ASSESSMENT OF BACTERIOLOGICAL QUALITY OF RAW MILK IN TRICHUR AND ITS PUBLIC HEALTH IMPORTANCE

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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1995

DECLARATION

I hereby declare that the thesis entitled **ASSESSMENT OF BACTERIOLOGICAL QUALITY OF RAW MILK IN TRICHUR AND ITS PUBLIC HEALTH IMPORTANCE** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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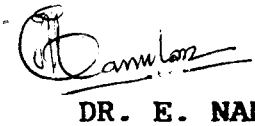
Certified that the thesis entitled **ASSESSMENT OF BACTERIOLOGICAL QUALITY OF RAW MILK IN TRICHUR AND ITS PUBLIC HEALTH IMPORTANCE** is a record of the research work done independently by **Miss. Anju Raghunathrao Kapre** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



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Dedicated to My inspiring Mummy and Most loving Daddy

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16.12.95

AKapre

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INTRODUCTION

Among the major milk producing countries of the world, India has registered the highest growth rate and is the largest producer among the developing countries of the world. The country has emerged as the second largest producer of milk in the world. Its annual milk production of about 53.6 million tonnes is next only to 66 million tonnes of the USA. As a result of increased milk production, the per capita consumption of the milk had gone upto 178 grams in 1990. Though the production has increased, the availability of milk is far below the Indian Council of Medical Research recommendation of 250 grams a day per capita.

In the southern region of India, Kerala exhibited higher production of cow's milk and has significantly contributed to the higher growth rate. The market milk industry in Kerala has grown very much and the major quantity of milk marketing is carried out through the local co-operative societies. The industry has its foundation in the family cow. The small quantities of milk produced in the individual households is brought to the societies by the producers, where its volume is measured

or weighed and pooled together in the milk cans. Many of these societies do not have refrigeration facilities and the pooled milk is either directly distributed to the consumers or it is taken to the processing plants. In the organised farms, milking is carried out mannualy by workers and the bacterial quality of milk depends on milking habits and hygienic practices of the individual milker, as well as the practices followed in the farms.

Milk is one of the most important of animal produce and its value is well known in human nutrition. It is highly perishable and the perishability is directly related to the type and extent of contamination of milk with bacteria. Milk produced from a clean and healthy udder contains only a few bacteria, but it aquires many more during milking, storage , transport and till it is distributed to consumers. If good milking practices are not followed, contamination of milk can occur from the coat of animal, milking utensils, air, feed, manure and other sources. Many types of organisms are represented in this type of contamination, like lactic acid bacteria, spore forming rods, coliforms and pseudomonas in particular. Milk from mastitic animal contains large number of bacteria. Such milk may contain streptococci, micrococci, staphylococci, pseudomonas and other organisms causing

mastitis. The milking and milk storage equipments generally contribute the largest proportion of microorganims found in farm milk supplies. Poorly designed equipment and improper cleaning also lead to high bacterial contamination.

The major proportion of bacterial contaminants in the milk are non-pathogenic but their continued biological activity will result in souring of milk and its spoilage and thus reduces its shelf-life. The products produced from such highly contaminated milk do not have the desired flavour. Hygienic control of milk is, therefore, necessary to provide safe and wholesome milk to the public.

Apart from being an ideal food, milk can also serve as a vehicle for transmission of certain disease causing agents. Milk may carry such organisms or their toxic metabolities, 'toxins', to the consumers. The number of persons affected in such cases are on the increase. Staphylococcus, Clostridium botulinum, Escherichia coli and Bacillus cereus are some of the toxin producing bacteria found in the milk. Staphylococci are capable of growing and producing enterotoxin in raw milk. The toxin production is much faster in milk with high bacterial

count. Contamination of milk with E. coli serotypes capable of producing enterotoxin can take place at any stage of the production, due to careless handling, transport and distribution .

Contamination of milk with various disease producing bacteria may cause health hazard to the consumers. Reports on the several out breaks of diphtheria, salmonellosis, sore throat, scarlet fever, typhoid and infectious diarrhoea has been traced to raw milk contaminated by milk handlers.

The use of antibiotics in animal agriculture has brought great benefits to man and animal. The wider use of it in animals has played a significant economic role in controlling losses due to infectious diseases and in helping to meet growing demands for animal protein foods. Its foremost use in animals is to save lives and relieve them from suffering. It has also been used for prophylaxis and growth promotion in animal husbandry.

Apart from the benefits, the extensive use of antibiotics has caused certain disadvantage also. The most important disadvantage is the antibiotic resistance being developed in bacterial population. The antibiotic resistant bacteria can contaminate the animal products and environment. Consumption of such animal products as

raw, or contamination of the finished or ready to eat product, may lead to infection with such resistant organisms in man and animals. This results in difficulty in treatment in susceptible animals and man. Therefore the bacterial isolates of milk should be tested for their antibiotic susceptibility.

The initial bacteriological quality of milk at the time of its production and collection has a tremendous effect on the quality of milk after the subsequent operations of transport, processing and distribution. The bacteriological quality of raw milk at production level alone will give first hand knowledge on the initial quality of raw milk.

Bacteriological quality of milk is assessed by enumerating bacteria. The detection of indicator organism is not just sufficient to establish the presence of bacterial pathogens. Hence the isolation and identification of such bacterial pathogen is necessary. The bacterial contamination of milk vary from source to source. Therefore the present study was undertaken with the following objectives.

1. To determine the bacterial load of fresh milk produced in the organised farm.

2. To assess the bacteriological quality of milk produced by individual farmers and also the pooled milk samples of the co-operatives societies.

3. To isolate and identify the bacterial pathogens such as Staphylococci and Escherichia.

4. To study the antibiotic sensitivity pattern of the isolates.

Review of Literature

REVIEW OF LITERATURE

Milk aseptically drawn from cow is not completely free from bacteria, as there are always organisms present in the teat canal and the udder. Milk produced from a normal healthy udder contains only a few numbers and types of organisms, but udder infection and contamination results in increased bacterial number. Thus the bacterial quality of milk depends on the hygienic status of its production and is assessed by different bacterial counts.

Total viable count

Among the counts, total viable count of raw milk obtained from various sources had been reported by many workers. Jain and Saraswat (1968) studied the bacteriological quality of milk obtained from different sources. They reported that the average standard plate count of bacteria in milk from dairy farm, city market and rural collection centre was 7.6×10^5, 8.6×10^6 and 1.1×10^7 CFU per ml respectively. The bacteriological quality of raw milk in Udaipur city was evaluated by Vijai and Saraswat (1968) and they found that the mean total viable count of milk from organised dairy farm was 1.4×10^6 per ml, while the count in city market sample was 3.0×10^6 and the corresponding count from rural collection centre was 6.0×10^6 per ml.

Mustafa and Idris (1976) made a survey of bacteriological quality of milk supplied in Sudan in 1973 and 1975. In 1973, milk samples from vendors had average colony count of 7.8×10^6 bacteria per ml while milk samples from dairies had 6.8×10^5 bacteria per ml. A survey conducted in 1975 revealed that 75.86 per cent of milk samples from vendors of three towns had total viable count of more than one million per ml.

Bacterial flora of 102 raw milk samples from buffaloes and cows were evaluated by Garg et al. (1977). Standard plate count per ml of buffalo's and cow's milk in winter ranged between 1.0×10^5 to 3.2×10^7 and 5.4×10^5 to 4.0×10^7 and in summer 3×10^4 to 1×10^7 and 4×10^5 to 2×10^8 CFU per ml respectively.

Misra et al. (1977) studied the bacteriological quality of market milk in Bhubaneswar. The standard plate count showed that milk from established dairy farms had lowest average count, followed by local gowalas, collection centres, local shops and milk booths. Desai and Natarajan (1981) assessed the bacteriological quality of raw milk collected from three societies situated in different areas around Banglore city. The average standard plate count varied between the areas, the counts being 205×10^5, 441×10^3 and 92×10^5 CFU per ml.

The microbiological quality of 100 raw milk samples from Polish state farms and 72 samples from private farms was investigated by Milko et al. (1981). The mean bacterial counts as reported by them were 50.6 x 10^6 and 41.0 x 10^6 bacterial cells per ml respectively. Bossuyt and Naudts (1982) observed that the average bacterial count of milk increased by a factor of 1.6 between leaving the farm and arriving at the dairy factory. The increase was higher in milk collected every three days (factor 1.8) than in milk collected every two days (factor 1.3). The bacterial quality of milk from seven specialized farms in Lubin and Chlem Province was assessed by Majewski and Rzaczynski (1983). Average mesophilic bacterial count ranged between 6.5 x 10^3 to 4.1 x 10^5 CFU per ml. They observed a considerable decrease in the count after disinfection of milking equipment.

