfu/ml). The count in the samples belonging to S_1 was at the level of 0.72 ± 0.31 log_{10} cfu/ml and the lowest count was seen in the samples of S_3 (0.54 ± 0.23 log_{10} cfu/ml).

Table 75. Mean bacterial counts of water samples collected from S_1, S_2 and S_3

Mean bacterial count (Mean ± SE (log_{10} cfu/ml)				
Bacterial counts	S_1	S_2	S_3	Overall
TVC	1.41 ± 0.20	2.24±0.13	1.82±0.18	1.82±0.16
CC	1.20 ± 0.14	1.58±0.14	1.05±0.09	1.28±0.08
EC	0.50 ± 0.05	0.68±0.07	0.51±0.04	0.56±0.06
FSC	0.72 ± 0.31	1.67±0.18	0.54±0.23	0.98±0.11

N = six from each society

4.5.3 Hand Wash

4.5.3.1 *Total Viable Count*

The mean total viable counts of hand wash samples collected from the three societies are given in table 76. The overall mean total viable count of the samples

of the three societies was 3.26 ± 0.07 log_{10} cfu/ml. Samples of S_2 had the highest mean count (3.67 ± 0.11 log_{10} cfu/ml). The lowest mean count was seen in samples of S_3 (2.97 ± 0.12 log_{10} cfu/ml). The count in the samples of S_1 was 3.14 ± 0.13 log_{10} cfu/ml.

4.5.3.2 *Coliform Count*

The mean coliform counts of hand wash samples collected from the three societies are given in table 76. The overall mean coliform count was 2.23 ± 0.15 log_{10} cfu/ml. Mean coliform count of the samples of S_1 was the highest (2.62 ± 0.13 log_{10} cfu/ml) followed by the count in the samples of S_2 (2.14 ± 0.15 log_{10} cfu/ml) and the lowest count was in the samples of S_3 (1.93 ± 0.17 log_{10} cfu/ml).

4.5.3.3 *Escherichia coli Count*

The mean *Escherichia coli* counts of hand washings obtained from the three societies are given in table 76. The samples belonging to the three societies had an overall mean *Escherichia coli* count of 0.62 ± 0.18 log_{10} cfu/ml. Samples of S_1 had the highest mean count (0.78 ± 0.28 log_{10} cfu/ml) and the lowest count was observed in the samples of S_3 (0.34 ± 0.24 log_{10} cfu/ml). The count in the samples of S_2 was 0.74 ± 0.31 log_{10} cfu/ml.

Table 76. Mean bacterial counts of hand washings collected from S_1, S_2 and S_3

Mean bacterial count (Mean ± SE (log_{10} cfu/ml)				
Bacterial counts	S_1	S_2	S_3	Overall
TVC	3.14 ± 0.13	3.67 ± 0.11	2.97 ± 0.12	3.26 ± 0.07
CC	2.62 ± 0.13	2.14 ± 0.15	1.93 ± 0.17	2.23 ± 0.15
EC	0.78 ± 0.28	0.74 ± 0.31	0.34 ± 0.24	0.62 ± 0.18
FSC	1.82 ± 0.21	2.15 ± 0.18	1.76 ± 0.06	1.91 ± 0.20

N = six from each society

4.5.3.4 *Faecal Streptococcal Count*

The mean faecal streptococcal counts of samples of hand wash from the

three societies are given in table 76. The overall mean faecal streptococcal count was 1.91 ± 0.20 log_{10} cfu/ml. The highest mean count of 2.15 ± 0.18 log_{10} cfu/ml was observed in the samples of S_2, followed by the count in the samples of S_1 (1.82 ± 0.21 log_{10} cfu/ml) and the lowest was seen in the samples of S_3 (1.76 ± 0.06 log_{10} cfu/ml).

4.5.4 Utensil Wash

4.5.4.1 *Total Viable Count*

The mean total viable counts of samples of utensil wash collected from the three societies are given in table 77. The count in samples of S_1, S_2 and S_3 were 2.06 ± 0.07, 2.81 ± 0.08 and 2.55 ± 0.13 log_{10} cfu/ml, respectively. The overall mean total viable count was 2.47 ± 0.06 log_{10} cfu/ml.

4.5.4.2 *Coliform Count*

The mean coliform count of samples of utensil wash collected from the three societies are given in table 77. The highest mean count (1.78 ± 0.31 log_{10} cfu/ml) was observed in the samples of S_3 and the lowest count was observed in the samples of S_1 (1.09 ± 0.09 log_{10} cfu/ml). The overall mean coliform count was 1.43 ± 0.12 log_{10} cfu/ml.

4.5.4.3 *Escherichia coli Count*

The mean *Escherichia coli* counts of samples of utensil wash from the three societies are given in table 77. The overall mean *Escherichia coli* count was 0.30 ± 0.14 log_{10} cfu/ml. Samples of S_2 had the highest mean count (0.42 ± 0.27 log_{10} cfu/ml). The lowest count was observed in the samples of S_3 (0.17 ± 0.15 log_{10} cfu/ml) and that of S_1 was 0.31 ± 0.19 log_{10} cfu/ml.

4.5.4.4 *Faecal Streptococcal Count*

The mean faecal streptococcal counts of samples of utensil wash from the three societies are given in table 77. Samples of S_2 had the highest mean count (1.95 ± 0.22 log_{10} cfu/ml) followed by the count in the samples of S_1 (1.43 ± 0.16

log_{10} cfu/ml). The lowest count was seen in the samples of S_3 (1.06 ± 0.08 log_{10} cfu/ml). The overall mean faecal streptococcal count was 1.48 ± 0.09 log_{10} cfu/ml.

Table 77. Mean bacterial counts of utensil washings collected from S_1, S_2 and S_3

Mean bacterial count (Mean ± SE (log_{10} cfu/ml)				
Microbial counts	S_1	S_2	S_3	Overall
TVC	2.06±0.07	2.81±0.08	2.55±0.13	2.47 ± 0.06
CC	1.09±0.09	1.43±0.20	1.78±0.31	1.43 ± 0.12
EC	0.31±0.19	0.42±0.27	0.17±0.15	0.30 ± 0.14
FSC	1.43±0.16	1.95±0.22	1.06±0.08	1.48 ± 0.09

N = six from each society

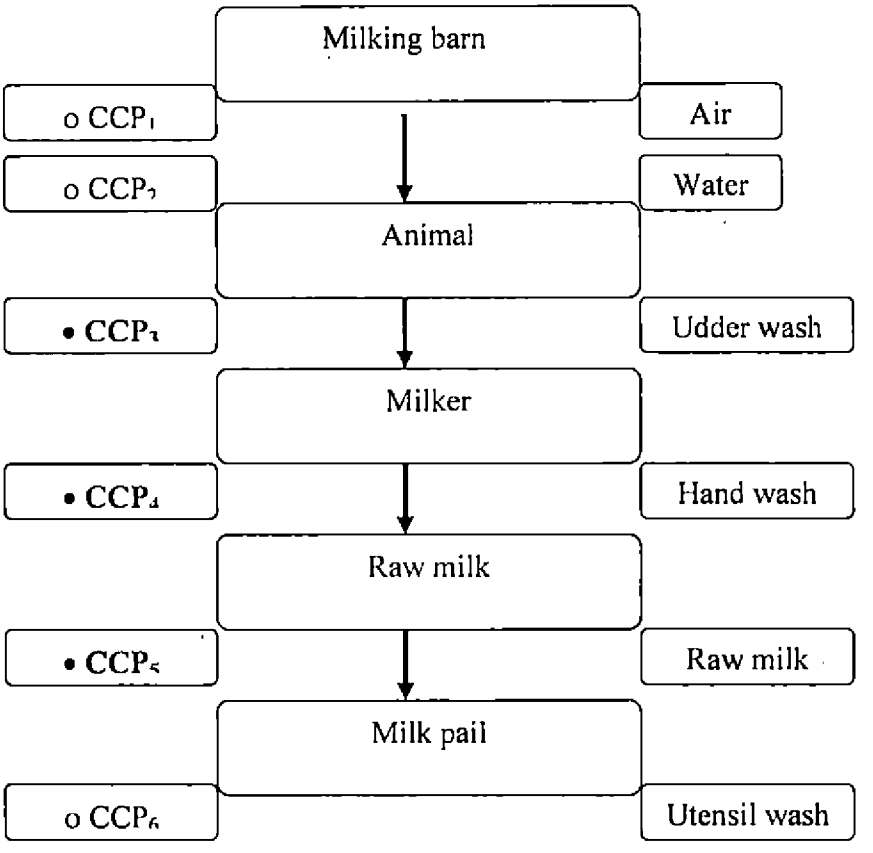
4.6 ADULTERANTS AND PRESERVATIVES IN THE MILK

All the 72 pooled milk samples were tested to determine the presence of adulterants *viz.*, starch and cane sugar and preservatives *viz.*, boric acid, formaldehyde and neutralizers. None of the samples were found positive for the adulterants and preservatives.

4.7 DETECTION OF *Escherichia coli* BY POLYMERASE CHAIN REACTION

The *Escherichia coli* isolates obtained from raw milk were confirmed by Polymerase Chain Reaction (PCR) following the procedure described by Daly *et al.* (2002). The expected 366 bp level amplification specific for *Escherichia coli* alanine racemase gene (alr) was obtained when DNA extracted from *Escherichia coli* were subjected to PCR. Agarose gel electrophoresis of the amplified PCR product was carried out along with a negative control and a molecular size marker in 1xTAE buffer. Analysis of the electrophoresed gel under UV transilluminator revealed the presence of a 366 bp band in 93.33 per cent isolates and particular band obtained was shown in fig. 13. In the negative control no amplification product was detected.

Flowchart 11. Critical control points in production of milk at farmer's level



• CCP - Major source of contamination

o CCP - Minor source of contamination

CCP_1= Environment sanitation

CCP_2=Chlorination, Environment sanitation

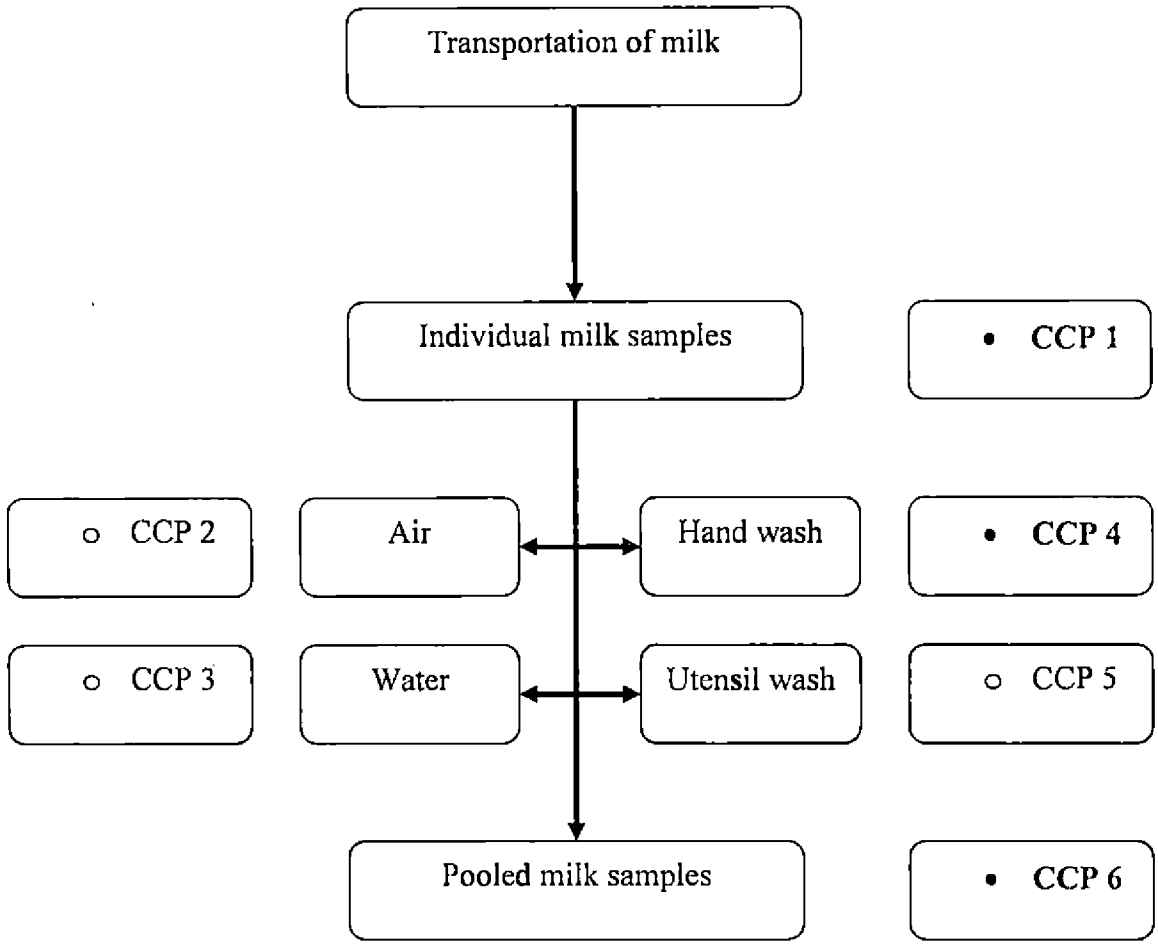
CCP_3=Healthy animal, desinfection of udder

CCP_4= Milkers personal hygiene, Hygienic milking process.

CCP_5= Hygienic production of milk

CCP_6=Periodic cleaning of utensils

Flow chart 12. Critical control points in production of milk at society level



- • CCP - Major source of contamination
- o CCP - Minor source of contamination

CCP 1- Better hygienic practices at the farm level, during handling and transportation

CCP2 - Environmental hygiene

CCP3 - Water sanitization, Use of disinfectants

CCP4 - Proper washing hands before handling of milk, Personnel hygiene.

CCP5 - Use of detergents and proper washing of utensils

CCP6 - Proper care while handling the milk, Hygiene of the milk handler

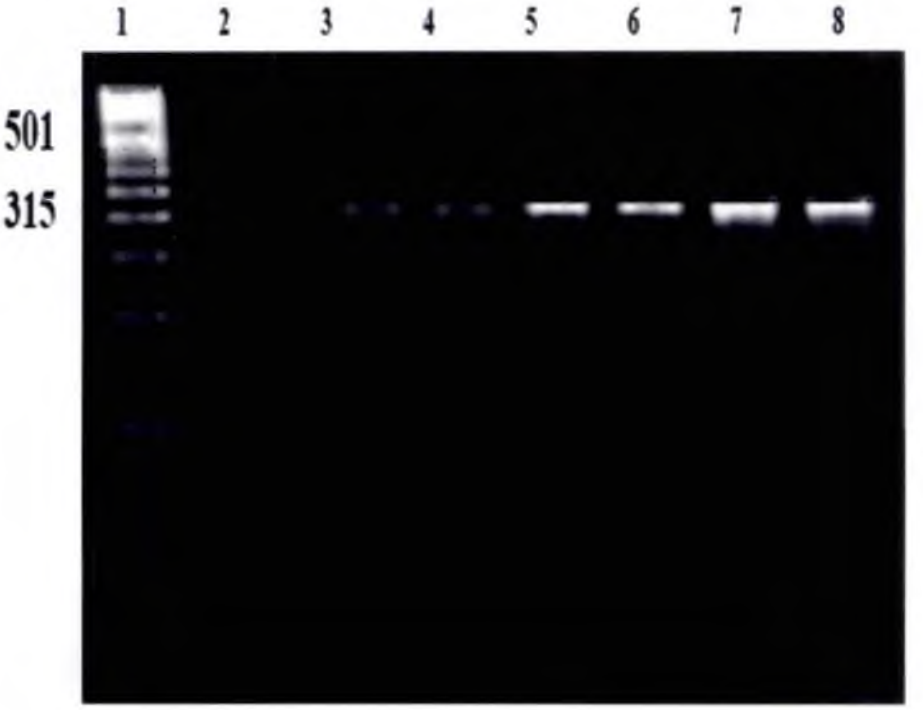


Fig.13. *E.coli* genus specific *alr* PCR III

Lane 1 pUC 19 DNA/ *Msp* 1 digest marker

Lane 2 Negative control

Lane 3 - 8 *E. coli* isolates

Discussion

5. DISCUSSION

Milk is the only food of young mammal during the initial period of its life, which provides both energy and building materials required for their growth. Though it is the nature's most ideal and perfect food, the possibility of it's being a source of biological and chemical hazards cannot be excluded. Unhygienic production of milk also leads to inferior keeping quality as a result of rapid microbial growth and multiplication and also serves as a potential health hazard to consumers. In view of the immense dietary importance of milk, the need for production of safe, clean and wholesome milk is emphasized. Moreover, in the present days consumers are more concerned about the quality of the product. Hence in the present study, microbial load of raw milk at the farmer's level and at society level were evaluated and isolated certain bacterial pathogens. In order to find out the source of bacterial contamination of milk and critical control points, samples of air, water, utensil rinsings, udder wash of the animal and hand washings of milker or milk handler were collected and evaluated the bacterial load. The pooled milk samples collected from society were also tested to determine the presence of adulterants and preservatives. Polymerase Chain Reaction was also employed to confirm *Escherichia coli* cultures isolated from milk.