Yadava et al. (1983) determined bacteriological quality of milk sold in Ranchi town. Milk from Ranchi Veterinary College had the lowest average standard plate count and highest Methylene Blue Reduction Time (MBRT), followed by local vendors and pasteurized milk from town booth. The standard plate count had no significant difference with season. Zangerl and Ginzinger (1986) reported that 73.5 per cent of 403 and 86.8 per cent of 438 fresh milk samples collected from the farms had total bacterial count less

than 50000 CFU per ml. Milk samples collected from two factories had total viable count of < 50000 CFU per ml in 48.8 per cent of 906 and 77 per cent of 742 samples.

Misra and Kuila (1989) estimated standarad plate count of 125 raw milk from organised dairy farm, city vendors and sweet meat shop and reported that the average standard plate count in milk from the above sources as 51 x 10^1, 71.73 x 10^5, 72.73 x 10^5 CFU per ml, respectively. Morgan et al. (1989) studied sanitary status of 100 raw market milk samples in Kaliobia Governorate and reported that the average total colony count was 39.46 x 10^{11} $\pm$ 17.13 CFU per ml. Rajmany et al. (1989) enumerated total viable bacterial count in raw milk. The number of total viable bacteria in raw milk samples varied between 11.6 x 10^6 and 98 x 10^6 CFU per ml, with a mean count of 53.4 x 10^6 CFU per ml.

Hamama and EL-Mouktafi (1990) studied the hygienic quality of raw milk produced in Morocco by analysing 42 samples from regional milk collection centres. The mean total aerobic mesophilic count was 2 x 10^7 CFU per ml. Rai et al. (1990) collected milk from various sources to study the bacteriological quality of milk supplied in Kanpur city. The average total plate count of the milk from University dairy farm, Milk board, Hawkers and Town dairies were 295.0 x 10^4, 33 x 10^4, 1142 x 10^4, and 429.12 x 10^4 CFU per ml, respectively.

Mahia et al. (1992) examined 113 milk samples collected from the province of La Coruna, Spain and reported that total aerobic bacterial count was < 7 x 10^5 per ml in 81 per cent of samples examined. Patel et. al. (1993) collected 21 samples of milk from buffaloe of Research Unit, Veterinary College, Anand. Total plate count varied from < 1000 to 1100000 with an average of 2.1 x 10^5 $\pm$ 0.7 CFU per ml.

Reddy et al. (1994) reported the least square mean value of standard plate count in the normal milk of cows as 2.6 x 10^6 $\pm$ 0.256 CFU per ml.

Coliform and faecal coliform counts

A count of coliform bacteria is usually made as an index of the general sanitation level of production and as an indicator of poor hygiene. Large number of coliforms in raw milk indicates gross contamination of the milk. Coliform count of the raw milk collected from different sources have been reported by various workers. Vijai and Saraswat (1968) enumerated coliforms in raw milk from different sources in Udaipur city. The counts in the organised dairy farm was 3.2 x 10^2 CFU per ml of milk, in city market sample was 2.2 x 10^3 CFU per ml and in samples from rural collection centre it was 1.8 x 10^4 CFU per ml of milk.

Mustafa and Idris (1976) reported that 64.65 per cent of milk samples from milk supplies in Sudan had coliforms in the range of 3×10^5 to 3×10^6 CFU per ml.

Misra et al. (1977) while studying the bacteriological quality of market milk in Bhubaneswar found lowest coliform counts in the samples from local shops, followed by collection centres, established dairy farms, local gowalas and milk booths. Singh and Ranganathan (1978) found that all the 128 samples of raw milk from institute herd and local market were positive for coliforms.

The mean coliform counts as reported by Desai and Natarajan (1981) were 1040×10^3, 80×10^3 and 282×10^3 CFU per ml of milk from three different areas around Bangalore city. Katona and Szita (1982) have stressed the importance of coliforms as indicator of raw milk contamination and unsatisfactory cleanliness of milking equipments.

Yadava et al. (1983) did not find any significant difference in coliform counts with the season in Ranchi. Palanniswamy et al. (1988) found that the farm utensils were the major source of the contamination of milk with coliform organisms, followed by personnel and teat ends. When sanitary practices were followed in the farm, the

individual cow's milk had four coliforms per ml and pooled milk had 30 coliforms per ml whereas it was 10 and 39000 per ml, before the sanitary practices were applied.

Coliforms other than Escherichia coli were found in 4 to 35 per cent of raw market milk samples from Kaliobia Governorate (Morgan et al., 1989). The count of coliform colonies averaged $10.99 \times 10^{11} \pm 9.34$ per ml. Hamama and EL-Mouktafi (1990) reported that total coliform count and faecal coliform count in raw milk collected from regional milk collection centres in Morocco were 1.8×10^5 and 7.5×10^3 CFU per ml, respectively. Rai et al. (1990) studied bacteriological quality of milk in Kanpur city. The average coliform count in milk from University dairy farm, Milk board, hawkers and Town dairies were 2437.5, 512.5, 21337.5 and 6412.5 CFU per ml, respectively. Patel et al. (1993) evaluated 21 samples of milk from buffaloe in Research Unit, Veterinary College, Anand and reported a mean coliform count of $1.9 \times 10^3 \pm 0.57$ CFU per ml.

The least square mean coliforms count of normal milk from cows was 28 ± 5.11 CFU per ml (Reddy et al., 1994).

Singh et al. (1994 a) studied bacteriological quality of raw milk and reported that the incidence of coliforms in the samples was 100 per cent. They found that the mean $\log_{10}$ number of coliforms in the samples was 4.447 CFU per ml.

Fecal-streptococcal count

Fecal-streptococci are consistently present in large numbers in faecal excreta. The presence of this organism in food is a useful indicator of the possible presence of enteric pathogens and their detection help in assessing the standard of hygiene followed during production.

In a study Pandey and Mandal (1980) reported the yield of streptococci in 7.5 per cent of raw milk samples collected from milk supply scheme, Patna.

The effect of season on faecal streptococcal count in milk from three different sources in Ranchi town was found to be significantly different (Yadava et al., 1983). Shah et al. (1984) studied the incidence of streptococci in milk obtained from 134 healthy cows and reported the presence of streptococcus spp. from 17 per cent of samples.

Hamama and EL-Mouktafi (1990) studied the hygienic quality of raw milk produced in Morocco by analysing 42 milk samples from regional collection centre and reported that average faecal streptococcal count was 1.2×10^4 CFU per ml of milk.

Staphylococcal count and *Staphylococcus aureus* count

Man is the main reservoir of staphylococci. The organism is located mainly in the nose and the most common

skin sources are the arms, hands and face. They may also be found in the eyes, throat and intestinal tract. From these sources the organisms find their way into air, dust and onto clothing from where they may contaminate the foods. The two most important sources of this organism to food are, nasal carriers and the individual whose hands and arms are inflicted with boils and carbuncle and who are permitted to handle foods.

It should be noted that most of the domesticated animals harbour staphylococci and staphylococcal mastitis is common among domesticated animals. It appears at least in low numbers, in any or all food products that are of animal origin or in those which are handled directly by man, unless heat processing steps are applied to effect their destruction. The presence of this organism in milk is reported by certain workers. Mustafa and Idris (1976) examined 113 milk samples collected from vendors of three towns and reported that 13 had Staphylococcus aureus. Pandey and Mandal (1980) studied the bacterial flora of raw milk samples collected from milk supply scheme, Patna. They found that 47.5 per cent of samples had staphylococci.

Coagulase positive staphylococci were found in 75 and 61.2 per cent raw milk samples from Polish state farms and private farms respectively (Milko et al., 1981). Shah et

al. (1984) studied the bacterial flora of milk from 134 healthy cows. It was observed that the incidence of Staphylococcus epidermidis was maximum (42%) followed by Staphylococcus aureus (20%).

Popovic et al. (1991) studied the hygienic quality of milk in Zajecar, Timok region of Serbia (Yugoslavia). He reported that 20 per cent of samples had coagulase positive staphylococci. Rajmany et al (1989) reported that total staphylococcal count varied from 20.5×10^3 to 104×10^3 CFU per ml, with an average count of 63.5×10^5 CFU/per ml. Hamama and EL-Mouktafi (1990) analysed raw milk obtained from regional collection centres in Morocco and reported that Staphylococcus aureus count averaged 4×10^4 per ml.

The normal milk of cows had a staphylococcal count of 58.5 ± 18.82 CFU per ml (Reddy et al., 1994).

Escherichia coli count

Escherichia coli are commonly found in the intestinal tract of man and animals. Their presence in food, particularly in large numbers, is taken to indicate faecal pollution or contamination. The presence of intestinal organisms in food indicate the possibility that etiological agent of intestinal disease may be present in such food. The occurrence of this organism in milk indicate lack of hygiene.

Few workers reported the presence of Escherichia coli in raw milk. Singh and Ranganathan (1978) studied the incidence and distribution of E. coli in milk and of the 128 samples of raw milk tested 92 were found positive for E. coli. Ten per cent of raw milk samples from milk supply scheme, Patna, yeilded E. coli (Pandey and Mandal 1980). E. coli titre of 100 raw milk samples from Polish state farms and 72 raw milk samples from private farms was investigated by Milko et al. (1981) and reported that it varied from 0.0001 to 0.00001 and 0.001 to 0.0001 respectively.

Katona and Szita (1982) stressed the importance of E. coli as an indicator for bacterial contamination of raw milk and cleanliness of milking equipments. Morgan et al. (1989) reported the presence of E. coli in 27 per cent of raw market milk samples obtained from Kaliobia Governorate. Othenhajmer (1989) studied Escherichia coli contamination as an indicator of hygienic condition in milk production. He found that seven per cent of hand milked samples, 89 per cent of machine milked samples, 20 per cent of can stored and 43 per cent of basin stored milk leaving the dairy were contaminated, but the percentage decreased to 35 when more hygienic procedures of milk production were practiced.

Popovic et al. (1991) detected E. coli in 81 per cent of milk samples collected from industrial plant and small scale producers in Timok market area in Yugoslavia.

Other bacterial counts

The average thermoduric and psychrophilic counts in dairy farm milk, city market milk and in milk from rural collection centre were 5.2×10^4 and 7.7×10^4; 4.0×10^5 and 2.9×10^6; 3.4×10^5 and 5.0×10^6 per ml, respectively (Jain and Saraswat, 1968).

Milk samples, collected from milk supply scheme, Patna, yielded Micrococci in 17 per cent, Bacillus spp. in 65 per cent, Alcaligenes, Pseudomonas, Providence and Citrobacter spp. in 1, 2, 2 and 2.5 per cent of samples (Pandey and Mandal 1980). Shah et al. (1984) studied the bacterial flora of milk from 134 healthy cows. They observed the incidence of Bacillus spp., gram negative bacilli and Micrococcus in 7.0, 3.9 and 1.3 per cent of samples, respectively. Das and Nag (1986) examined 162 raw milk samples collected from vendors and reported the isolation of two strains of Salmonella typhimurium and three strains of Salmonella paratyphi B.

Hamama and El-Mouktafi (1990) reported the presence of salmonella in 21 per cent of raw milk samples and Yersinea enterocolitica in 40.4 per cent of samples. The average Bacillus cereus count reported from raw milk was 5.7×10^2 per ml.

Bacterial quality and grading of milk:

The bacterial count of milk is an index of the sanitary quality of milk. A low count does not necessarily mean that pathogenic organisms are absent. A high count certainly indicates that the milk has either come from a diseased udder or has been handled under undesirable conditions or has been kept warm enough to permit the growth of bacteria. In the first two cases there are greater chances that milk may contain undesirable organisms. In the last case such undesirable organisms having got entrance to the milk, there is greater chance of it growing to more dangerous proportions. Hence the necessity of grading milk on the basis of the bacterial count. The requirements for the different grades of milk vary according to the standards set up by the local public health authorities, at national and international levels. For raw milk, a widely adopted standard for grade A or Grade I raw milk is the standard plate count of less than 1×10^5 /ml and this is for raw milk intended for heat treatment before consumption. But for raw milk to be consumed directly, a more stringent standard is required (Yadav et al., 1993). In North America standard plate count of less than 3×10^6 /ml or equivalent is acceptable for manufacturing grade milk, but in UK no distinction is made between raw milk going for manufacture and that for liquid consumption. According to International

Dairy Federation (IDF), standard plate count of less than 10000 per ml for raw milk reflects good hygienic practices during production.

In India, the Bureau of Indian Standards (BIS) prescribes bacteriological standard for raw milk and its products. According to IS : 1479 (Part III) 1977 the standard plate count not more than 200000, between 200000 and 1000000, 1000000 and 5000000 and above 5000000 CFU per ml are recommended for grading of milk as very good, good fair and poor respectively. The standards also state that satisfactory raw milk should be free from coliforms count in 1:100 dilution.

The United States Department of Agriculture (USDA) / Federal Drug Administration (FDA) standards specified that the maximum standard plate count per ml of raw milk (pick-up) is 1×10^5 and raw milk (co-mingled) is 3×10^5 (Yadav et al., 1993).

Microbiological specifications given by the Military Federal Purchases specifies that the standard plate count of milk (fresh) should not be more than 2×10^4 per ml and coliforms count should not be more than 10 /ml (Powers, 1976).

Milk sample from the organised dairy farm vendor, sweet meat shop and dairy plant were tested for its

bacterial quality and catogorised into four grades (Misra and Kuila, 1989). Th per cent of milk graded as good, fair, poor and very poor was 11.3, 37.3, 36.7 and 14.7, respectively. They considered the count in range of 2 x 10^3 to 19 x 10^6 as good to very poor. Singh et al. (1994 a) analysed the bacterial quality of raw milk and catogorised the samples based in standard plate count prescribed by BIS (1969). They found that 28.75, 14.28, 21.43 and 37.73 per cent samples fell into very good, good, fair and poor grade, respectively.

Isolation of Staphylococcus aureus

Various workers have isolated and characterised staphylococci.

Mohan and Misra (1967) reported the isolation of both coagulase positive and coagulase negative staphylococci from milk supplied to Patna milk supply scheme. From 200 samples examined, 33 coagulase positive and 38 coagulase negative staphylococci were isolated.

Garg et al. (1977) isolated Staphylococcus aureus from a significant proportion of raw market milk. Butkus et al. (1978) isolated staphylococcal spp. from bulk milk and milk for Rossiski cheese manufacture. Out of the 97 isolates of staphylococci, 84 were coagulase positive.

Bacteriological quality of raw buffalo milk marketed in and around Patna was studied by Kumar et al. (1978 b). From 240 milk samples 144 staphylococcal isolates were recovered. Kostadimov (1980) examined milk samples collected from cows and out of 73 isolates from the samples, 54 were Staphylococcus aureus. Terayama et al. (1980) reported the isolation of S. aureus from 112 of 170 milk samples collected from milk tanks and in 34 of 52 samples from storage tank of seven dairy plants. All 202 isolates coagulated rabbit plasma and fermented mannitol. In an investigation, Araujo (1984) isolated S. aureus in 50 out of 100 raw milk samples examined on receipt at pasteurization plant in Sau Paulo, Brazil. S. aureus was isolated from raw milk samples and 18 of 94 isolates produced enterotoxin A, B and C (Blostridge and Roth, 1985).

The isolation of Staphylococcus aureus from 19.04 per cent of pathogenic flora in raw market milk samples was reported by Yadava et al. (1985). Ahmed et al. (1988) investigated the occurrence of staphylococci in raw milk. They found that 36.4 per cent of the samples yielded S. aureus. Dedyukhina and Lyakhova (1988) reported the isolation of staphylococci from 68 samples of raw milk collected from stall and from bulk. Of the 272 isolates 26 showed the characteristics of toxigencity and a further 13 isolates had properties indicating that they were S. aureus.

Filho et al. (1988) examined 20 samples of raw bulk milk, collected from a dairy processing milk obtained from 17 dairy herds in Sao Paulo state and reported that all samples yielded S. aureus and the mean count was 1190 per ml. Two of the 99 isolates of S. aureus produced enterotoxin B.

Coagulase positive staphylococci were isolated from all the raw milk samples examined by Rajmany et al. (1989). Of the 178 apparently healthy cow's milk samples, 24.1 per cent yielded S. aureus. Among the isolates 42.8 per cent were coagulase positive, 50 per cent of which showed severe enterotoxigenic effect in kittens (Sen et al., 1989). Popovic et al. (1991) reported the isolation of coagulase positive staphylococci from 20 per cent of milk samples collected from industrial plants and small scale producers in Timok market area in Yugoslavia.