5.1 MICROBIAL QUALITY OF MILK

Microbial quality of the 108 individual milk samples collected from the farmers and the 72 pooled milk samples from society were assessed by determining the total viable count, coliform count, *Escherichia coli* count, faecal streptococcal count and yeast and mould count.

5.1.1 Microbial count of individual milk samples from S_1, S_2 and S_3

5.1.1.1 *Total Viable Count*

Total viable count of any food item reflects its microbial quality and degree of freshness and it also indicates hygiene practices applied during the production and further handling.

The mean total viable count of samples from the three societies showed highly significant ($P<0.01$) difference (Table 2). The overall mean count of the samples obtained from the farmers of three societies was 6.01 ± 0.07 $\log_{10}$ cfu/ml, which was in accordance with the count reported Oliveira *et al.* (2000), Chacko (2006) and Nanu *et al* (2007). The count in the present study was about two log higher than that observed in the samples of source A (Raj *et al.*, 2003), whereas the count was two log lower than that reported by Lues *et al.* (2003). High total viable count in the samples might be due to the poor hygienic practices followed during milking or subclinical infections of the cow, which is difficult to understand by general appearance. The counts in the samples of S_1 and S_3 were almost similar. But the count in the samples of S_2 was significantly ($P<0.05$) higher than the other two societies. This indicates the difference in the hygienic practices followed between societies. Difference between the mean total viable count of the samples from societies were also reported by Jolly *et al.* (2000), Raj *et al.* (2003) and Chacko (2006). The count at the level of 10^7 cfu/ml was seen in 11.11 per cent samples of the present study and 40.74 per cent samples had count at the level of 10^6 cfu/ml. As the total number of microorganisms increases, shelf life of the product decreases and increases the risk of milk borne infections to the consumers.

5.1.1.2 *Coliform Count*

Presence of coliform organisms in milk indicates the unhygienic conditions prevailing during production and processing of milk. It also indicates the possibility of carrying pathogenic coliforms *viz.* salmonella spp. and *Escherichia coli*. The overall mean coliform count of the samples was 4.44 ± 0.07 $\log_{10}$ cfu/ml (Table 4) and was in accordance with the count reported by Desai and Natarajan (1981) and Singh *et al.* (1994b). The count was also in agreement with the count recorded as 4.8×10^4 cfu/ml of samples from S_2 (Kapre, 1995). Coliform count in the present study was three log higher than that reported by Prejit (2005) but the count was also too low as compared to the count reported as 6.7×10^7 cfu/ml (Lues *et al.*, 2003). Vijai and Saraswat (1968) reported that milk produced under controlled and strict clean conditions had coliform count at the level of 410/ml.

Therefore, it may be inferred that high coliform count in milk samples is due to the poor sanitary conditions prevailing during the production and further handling of milk. It also indicates the possible risk of the presence of certain pathogenic microorganisms, which may be transferred to the consumers.

During the investigation it was observed that, most of the farmers are not giving proper attention for cleaning udder before milking and most of them are washing milking utensils only with water, without the use of sanitizers. As the chances of dung and dust gain entry into milk will increase the coliforms count in the milk samples. Coliforms can easily establish on milking equipment and utensils and act as a reservoir, if not cleaned and sanitized properly. The high coliform count of the samples might be due to improper cleaning of utensils and udder.

As in the case of the present study, Chacko (2006) encountered coliform count in 100 per cent samples, whereas Jolly *et al.* (2000) reported that the presence of organism in 96.67 per cent samples and the organism was recovered from 89.9 per cent samples examined by Chye *et al* (2004). Of the samples, count at the level of 10^3 cfu/ml was observed in 25.00 per cent samples, whereas the count at the level of 10^4 and 10^5 cfu/ml was observed in 52.78 per cent and 22.22 per cent samples, respectively. The count in the present study did not concur with the count at the level of 10^{-2} ml in 80.6 per cent samples of churn supplies (Davies, 1977).

Coliforms are one of the important spoilage organisms; grow rapidly in milk, especially at temperature above 20° C, attack protein and lactose, resulting in production of gas and undesirable flavour. Some coliforms are reported to grow at temperatures as low as –2° C and thus lead to spoilage even at refrigerated storage. So higher the coliform count, the faster will be the spoilage of milk. The count observed in the three societies was almost similar. The highest mean coliform count of 4.63 ± 0.11 $\log_{10}$ cfu/ml was observed in samples of S_2, whereas the lowest count was seen in the samples of S_3 (4.31 ± 0.10 $\log_{10}$ cfu/ml). According to Bureau of Indian standards (1977), coliforms should be absent in 0.01 ml of raw milk. Cent per cent of the samples of present study did not meet the coliform

standards prescribed by BIS (1977), whereas 45 per cent samples analyzed by Gopi *et al.* (2001) and 12.04 per cent of the samples analyzed by Chacko (2006) met the standards, but Raj *et al* (2003) reported that all samples from source A did not meet the standards.

5.1.1.3 *Escherichia coli Count*

Primary habitat of *Escherichia coli* is the intestinal tract of most warm-blooded animals and the organism is being the widely used as an indicator of faecal contamination in food and water. As the number of the organism increases in milk, the risk of occurrence of a variety of gastro enteric illness and enterotoxaemia become eminent. Presence of the organism was also used to address the quality and shelf life of the food.

Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between mean counts of the samples from the three societies (Table 6) and the overall mean *Escherichia coli* Count from the samples of three societies was 0.86 ± 0.11 $\log_{10}$ cfu/ml. The count was almost similar to the overall mean count reported by Prejit (2005) and Chacko (2006), whereas the count of the current study was two log higher than that recorded by Jolly *et al.* (2000) and Chye *et al.* (2004). The mean count of the samples of S_2 was one log greater than that observed in the samples of S_1 and S_3. The organism was present in 36.11 per cent of the total samples and was much lower than that reported by Chacko (2006), who recoded the isolation of the organism from 41.66 per cent of the samples. Because *Escherichia coli* is generally regarded as an indicator of faecal contamination, it can be concluded that the samples were contaminated with faecal mater either directly or indirectly. The organism can easily become establish on equipment and utensils, so the milking utensils if not properly sanitized will form a major source of contamination.

5.1.1.4 *Faecal Streptococcal Count*

Presence of faecal streptococci (Enterococci) in food has sanitary significance since the organism is found in the intestinal tract of man and animals and the organisms can cause proteolysis, bitterness and other defects in milk.

Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between mean faecal streptococcal counts of the samples from the three societies (Table 8). The overall mean count of the samples was 3.14 ± 0.10 $\log_{10}$ cfu/ml and was almost similar to the counts observed in the individual milk samples belonging to S_2 (2.1×10^3 cfu/ml) and S_3 (1.7×10^3 cfu/ml) by Kapre (1995) and the overall mean count reported by Chacko (2006). The count observed in the current study was about one log greater than that observed in the individual milk samples (Jolly *et al.*, 2000). The highest mean count, 3.66 ± 0.10 $\log_{10}$ cfu/ml, was observed in the samples of S_2 and the lowest mean count was seen in samples of S_1 (2.86 ± 0.20 $\log_{10}$ cfu/ml). Critical difference test of the data showed significant ($P<0.05$) difference between the mean counts of the samples from S_1 and S_2 and that of the samples from S_2 and S_3. Disparity between the mean count of the samples belonging to different societies was also reported by Jolly *et al.* (2000) and Nanu *et al.* (2007). The reason for such disparity might be attributed to the difference in sanitary practices followed in these societies. The organism was detected in 93.52 per cent of the samples and was much higher than that observed by Jolly *et al.* (2000), who reported the presence of the organism in 80 per cent samples, whereas Chacko (2006) recorded that the organism was present in cent per cent of samples.

Faecal streptococci are present in large numbers in faeces, sewage and also in dust. The presence of organism at high levels in the samples of present study indicates contamination of milk either with faecal material or contaminated environment. Some of the organism like *Enterococcus faecalis* and *Enterococcus faecium* are able to grow at temperatures between 0 to 6 °C and thus the presence of the organism can cause certain defects in milk even under refrigerated storage.

5.1.1.5 *Yeast and Mould Count*

Yeast and moulds are widely distributed in the environment and its presence in excess in food articles indicates unsanitary conditions of handling and contamination from air. Some of these organisms produce toxins, which can resists heat treatment, and thus become public health hazard to the consumers.

The overall mean yeast and mould count of samples was 2.09 ± 0.12 $\log_{10}$ cfu/ml (Table 10), which was in agreement with the reports of Nanu *et al.* (2007), who observed a count of 2.77 ± 0.05 $\log_{10}$ cfu/ml in the individual milk samples of the source FS_3. The count was much lower (2.3 x 10^6 cfu ml^{-1}) than the mean yeast count reported by Lues *et al.* (2003) and the count, 3.75 ± 0.06 $\log_{10}$ cfu/ml, reported by Chacko (2006), whereas the count of the present study was much higher than that reported by Prejit (2005) in the samples collected from dairy plant (1.58 ± 0.27 $\log_{10}$ cfu/ml). The organism was not detected in 24.07 per cent samples and was present in 75.93 per cent samples at and above the level of 10^2 cfu/ml, whereas Mutukumira *et al.* (1996) reported the count above 100 cfu/ml in 30.00 per cent samples. Count at the level of 10^3 cfu/ml was present in 19.44 per cent samples, which was much lower than that observed by Chacko (2006), who reported 45.37 percent of samples with that count. Fungal count in excess degrades the sensory quality of milk, which indicates the unhygienic handling of the utensils and unsatisfactory environmental conditions.

5.1.1.6 *Correlation between microbial counts of individual milk samples of S_1, S_2 and S_3*

A significant ($P<0.05$) and positive correlation was observed between the total viable count and coliform count (Table 12), which was in agreement with the reports of Vijai and Saraswat (1968), Patel *et al.* (1993) and Siva *et al.* (1993). The association between the total viable count and faecal streptococcal count was also revealed highly significant ($P<0.01$) significance. Similar association was also reported by Jolly *et al.* (2000) and Chacko (2006). A significant ($P<0.05$) correlation was observed between coliform count and Faecal Streptococcal Count, which were similar to the association reported by Chacko (2006).

5.1.2 Microbial count of individual milk samples from S_1

5.1.2.1 *Total Viable Count*

The overall mean total viable count of the samples obtained from the farmers of S_1 was 5.99 ± 0.09 $\log_{10}$ cfu/ml (Table 13) and the count was almost in conformity with the count, 5.09 ± 0.24 $\log_{10}$ cfu/ml, observed by Raj *et al.* (2003) in the samples from the source B and also with the overall mean count, 5.14 ± 0.13 $\log_{10}$ cfu/ml, observed by Prejit (2005) in the individual milk samples collected from dairy farm. The mean count at the level of six $\log_{10}$ cfu/ml was present in the samples of F_1, F_3 and F_6 and almost identical counts was reported by Chacko (2006) in the samples collected from the three farmers of S_1. The mean count at the level of 10^5 cfu/ml was present in samples collected from F_2, F_4 and F_5 and almost similar counts was observed in the individual samples belonging to S_2 and S_3 examined by Kapre (1995) and the count, 5.14 ± 0.13 $\log_{10}$ cfu/ml, reported by Prejit (2005). The count at the level of 10^6 and 10^5 cfu/ml was present in 58.33 and 38.89 per cent samples, respectively. Only 2.78 per cent samples had count at the level of 10^4 cfu/ml. The higher the count in raw milk, the greater will be the number of microbes that can survive heat treatment and thus impose health hazards to consumers and earlier spoilage of the product.

5.1.2.2 *Coliform Count*

The samples of S_1 had an overall mean count of 4.41 ± 0.16 $\log_{10}$ cfu/ml (Table 15) and the count was almost similar to the mean count, 4.74 ± 0.54 $\log_{10}$ cfu/ml in the pooled samples from the source A, observed by Jolly *et al.* (2000) and the overall mean count, 17.0 x 10^4 cfu/ml, reported by Chye *et al.* (2004), whereas the count was about one log greater than that recorded by Chacko (2006) and was about three log lower than that reported by Lues *et al.* (2003). Coliforms were detected in all the samples collected from the samples of S_1. Except the samples from F_2, the samples from other five farmers had count at the level of four $\log_{10}$ cfu/ml, which was in agreement with the reports of Reddy *et al.* (1984), who recorded a count of 2.8 x 10^4 cfu/ml and with the counts observed in the samples obtained from F_4 and F_5 belonging to S_1 (Chacko, 2006). Samples from F_2 of S_1 in

the present study had the count of 3.52 ± 0.17 log_{10} cfu/ml and was similar to the count 3.96 x 10^3 cfu/ml reported by Misra and Kuila (1989). The higher counts of the organism in the present study indicate the unhygienic practices prevailing in that area during the production and handling of milk.

5.1.2.3 *Escherichia coli Count*

The mean *Escherichia coli* Count of the samples from S_1 was 0.78 ± 0.19 log_{10} cfu/ml (Table 17) and was almost similar to that of the count, 0.63 ± 0.31 log_{10} cfu/ml observed by Prejit (2005) and the count, 0.90 ± 0.17 log_{10} cfu/ml reported by Chacko (2006) in the samples collected from farmers of S_1. The count of the present study was much lower than that reported by different workers like Gran *et al.* (2003), Lues *et al.* (2003) and Chye *et al.* (2004). The samples collected from F_5 had the highest mean count of 1.50 ± 0.49 log_{10} cfu/ml but the samples of F_6 did not reveal the presence of the organism. Such difference in the counts of the samples from the farmers belonging same society was also reported by Chacko (2006). Of the samples, 30.56 per cent had the count at the level of 10^2 cfu/ml and 2.78 percent sample showed the count at the level of 10^3 cfu/ml. The variation in the counts of the organism in the samples obtained from the farmers indicates the difference in the hygienic and sanitary practices followed during milking.