Ombui et al. (1992) isolated S. aureus from 183 of 300 raw milk samples. Seventy two of 97 isolates assayed for the production of enterotoxins were found to be positive. They reported that raw milk is a potential source of enterotoxigenic S. aureus. Rahman et al. (1992) investigated occurrence of pathogenic bacterial flora in raw market milk in Gawahati city and reported staphylococci (56.13 %) were the chief bacterial flora isolated.

Masud et al. (1993) reported that 48 of the 85 isolates of S. aureus were enterotoxigenic. The isolates produced enterotoxin A, B, C and D.

The presence of S. aureus in 18.71 per cent of market milk of cows and buffaloes was reported by Gill et al. (1994). Of the various bacterial isolates of cow's milk; 12.24 per cent were S. aureus but the per cent of S. aureus isolates from buffaloes milk was 15.09.

Singh et al. (1994 b) carried out bacteriological analysis of milk samples and reported the isolation of S. aureus.

Isolation of Escherichia coli.

Garg et al. (1977) isolated Escherichia coli from raw market milk. Johnston et al. (1983) reported that 10.6 per cent of milk samples collected from 998 farms in West of Scotland were contaminated with coliforms from which E. coli were isolated and identified. Yadava et al. (1985) reported the isolation of E. coli from 78 per cent of raw milk samples. Rahman et al. (1992) isolated six strains of E. coli from raw milk marketed in Gawahati city and reported that the isolates belonged to four different sero groups.

Sharma and Joshi (1992) collected 69 samples of milk from Ludhiana market, and analysed its bacteriological quality. They reported the isolation of E. coli from nine samples and suggested that the presence of these organisms in milk might be due to contamination through containers, water or due to unhygienic handling. Gill et al. (1994) examined market milk of cows and buffaloes and reported the isolation of various bacteria. The per cent of E. coli isolates from cows and buffaloes milk was 10.20 and 7.55 respectively. Singh et al. (1994 b) reported the isolation of E. coli from milk samples.

Other bacteria isolated

Various other organisms were isolated from milk by different workers.

Garg et al. (1977) isolated pathogenic organisms from raw milk, among the isolates were Salmonella hvittingfoss, Shigella spp. Klebsiella, Bacillus cereus, Proteus spp. and Enterobacter Citrobacter spp. Kumar et al. (1978 b) isolated Micrococcus spp, Streptococcus spp, Bacillus spp., Coliforms, Proteus, Pseudomonas aeruginosa and Aeromonas spp, from 240 samples of raw buffalo milk marketed in Patna. Devriese and Keyser (1980) studied the prevalence of different species of coagulase negative staphylococci in milk samples collected from dairy cows and reported the

presence of Staphylococcus xylosus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hyicus sub spp. chromogenes and Staphylococcus simulans.

Kostadimov (1980) reported the isolation of bacteria from milk of cows and the isolates consisted of Staphylococcus epidermidis, Staphylococcus dysagalactiae, Corynebacterium spp. and Bacillus cereus. Yadava et al. (1985) reported the isolation of Streptococcus lactis, Streptococcus cremoris, Streptococcus fecalis, Staphylococcus epidermidis, Micrococcus spp., Proteus mirabilis, Providence spp., Pseudomonas aeruginosa, Corynebacterium bovis and Corynebacterium pyogenes from raw milk and pasteurized milk. Rahman et al. (1992) isolated Klebsiella spp. and Enterobacter spp. each from 12.2 per cent of raw market milk samples in Gawahati city. Sharma and Joshi (1992) reported isolation of Klebsiella and Pseudomonas from milk marketed in Ludhiana.

ANTIBIOGRAM

The indiscriminate use of antibiotics for farm livestock, particularly at sub therapeutic levels and also its use as growth promoter along with feed or for prophylaxis, posed certain hazards to human and animal health. The most important hazard caused by such use of

antibiotics is the increase in the resistance of bacteria of animal origin to one or more antibiotics. The resistance acquired by the organism can be transferred from one bacterium to another as genetic elements on plasmids. Food products containing such bacteria, particularly pathogenic organisms, can cause disease in human beings and the treatment of such disease is a great problem. Thus the test of antibiotic sensitivity of organism isolated from livestock product is of great public health importance. A few investigators have reported the antibiogram of staphylococci and Escherichia coli isolated from various sources.

Butkus et al. (1978) reported that 97 isolates of staphylococci from milk and milk products were sensitive to neomycin, monomycin and erythromycin, but resistant to streptomycin, tetracycline, levomycetin and penicillin. Kumar et al. (1978 a) studied antibiotic sensitivity pattern of staphylococci and micrococci isolated from healthy and subclinical bovine udders. They reported that 28.3 per cent of staphylococcal isolates were resistant to pencillin.

Gupta et al. (1979) studied in vitro-sensitivity of Staphylococcus aureus isolated from mastitis milk, milk products, meat and human sources and reported that

nitrofurantion inhibited the growth of 99.36 per cent of the isolates while streptomycin inhibited the growth of 96.82 per cent of isolates. Chloramphenicol and tetracycline inhibited the growth of 97.45 and 94.9 per cent of the isolates respectively. Penicillin-G and nalidixic acid inhibited growth of 72.61 and 44.58 per cent of Staphylococcus aureus, respectively Sulphathiazole was found to be the least effective drug.

In vitro sensitivity test of S. aureus isolates from mastitic milk revealed that chloramphenicol was the most effective drug with 94.44 per cent sensitivity, while the percent of sensitivity to pencillin was zero (Rao et al., 1982).

Rahman and Baxi (1983) reported that there was no significant difference in the susceptibility pattern of Staphylococcus aureus and Staphylococcus epidermidis isolated form clinical and sub-clinical cases of mastitis in cows tested against nine antimicrobial agents. The isolates showed a high susceptibility to neomycin, followed by chloramphenicol and nitrofurantion, but showed high resistance to penicillin and streptomycin. The isolates which were resistant to streptomycin were also resistant to penicillin.

Sreedharan (1983) reported that more than 90 per cent of staphylococci isolated from the clinical cases of bovine mastitis were found resistant to ampicillin, penicillin and 80 per cent showed resistance to tetracycline, streptomycin and erythromycin. Mathew (1986) reported the emergence of drug resistant staphylococci causing mastitis. Mackie et al. (1988) studied the antibiotic sensitivity of Staphylococcus aureus isolated from clinical and sub-clinical cases of mastitis for over a period of four years. They recorded that all isolates were highly sensitive to cephaloridine and cloxacillin through out the study and that a very high percentage of isolates were sensitive to novobiocin in the two years period that was tested. Erythromycin, neomycin and tetracycline were effective with little variation over four years. Ampicillin and Penicillin G inhibited growth of 62 and 55 per cent of isolates, in 1984, but in 1986 figures dropped to 19 and 33 per cent, respectively and then returned to sensitivity level observed in 1984.

Bansal et al. (1990) studied antibiogram of staphylococcal isoates from cows and buffaloes suffering from sub clinical mastitis and reported that 89.34, 85.24 and 59.84 per cent of the isolates were sensitive to cloxacillin, cholaramphenicol and penicillin respectively.

Buragohain and Dutta (1990) studied the antibiotic sensitivity of 131 S. aureus isolates from the milk of cows suffering from subclinical mastitis and reported 15, 7.87 , 1.52 and 4.58 per cent of isolates were resistant to penicillin-G, cloxacillin and gentamicin and chloramphenicol.

Harne et al. (1990) reported that 77.77 and 44.44 per cent staphylococcal isolates from milk of cows with sub clinical mastitis were sensitive to gentamicin and penicillin respectively. While 50 and 20 per cent isolates from the milk of cows with clinical mastitis were sensitive to genetamicin and penicillin respectively.

Saxena et al. (1993) isolated different bacteria from milk obtained from cows with subclincial mastitis. The predominant bacterial isolates was staphylococci. The in vitro antibiotic sensitivity test of the isolates found that cholaramphenicol was the most effective drug with 98.62 per cent sensitivity. The sensitivity of the isolates to gentamicin was 96.55 per cent. Pencillin - G was the least effective chemotherapeutic agent and its sensitivity varied between 57.24 and 62.75 per cent.