5.1.2.4 *Faecal Streptococcal Count*

The difference between the mean count of samples collected from six farmers belonging to S_1 was highly significant ($P<0.01$) (Table19). The mean faecal streptococcal count of raw milk samples belonging to S_1 was 2.86 ± 0.20 log_{10} cfu/ml and the count was in agreement with the reports of Prejit (2005), who recorded a count of 2.59 ± 0.11 log_{10} cfu/ml in the samples collected at various stages of pasteurization and the counts recorded by Nanu *et al.* (2007) in the samples collected from FS_2 and FS_3. The count in the samples of the current study was about one log lower than the count reported by Chacko (2006) in the samples of S_1, S_2 and S_3 and that reported by Kapre (1995) in the individual milk samples collected from S_2 and S_3, whereas the count was about one log higher than that

observed by Jolly *et al.* (2000) in the individual milk samples collected from the source B.

The organism was detected in 88.89 per cent samples of the present study, whereas Jolly *et al.* (2000) reported its presence only in 80 per cent of the individual milk samples. The lowest count was observed in samples belonging to F_5 (1.80 ± 0.59 $\log_{10}$ cfu/ml), which was about one log lower than that of the overall mean count, 2.59 ± 0.11 $\log_{10}$ cfu/ml (Prejit, 2005), whereas the samples from F_6 had the highest (3.97 ± 0.09 $\log_{10}$ cfu/ml) faecal streptococcal count among the samples of S_1. Such difference between the samples collected from the farmers within a society was also recorded by Chacko (2006).

5.1.2.5 *Yeast and Mould Count*

The mean count of the sample was 2.06 ± 0.21 $\log_{10}$ cfu/ml (Table 21) and was almost similar to the counts reported by Prejit (2005) in the pooled milk (1.86 ± 0.19 $\log_{10}$ cfu/ml) and chilled milk (1.84 ± 0.24 $\log_{10}$ cfu/ml) samples. But the count of the present study was about two log lower than the overall mean count observed by Chacko (2006) in the individual milk samples and about one log lower than the count reported by Nanu *et al.* (2007) in the samples belonging to FS_1 and FS_2. The samples belonging to F_3 had the highest mean count (2.74 ± 0.25 $\log_{10}$ cfu/ml) and the lowest mean count (1.75 ± 0.56 $\log_{10}$ cfu/ml) was observed in the samples of F_5. The organism was detected in only 75.00 per cent samples of the present study. Such difference in the mean count between the samples collected from farmer belonging to the same society was also reported by Chacko (2006) and reported that cent per cent of the individual samples had revealed the presence of the organism.

5.1.3 Microbial count of individual milk samples from S_2

5.1.3.1 *Total Viable Count*

The mean total viable count of the samples collected from six farmers of S_2 showed highly significant ($P<0.01$) variation (Table 23) and the overall mean count

was 6.37 ± 0.13 log_{10} cfu/ml, which was in agreement with the reports of Jolly *et al.* (2000), who reported the count of 6.08 ± 0.02 log_{10} cfu/ml, Olivera *et al* (2000), who recorded the count of 8.1 x 10^6 cfu/ml and the count, 6.14 ± 0.09 log_{10} cfu/ml, observed by Chacko *et al.* (2006). The mean count of F_2 was at the level of 10^7 cfu/ml, which was similar to the count recorded by Aaku *et al.* (2004) from the source A. The samples of F_1, F_3 and F_6 in the present study had the mean count at the level of six log_{10} cfu/ml and these counts almost coincides with the counts reported by Chye *et al.* (2004) in the samples collected from four different regions of Malaysia. The counts of F_4 and F_5 were at the level of five log_{10} cfu/ml. Count at the level of 10^7, 10^6, 10^5 and 10^4 cfu/ml was present in 25.00, 47.22, 25.00 and 2.78 per cent samples, respectively. The disparity in the mean counts of the samples collected from the same locality clearly indicates difference in the hygienic practices between farmers during milk production.

5.1.3.2 *Coliform Count*

Mean coliform count of the samples from S_2 was 4.63 ± 0.11 log_{10} cfu/ml (Table 25) and the count was almost similar to the count, 80 x 10^3 cfu/ml reported by Desai and Natarajan (1981) in the samples from the source B and the count reported by Kapre (1995), who recorded a count of 4.8 x 10^4 cfu/ml in the samples collected from the society S_2. The count was also similar to the overall mean count observed by Chye *et al.* (2004), whereas other investigators (Chacko, 2006 and Nanu *et al.*, 2007) reported lower counts than that of the present study. Samples from F_2 and F_3 had count at level of five log_{10} cfu/ml and that of F_5 was 3.89 ± 0.18 log_{10} cfu/ml. Samples from other three farmers had coliform at the level of four log. Chacko (2006) was also reported such variation in the coliform count of the samples collected from six different farmers belonging to same locality similar to that recorded in the present study. From the observations it may be concluded that the hygienic practices followed by the farmer F_5 was much better than that of F_2 and F_3. Counts similar to the samples from F_2 and F_3 were also reported by Yadava *et al.* (1983) and Jolly *et al.* (2000) from individual milk samples.

5.1.3.3 *Escherichia coli Count*

The mean count of 1.25 ± 0.22 $\log_{10}$ cfu/ml was observed in the samples of S_2 and the analysis of variance test of the data revealed highly significant ($P<0.01$) difference between mean counts of the samples from the six farmers (Table 27). The mean count was almost in accordance with the count, 1.01 ± 0.21 $\log_{10}$ cfu/ml, reported by Prejit (2005) in individual samples of the dairy farm and the count, 1.11 ± 0.19 $\log_{10}$ cfu/ml, observed by Chacko (2006) in the samples of S_2, whereas the count was much lower than the counts reported by Gran *et al.* (2003) and Chye *et al.* (2004). Organism was present in 50.00 per cent samples of S_2. The samples of F_2 did not reveal the presence of the organism, whereas the samples of F_6 had count at the level of 2.23 ± 0.49 $\log_{10}$ cfu/ml. Such disparity in the counts of samples obtained from the farmers of a society were also reported by Chacko (2006), who reported the count as high as 2.06 ± 0.41 $\log_{10}$ cfu/ml in the samples of F_4 and zero in the samples of F_3 belonging to S_1.

5.1.3.4 *Faecal Streptococcal Count*

The difference between the mean count of samples collected from six farmers belonging to S_2 was highly significant ($P<0.01$) (Table 29). The overall mean count of the samples collected from six farmers was 3.66 ± 0.10 $\log_{10}$ cfu/ml. The counts reported by Kapre (1995) in the individual milk samples of S_2 and S_3 and the overall mean count (3.62 ± 0.05 $\log_{10}$ cfu/ml) reported by Chacko (2006) were agreeing with the mean count of the samples S_2. The mean count observed in the samples of S_2 of the present study was much higher than that of the count observed by Raj *et al.* (2003), Prejit (2005) and Nanu *et al.* (2007). The samples from F_3 had highest count of 4.10 ± 0.18 $\log_{10}$ cfu/ml and the samples from other five farmers lies within three log values. Count at the level of 10^4 cfu/ml was present in 33.33 per cent samples, which was higher than that reported by Chacko (2006), who recorded the count at the level of 10^4 cfu/ml in 27.78 per cent samples from S_1. These higher count of the organism indicate the unsanitary conditions prevailing in that particular area.

5.1.3.5 *Yeast and Mould Count*

The overall mean yeast and mould count of the samples collected from S_2 was 2.00 ± 0.20 $\log_{10}$ cfu/ml (Table 31) which was in agreement with the counts reported by Prejit (2005) in the pooled milk (1.86 ± 0.19 $\log_{10}$ cfu/ml) and chilled milk (1.84 ± 0.24 $\log_{10}$ cfu/ml) samples. The count of the present study was about one log lower than the count reported by Nanu *et al.* (2007) in the samples collected from FS_1 and FS_2. Organism was not detected in 25.00 per cent samples of the present study, while Chacko (2006) reported count in all the samples of S_1, S_2 and S_3. Seventy five per cent samples of the present study had the organism above level of 10^2 cfu/ml, whereas Mutukumira *et al.* (1996) reported count more than 100 cfu/ml in 30 per cent samples.

5.1.3.6 Correlation between microbial counts of individual milk samples of S_2

A significant ($P<0.05$) and positive correlation was observed between the total viable count and coliform count (Table 33), which was in agreement with the reports of Vijai and Saraswat (1968), Patel *et al.* (1993) and Siva *et al.* (1993). Significant correlation was also observed between total viable count and faecal streptococcal count and between coliform count with faecal streptococcal count and yeast and mould count.

5.1.4 Microbial count of individual milk samples from S_3

5.1.4.1 *Total Viable Count*

Highly significant ($P<0.01$) variation was observed between the mean total viable counts of the samples collected from six farmers of S_3 (Table 34). The overall mean count was 5.67 ± 0.13 $\log_{10}$ cfu/ml, which corroborate with the count, 5.14 ± 0.13 $\log_{10}$ cfu/ml, reported in the individual samples by Prejit (2005) and the count, 5.59 ± 0.16 $\log_{10}$ cfu/ml observed in samples of S_3 by Chacko (2006), whereas the count was much lower than the counts, 7.30 $\log_{10}$ cfu/ml (Singh *et al.*, 1994b) and 8.6 x 10^8 $\log_{10}$ cfu/ml (Lues *et al.*, 2003). In the samples of S_3, 22.22, 52.78, 16.67 and 8.33 per cent samples had count at the level 10^4, 10^5, 10^6 and 10^7 cfu/ml, respectively. The highest mean total viable count (6.66 ± 0.38

$\log_{10}$ cfu/ml) was observed in samples from F_6, which was about one log greater than the overall mean count of the samples from S_3 and the samples from F_2 had the lowest mean count (4.85 ± 0.18 $\log_{10}$ cfu/ml). Such variation in the mean counts of the samples collected from an area was also reported by Homhual and Jindal (2001), who recorded that the count was ranged between 6.5 x 10^4 to 1.2 x 10^8 cfu/ml. The difference in the mean count of samples within societies was also reported by Kapre (1995), Chacko (2006) and Jaibi (2006).

5.1.4.2 *Coliform Count*

Mean coliform count of the samples from S_3 was 4.31 ± 0.10 $\log_{10}$ cfu/ml (Table 36), which was in agreement with the count, 17 x10^4 cfu/ml, observed by Chye *et al.* (2004). The count of the present study was about one log greater than that reported by Chacko (2006), whereas Nanu *et al.* (2007) reported two log lower count in the samples of FS_1 than that of the present study. Samples belonging to F_1 and F_2 had count at the level of 10^3 cfu/ml and the samples from other four farmers had count at the level of four log. These counts were much greater than that recorded by Prejit (2005), who reported the count of 1.83 ± 0.23 $\log_{10}$ cfu/ml in individual milk samples collected from dairy farm. Count at the level of 10^4 and 10^5 cfu/ml was present in 55.56 and 11.11 per cent samples. The samples from S_1 and S_2 of the present study also had almost similar per cent of samples within these counts.

5.1.4.3 *Escherichia coli Count*

The samples collected from S_3 had the mean count of 0.54 ± 0.16 $\log_{10}$ cfu/ml (Table 38) and this count was in agreement with the count (0.63 ± 0.31 $\log_{10}$ cfu/ml) reported by Prejit (2005) in pooled milk samples and the count observed by Chacko (2006) in the individual milk samples of S_3 (0.57 ± 0.15 $\log_{10}$ cfu/ml). The count in the present study was about three and four log lower than the mean count reported by Jolly *et al.* (2000) in the individual and pooled samples collected from three sources, respectively. Organism was present only in 25 per cent samples belonging to S_3, and was much lower than the 33.34 and 50.00 per cent isolation in

the samples collected from S_1 and S_2 of the present study. This indicates that the farmers of S_3 are more aware of the importance of hygienic practices need to follow in the production of quality milk than the other two societies.

5.1.4.4 *Faecal Streptococcal Count*

The overall mean count of the samples collected from the society was 2.92 ± 0.17 $\log_{10}$ cfu/ml (Table 40). The count was almost similar to that recorded by Nanu *et al.* (2007), who observed a count of 2.80 ± 0.28 $\log_{10}$ cfu/ml in the individual milk samples of FS_3, while the count was much greater than the count reported by Yadava *et al.* (1983) in the samples collected during monsoon and winter. But the count of the present study was about one log lower than the counts recorded by Kapre (1995) in the samples belonging to S_2 and S_3. Samples from F_4 had the highest mean count (3.66 ± 0.14 $\log_{10}$ cfu/ml), while lowest mean count (2.13 ± 0.68 $\log_{10}$ cfu/ml) was observed in the samples of F_3. Similar to the samples of S_2, S_3 were also not revealed the presence of the organism in 8.33 per cent samples, whereas Jaibi (2006) recorded that cent per cent of the samples examined had the organism.

5.1.4.5 *Yeast and Mould Count*

The mean count of the samples collected from the farmers of S_3 was 2.21 ± 0.20 $\log_{10}$ cfu/ml (Table 42), which was in accordance with the count, 2.77 ± 0.05 $\log_{10}$ cfu/ml, observed by Nanu *et al.* (2007) in the samples of FS_3. The count in the present study was about two log higher than the count in the samples of S_1 reported by Chacko (2006), whereas it was about one log lower than that reported by Prejit (2005) in the individual milk samples. Highest mean count, 2.77 ± 0.11 $\log_{10}$ cfu/ml was observed in the samples belonging to F_3 and was similar to the count in the samples belonging to FS_3 (Nanu *et al.*, 2007). The samples of F_1 had the lowest mean count (1.41± 0.64 $\log_{10}$ cfu/ml) and that agrees with the count of 1.58 ± 0.27 $\log_{10}$ cfu/ml reported by Prejit (2005).

5.1.4.6 Correlation between microbial counts of individual milk samples of S_3

A significant ($P<0.05$) and positive correlation was observed between the total viable count and coliform count (Table 44), which was in agreement with the reports of Vijai and Saraswat (1968), Patel *et al.* (1993) and Siva *et al.* (1993).

5.1.6 Microbial count of pooled raw milk samples from S_1, S_2 and S_3

5.1.6.1 Total *Viable Count*

Analysis of variance test of the data revealed highly significant ($P<0.01$) difference between mean counts of the pooled samples from the three societies (Table 45). The overall mean count of the samples was 6.19 ± 0.09 $\log_{10}$ cfu/ml and the count was in agreement with the count, 6.30 ± 0.20 $\log_{10}$ cfu/ml, in the pooled milk samples (Jolly *et al.*, 2000) and the overall mean count of 6.52 ± 0.08 $\log_{10}$ cfu/ml (Jaibi, 2006). But the count of the present study was greater than the count observed in the pooled samples of S_1 and S_3 (Kapre, 1995) and that reported by Raj *et al.* (2003) in samples from the source A. The count in the present study was much lower than the count, 8.6×10^8 cfu/ml, reported by Lues *et al.* (2003) and the count, 3×10^7 cfu /ml, in the pooled samples belonging to the source A (Aaku *et al.*, 2004). The samples of S_2 had the highest mean count (6.49 ± 0.11 $\log_{10}$ cfu/ml), while the lowest count was in the samples of S_3 (5.73 ± 0.08 $\log_{10}$ cfu/ml). Such difference of about one log in the mean count of samples belonging different societies was also reported by Jaibi (2006). The difference in the counts might be attributed to the low hygienic status at the production site and improper handling during the transportation of milk to societies. During the investigation it was observed that farmers used to keep milk at room temperature even though there is enough time lag between milking and transportation to milk collection centers. This helps the mesophilic micro flora of milk to get multiplied in large numbers.

In the present study, the count at high levels of 10^8, 10^7 and 10^6 cfu/ml were present in 1.39, 8.33 and 50.00 per cent samples (Table 46). Count at the level of 10^5 cfu/ml was observed in 34.72 per cent of the samples, whereas only 5.56 per cent of the samples had count at the level of 10^4 cfu/ml. The count at the level of 10^7 and 10^8 cfu/ml were recorded only in the samples of S_1, while the samples of S_2

and S_3 had count belonging to the levels of 10^6, 10^5 and 10^4 cfu/ml. This indicated that the bacterial quality of milk samples collected from S_1 was much lower than that of S_2 and S_3. This difference might be attributed to the difference in hygienic practices followed by the farmers and milk handlers of the societies.

5.1.6.2 *Coliform Count*

The overall mean count of pooled milk samples was 4.65 ± 0.09 $\log_{10}$ cfu/ml (Table 47) and the count was in agreement with the reports of Kapre (1995), who recorded the count, 4.8 x 10^4 cfu/ml, in the individual samples of the source S_2 and the count, 4.74 ± 0.54 $\log_{10}$ cfu/ml, reported by Jolly *et al.* (2000) in the pooled samples of the source A. The count of the present study was about one log lower than the count, 2.0 x 10^5 cfu/ml, in the pooled samples of S_2 (Kapre, 1995) and the overall mean count of 5.31 ± 0.09 $\log_{10}$ cfu/ml, reported by Jolly *et al.* (2000). But the count was about one log higher than the count, 3.47 ± 0.07 $\log_{10}$ cfu/ml, reported by Jaibi (2006) in the pooled milk samples. The organism was detected in 98.16 per cent samples of the present study and was almost similar to the reports of Jolly *et al.* (2000), who observed that the organism was present in 96.67 per cent of the pooled milk samples.

The count at the level of 10^5 cfu/ml was highest in the samples of S_1 (54.17 per cent) followed by S_2 (21.17 per cent) and S_3 (20.83 per cent). Unhygienic milking procedures and practices of milk handler, use of unclean utensils and water together with storage of milk at elevated temperature helps the entry and subsequent multiplication of organisms resulting in higher bacterial counts. As the number of spoilage organisms like coliforms increases, the quality of the milk decreases leading to earlier spoilage and shorter shelf life. Some of the coliforms can grow at and below refrigeration temperature and thus leads to spoilage even under refrigerated storage.

5.1.6.3 *Escherichia coli Count*

The overall mean *Escherichia coli* count of the samples was 1.27 ± 0.16 $\log_{10}$ cfu/ml (Table 49) and was in accordance with the overall mean count (1.52 ±

0.27 $\log_{10}$ cfu/ml) of pooled samples reported by Jaibi (2006). The count in the present study was lower than the counts reported by Kapre (1995) and Jolly *et al.* (2000) in the pooled milk samples, whereas the count was greater than the count (0.63 ± 0.31 $\log_{10}$ cfu/ml) reported by Prejit (2005) in the pooled samples of the dairy farm. The count at the level of 10^2 and 10^3 cfu/ml was observed in 40.28 and 9.72 per cent samples, respectively. The count in 12.50 per cent samples each from S_1 and S_2 was at the level of 10^3 cfu/ml.

Escherichia coli was detected in 50.00 per cent of the pooled milk samples (Table 50) of the three societies and was similar to the reports of Jaibi (2006), who recorded that the organism was present in 47.22 per cent of the pooled samples, whereas the per cent of isolation of the organism in the present study was much lower than that reported by Jolly *et al.* (2000), who recorded the presence of organism in 96.67 per cent of the pooled samples. The presence of this organism in about 50 per cent samples indicates the unhygienic practices and possible faecal contamination in the production and retailing of milk.

5.1.6.4 *Faecal Streptococcal Count*

The faecal streptococcal counts in samples of the three societies showed highly significant ($P<0.01$) difference (Table 51). The overall mean faecal streptococcal count of samples was 3.50 ± 0.06 $\log_{10}$ cfu/ml and the count was almost similar to that, 6.3 x 10^3 cfu/ml, observed in the samples of S_2 (Kapre, 1995) and the overall mean count, (3.37 ± 0.07 $\log_{10}$ cfu/ml), in pooled milk samples (Jaibi, 2006). But the count observed in the present study was higher than the count (2.49 ± 0.04 $\log_{10}$ cfu/ml) in the pooled samples from societies (Jolly *et al.* 2000) and the count, 1.30 ± 0.45 $\log_{10}$ cfu/ml in the samples of society B (Raj *et al.*, 2003). The samples of S_2 and S_3 had almost same count, whereas the count in the samples from S_1 was lower than other two societies.

The organism was present in all the pooled milk samples of the present study, similar to that reported by Jaibi (2006). But Jolly *et al.* (2000) detected the organism only in 83.33 per cent pooled samples. The presence of faecal

streptococci in milk samples indicates contamination of milk with faecal mater of either man or animals directly and indirectly and/or contaminated water. It also gives indication about the unhygienic practices followed by the farmers and workers of the cooperative societies.

5.1.6.5 *Yeast and Mould Count*

The overall mean yeast and mould count of the samples was 2.37 ± 0.11 $\log_{10}$ cfu/ml (Table 53) and was much lower than the count, 2.3 x 10^6 cfu/ml, in the samples from household (Lues *et al.*, 2003) and the count 3.85 ± 0.09 $\log_{10}$ cfu/ml, in the pooled milk samples (Jaibi, 2006). But the count in the present study was much higher than the count, 1.86 ± 0.19 $\log_{10}$ cfu/ml, in the pooled milk samples from farm (Prejit, 2005). In the samples of the present study, 87.5 per cent samples had count greater than 100 cfu/ml, which was much greater than that reported by Mutukumira *et al.* (1996), who recorded the count above 100 cfu/ml only in 30.00 per cent of the samples.

5.1.7 Correlation between microbial counts of pooled milk samples of S_1, S_2 and S_3

Significant ($P<0.05$) correlation was observed between the mean total viable count and coliform count in the pooled samples of the present study (Table 55). Such association was also reported by Vijai and Saraswat (1968), Siva *et al.* (1993), Patel *et al.* (1993) and Jaibi (2006).

5.2 ISOLATION AND IDENTIFICATION OF BACTERIA

Milk provides nutrients for the support and growth of microorganisms and may serve as a potential source of milk borne infection and intoxication to the consumers. Some of the pathogens having major public health significance like *Escherichia coli, Staphylococcus aureus* and *Yersinia* revealed their presence in the milk samples of present study.

5.2.1 Isolation and identification of bacteria from individual milk samples

5.2.1.1 *Escherichia coli*

A total of 39 *Escherichia coli* was isolated from 108 individual samples (36.11 per cent) (Table 56), which was almost in agreement with the 41.67 per cent isolates reported by Chacko (2006). On serotyping, 15 isolates were fell into 10 different serotypes (O12, O29, O68, O75, O79, O107, O116, O131, O160 and O172) and 12 isolates each were belonged to untypable and rough strains (Table 57). The serotypes O116 and O172 belonged to the enterohaemorrhagic *Escherichia coli* (EHEC) group. They produce one or two Shiga like toxins. Infection with enterohaemorrhagic group of *Escherichia coli* in man causes diarrhoea, haemorrhagic colitis and hemolytic uraemic syndrome (HUS). Chacko (2006) and Nanu *et al.* (2007) also reported the isolation of O116 and O172 serotypes from raw milk. Serotype O29 is enteroinvasive *Escherichia coli* (EIEC). They can invade and multiply in the intestinal epithelial cells especially colon, resulting in cell death and leads to nonbloody diarrhoea and dysentery. The isolate O75 belongs to the group of diffusely adhering *Escherichia coli* (DAEC). Members of this group have been associated with diarrhoea mainly in young children. The serotype O68 is belonging to enteroaggregative *Escherichia coli* (EAEC) and they are associated with persistent diarrhoea in infants and childrens and Nanu *et al.* (2007) also reported the isolation of serotype O68 from raw milk. Some of the O79 strains can able to produce heat stable toxins.

The per cent of isolation of the organism in the present study was much lower than that of the 76.19 per cent isolates reported by Kapre (1995) and 72.2 per cent isolates observed by Chye *et al.* (2004), whereas Raj *et al.* (2003) isolated *Escherichia coli* only in 12.5 per cent of the samples, which was much lower than that of the present study.

Of the 15 serotyped *Escherichia coli* 9 isolates had Congo red binding property, which indicated the pathogenic property of the isolates. The characteristics of Congo red binding constitutes a moderately stable, reproducible and easily distinguishable phenotypic marker (Rajil *et al.*, 2003). A good

correlation between pathogenic potential and Congo red binding property of the organism was also reported by Abhilasha *et al.* (2001).

Escherichia coli is a commensal organism found in the intestine of humans and animals and is associated with various disease conditions in human beings and animals. The presence of the organisms in milk indicates either the contamination with faecal matter, contaminated water or the contamination from the milk handlers.

5.2.1.2 *Staphylococcus aureus*

Staphylococcus aureus was isolated from 40.74 per cent of 108 individual milk samples examined (Table 56). The isolates obtained in the study was almost similar to the reports of Jolly *et al.* (2000), who recorded that the isolation of organism in 36.67 per cent samples and also to the per cent of isolation of the organism in 37.5 per cent samples (Raj *et al.*, 2003). But the per cent of isolation of the organism in the present study was lower than that of the isolation reported by Chye *et al.* (2004) and Prejit (2005). However, the per cent of isolation of the organism in the present study was greater than that reported by Shah *et al.* (1984), Yadava *et al.* (1985), Sen *et al.* (1989) and Chako (2006), whereas Aaku *et al.* (2004) reported that none of the 43 samples examined had *Staphylococcus*.

Staphylococcus aureus is one of the bacterial agents associated with food poisoning, which produce unique thermostable enterotoxins and are responsible for food poisoning outbreaks. Carmo *et al.*, (2002) reported the possible association of *Staphylococcus aureus* in food poisoning with the consumption of raw milk. Since humans are natural carriers of the organism, milk handlers and poor personal hygiene of the farmers attribute the cause of its presence in milk. Infected udder also contribute considerable amount of the organism to milk. So attention must be paid to the sanitation and personnel hygiene to minimize the contamination of the milk with the organism.

5.2.1.3 *Yersinia.*

Yersinia enterocolitica and *Yersinia pseudotuberculosis* are emerging foodborne pathogen of public health significance and is found in the intestinal tract of animals, which contaminates milk through faeces, urine, insects and some times leads to food poisoning outbreaks.

The individual milk samples from the three societies were tested and *Yersinia* was isolated from 22.22 per cent samples (Table 59), while Nanu *et al.* (2007) reported isolation of the organism from 49 per cent of the samples, which was higher than the reports of the present study. Among the samples, *Yersinia enterocolitica* was isolated from 4.63 per cent samples, whereas *Yersinia pseudotuberculosis* was isolated from 0.93 per cent samples. The isolation of *Yersinia enterocolitica* from the individual milk samples of the present study was almost similar to that of 6.1 per cent reported by Jayarao and Henning, 2001, whereas Schiemann and Toma (1978) reported the isolation of the organism from 14.3 per cent of the milk samples. The samples analyzed by Gran *et al.* (2003) and Chacko (2006) did not revealed the presence of the organism.

Other isolates of *Yersinia* in the samples of the present study fell into species *viz., Yersinia frederiksenii, Yersinia kristensenii, Yersinia intermedia* and *Yersinia aldovae.* Nihal and Huriye (2006) reported the isolation of *Yersinia frederiksenii, Yersinia kristensenii, Yersinia intermedia* and atypical *Yersinia* spp. in 31.0, 12.7, 7.2 and 1.8 per cent from the milk samples, respectively. *Yersinia enterocolitica* can grow and multiply at temperature as low as 4°C and readily withstand freezing, thus pose potential risk as a foodborne pathogen to the consumers.

5.2.2 Isolation and identification of bacteria from pooled raw milk samples

5.2.2.1 *Escherichia coli*

Pooled milk samples of the three societies yielded 36 (50.00 per cent) isolates from 72 samples (Table 60). The observation of the study was comparable with the reports of Jaibi (2006) who isolated the organism from 47.22 per cent of

raw milk samples, whereas lower per cent of isolates than that of the present study were reported by Kapoor *et al.* (2002), Aaku *et al.* (2004) and Prejit (2005). The per cent of samples which yielded the organism in the study was much less than that reported by Kapre (1995), who reported that all samples examined had *Escherichia coli,* while the organism was isolated from 78.09 and 96.67 per cent samples tested by Yadava *et al.* (1985) and Jolly *et al.* (2000), respectively.

The organism was present in 54.17 per cent samples belonging to S_3, followed by the samples of S_1 (50.00 per cent) and S_2 (45.83 per cent). The per cent of isolation of the organism from pooled samples was more than that of individual samples, which was in agreement with the observations of Jolly *et al.* (2000) and Jaibi (2006).

Of the 36 isolates, eighteen isolates were serotyped into five different groups and they are O116 (10), O68 (3), O75 (2), O60 (2) and O96 (1) (Table 61). Three of the isolates were untypable and fifteen were rough. The serotype O116 are Enterohaemorrhagic *Escherichia coli* (EHEC), which causes diarrhea, colitis and haemolytic uraermic syndrome in human being. The serotype was also isolated from raw milk by Chacko (2006), Jaibi (2006) and Nanu *et al.* (2007). The serotype O68 was belonging to enteroaggregative *Escherichia coli* (EAEC) and they are associated with persistent diarrhoea in infants and childrens. Nanu *et al.* (2007) also reported the isolation of serotype O68 from raw milk. The isolates belonging to serotypes O116, O96, O68 and O75, revealed Congo red binding property that indicates the pathogenic characteristic of the organism.

5.2.2.2 *Staphylococcus aureus*

Pooled raw milk samples collected from three societies was tested for the presence of *Staphylococcus aureus* and the organism was isolated 34.72 per cent of 72 samples (Table 60). The per cent of isolation of the organism in the present study was greater than that reported by Yadava *et al.* (1985), Sen *et al.* (1989) and Jaibi (2006), whereas Chacko (2006) and Nanu *et al.* (2007) reported almost similar per cent isolation of the organism from the milk samples. The per cent of

isolation of the organism reported by Kapre (1995) and Jolly *et al.* (2000) from pooled milk samples was much more than that of the present study.

Of the 25 *Staphylococcus aureus* isolated, 45.83 per cent was isolated from the samples of S_2. The organism was isolated from 29.17 per cent samples each belonging to S_1 and S_3. The presence of this organism indicates poor hygienic practices and sanitary measures followed during the production and handling of milk. The disparity in the per cent of isolation of the organism indicates the difference in the hygienic practices followed during the milk production and further handling.

The per cent of isolation of the organism was more in individual milk samples compared to pooled milk samples. The observation of the current study did not corroborate with the observation of Jolly *et al.* (2000), who recorded the isolation of the organism from 50.00 per cent of the pooled milk samples and only 36.67 per cent of individual milk samples.

5.2.2.3 *Yersinia.*

The pooled raw milk samples from the three sources were analyzed for the presence of *Yersinia* and the organism was isolated from 29.17 per cent samples (Table 63). Of the samples, 1.39 per cent had *Yersinia enterocolitica* and the isolation of the organism in the present study was lower than that of the 31.1 per cent reported by Schiemann and Toma (1978) from pooled milk samples, 81.4 per cent (Vidon and Delmas, 1981) and 15.1 per cent (Rohrbach *et al.*, 1992), whereas Jaibi (2006) did not isolate the organisms from pooled milk samples. Other isolates of *Yersinia* in the present study were belonged to different species *viz.*, *Yersinia frederiksenii* (8.33 per cent), *Yersinia kristensenii* (4.17 per cent), *Yersinia aldovae* (4.17 per cent) and *Yersinia intermedia* (11.11 per cent). Nihal and Huriye (2006) isolated *Yersinia enterocolitica, Yersinia frederiksenii, Yersinia kristensenii, Yersinia intermedia* and atypical *Yersinia* spp. from 47.3, 31.0, 12.7, 7.2 and 1.8 per cent samples, respectively.