Umoh et al. (1990) studied resistance pattern of 248 staphylococcal isolates from milk and milk products to eight antimicrobial agents. A significant percentage of isolates

from raw milk were resistant to erythromycin, sulphafurazole, cloxacillin, penicillin-G and streptomycin, as compared to isolates from fermented milk.

Ogawa and Endo (1991) determined the sensitivity of 124 Staphylococcus aureus isolates from milk against eight antibiotics. None of the isolates were sensitive to methecillin. The Minimum Inhibition Concentration (MIC), of benzylpenicillin, cloxacillin, cefalonium, cefuroxime, streptomycin, kanamycin, erythromycin and oxytetracycline ranged from 0.14 to 7.69 µg/ml. Singh et al. (1994 a) reported that all the Staphylococcus aureus isolated were sensitive or intermediate sensitive to tetracycline, kanamycin, streptomycin and neomycin.

Singh et al. (1994 b) reported that Staphylococcus aureus from raw milk samples showed maximum resistance against amoxycillin followed by pencillin. High sensitivity against chloramphenicol was also observed. All S. aureus isolates were resistant to either one or more drugs under investigation.

Some workers have studied antibiotic sensitivity of Escherichia coli isolates from different sources. Fein et al. (1974) found strong association between E. coli isolated from farm animals and their attendants in respect to

antibiotic resistance. Mc Donald et al. (1977) studied antibiotic sensitivity of E. coli isolates from bovine udder infection and reported that 100 per cent isolates were sensitive to gentamicin and 92 per cent of organisms were found susceptible to carbinicillin.

Coates and Hoopes (1979) reported that 32 per cent of E. coli isolates from bovine enteric infections were sensitive to ampicillin and the percent of sensitivity of porcine isolates was 42. The corresponding per cent of sensitivity of bovine and porcine isolates to carbiniclin was 31 and 47. All bovine and porcine isolates showed 100 per cent sensitivity to gentamicin.

Raw milk samples were examined to assess the total aerobic plate count and percentage of bacteria resistant to seven antibiotics (Hankin et al., 1979). A significant negative correlation was found between the total aerobic plate count in milk and concentration of bacteria showing resistance to each of the antibiotics tested. Three of 42 gram negative isolates were capable of transferring their antibiotic resistance to E. coli.

Jackson (1981) studied antibiogram of E. coli. isolates from farm animals and reported that 10.9 per cent and 27.8 per cent of the isolates shgowed resistance against furuzolidone and ampicillin, respectively.

Tripathy and Soni (1981) studied the antibiotic sensitivity of E. coli. isolates from cases of neonatal calf diarrhoea. They reported that 69.7 per cent of E. coli were sensitive 18.18 per cent were intermidiately sensitive and 12.12 per cent were resistant to ampicillin.

Johnston et al. (1983) reported the presence of antibiotic resistant Escherichia coli in 10.6 per cent of milk samples. The incidence of antibiotic resistant Escherichia coli in milk was higher when cattle were housed day and night than when they were out doors.

Mir Shams U-Din et al. (1989) studied sensitivity of E. coli isolates from rectal swabs of neonatal kids with entric collibacillosis. They reported 100 per cent sensitivity of the isolates to gentamicin but the per cent sensitivity of the isolates to ampicillin was 20.

Kulshrestha (1990) reported that 88 per cent of E. coli isolated from milk products were resistant to ampicillin.

Materials and Methods

MATERIALS AND METHODS

In the present investigation a total of 84 raw milk samples, consisting of 28 each from University Livestock Farm, Mannuthy (S_1), Ollukkara Ksheera Vyavasaya Co-operative Society (S_2) and Panannchery Ksheera Udpathaka Sahakarana Sangham (S_3), were examined to determine their bacteriological quality. The samples were also tested for the isolation and identification of bacterial pathogens such as, Staphylococcus aureus and Escherichia coli. Every isolate was tested against five commonly used antibiotics.

Collection of raw milk samples

Milk samples were collected from University Livestock Farm, mannuthy (S_1), Ollukara Ksheera Vyavasaya Co-operative Society (S_2) and Pannanchery Ksheera Udpathaka Sahakarana Sangham (S_3). From each of the above sources, 21 individual and seven pooled samples were collected for investigation. At a time three individual and one pooled sample were collected from a centre. Each sample consisted of 250 ml. Replicate samples were collected from each centre seven times during February to June 1995.

From S_1 source, the individual samples were collected randomly from the milk of each animal immediately after

milking, in a sterile 500 ml flask and pooled samples were collected after pooling the milk of ten animals. The samples were brought to laboratory within 15 minutes after collection and processed for bacteriological examination. The milk brought by every member of Ollukkara Ksheera Vyavasaya Co-operative Society (S_2) and Panannchery Ksheera Udpathaka Sahakarana Sangham (S_3) is considered as individual animal's milk. From the S_2 and S_3 sources, the individual samples were collected randomly from the milk brought by its members and pooled samples were collected after pooling the milk brought by 10 members.

Two hundred and fifty ml of each milk samples thus collected were immediately brought to laboratory in thermocool containers and processed for further bacteriological examination.

Preparation of raw milk samples

Every sample brought to the laboratory was individually processed for bacteriological examination. Each sample was thoroughly mixed for uniform dispersion of bacteria present in it. Aseptic conditions were followed through out the preparation of the sample. Serial dilution of each sample was made following the procedure described in IS: 5401 (1969).

From a mixed sample, 11 ml of milk was transferred into 99 ml of sterile 0.85 per cent sodium chloride solution (NSS) with a pipette so as to make one in 10 dilution. Then the diluted sample was mixed thoroughly, one ml was transferred into another test tube containing nine ml sterile 0.85 per cent sodium chloride solution (diluent), so as to form one in 100 dilution. From this further 10 fold serial dilutions were made. From each sample, selected dilution and/or undiluted raw milk was used for further bacteriological examination.

Bacterial count

The samples were inoculated into specific culture media by pour plate method for estimating total viable count and by surface plating, for coliform at 37°C and 44°C, staphylococci, streptococci, Staphylococcus aureus and Escherichia coli counts. Duplicate plates were inoculated. One ml of inoculum was used for pour plate method and 0.1 ml of inoculum was used for surface plating. In spread plate technique the inoculum was evenly distributed on the surface of the media with 'L' shaped glass rod. The inoculated plates were incubated at the appropriate temperature for a specified periods of time. Sterile precautions were taken throughout the bacteriological

colonies between 30 and 300 were selected for counting. The bacterial load was estimated by multiplying the mean count of duplicate plates by the dilution factor and was expressed as colony forming units per milli litre (CFU per ml) of the sample.

Total viable count

Total viable count (TVC) of each individual and pooled raw milk sample was determined according to the procedure recommended by American Public Health Association (APHA) (1976). To each of the plates containing the inoculum, about 15 to 20 ml molten, plate count agar (Hi-media), maintained at 45°C, was poured and mixed with the inoculum by gentle rotatory movements, viz, clockwise, anticlockwise, forward and backward. The plates were then left at room termperature for solidification. The inoculated plates were then incubated at 37°C for 24 h. and the TVC was estimated as mentioned earlier. All the milk samples from different sources were graded as very good, good, fair and poor quality according to IS: 1479 (1977).

Coliforms and Faecal coliforms count

Violet Red Bile Agar (VRBA) was used to estimate the coliforms and faecal coliforms present in the samples.

Plates containing media were inoculated with inoculum. These plates were incubated at 37°C for 24 h. At the end of the incubation period, purplish red colonies with a diameter of 0.5 mm or more surrounded by red percepitation zone were counted as coliforms. The total number of CFU per ml of sample was then calculated. For estimating the faecal coliform, the above procedure was followed, except that the temperature of incubation was 44°C. The method followed was that recommended by Nordic committee on Food Analysis (1966)

Faecal-Streptococcal count

Faecal streptococcal count in the individual and pooled samples was determined following the spread plate technique described by Nordic Committee on Food Analysis (1968). Sterile KF-streptococcal agar (Hi-media) was inoculated with the inoculum. These inoculated plates were incubated at 37°C for 48 h. After the period of incubation, colonies with pink to dark red colour surrounded by narrow white zone were counted as faceal streptococci and the number of CFU per ml of sample was estimated.

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Staphylococcal count

The number of staphylococci present in every individual and pooled milk sample was estimated according to Difco Manual (1977). The inoculum from each sample was inoculated on to Mannitol Salt Agar (MSA), (Hi-media). The inoculated plates were then incubated at 37°C for 24 h. At the end of incubation period smooth, circular, convex, yellow colonies were counted as staphylococci. The number of staphylococci present per ml of sample was estimated as described earlier.