5.3 GRADING OF MILK BASED ON TOTAL VIABLE COUNT

Based on total viable count, the milk samples collected from the three societies were graded as very good, good, fair and poor following the criteria prescribed by Indian Standards (1977).

5.3.1 Individual samples from S_1, S_2 and S_3

Out of the 108 individual raw milk samples, 15.74 per cent were graded as very good (Table 64). The observation was much less than that reported as 80.95 per cent of the individual samples belonging to S_3 (Kapre, 1995) and 90.00 per cent of individual samples of the society A (Raj *et al.*, 2003). The per cent of very good samples obtained in the present study was much greater than that of the 2.33 per cent reported by Garg and Mandokhot (1997).

The per cent of good samples in the present study was 34.26, which was in agreement with the reports of Chacko (2006) and Nanu *et al.* (2007), whereas it was far less than that of the 60.00 and 50.00 per cent of the samples of the society A and C, respectively (Jolly *et al.*, 2000). The per cent of good samples observed in the current study was much higher than that of the 14.28 per cent recorded by Singh *et al.* (1994b), 4.76 per cent samples collected from S_1 (Kapre, 1995), 13.95 per cent reported by Garg and Mandokhot (1997) and 20.00 per cent in machine milked samples (Raj *et al.*, 2003).

In the present study, 32.41 per cent samples were graded as fair and was much less than that of the 40.00 per cent each of individual samples belonging to societies A and C (Jolly *et al.*, 2000). The observation of the current study was much lower than that of the 48.00 per cent of samples from sweet meat shops (Misra and Kuila, 1989) and 50.00 per cent individual samples belonging to society B (Jolly *et al.* 2000).

In the present study, 17.59 per cent individual samples were graded as poor while Kapre (1995) and Raj *et al.* (2003) reported that none of the individual samples collected from the societies was graded as poor. The observation in the

present study was much less than that of the 45.00 per cent of the samples of city vendors (Misra and Kuila, 1989) and 74.4 per cent of the raw milk samples (Garg and Mandokhot, 1997).

Among the samples of the societies, 33.33 per cent samples of S_3 was graded as very good and 11.11 per cent samples of S_2 was also belonging to that grade. In the samples of S_3, 41.67 per cent was graded as good and 36.11 and 25.00 per cent samples of S_1 and S_2 was also graded as good. In the samples of S_1, 52.78 per cent were fell in the grade fair, whereas only 16.67 per cent from S_3 was belonged to that grade. The per cent of poor samples was highest (36.11 per cent) in S_2 while only 8.33 per cent each of the samples of S_1 and S_3 belonged to that grade. Hence, it can be inferred that the hygienic practices followed in S_3 was much better than that of S_1 and S_2. The difference in the quality of individual milk samples obtained from the three sources indicated the difference in the hygienic and sanitary practices followed by the farmers and the societies. According to BIS (1977) almost 50.00 per cent samples of the present study did not meet the criteria for good samples.

5.3.2 Pooled milk samples from S_1, S_2 and S_3

Of the pooled samples, 9.72 per cent was graded as very good (Table 65), which was much lower than that observed by Kapre (1995) who reported that 42.84 and 57.14 per cent of pooled samples from S_2 and S_3, respectively, were graded as very good. However, contrary to the present study Jolly *et al.* (2000) and Jaibi (2006) reported that none of the pooled milk samples was graded as very good. During the investigation, 31.94 per cent samples was graded as good and was lower than that of the 40.00 per cent samples belonging to society A and C (Jolly *et al.*, 2000). The observation of the current study was much higher than that reported as 14.28 per cent (Singh *et al.* 1994b), 10.00 per cent of pooled samples from society B (Jolly *et al.* 2000) and 16.67 per cent (Jaibi, 2006).

Of the 72 pooled milk samples, 40.28 per cent was graded as fair. The finding of the present study corroborate with the observation of the samples

belonging to society C but was higher than that of the 50.00 per cent of samples each belonging to the societies A and B (Jolly *et al.* 2000) and 58.33 per cent of the pooled samples reported by Jaibi (2006).

In the present study, 18.06 per cent of the samples were graded as poor. However, Kapre (1995) reported that none of the pooled milk samples belonging to S_3 was graded as poor. The per cent of samples graded as poor in the present study was much lower than that of the 45.00 per cent of samples from vendors (Misra and Kuila, 1989), 74.4 per cent samples (Garg and Mandokhot, 1997) and 25.00 per cent samples (Jaibi, 2006) which indicated that the hygienic practices followed by the societies under the present study was satisfactory.

During the investigation, none of the pooled samples belonging to S_2 was graded as very good but 16.67 and 12.50 per cent samples from S_1 and S_3 were belonging to that grade. Compared to S_1 and S_2, a higher per cent of samples of S_3 (58.33 per cent) was graded as good. None of the samples from S_3 was graded as poor, whereas 33.33 per cent samples of S_1 and 20.83 per cent samples of S_2 was fell in to that grade. This indicated that the hygienic measures followed by the society S_3 was much better compared to the other two societies.

5.3.3 Individual milk samples from S_1

Out of the 36 samples belonging to S_1, 2.78 per cent was graded as very good (Table 66) and was much lower than that reported by Kapre (1995), Raj *et al.* (2003) and Chacko (2006). But the observation of the present study was almost similar to that of the 2.33 per cent recorded by Garg and Mandokhot (1997).

In the current study, 36.11 per cent samples was graded as good, but it was greater than 23.80 per cent reported by Kapre (1995) from individual samples belonging to S_2, whereas none of the pooled milk samples from S_1 and S_2 examined by Jaibi (2006) fell into the grade good.

The per cent of fair samples in the current study was 52.78 and was almost comparable with the 48 per cent of samples from sweet meat shops (Misra and Kuila, 1989) and 50.00 per cent of individual milk samples (Jaibi, 2006).

In the present study, only 8.33 per cent samples were graded as poor. The observation in the present study was much lower than that of the 37.73 per cent reported by Singh *et al.* (1994b) and 74.4 per cent reported by Garg and Mandokhot (1997), while Jolly *et al.* (2000) reported that none of the individual samples belonging to the society A was graded as poor. Of the samples belonging to F_2, 16.67 per cent were graded as very good, whereas 50.00 per cent of the samples each from F_4, F_5 and F_6 were graded as good. However, 66.67 per cent each of the samples obtained from F_1, F_2 and F_3 was graded as fair. From the results it may be inferred that there is not much variation between the samples from S_1.

5.3.4 Individual milk samples of S_2

Only 11.11 per cent samples from the society S_2 was graded as very good (Table 67). However, Chacko (2006) reported that 16.67 per cent of samples belonging to S_1 were graded as very good, whereas Jolly *et al.* (2000) reported that none of the individual samples from societies A and C fell into that grade. Out of the samples, 25.00 per cent were graded as good and was much lower than that of the 36.11 per cent reported by Chacko (2006), 80.95 per cent of samples of S_3 (Kapre, 1995) and 60.00 per cent in the samples of society A (Jolly *et al.*, 2000).

In the present study 27.78 per cent of the samples was graded as fair, which was almost in agreement with the 30.56 per cent reported by Chacko (2006) from the samples of S_1 but was much lower than that of the 40.00 per cent observed in the samples of society A (Jolly *et al.*, 2000). In the samples of the present study, 36.11 per cent was graded as poor and was much lower than that of 50.00 per cent reported by Chacko (2006). The observation of the present study was much higher than that of the 10.00 per cent of the individual samples belonging to society B (Jolly *et al.*, 2000). None of the samples from F_1, F_2 and F_6 yielded very good milk. The samples collected from F_5, 33.33 per cent was graded as very good. Among the

samples belonging to F_4 and F_5, 50 per cent each were graded as good. Of the samples of F_2, 83.33 per cent samples were graded as poor. The observations of the current study indicate that the hygienic practices followed by the farmers of the society during production of milk varied considerably.

5.3.5 Individual milk samples of S_3

In the samples of S_3, 33.33 per cent was graded as very good (Table 68), which was similar to that recorded from individual milk samples of S_3 by Jaibi (2006) and was higher than that of the 28.75 per cent reported by Singh *et al.* (1994b) and that recorded as 20.00 per cent in the individual samples of society B (Jolly *et al.*, 2000). The observation of the present study was much less than that of the 76.20 per cent of individual samples of S_2 (Kapre, 1995) and 90.00 per cent of the individual samples of society A (Raj *et al.*, 2003).

In the current study, 41.67 per cent samples was graded as good and was much greater than that of the 13.95 per cent reported by Garg and Mandokhot (1997) and 33.33 per cent observed from individual milk samples of S_3 by Jaibi (2006). The observation of the present study was much lower as compared to the 60.00 per cent observed in individual samples belonging to society A (Jolly *et al.*, 2000). Only 16.77 per cent of the samples were graded as fair, which was much lower than that of the 33.33 per cent observed in the samples of the organised dairy farm (Misra and Kuila, 1999). Among the samples of S_3, 8.33 per cent was graded as poor, which was much lower than that reported as 74.4 per cent poor samples (Garg and Mandokhot, 1997). However Raj *et al.* (2003) reported that none of the individual samples from the society fell in the grade poor.

During the investigation, 83.33 per cent of the samples belonging to F_2 and F_3 were graded as very good but none of the samples from F_4, F_5 and F_6 belonging to that grade. But 50.00 per cent samples each from the later three farmers fell in the grade good. None of the samples from F_1, F_2 and F_3 was graded as fair or poor. This indicates that the quality of milk produced by F_1, F_2 and F_3 was microbiologically much superior than that produced by the other farmers of S_3. The

difference in the microbial quality of milk samples belonging to the farmers of the society indicated the variation in hygienic practices and sanitary measures taken by the farmers during the production and distribution of milk.

5.4 ASSESSMENT OF CRITICAL CONTROL POINTS OF BACTERIAL CONTAMINATION OF MILK AT THE POINT OF PRODUCTION

Bacterial load of air, water, utensil rinsings, hand wash of the milker and udder wash of the animal were determined to identify the critical control points of bacterial contamination of milk during its production at the farmer's households.

5.4.1 Air

Microbiological load of milk has direct relationship with the quality of the environment in which it is produced. The mean total viable count of air samples was 149.83 ± 13.21 cfu/ft^2/min (Table 69) and was higher than that of the count, 127.83 ± 3.90 cfu/ft^2/min observed in the samples obtained from milking barn after milking (Prejit, 2005), whereas the count was lower (176.61 ± 16.25 cfu/ft^2/min) than that of the count of samples belonging to societies (Jaibi, 2006). The highest mean count (217.43 ± 21.41 cfu/ft^2/min) was seen in the samples belonging to F_2 of the S_1 and the lowest count (119.43 ± 15.22 cfu/ft^2/min) was observed in the samples of F_1 of the S_2. The counts in F_1 of S_1, F_2 of S_2 and F_1 and F_2 of S_3 were 143.67 ± 19.33, 163.34 ± 14.67, 121.87 ± 22.48 and 133.22 ± 15.21 cfu/ft^2/min, respectively. The finding of the study showed that the air samples of F_2 belonging to S_1 were highly contaminated and can play significant role in contaminating milk. Disinfection of the environment of the society can considerably reduce the bacterial load in air and thereby limit the bacterial contamination of milk.

5.4.2 Water

The overall mean total viable count of water samples was 1.65 ± 0.09 $\log_{10}$ cfu/ml and its overall mean coliform count was 0.97 ± 0.14 $\log_{10}$ cfu/ml (Table 70). The mean *Escherichia coli* count in the samples was 0.10 ± 0.13 $\log_{10}$ cfu/ml and the mean faecal streptococcal count was 0.91 ± 0.22 $\log_{10}$ cfu/ml. The highest mean

total viable count, coliform count and faecal streptococcal count were observed in the water samples collected from F_2 of S_1, whereas the highest *Escherichia coli* count was seen in the samples of F_1 of S_2. Well water is used for cleaning utensils, udder and hands of milker. Therefore, quality of water has important role in determining the quality of milk and any practices which decreases quality of water will indirectly affects quality of milk. The use of wholesome water and good hygienic practices can reduce the microbial load of milk and increase consumer safety and improve the keeping quality of milk.

5.4.3 Hand wash

Hand wash of the milker's had an overall mean total viable count, coliform count, *Escherichia coli* count and faecal streptococcal count of 3.38 ± 0.08, 2.03 ± 0.27, 0.24 ± 0.21 and 2.01 ± 0.18 $\log_{10}$ cfu/ml, respectively (Table 71). The highest mean total viable count and coliform count were observed in the samples collected from F_1 belonging to S_2, whereas highest *Escherichia coli* count and faecal streptococcal count was seen in the samples of F_2 of S_2. The high level of bacterial load in the samples of hand wash, particularly the presence of *Escherichia coli* and faecal streptococci indicate poor personal hygiene. Therefore, the farmers should be educated about the importance of personal hygiene in the production of good quality milk and the potential risk of pathogen that may enter into milk via poor personal hygiene and thus lead to food poisoning outbreaks.

5.4.4 Utensil wash

The samples had an overall mean total viable count, coliform count, *Escherichia coli* count and faecal streptococcal count of 2.91 ± 0.12, 1.14 ± 0.16, 0.33 ± 0.24 and 1.81 ± 0.35 $\log_{10}$ cfu/ml, respectively (Table 72). The samples collected from F_2 of S_1 had the highest mean total viable count, coliform count, *Escherichia coli* count and Faecal Streptococcal Count. As unhygienic utensils can contribute contamination to milk, proper cleaning of the utensils with suitable detergents and effective sanitizers is essential to reduce contamination and ensure safe milk to the consumers.

5.4.5 Udder Wash

The samples had an overall mean total viable count, coliform count, *Escherichia coli* count and faecal streptococcal count of 3.06 ± 0.13, 1.31 ± 0.20, 0.43 ± 0.24 and 1.50 ± 0.16 $\log_{10}$ cfu/ml, respectively (Table 73). The highest mean total viable count (3.31 ± 0.21 $\log_{10}$ cfu/ml) and faecal streptococcal count (2.23 ± 0.06 $\log_{10}$ cfu/ml) was observed in the samples of F_1 of S_1. The samples from F_1 of S_2 had the highest mean coliform count (1.68 ± 0.17 $\log_{10}$ cfu/ml), whereas the highest mean *Escherichia coli* count (0.54 ± 0.22 $\log_{10}$ cfu/ml) was observed in the samples of F_1 of S_3.

The level of *Escherichia coli* and faecal streptococci in the samples give an index of contamination of udder with faecal matter of the animals and thereby increase the chance of these organism gain entry into the milk and therefore, it is necessary to educate the farmers regarding the importance of cleaning the udder of animals before milking.

The assessment of various critical points of bacterial contamination of milk revealed that contamination of milk can occurred from hand of milker's, udder of the animal, utensil and water used during the production of milk in the descending order of preference. Similar observation was also reported by Chacko (2006) in the samples collected from the production site of milk.

5.5 ASSESSMENT OF CRITICAL CONTROL POINTS OF BACTERIAL CONTAMINATION OF MILK AT THE SOCIETY LEVEL

5.5.1 Air

The samples from S_1 had the highest mean count of 176.67 ± 23.16 cfu/ft^2/min (Table 74) and was greater than that of the count, 127.83 ± 3.90 cfu/ft^2/min, observed in the samples obtained from milking barn after milking (Prejit, 2005), whereas lower than that reported by Jaibi (2006) in the samples of S_2 (234.33 ± 22.03 cfu/ft^2/min). In the present study lowest count was observed in the samples of S_2 (112.34 ± 14.12 cfu/ft^2/min). The observation of the study showed

that the air samples of S_1 was highly contaminated and can play significant role in contamination of milk. Disinfection of the environment of the society can considerably reduce the bacterial load in air and thereby limit the bacterial contamination of milk.