Staphylococcus aureus count

Staphylococcus aureus count was determined using Tellurite Polymixin Egg Yolk Agar (TPEYA) according to the procedure described by Leninger (1976). The inoculated plates were incubated at 37°C for 24 h. After the period of incubation, only the black, circular, convex colonies having 1 to 1.5 mm diameter and with a zone of precipitation around the colony, or colonies with a clear zone or halo around the it and a white precipitate beneath the colony, or the colony with no zone or halo but precipitate beneath the colony was counted as Staphylococcus aureus and the count was estimated as described earlier.

Escherichia coli count

Escherichia coli count was carried out according to the method described by Varadraj (1993). The diluted sample was inoculated on to Eosin Methylene Blue Agar (EMB Agar). These inoculated plates were incubated at 37°C for 24 h. After the period of incubation colonies with dark centre giving a distinct indelible-ink, greenish-black metallic sheen on deflected light were counted as E. coli. The number of E. coli present per ml of sample was estimated as described earlier.

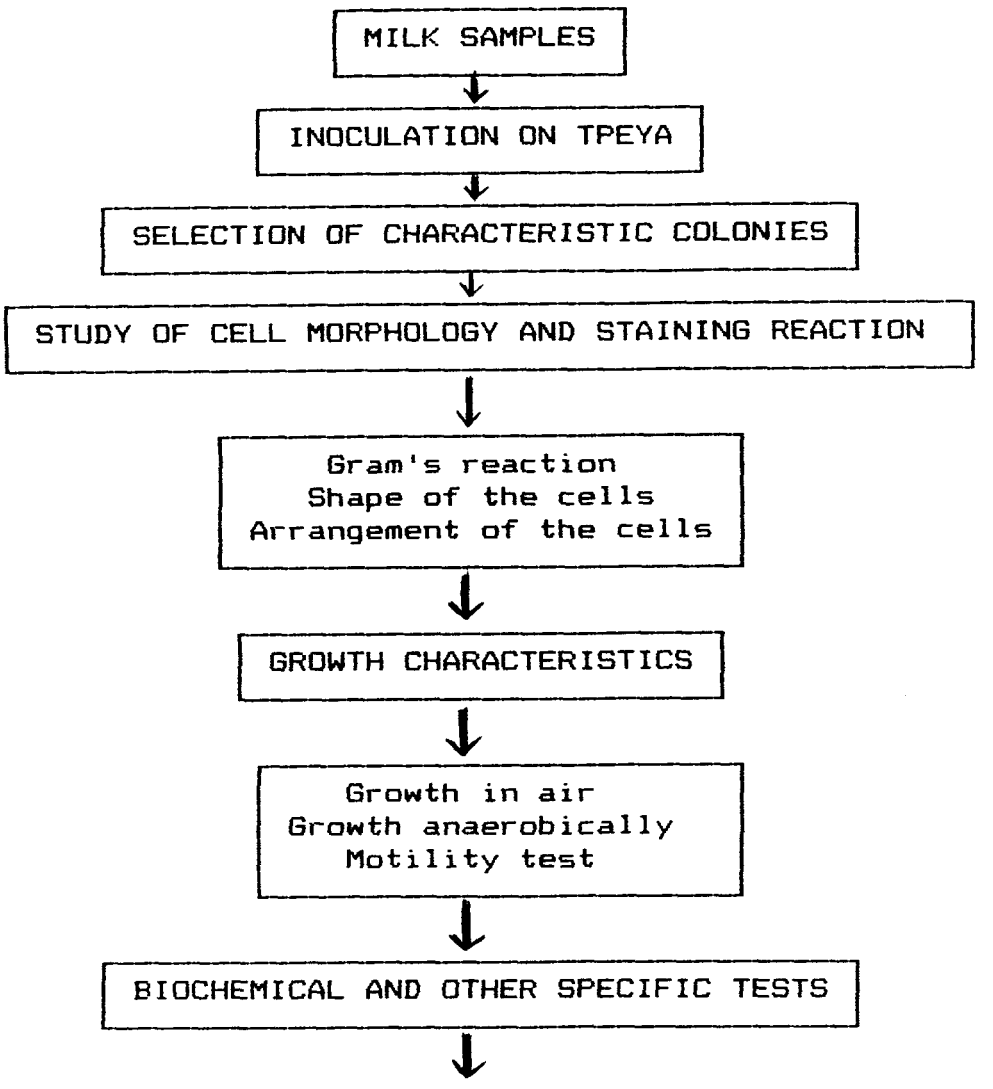
Statistical analysis

Data were subjected to analysis of variance test and critical difference test following the procedure described by Snecdecor and Cochran, 1967.

Isolation, identification and characterization of Staphylococcus aureus

From every milk sample, 0.1 ml of the undiluted and/or diluted sample was inoculated on the surface of Tellurite Polymyxin Egg Yolk Agar (TPEYA) and incubated at 37°C for 24 h. From a sample two or three individual characteristic colonies were selected and transferred onto nutrient agar slants with the help of a sterile loop. These inoculated

FLOW CHART FOR ISOLATION AND IDENTIFICATION OF
Staphylococcus aureus



tubes were incubated at 37°C for overnight. At the end of incubation period the agar slant cultures were stored in a refrigerator for further characterisation as detailed below.

Gram's reaction and cell morphology

A thin smear of each isolate was made from an overnight culture on a clean microscopic slide. This smear was stained by Gram's method as described by Cruickshank et al. (1975). The stained smears were examined under the oil immersion of a microscope to study the morphology and gram's reaction of the isolates. Gram positive, spherical cells arranged in irregular clusters resembling bunches of grapes were regarded as Staphylococci. All staphylococcal isolates were subjected to biochemical and other specific tests as decribed by Cowan (1974).

Oxidation - Fermentation (O-F) test

This test was done to find out whether the isolates attacked on sugar by oxidation and/by fermentation. The OF test was made by growing each isolate in duplicate tubes of Hugh and Leifson medium. The tubes were inoculated by stabbing with a straight wire. To one of these tubes, a layer of sterile liquid paraffin was added up to a depth of about one cm. The inoculated tubes were incubated at

37°C and examined daily for 14 days. A change in colour of the medium in open tube from blue or green to yellow indicated oxidation and the change of colour in sealed tubes indicated fermentation. No change in the colour of the medium indicated no action on carbohydrate. The motility of the organism was assessed from its growth in Hugh and Leifson's medium. Motility of the organism was indicated by spreading growth from the line of stab and the growth limited to the stab only indicated non-motility.

Catalase test

Each isolate was inoculated into nutrient broth (Hi-media) and incubated at 37°C for overnight. To this broth culture, one ml of three per cent. H_2O_2 was added and examined immediately and after five minute for gas production. Evolution of gas bubbles during the period of observation was regarded as catalase positive.

Oxidase activity

On a piece of filter paper placed in petri-dish, three drops of one per cent tetramethyl - P - phenylenediamine dihydrochloride were add . Before it could dry fresh culture of the isolate was smeared across the surface of the impregenated filter paper, with the help of inoculating

loop. Development of a dark purple colour with in 10 seconds indicated positive reaction.

Voges - Proskauer reaction

Every isolate inoculated in MR-VP broth (Hi-media) was incubated at 37°C for 48 h. At the end of incubation 0.6 ml of five per cent alpha-naphthol solution and 0.2 ml of 40 per cent KOH aqueous solution were added, test tube was shaken and kept in a slanting position. Tubes were examined after 15 minutes and one h. A positive reaction is indicated by strong red colour.

Acid from carbohydrates

The ability of the isolate to utilise sugar under aerobic and anaerobic condition was tested. The ability of the organism to utilize sugars aerobically (oxidation), was tested by inoculating it in basal medium (peptone water), containing one per cent of the appropriate sugar and incubated at 37°C. The tubes were examined daily for seven days. Oxidation of sugar and production of acid was indicated by a change in the colour of the medium from blue to yellow. To test the ability of the organism to utilise sugar under anaerobic condition (fermentation), the above

procedure was followed except that the inoculated tubes were sealed with a layer of liquid parafin to a depth of about one cm.

Arginine Hydrolysis

Five ml of Arginine broth (Hi-media) was inoculated and after incubation for 24 h at 37°C, 0.25 ml of Nessler's reagent was added. Arginine hydrolysis was indicated by the development of a brown colour.