5.5.2 Water

The mean total viable count of water samples was 1.82 ± 0.16 $\log_{10}$ cfu/ml (Table 75) and was lower than the count, 2.68 ± 0.04 $\log_{10}$ cfu/ml, in the samples obtained from societies (Jaibi, 2006). The mean coliform count in the water samples was 1.28 ± 0.08 $\log_{10}$ cfu/ml. The mean *Escherichia coli* count in the samples was 0.56 ± 0.06 $\log_{10}$ cfu/ml and the mean faecal streptococcal count was 0.98 ± 0.11 $\log_{10}$ cfu/ml. The samples collected from S_2 had the highest mean total viable count, coliform count, *Escherichia coli* count and Faecal Streptococcal Count. During the investigation it was observed that bare water is using for cleaning utensils and hands of milk handler in the societies. Therefore, the use of contaminated water increases the microbial load of milk. The use of wholesome water along with good hygienic practices will reduce the microbial load of milk.

5.5.3 Hand wash

Hand wash samples of milk handlers of the societies were evaluated to determine their contribution to the total bacterial load to milk. The overall mean total viable count of the samples was 3.26 ± 0.07 $\log_{10}$ cfu/ml (Table 76), which was higher (2.98 ± 0.10 $\log_{10}$ cfu/ml) than that reported by Jaibi (2006). In the present study the samples of S_2 had highest mean count, 3.67 ± 0.11 $\log_{10}$ cfu/ml. The overall mean coliform count of the samples was 2.23 ± 0.15 $\log_{10}$ cfu/ml and the mean *Escherichia coli* count was 0.62 ± 0.18 $\log_{10}$ cfu/ml. The later count was lower than that of 1.45 ± 0.32 $\log_{10}$ cfu/ml, reported by Jaibi (2006). The highest mean coliform count and *Escherichia coli* count was observed in the samples of S_1. The mean faecal streptococcal count of the samples was 1.91 ± 0.20 $\log_{10}$ cfu/ml and the highest mean count was seen in the samples of S_2. The high level of bacterial load in the samples of hand wash, particularly the presence of *Escherichia*

coli count and faecal streptococci indicate poor personal hygiene. Therefore, the farmers should be educated on the impact of bacterial contamination of milk and the role of personal hygiene in reducing the contamination of milk and thus the safety of consumers.

5.5.4 Utensil wash

The overall mean total viable count of the samples was 2.47 ± 0.06 $\log_{10}$ cfu/ml and the highest mean count, 2.81 ± 0.08 $\log_{10}$ cfu/ml, was observed in the samples of S_2 (Table 77). The overall mean coliform count of the samples in the present study was 1.43 ± 0.12 $\log_{10}$ cfu/ml and the highest mean count (1.78 ± 0.31 $\log_{10}$ cfu/ml) was observed in the samples of S_3. The overall mean *Escherichia coli* count in the samples was 0.30 ± 0.14 $\log_{10}$ cfu/ml, which was much lower than that reported by Jaibi (2006) from the utensil wash samples collected from the societies. The overall mean faecal streptococcal count was 1.48 ± 0.09 $\log_{10}$ cfu/ml. Similar to total viable count highest *Escherichia coli* count and faecal streptococcal count was also seen in the samples of S_2. Since many of the organism can easily establish on equipment and milk cans proper cleaning of the same with detergents and sanitizers are essential to reduce contamination of milk via utensils.

Based on the analysis of the bacterial load of various samples it was concluded that hand wash of the milk handler is the major contributing source of contamination of milk, followed by utensil wash and water. This was in agreement with the reports of Jaibi (2006), who observed that the hand wash was a major source of contamination to milk.

5.6 ADULTERANTS AND PRESERVATIVES IN THE MILK

None of the pooled samples had revealed the presence of the adulterants; starch and cane sugar and preservatives; boric acid, formaldehyde and bicarbonates. The observation was similar to the reports of Jolly *et al.* (2000), who recorded that all the milk samples were found to be free of the preservative and adulterants.

5.7 DETECTION OF *Escherichia coli* BY POLYMERASE CHAIN REACTION

The *Escherichia coli* isolates obtained from raw milk were confirmed by Polymerase Chain Reaction (PCR) following the procedure described by Daly *et al.* (2002). Analysis of the electrophoresed gel under UV transilluminator revealed the presence of a 366 bp band in 93.33 per cent isolates.

Milk is an ideal food for all human beings and it form an important raw material used for the manufacture of various food products. The initial microbial quality of milk has paramount importance since it decides the final product quality and its shelf life. The study provided the basic information on the microbial quality of milk and also the level of bacteria present in the various sources that can contaminate the milk. Further, the study also revealed significant difference between the microbial quality of milk supplied by the societies and also the quality of milk produced by the farmers who supply milk to the societies. The study revealed that adopting clean milk production, practicing good personal hygiene and sanitary practices could improve the microbial quality of milk and thereby safeguard consumer health and extent the shelf life of milk.

Summary

6. SUMMARY

In the present investigation, a total of 180 raw milk samples consisted of 108 individual milk samples at the point of production from farmers belonging to three co operative societies *viz.* S_1, S_2 and S_3 and 72 pooled milk samples from the three society were collected and tested to assess the microbial quality. From each society, individual samples were collected from six farmers and collection from each farmer was repeated six times. Similarly, four pooled samples were collected from a society and the collection was repeated six times. From each society, 36 individual and 24 pooled milk samples were collected and the microbial load of each sample was tested by estimating Total Viable Count (TVC), Coliform Count (CC), *Escherichia coli* Count (ECC), Faecal Streptococcal Count (FSC) and Yeast and Mould Count (YMC). All samples were subjected to isolation and identification of *Escherichia coli, Staphylococcus aureus* and *Yersinia*. The isolated *Escherichia coli* cultures were confirmed by employing Polymerase Chain Reaction (PCR) technique. The pooled milk samples obtained from the societies were also tested to detect the adulterants and preservatives added in the milk. To assess the sources of contamination, bacterial load of the samples of air, water, hand wash of milker or milk handler, udder wash and utensil wash were analyzed.

Highly significant ($P<0.01$) difference was observed between the mean total viable count of the individual milk samples of the three sources. The analysis of the data by Least Significant Difference (LSD) test showed significant ($P<0.05$) difference between the mean total viable count of samples between S_1 and S_2 and the mean count of samples of S_2 and S_3. Overall mean total viable count of the samples was 6.01 ± 0.07 $\log_{10}$ cfu/ml. The highest mean count, 6.37 ± 0.13 $\log_{10}$ cfu/ml, was observed in the samples of S_2 and the lowest count was seen in the samples of S_3 (5.67 ± 0.13 $\log_{10}$ cfu/ml). Of the samples, 51.85 per cent of the samples had count at and above the level of 10^6 cfu/ml.

The highest mean coliform count was observed in the samples of F_3 (4.77 ± 0.19 $\log_{10}$ cfu/ml). The lowest count was in the samples of F_2 (3.52 ± 0.77 $\log_{10}$ cfu/ml).

Escherichia coli was not detected in 66.67 per cent samples of S_1. Samples of F_5 had the highest mean count (1.50 ± 0.49 $\log_{10}$ cfu/ml). The lowest count was observed in the samples of F_1 (0.67 ± 0.42 $\log_{10}$ cfu/ml). None of the samples of F_6 revealed the presence of the organism.

Faecal streptococcal count of the individual milk samples from farmers of S_1 revealed highly significant ($P<0.01$) difference. Samples of F_6 had the highest mean count (3.97 ± 0.09 $\log_{10}$ cfu/ml) followed by F_3 (3.21 ± 0.27 $\log_{10}$ cfu/ml) and the lowest count was observed in the samples of F_5 (1.80 ± 0.59 $\log_{10}$ cfu/ml).

The mean yeast and mould count of the samples was highest in samples of F_3 (2.74 ± 0.25 $\log_{10}$ cfu/ml) and the lowest count was seen in the samples of F_5 (1.75 ± 0.56 $\log_{10}$ cfu/ml).

The total viable count of individual samples of S_2 showed highly significant ($P<0.01$) difference. Samples of F_2 had the highest mean count (7.08 ± 0.20 $\log_{10}$ cfu/ml) followed by the samples of F_1 (6.72 ± 0.24 $\log_{10}$ cfu/ml). The lowest mean count was observed in the samples of F_5 (5.47 ± 0.16 $\log_{10}$ cfu/ml).

Highly significant ($P<0.01$) difference was observed between the mean coliform count of individual samples belonging to S_2. The highest mean count was observed in the samples of F_2 (5.33 ± 0.15 $\log_{10}$ cfu/ml) and the lowest count was seen in the samples of F_5 (3.89 ± 0.18 $\log_{10}$ cfu/ml).

The *Escherichia coli* Count of individual raw milk samples obtained from S_2 revealed highly significant ($P<0.01$) difference. The organism was not detected in 50.00 per cent of the individual samples of S_2. The samples collected from F_6 had the highest mean count (2.33 ± 0.49 $\log_{10}$ cfu/ml) and the samples from F_5 had the lowest

mean count (0.72 ± 0.46 log_{10} cfu/ml). None of the samples from F_2 had revealed the presence of the organism.

The faecal streptococcal count of samples from the farmers of S_2 showed highly significant ($P<0.01$) difference. The highest mean count was observed in the samples of F_3 (4.10 ± 0.18 log_{10} cfu/ml) and the lowest count was seen in the samples of F_5 (3.13 ± 0.21 log_{10} cfu/ml). The lowest mean yeast and mould count was observed in the samples of F_6 (0.96 ± 0.61 log_{10} cfu/ml) and the highest mean count was observed in the samples of F_3 (2.99 ± 0.24 log_{10} cfu/ml).

Significant ($P<0.05$) and positive correlation was observed between coliform count and other bacterial counts of the samples obtained from S_2. The association between total viable count and faecal streptococcal count was also significant ($P<0.05$).

Total viable count of the samples of farmers belonging to S_3 showed highly significant ($P<0.01$) difference. The samples of F_2 had the lowest count (4.85 ± 0.18 log_{10} cfu/ml) and the highest count was observed in the samples of F_6 (6.66 ± 0.38 log_{10} cfu/ml).

Coliform count of the samples collected from S_3 showed highly significant ($P<0.01$) difference. The samples from F_4 had the highest mean count (4.96 ± 0.17 log_{10} cfu/ml) and the lowest mean count was observed in the samples of F_1 (3.74 ± 0.13 log_{10} cfu/ml).

Escherichia coli was not detected from 75.00 per cent of the samples of S_3. The mean count of samples from F_1 and F_5 was 0.80 ± 0.51 log_{10} cfu/ml and the count in the samples of F_2, F_4 and F_6 was 0.33 ± 0.33 log_{10} cfu/ml.

Samples collected from F_4 had the highest mean faecal streptococcal count (3.66 ± 0.14 log_{10} cfu/ml). The lowest mean count was seen in the samples of F_3 (2.13 ± 0.68 log_{10} cfu/ml).

The samples collected from F_3 had the highest mean yeast and mould count (2.77 ± 0.11 log_{10} cfu/ml) and the lowest count was observed in the samples of F_1 (1.41 ± 0.64 log_{10} cfu/ml).

Significant ($P<0.05$) and positive correlation was observed between coliform count and total viable count of the samples obtained from S_3.

Microbial count of 72 pooled milk samples from the three sources were evaluated. The mean total viable count of pooled milk samples belonging to the three sources revealed highly significant ($P<0.01$) difference. Least Significant Difference test of the data showed significant ($P<0.05$) difference between the mean count of the samples from S_1 and S_3 and S_2 and S_3. The overall mean count of the samples was 6.19 ± 0.09 log_{10} cfu/ml. The highest mean count was observed in the samples of S_2 (6.49 ± 0.05 log_{10} cfu/ml) and the lowest count was seen in the samples of S_3 (5.73 ± 0.08 log_{10} cfu/ml).

The overall mean coliform count of the samples was 4.65 ± 0.09 log_{10} cfu/ml. Samples of S_2 had the highest count (4.75 ± 0.09 log_{10} cfu/ml). The lowest mean count was observed in samples of S_1 (4.33 ± 0.12 log_{10} cfu/ml).

Escherichia coli was not detected in 50.00 per cent of the samples. The overall mean *Escherichia coli* count of the samples was 1.27 ± 0.16 log_{10} cfu/ml. The highest mean count of 1.33 ± 0.43 log_{10} cfu/ml was observed in the samples of S_3 and the lowest was in the samples of S_1 (1.24 ± 0.27 log_{10} cfu/ml).

The mean faecal streptococcal count of the pooled milk samples from three sources showed highly significant ($P<0.01$) difference The mean count of the samples from the three societies was analyzed by Least Significant Difference test and the results showed significant ($P<0.05$) difference between the mean count of the samples of S_1 and S_2 and also between the mean count of the samples of S_1 and S_3. The overall mean faecal streptococcal count of the samples was 3.50 ± 0.06 log_{10} cfu/ml. The

samples of S_2 had the highest mean count (3.74 ± 0.10 log_{10} cfu/ml) followed by samples of S_3 (3.63 ± 0.09 log_{10} cfu/ml) and S_1 (3.13 ± 0.09 log_{10} cfu/ml).

The overall mean yeast and mould count of the samples was 2.37 ± 0.11 log_{10} cfu/ml. The samples of S_3 had the highest mean count (2.73 ± 0.08 log_{10} cfu/ml). The count in the samples of S_1 and S_2 were 2.20 ± 0.21 log_{10} cfu/ml and 2.16 ± 0.24 log_{10} cfu/ml, respectively.

Significant ($P<0.05$) and positive correlation was observed between total viable count and coliform count of the pooled samples obtained from the three societies.

Escherichia coli was isolated from 39 (36.11 per cent) individual milk samples. Out of the 39 isolates, 15 were serotyped into 10 serotypes, *viz.* O12 (2), O29 (2), O68 (2), O75 (1), O79 (1), O107 (1), O116 (3), O131 (1), O160 (1) and O172 (1). Out of the 39 isolates 12 each were untypable and rough. Congo red binding property was shown by 9 serotyped isolates.

Staphylococcus aureus was isolated from 44 (40.74 per cent) individual milk samples. Of the 108 individual milk samples, 24 (22.22 per cent) samples showed the presence of *Yersinia*, of which, five were *Yersinia enterocolitica* and one was *Yersinia pseudotuberculosis.*

Of the 72 pooled raw milk samples, 36 (50.00 per cent) samples showed the presence of *Escherichia coli.* Out of the 36 isolates, 18 were serotyped into five different serotypes, *viz.* O116 (10), O68 (3), O75 (2), O60 (2) and O96 (1). Three isolates were untypable and fifteen were rough. Congo red binding property was shown by sixteen serotyped isolates.

Staphylococcus aureus was present in 25 (34.72 per cent) pooled milk samples. *Yersinia* was isolated from 21 (29.17 per cent) samples, among them one was *Yersinia enterocolitica.*

Grading of individual milk samples based on total viable count as per the standards prescribed by Indian Standards (1977) revealed that 34.26 per cent samples were graded as good. Only 15.74 per cent samples was graded as very good whereas 32.41 and 17.59 per cent samples were graded as fair and poor, respectively. The percent of very good samples was more in S_3 whereas highest per cent of poor samples were observed in the samples of S_2.

Of the 72 pooled milk samples, only 9.72 were graded as very good. The per cent of good samples was 31.94 per cent, whereas 40.28 per cent was graded as fair and 18.06 per cent was graded as poor.

Environmental samples, rinsings of utensils and hand wash of the milker were collected and their bacterial load were estimated in order to determine the critical control points of bacterial contamination of milk at the production point. Air samples had an average total viable count of 149.83 ± 13.21 cfu/ft^2/min. Samples of F_2 of the S_1 had the highest mean count (217.43 ± 21.41 cfu/ft^2/min) whereas the lowest mean count was seen in the samples obtained from F_1 of the S_2 (119.43 ± 15.22 cfu/ft^2/min).

The overall mean total viable count of water was 1.65 ± 0.09 $\log_{10}$ cfu/ml. The highest mean total viable count, coliform count and faecal streptococcal count were observed in the water samples collected from F_2 of S_1, whereas the highest *Escherichia coli* count was seen in the samples of F_1 of S_2.

The overall mean total viable count of hand wash was 3.38 ± 0.08 $\log_{10}$ cfu/ml. The highest mean total viable count and coliform count were observed in the samples of F_1 belonging to S_2, whereas highest *Escherichia coli* count and faecal streptococcal count of the samples was seen in the samples of F_2 of S_2. The overall mean total viable count of utensil wash was 2.91 ± 0.12 $\log_{10}$ cfu/ml. The highest mean total viable count, coliform count, *Escherichia coli* count and faecal streptococcal count of the utensil wash were observed in the water samples collected from F_2 of S_1. The mean total viable count (3.31 ± 0.21 $\log_{10}$ cfu/ml) and faecal streptococcal count of the udder

wash was highest in the samples from F_1 of S_1. The samples from F_1 of S_2 had the highest mean coliform count, whereas the highest mean *Escherichia coli* count was observed in the samples of F_1 of S_3.

The result revealed that hand wash of the milker was the major sources of contamination, followed by the udder wash.

The various sources of contamination of milk at the society level were identified and their bacterial load was determined. Air samples had an average total viable count of 151.12 ± 19.15 cfu/ft^2/min. The samples from S_1 had the highest mean count (176.67 ± 23.16 cfu/ft^2/min) and the lowest count was seen in the samples of S_2 (112.34 ± 14.12 cfu/ft^2/min). The overall mean total viable count of water was 1.82 ± 0.16 $\log_{10}$ cfu/ml. The highest mean total viable count, coliform count, faecal streptococcal count and *Escherichia coli* count were observed in the water samples collected from S_2. The overall mean total viable count of hand wash was 3.26 ± 0.07 $\log_{10}$ cfu/ml. The highest mean total viable count and faecal streptococcal count were observed in the samples of S_2, whereas highest *Escherichia coli* count and coliform count was seen in the samples of S_1. The overall mean total viable count of utensil wash was 2.47 ± 0.06 $\log_{10}$ cfu/ml. The highest mean total viable count, *Escherichia coli* count and faecal streptococcal count of the utensil wash was observed in the water samples of S_2. But the samples from S_3 had the highest coliform count.

According to the study, hand wash of the milk handler was the major sources of contamination.

All pooled milk samples were tested to determine the presence of adulterants *viz.*, starch and cane sugar and preservatives *viz.*, boric acid, formaldehyde and neutralizers. None of the samples had revealed the presence of the adulterants and preservatives.

The *Escherichia coli* isolates obtained from raw milk were confirmed by Polymerase Chain Reaction (PCR) and the analysis of the electrophoresed gel under UV transilluminator revealed the presence of a 366 bp band in 93.33 per cent isolates.

High microbial count in milk samples and presence of pathogens like *Escherichia coli*, *Staphylococcus aureus* and *Yersinia* are indication of poor hygienic practices followed by the farmer and workers of co-operative society. Coliform count, *Escherichia coli* count and faecal streptococcal count are indication of faecal contamination from animals and/or man. Therefore, strict hygienic measures should be followed to reduce the microbial contamination and to produce good quality milk. Hand wash was the major source of contamination both at the farmer level and at the society level. Hygiene of the milk handler should be ensured to improve the quality of milk collected at the co-operative society level.

Reference

7. REFERENCES

Aaku, E.N., Collison, E.K., Gashe, B.A. and Mapuchane, S. 2004. Microbiological quality of milk from two processing plants in Gaborone Botswana. *Fd. Control.* 15: 181-186

Abhilasha, Singh, S.P. and Gupta, R.S. 2001. Pathogenicity of *Escherichia coli* strains by congo red binding test. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 22: 172-173

Adesiyun, A.A., Webb, L.A. and Romain, H.T. 1998. Prevalence and characteristics of *Staphylococcus aureus* strains isolated from bulk and composite milk and cattle handlers. *J. Fd. Prot.* 61: 629-632

Allmann, M., Hofelein, C., Koppel, E., Luthy, J., Meyer, R., Niederhäuser, C., Wegmuller, B. and Candrian, U. 1995. Polymerase chain reaction (PCR) for detection of pathogenic microorganisms in bacteriological monitoring of dairy products. *Res. Microbiol.* 146: 85-97

Arora, S., Sharma, V., Raj, D., Ram, M. and Kishore, K. 2004. Status of milk adulteration in some states of North India. *Indian J. Dairy Sci.* 57 : 65-66

Barrow, C.J. and Feltham, R.K.A. 1993. *Cowan and Steel's Manual for the Identification of Medical Bacteria.* Third edition. Cambridge Press, London, p.238

Beuchat, L.R. and Cousin, M.A. 2001. Yeasts and Molds. *Compendium of Methods for the Microbiological Examination of Foods* (eds. Downes, F.P. and Ito, K.). Fourth edition. American Public Health Association, Washington DC, pp. 209-213

Carmo, L.S., Dias, R., Linardi, V.R., Sena, M.J., Santos, D.A.D., Faria, M.E., Pena, E.C., Jett, M. and Heneine, L. 2002. Food poisoning due to

enterotoxigenic strains of *Staphylococcus* present in Minas Cheese and Raw milk in Brazil. *Fd. Microbiol.* 19: 9-14

Chacko, L.K. 2006. Bacterial quality of milk at the point of production with special emphesis on the quality assurance programme. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, p.176

Chye, F. Y., Abdullah, A. and Ayob, M.K. 2004. Bacteriological quality and safety of raw milk in Malaysia. *Fd. Microbiol.* 21: 535-541

Daly, P., Collier, T. and Doyle, S. 2002. PCR-ELISA detection of *Escherichia coli* in milk. *Lett. Appl. Microbiol.* 34: 222-226

Das, R. and Nag, N.C. 1986. Examination of market milk collected from Calcutta and neighbouring places with special reference to isolation of Salmonellae. *Indian. J. Hlth.* 25:145-149

Davies, D.G. 1977. The bacteriological quality of bulk and churn collected milk supplies in Wales. *J. Soc. Dairy Technol.* 30: 52-59

Desai, S.C. and Natarajan, A.M. 1981. Bacteriological quality of raw milk collected from societies for transportation to chilling centers. *Cheiron.*10: 146-150

Desmarchelier, P.M., Bilge, S.S., Fegan, N., Mills, L., Vary, J.C. Jr. and Tarr, P.I. 1998. A PCR specific for *Escherichia coli* O157 based on the *rfb* locus encoding O157 lipopolysaccharide. *J. clin. Microbial.* 36: 1801-1804

Dontorou, C., Papadotoulou, C., Filioussis, G., Economu, V., Apostolou, I., Zakkas, G., Salamoura, A., Kansouzidou, A. and Levidiotou, S. 2003. Isolation of *Escherichia coli* O157: H7 from foods in Greece. *Int. J. Fd. Microbiol.* 82: 273-279

Edwards, P.R. and Ewing, W.H. 1972. *Identification of Enterobacteriaceae.* Third edition. Burgess Publishing Company, Georgia, p.362

Evancho, G.M., Sveum, W.H., Moberg, L.J. and Frank, J.F. 2001. Microbiological monitoring of the food-processing environment. *Compendium of Methods for the Microbiological Examination of Foods* (eds. Downes, F.P. and Ito, K.). Fourth edition. American Public Health Association, Washington D.C, pp. 25-35

Garg, D.N., Bhargava, D.N. and Narayan, K.G. 1977. Pathogenic bacterial flora of raw market milk. *Indian J. Dairy Sci.* 30: 36-39

Garg, S. R. and Mandokhot, U.V. 1997. Reliability of rapid and routine quality control tests for grading raw milk under Indian conditions. *J. Fd. Sci. Technol.* 34: 357-359

Gill, J.P.S., Joshi, D.V. and Kwatra, M.S. 1994. Qualitative bacteriological survey of milk and milk products with special reference to *Staphylococcus aureus*. *Indian J. Dairy Sci.* 47: 680-682

Gopi, H., Parthiban, M. and Dhanalakshmi, B. 2001. Bacteriological quality of private brands of milk in Chennai city. *Cheiron*. 30:106-107

Gran, H.M., Wetlesen, A., Mutukumira, A.N., Rukure, G. and Narvhus, J.A. 2003. Occurrence of pathogenic bacteria in raw milk; cultured pasteurized milk and naturally soured milk produced at small-scale dairies in Zimbabwe. *Fd. Control.* 14: 539-544

Gupta.S. 2007. *Dairy Industry Profile 2005.* Sixth edition. Thomson Press, India, p. 836

Heuvelink, A.E., Bleumink, B., Biggelaar,V., Giffel, M.C.T., Beumer, R.R and Boer, E. 1998. Occurence and survival of verotoxin – producing *Escherichia coli* O157 in raw cow's milk in the Netherlands. *J. Fd. Prot.* 61: 1597-1601

Homhual, S. and Jindal, V.K. 2001. Simple tests for rapid assessment of the quality of raw milk. *J. Fd. Prot.* 64: 1996-2000

Hughes, D. and Jensen, N. 1981. *Yersinia enterocolitica* in raw goat's milk. *Appl. Environ. Microbiol.* 41: 309-310

Indian Standards. 1977. IS: 1479. *Methods for Test for Dairy Industry. Part III. Bacteriological Analysis of Milk* (First revision). Bureau of Indian Standards, Manak Bhavan, 9, Bahadur Shah Zafar Marg, New Delhi – 1. p. 25

Indian Standards. 1978. IS: 1622-1964. *Methods of Sampling and Test for Microbiological Examination of Water Used in Industry.* Bureau of Indian Standards. Manak Bhavan, 9, Bahadur Shah Zafar Marg, New Delhi-1.p.39

Indian Standards. 1980. SP: 18. *ISI Hand Book of Food Analysis. Part I.* Bureau of Indian Standards. Manak Bhavan, 9, Bahadur Shah Zafar Marg, New Delhi- 1. p. 85

Indian Standards. 1981. SP: 18. *Handbook of food analysis - Part XI. Dairy Products.* Bureau of Indian Standards. Manak Bhavan, 9, Bahadur Shah Zafar Marg, New Delhi-1. p.171

Jaibi, K. 2006. Bacterial quality of raw milk at the co-operative society level with special reference to quality assurance programme. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, p.199

Jayarao, B.M. and Henning, D.R. 2001. Prevalence of Foodborne pathogen in bulk tank milk. *J. Dairy Sci.* 84: 2157- 2162

Jolly, D., Nanu, E. and Sunil, B. 2000. Evaluation of certain quality characteristics of raw market milk. *Smallholder Livestock Production System in Developing Countries.* 451-458

Jothikumar, N. and Griffiths, M.W. 2002. Rapid detection of *Escherichia coli* O157: H7 with multiplex real-time PCR assays. *Appl. Environ. Microbiol.* 68: 3169-3171

Kapoor, K.N., Rathore, R.S. and Agarwal, R.K. 2002. Incidence of enterotoxigenic *Escherichia coli* in different foods. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 23: 71-72

Kapre, A.R. 1995. Assessment of bacteriological quality of raw milk in Trichur and its public health importance. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, p.107

Kessel, J.S.V., Carns, J.S., Gorski, L., McCluskey, B.J. and Perdeue, M.L. 2004. Revalence of salmonellae, *Listeria monocytogenes* and fecal coliforms in bulk tank milk on US dairies. *Am Dairy Sci. Ass.* 87: 2822-2830

Khalilur, M., Khan, R. and Malik, A. 2002. Microbiological quality of milk, vegetables and fruit juices. *J. Fd. Sci. Technol.* 39: 120-123

Kornacki, J.L. and Johnson, J.L. 2001. *Enterobacteriaceae*, coliform and *Escherichia coli* as quality and safety indicators. *Compendium of Methods for the Microbiological Examination of Foods* (eds. Downes, F.P. and Ito, K.). Fourth edition. American public health association. Washington, DC, pp. 75-92

Kumar, S.S., Batish, V.K. and Grover, S. 2001. Rapid detection of *Escherichia coli* in milk by 'uidR' targeted PCR assay. *Indian J. Dairy Sci.* 54: 129-137

Kushal, R. and Anand, S.K. 2001a. Isolation, biochemical characterization and antibiotic susceptibility of *Yersinia enterocolitica* isolates from milk. *J. Fd. Sci. Technol.* 38: 129-134

Lancette, G.A. and Bennett, R.W. 2001. *Staphylococcus aureus* and staphylococcal enterotoxins. *Compendium of Methods for the Microbiological Examination of Foods* (eds. Downes, F.P. and Ito, K.). Fourth edition. American public health association, Washington DC, pp. 387-403.

Lues, J.F.R., Venter, P. and Westhuizen, H.V. 2003. Enumeration of potential microbiological hazard in milk from a marginal urban settlement in central South Africa. *Fd. Microbiol.* 20: 321-326

Mankar, A.M., Ingole, A.S. and Murkute, J.S. 2005. Survey on adulteraton of milk received at Government milk scheme, Nagpur. *Indian J. Dairy Sci.* 58 : 71-72

Manna, S.K., Brahmane, M. P., Das, R., Chandana, M. and Batabyal, S. 2006. Detection of *Esherichia coli* O157 in foods of animal origin by culture and multiplex polymerase chain reaction. *J. Food Sci. Technol.* 43 : 77-79

Matta, H. and Punj, V. 1996. Isolation and identification of proteolytic psychrotrophic spore forming bacteria from milk. *Indian J. Dairy Sci.* 49: 695-699

Misra, A.K. and Kuila, R.K. 1989. Bacteriological quality of market milk. *Indian Dairyman.* 101: 487-491

Mohan, K. and Misra, S.K. 1967. *Staphylococcus aureus* in milk supplied to Patna milk supply scheme. *Indian J. Dairy Sci.* 20: 178-180

Mortan, D.R. 2001. Aerobic plate count. *Compendium of Methods for the Microbiological Examination of Foods* (eds. Downes, F.P. and Ito, K.). Fourth edition. American Public Health Association, Washington DC, pp. 63-68

Mutukumira, A.N., Feresu, S.B., Narvhus, J.A. and Abrahamsen, R.K. 1996. Chemical and microbiological quality of raw milk produced by smallholder farmers in Zimbabwe. *J. Fd. Prot.* 59: 984-987

Nanu, E., Latha,C., Sunil, B., Prejit., Thomas, M. and Menon, K. V. 2007. Quality assurence and public health safety of raw milk at the production point. *Am. J. Food Tech.* 2 : 145 -152

Nihal and Huriye. 2006. A Turkey survey of hygiene indicator bacteria and *Yersinia enterocolitica* in raw milk and cheese samples. *Food control.* 17:383-388

Nordic Committee on Food Analysis. 1968. *Determination of Faecal Streptococci in Foods.* 68. UDC. 576, 851, 21

Oksuz, O., Arici, M., Kurultay, S. and Gumus, T. 2004. Incidence of *Escherichia coli* 0157 in raw milk and white pickled cheese manufactured from raw milk in Turkey. *Fd. Control* 15: 453-456

Oliveira, C.A.F, Mestier,L., Santos,M.V., Moreno, J.F.G., Spers, A., Germano, P.M.L. 2000. Effect of microbial characteristics of raw milk on the quality of raw milk powder. *Brazilian. J. Microbiol.* 31:95-98