Phosphatase test:

Phenolphthalein agar (Hi-media) was lightly inoculated to obtain discrete colonies and incubated 37°C for 18 h. Placed 0.1 ml of ammonia solution (specific gravity 0.80) in the lid of the petri-plate and inverted the inoculated medium containing discrete colonies above it. The free phenolphthalein liberated by phosphatase react with the ammonia and Phosphatase - positive colonies become bright pink.

Gelatin Hydrolysis

Inoculated every isolate to nutrient gelatin medium and incubated the inoculated tubes at 37°C for 14 days. After every 2-3 days of incubation the tubes were cooled in refrigerator, for two h and examined for gelatin liquefaction.

Coagulase test.

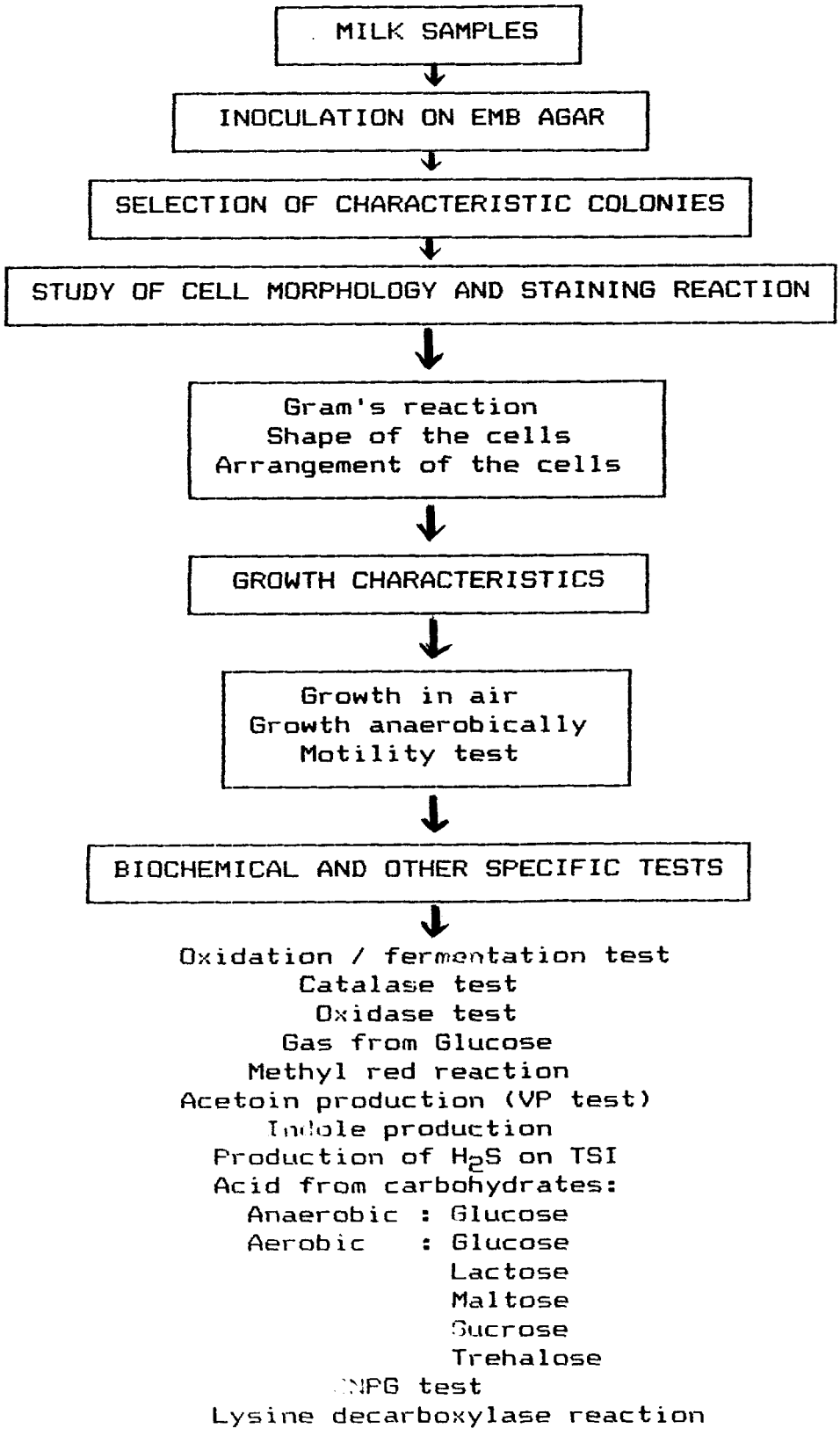
All staphylococcal isolates were subjected to both slide and tube coagulase test. In slide method, a colony was emulsified in a drop of water on a microscopic slide to produce a thick suspension with the minimum spreading. The bacterial suspension was stirred with wire which has been dipped into rabbit plasma. A positive result is indicated by macroscopical clumping with in five seconds.

In tube method 0.5 ml of undiluted rabbit plasma was mixed with equal volume of an 18 to 24 h broth culture and incubated at 37°C and examined after one h and four h. The negative tubes were left at room temperature for overnight and then re-examined for a coagulam.

Isolation, Identification and characterization of <u>Escherichia coli</u>

From every milk sample, 0.1 ml of the diluted and/or undiluted sample was inoculated on the surface of Eosin Methylene Blue Agar (EMB Agar) and incubated at 37°C for 24 h. From a sample, two or three individual characterstic colonies were selected and transferred onto nutrient agar slants using a sterile loop. These inoculated tubes were incubated at 37°C for overnight. At the end of incubation period the agar slant cultures were stored in a refrigerator for further characterization as follows.

FLOW CHART FOR ISOLATION AND IDENTIFICATION OF *Escherichia coli*



Gram's reaction and cell morphology, growth characteristic, Oxidation-Fermentation (OF) test, catalase test, Voges-Proskauer reaction and prodution of acid by utilisation of carbohydrate were studied following the procedure described earlier in the isolation, identification and characterization of Staphylococcus aureus. Other tests performed were:

Methyl red reaction

Every isolate was inoculated into MR-VP broth (H1-meida) at 37°C for two days. After the period of incubation, two drops of methyl red solution was added into each tube, shaken and examined. Methyl red positive reaction is indicated by the development of a red colour in the medium.

Indole production

A 48 h old, nutrient broth culture of the organism was taken in a test tube and mixed with one ml xylol. To this tube, 0.5 ml of Ehrlich's reagent was run down through its side. Development of a pink or red colour in the solvent indicated the producion of indole.

Hydrogen Sulphide production

The tubes of triple sugar iron agar were inoculated with the organism by stabbing the butt and streaking the slope. The tubes were observed daily up to seven days for blackening due to H_2S production. No change in colour along the stabb and streak line indicated negative results of the test.

Decarboxylase reaction

Every isolate was inoculated into a test tube containing Moller's decarboxylase broth (Hi-media) with one per cent L-lysine hydrochloride and a tube with out L-lysine hydrochloride. The inoculated tubes were incubated at 37°C for four days. First the media becomes yellow then turns to violet, indicating decarboxylation. The tubes with out L-lysine (control) remains yellow.

ONPG test

Tubes of ONPG broth (Hi-media) were inoculated and incubated for 24 h. Appearence of yellow colour due to O-nitrophenol indicated ß-galactosidase activity.

Antibiotic Sensitivity Test

Every isolate identified as Staphylococcus aureus and Escherichia coli was tested against five different

antimicrobial agents, to detect their sensitivity to these agents, by agar diffussion method, as per the procedure described by Barry (1976). Staphylococcal isolates were tested against cloxacillin, gentamicin, amoxycillin, chloramphenicol and penicillin-G, while Escherichia coli isolates were tested against gentamicin, ampicillin, furazolidone, carbenicillin and doxycycline antibiotic discs.

Preparation of Mac Farland standard

The turbidity standard solution was prepared by adding 0.5 ml of 0.048 M $BaCl_2$ (1.175%, w/v. $BaCl_2 . 2H_2O$) to 99.5 ml of 0.36 N H_2SO_4 (1% v/v). This solution is equal to half the density of No. 1 Mac Farland standard solution. This solution was taken into glass tube, sealed tightly and kept in the dark, at room temperature for further use. The tube was vigourously agitated just before each use.