Palanniswami, K.S., Venugopalan, A.T. and Masillamony, P.R. 1988. Studies of coliform of farm milk and its environment. *Indian J. Dairy Sci.* 41: 149-153

Patel, D.A., Siva, C.V. and Sannabhadti, S.S. 1993. Sources of microbial contamination of raw milk. *Indian J. Dairy Sci.* 46 : 65-70

Prejit. 2005. Microbial quality assurance of milk in its production, processing and storage. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, p.157

Rahman, H., Nath, N.C. and Boro, B.R. 1992. Bacterial flora and insecticidal residue in raw milk marketed in Guwahati city, Assam. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 13: 105-108

Rai, C.K., Bihari, H and Dwivedi, H.B. 1990. Study of the bacterial quality of milk supplied in Kanpur city by different sources. *Indian Dairyman.* 102: 520-523

Raj, B.R., Nanu, E., Sunil, B. and Sujatha, K.S. 2003. Evaluation of bacterial quality and detection of bacterial pathogens from raw milk. *Proceedings of fifteenth Kerala Science Congress. 29-31 January 2003.* Thiruvanthapuram, pp. 319-325

Rajil, M.A., Adekeyel, J.O., Kwaga, J.K.P. and Bale, J.O.O. 2003. In vitro and in vivo pathogenicity studies of *Escherichia coli* isolated from poultry in Nigeria. *Israel J. Vet. Med.* 58:81-87

Rajmany, L. Garg, S. K., Yadav, R.K. 1989. Incidence of Staphylococci in milk and milk products. *Indian J. Dairy Sci.* 42: 136-138

Raju, V.V.R. and Nambudripad, V.K.N. 1987. Incidence and growth of heat resistant coliforms bacteria in milk and other media. *Indian J. Dairy Sci.* 40 : 354-358

Rangaswamy, R. 1995. *A Textbook of Agricultural Statistics.* New Age International publishers Ltd, New Delhi, p. 495

Rao,L.V., Ranganadham, M. and Rao, B.V.R. 2002. Quality of milk andmilk products marketed in Hyderabad city part 1- chemical quality of milk and fermented milk. *Indian J. Dairy Sci.* 55 : 338-341

Reddy, N.S., Rao, V.J. and Venkayya, D. 1984. Studies on bacteriological quality of dried whole milk in relation to the initial quality of raw milk. *J. Fd. Sci. Technol.* 21: 415-417

Reid, S.D., Betting, D.J. and Whittam, T.S. 1999. Molecular detection and identification of intimin alleles in pathogenic *Escherichia coli* by multiplex PCR. *J. Clin. Microbiol.* 37: 2719-2722

Rohrbach, B.W., Draughton, F.A., Davidson, P.M. and Oliver, S.P. 1992. Prevalence of *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Salmonella* in bulk tank milk: risk factors and risk of human exposures. *J. Fd. Prot.* 55: 93-97

Sakkarvarthi, S., Mathur, D.K. and Malik, R.K. 1990. Bacteriological quality of buffalo milk in relation to processing at high temperatures. *Indian J. Dairy Sci.* 43:207-212

Saxena , G. and Agrawal, M. 2004. Quality assessment of market milk available in Jaipur. *The Ind. J. Nurt. Dietet.* 41 : 358-364

Schiemann, D.A. 1978. Association of *Yersinia enterocolitica* with manufacture of cheese and occurrence in pasteurized milk. *Appl. Environ. Microbiol.* 36 : 274-277

Schiemann, D.A. and Toma, S. 1978. Isolation of *Yersinia enterocolitica* from raw milk. *Appl. Environ. Microbiol.* 35: 54-58

Sen, G.P., Haldar, S.K. and Aich, A.C. 1989. Healthy bovine udder as a source of enterotoxigenic Staphylococcus. *Indian J. Al. Hlth.* 28: 163-165

Shah, N.M., Rajput, H.A., Dholakia, P.M. and Simaria, M.B. 1984. Studies on bacterial flora from milk secretion of healthy cows. *Indian Dairyman.* 37: 323-324

Singh, K. and Sinha, R.N. 1981. Rapid detection of coliforms in pasteurized milk. *Indian J. Dairy Sci.* 34: 305-309

Singh, R.S. and Ranganathan, B. 1978. Incidence and distribution of *Escherichia coli* in dairy products. *Indian J. Dairy Sci.* 31: 82-84

Singh, Y., Kumar, A. and Sharma, V.D. 1994b. Sanitary analysis of milk, cream and burfi. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 15: 12-14

Singh, Y., Kumar, A. and Thapliyal, D.C. 1994c. Sanitary analysis of milk, cream and burfi. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 15: 35-37

Siva, C.V., Patel, D.A. and Sannabhadti, S.S. 1993. Microbiological status of raw and pasteurized milk. *Indian J. Dairy Sci.* 46: 62-66

Steel, M.L., McNab, W.B., Poppe, C., Griffiths, M.W., Chen, S., Degrandis, S.A., Fruhner, L.C., Larkin, C.A., lynch, J.A. and Odumeru, J.A. 1997. Survey of Ontario bulk tank raw milk for food-borne pathogens. *J. Fd. Prot.* 60: 1341-1346

Vidon, D.J.M. and Delmas, C.L. 1981. Incidence of *Yersinia enterocolitica* in raw milk in eastern France. *Appl. Environ. Microbiol.* 41: 355-359

Vijai, R.G. and Saraswat, D.S. 1968. Studies on bacteriological quality of market milk in Udaipur city-1. Enumeration of standard plate and coliform counts in raw and pasteurized milk. *Indian J. Dairy Sci.* 21: 233-237

Weagant, S.D. and Feng, P. 2001. *Yersinia. Compendium of Methods for the Microbiological Examination of Foods* (eds. Downes, F.P. and Ito, K.). Fourth edition. American public health association, Washington DC, pp. 421-438.

Yadava, J.S., Grover, S. and Batish, V.K. 1993. *A Comprehensive Dairy Microbiology.* First edition. Metropolitan Book Co. Pvt. Ltd. New Delhi P.700

Yadava, R., Choudhary, S.P. and Narayan, K.G. 1983. Bacteriological quality of market milk at Ranchi. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 4: 215-219

Yadava, R., Choudhary, S.P. and Narayan, K.G. 1985. Bacterial flora of market milk and its public health importance. *Indian J. Dairy Sci.* 38: 235-237

Yadava, R., Choudhary, S.P. and Narayan, K.G. 1987. Serotypes of *Escherichia coli* in market milk. *Indian J. Dairy Sci.* 23: 466-467

Yokoigawa, K., Inoue, K., Okubo, Y. and Kawai, H. 1999. Primers for amplifying an alanine racemase gene fragment to detect *Escherichia coli* strains in foods. *J. Fd. Sci.* 64: 571-575

MICROBIAL QUALITY AND SAFETY OF RAW MILK WITH REFERENCE TO SOURCES OF CONTAMINATION

GINI GEORGE

Abstract of the thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2007

Department of Veterinary Public Health
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

ABSTRACT

In the present investigation a total of 180 raw milk samples, consisted of 108 individual milk samples obtained from farmers belonging to three co- operative societies (S_1, S_2 and S_3) and 72 pooled milk samples from the three societies were collected and evaluated the microbial quality. The samples were also tested to detect the presence of *Escherichia coli, Staphylococcus aureus* and *Yersinia.* The isolated *Escherichia coli* cultures were confirmed using Polymerase Chain Reaction (PCR) technique. The pooled milk samples obtained from the societies were also tested to detect the adulterants and preservatives added in the milk. During the investigation the factors contributing the bacterial contamination of milk from various sources were also evaluated to identify the critical control points.

Statistical analysis of the data revealed highly significant difference ($P<0.01$) in microbial counts of individual samples of the three sources. The overall mean total viable count, coliform count, *Escherichia coli* count, faecal streptococcal count and yeast and mould count was 6.01 ± 0.07, 4.44 ± 0.07, 0.86 ± 0.11, 3.14 ± 0.10 and 2.09 ± 0.12 $\log_{10}$ cfu/ml, respectively. Samples of S_2 had the highest mean count on the basis of total viable count, coliform count, *Escherichia coli* count and faecal streptococcal count. Milk samples from S_2 revealed maximum contamination. *Escherichia coli* was not detected in 63.89 per cent of individual samples. Analysis of the data revealed significant ($P<0.05$) and positive correlation between total viable count and faecal streptococcal count and also between total viable count and coliform count. A similar correlation was observed between coliform count with faecal streptococcal count.

Microbial analysis of milk samples collected from six farmers of S_1 revealed that samples from F_1 had highest mean total viable count (6.29 ± 0.15 $\log_{10}$ cfu/ml) and the lowest count was observed in samples of F_5 (5.58 ± 0.37 $\log_{10}$ cfu/ml). Highest mean coliform count (4.77 ± 0.19 $\log_{10}$ cfu/ml) and yeast and mould count (2.74 ± 0.25 $\log_{10}$ cfu/ml) were seen in the samples of F_3, whereas lowest coliform count (3.52 ± 0.77 $\log_{10}$ cfu/ml) and yeast and mould count (1.75 ± 0.56 $\log_{10}$ cfu/ml) was observed in the samples of F_2 and F_5, respectively. Samples

obtained from F_6 did not revealed the presence of *Escherichia coli,* but the count was highest in the samples of F_5 (1.50 ±0.49 $\log_{10}$ cfu/ml). The highest (3.97 ± 0.09 $\log_{10}$ cfu/ml) and lowest (1.80 ± 0.59 $\log_{10}$ cfu/ml) faecal streptococcal count was observed in the samples of F_6 and F_5, respectively. Critical difference test of the data revealed that none of the bacterial association was significant in the samples of S_1.

Microbial analysis of individual milk samples collected from the farmers of S_2 revealed that samples from F_2 had highest mean total viable count (7.08 ± 0.20 $\log_{10}$ cfu/ml) and coliform count (5.33 ± 0.15 $\log_{10}$ cfu/ml) and the samples from F_5 showed lowest values for the above two counts. Similarly, the bacterial counts, *viz.*, faecal streptococcal count (4.10 ± 0.18 $\log_{10}$ cfu/ml) and yeast and mould count (2.99 ± 0.24 $\log_{10}$ cfu/ml) were highest in the samples of F_3. Lowest values for faecal streptococcal count (3.12 ± 0.31 $\log_{10}$ cfu/ml) and yeast and mould count (0.96 ± 0.61 $\log_{10}$ cfu/ml) were in the samples of F_4 and F_6, respectively. Samples obtained from F_2 did not revealed the presence of *Escherichia coli,* but the count was highest in the samples of F_6 (2.23 ± 0.49 $\log_{10}$ cfu/ml). Analysis of the data of the samples obtained from S_2 revealed that significant ($P<0.05$) and positive correlation between total viable count and faecal streptococcal count and also between total viable count and coliform count. A similar correlation was observed between coliform count with faecal streptococcal count and yeast and mould count.

Analysis of variance test of the data of the samples belonging to the farmers of S_3 revealed highly significant ($P<0.01$) difference between the mean total viable count and coliform count. The samples of F_2 had lowest total viable count (4.85 ± 0.18 $\log_{10}$ cfu/ml), but the highest count was in the samples of F_6 (6.66 ± 0.38 $\log_{10}$ cfu/ml). The samples belonging to F_4 had the highest mean coliform count (4.96 ± 0.17 $\log_{10}$ cfu/ml) while the lowest count was observed in samples of F_1 (3.74 ± 0.13 $\log_{10}$ cfu/ml). The highest mean *Escherichia coli* count (0.80 ± 0.51 $\log_{10}$ cfu/ml) was seen in the samples belonging to F_1 and F_5. The samples belonging to F_2, F_4 and F_6 had the mean count of 0.33 ± 0.33 $\log_{10}$ cfu/ml. The highest mean faecal streptococcal count (3.66 ± 0.14$\log_{10}$ cfu/ml) was seen in the samples of F_4.

The samples of F_3 had the lowest mean count (2.13 ± 0.68 log_{10} cfu/ml). The samples belonging to F_3 had the highest mean yeast and mould count (2.77 ± 0.11 log_{10} cfu/ml) and the lowest mean count was observed (1.41 ± 0.64 log_{10} cfu/ml) in the samples from F_1. A significant ($P<0.05$) and positive correlation was observed only between the total viable count and coliform count of the samples of S_3.

Analysis of variance test of the data revealed highly significant ($P<0.01$) difference in the bacterial count of the 72 pooled milk samples obtained from the three sources. The overall mean total viable count, coliform count, *Escherichia coli* count, faecal streptococcal count and yeast and mould count was 6.19 ± 0.09, 4.65 ± 0.09, 1.27 ± 0.16, 3.50 ± 0.06 and 2.37± 0.11 log_{10} cfu/ml, respectively. *Escherichia coli* was not detected in 50.00 per cent of pooled samples. Samples of S_2 had the highest mean count based on total viable count, coliform Count and Faecal Streptococcal Count. *Escherichia coli* count and yeast and mould count showed highest values in the samples of S_3. Significant ($P<0.05$) and positive correlation was observed only between total viable count and coliform Count of the pooled milk samples.

Escherichia coli was isolated from 36.11 per cent individual samples and 50.00 per cent of pooled milk samples. Fifteen isolates from individual samples and eighteen isolates from pooled milk samples were serotyped. The serotypes obtained were O12, O29, O60, O68, O75, O79, O96, O107, O116, O131, O160 and O172. Twelve isolates each from individual samples were untypable and rough. Among the pooled samples three isolates were untypable and fifteen isolates were rough. Congo red binding property was shown by nine and sixteen serotyped isolates obtained from individual and pooled milk samples, respectively.

Staphylococcus aureus was isolated from 40.74 per cent of individual and 34.72 per cent of pooled milk samples. *Yersinia* was isolated from 22.22 per cent of individual samples and 29.17 per cent of pooled milk samples. From the individual samples, five isolates of *Yersinia enterocolitica* was obtained. *Yersinia frederiksenii, Yersinia intermedia, Yersinia aldovae* and *Yersinia kristensenii* was isolated from ten, five, two and 1 of the individual milk samples. *Yersinia*

pseudotuberculosis was obtained from one of the individual sample. From the pooled milk samples, three isolates each of *Yersinia kristensenii* and *Yersinia aldovae* was obtained. *Yersinia intermedia* and *Yersinia frederiksenii* was isolated from eight and six samples, respectively. *Yersinia enterocolitica* was obtained from one of the sample.

Grading of individual milk samples based on total viable count as per the standards prescribed by Indian Standards (1977) revealed that 34.26 per cent samples were graded as good. The per cent of samples graded as fair was 32.41. Only 15.74 per cent samples was graded as very good, whereas 17.59 per cent was graded as poor. The highest (40.28) per cent of pooled milk samples was graded under the category fair, while 9.72, 31.94 and 18.06 per cent samples were graded as very good, good and poor, respectively.

The various critical control points of microbial contamination of milk was evaluated by collecting samples of air, water, hand wash of the milker or milk handler and utensil wash and subjecting to estimation of the bacterial load. Hand wash was found to be a major source of contamination. The highest microbial count in the samples of water, utensil wash and hand wash was observed in the samples obtained from S_2. The higher mean microbial count in the milk samples of S_2 might be attributed to the contamination from these sources.

Adulterants (starch and cane sugar) and preservatives (carbonates, formaldehyde and boric acid) were not detected in any of the 72 pooled milk samples examined.

The *Escherichia coli* isolates obtained from raw milk were confirmed by Polymerase Chain Reaction (PCR) and the analysis of the electrophoresed gel under UV transilluminator revealed the presence of a 366 bp band in 93.33 per cent isolates.