Preparation and standardization of inoculum

Three to four well isolated colonies were selected from a pure culture and transferred into sterile nutrient broth (Hi-media) and incubated at 37°C, overnight. The turbidity of culture was adjusted using solution having half the density of Mac Farland standard No. 1. When the broth

culture was found to be more turbid, it was diluted with nutrient broth and when the turbidity was found to be less, culture was incubated for more time to achieve the required turbidity.

Inoculation

The swab was dipped into standardized inoculum and excess inoculum was removed from the swab by rotating it several times with a firm pressure on the inside wall of the inoculum containing test tube, above the fluid level. The nutrient agar was inoculated by streaking the swab over its entire surface, with in 15 minutes after adjusting the density of inoculum.

The streaking procedure was repeated two more times, rotating the plate approximately 60°C at each time, so as to ensure an even distribution of inoculum.

Application of antibiotic discs

The inoculated plate was left for not more than 15 minutes at room temperature to absorb any excess surface moisture before applying the drug - impregnated discs. The discs were applied to the surface of the inoculated agar with a sterile forceps. With the tip of forceps, each disc was gently pressed down to ensure complete contact with the

agar surface. During the application of discs care was taken not to place it closer than 15 mm from the edge of the plate and the distance between the centre's of two such discs was not less than 24 mm. The inoculated plates were inverted and incubated at 37°C for 18 hours, within 15 minutes after the application of the discs.

Reading and Interpretation

At the end of the incubation period, the plates were examined and the diameter of the zones of complete inhibition was measured to the nearest whole millimeter with a scale held on the back of the petri-plate which was illuminated with a reflected light.

The zone of inhibiton of each disc was measured in three different directions keeping the mid point of disc as the centre of the zone. The mean of the measurement of inhibition was used for the interpretation of the results. The interpretation of the results was made by comparing diameter of the zone of inhibition with standard zone of inhibition chart provided by the disc manufacturing company (Hi-media) and isolates were grouped as sensitive, intermediary sensitive and resistant, against each antibiotic.

Results

RESULTS

In the present investigation, individual and pooled milk samples were collected from Kerala Agricultural University Livestock Farm, Mannuthy (S_1), Ollukkara Ksheera Vyavasaya Co-operative Society (S_2) and Pannancherry Ksheera Udpathaka Sahakarana Sangham (S_3). From each of the above surces 21 individual and seven pooled samples were collected. The bacteriological quality of samples were assessed on the basis of various bacterial counts and the isolation and identification of Staphylococcus aureus and Escherichia coli. The antibiogram of these isolates were also studied.

BACTERIAL COUNTS OF INDIVIDUAL MILK SAMPLES

The mean CFU per ml of individual milk samples obtained from S_1, S_2 and S_3 sources are shown in Table 1 and the analysis of variance of the counts is shown in Table 2. Analysis of variance of the total viable count of samples obtained from S_1, S_2 and S_3 sources showed highly significant difference ($P < 0.01$). Critical difference test (Table 3) revealed that the counts in the samples from S_2 and S_3 were significantly ($P < 0.01$) more than that of the count of samples from S_1. Of the 21 individual samples from S_1, 16 (76.20 %) had a count of 10^4 per ml. In three

Table 1. Mean bacterial count of individual samples collected from S_1, S_2 and S_3 sources

Bacterial counts	Mean colony forming unit per ml of samples from		
	S_1	S_2	S_3
Total viable count	7.5×10^4	1.4×10^5	2.0×10^5
Coliforms count at 37°C	2.4×10^1	4.8×10^4	3.8×10^3
Coliforms count at 44°C	2.2×10^1	2.4×10^4	2.4×10^3
Faecal streptococcal count	1.5×10^2	2.1×10^3	1.7×10^3
Staphylococcal count	5.7×10^2	2.8×10^3	6.8×10^3
Staphylococcus aureus count	8.5×10^1	1.8×10^2	7.1×10^1
Escherichia coli count	2.0×10^2	1.2×10^4	1.5×10^3

Table 2. ANOVA of different bacterial counts of individual samples from S_1, S_2 and S_3 sources

Degree of freedom		TVC	Coliforms at 37°C	Coliforms at 44°C	Faecal Streptococci	Staphylococci	*S. aureus*	*E. coli*
Treatment	2	1.938	74.261	46.076	5.411	8.214	1.748	24.040
Error	60	0.172	0.697	1.725	0.653	1.048	1.476	0.787
F value		11.28**	106.544**	26.705**	8.279**	7.835**	1.184	30.562**

** P<0.01

Table 3. Critical difference test of different bacterial counts of individual samples from S_1, S_2 and S_3 sources

Source of samples	Mean bacterial counts in milk (Mean ± SE in $\log_{10}$ CFU per ml)						
	T V C	Coliforms at		Faecal streptococci	Staphylococci	S. aureus	E. coli
		37°C	44°C				
S_1	4.503 ± 0.098[a]	0.655 ± 0.2007[a]	0.465 ± 0.2116[a]	1.984 ± 0.1527[a]	2.142 ± 0.274[a]	1.117 ± 0.244[a]	1.489 ± 0.257[a]
S_2	4.950 ± 0.098[b]	4.296 ± 0.139[b]	3.045 ± 0.397[b]	2.743 ± 0.229[b]	3.147 ± 0.194[b]	1.269 ± 0.292[a]	3.566 ± 0.189[b]
S_3	5.083 ± 0.0741[b]	3.290 ± 0.2007[c]	3.016 ± 0.2094[b]	2.947 ± 0.1309[b]	3.289 ± 0.192[b]	0.730 ± 0.255[a]	2.973 ± 0.098[c]
Critical difference	0.2559	0.5152	0.8106	0.4987	0.6318	0.7498	0.5475

Figures with the same superscript within the column do not differ significantly.

(14.28%) samples the count was 10^5 per ml and in two (9.52%) it was 10^3 per ml.Eleven (52.38 %) samples from the S_2 source had a count of 10^5 per ml but in 10(47.62%)samples the count was 10^4 per ml. Amoong the samples obtained from S_3 source 12 (57.14 %) had a count of 10^5 per ml and in remaining nine (42.86 %) samples the count was 10^4 per ml.

Highly significant difference ($P < 0.01$) in coliforms count at 37°C was observed in the samples collected from S_1, S_2 and S_3 sources. Critical difference test revealed a highly significant difference ($P < 0.01$) in the mean counts between the samples of S_1 and S_2 ; S_1 and S_3; and S_2 and S_3. Eight (38.1 %) samples collected from S_1 did not yield coliforms. Twelve ((57.14%)samples revealed a count of 10 per ml, whereas in one (4.76 %) sample the count was 10^2 per ml. All the samples collected from S_2 source yielded coliforms at 37°C. Of the samples, nine (42.86 %), eight (38.1 %) and four (19.04 %) had coliforms count of 10^4 per ml, 10^3 per ml and 10^5 per ml, respectively. Except one (4.76%) all the samples from the S_3 source revealed the presence of coliforms at 37°C. The counts in 17 (80.96 %), two (9.52 %) and one (4.76 %) samples was 10^3 per ml, 10^2 per ml and 10^4 per ml respectively.

Highly significant difference ($P < 0.01$) in faecal coliforms count at 44°C of incubation was observed in the samples obtained from S_1, S_2 and S_3 sources. Critical difference test revealed that the count was significantly ($P < 0.01$) more in the samples of S_2 and S_3 sources as compared to S_1 source. There was no significant difference between the counts of S_2 and S_3 sources. Only nine (42.86 %) samples from S_1 source yielded coliform at 44°C. Eight (38.10 %) samples had a count of 10 per ml and one (4.76%) had a count of 10^2 per ml. Of the samples from S_2 source, 17 (80.96 %) yielded faecal coliforms. The counts in eight (38.09 %), five (23.80 %), two (9.52 %) and other two (9.52 %) samples was 10^3 per ml, 10^4 per ml 10^2 per ml and 10^5 per ml, respectively. Faecal coliforms were found in 20 (95.23 %) samples collected from S_3 source. The count in 14 (66.67 %) samples was 10^3 per ml and in four (19.04 %) it was 10^2 per ml. One (4.76 %) sample each had a count of 10 per ml and 10^4 per ml.

Highly significant difference ($P < 0.01$) in faecal streptococcal count was observed in samples collected from S_1, S_2 and S_3 sources. The mean count of samples from the three sources was subjected to critical difference test. The test revealed highly significant difference ($P < 0.01$) between the count of samples from S_1 and S_2 sources and also from S_1 and S_3 sources. The count of the

samples collected from S_2 and S_3 sources did not show any significant difference. All the samples collected from S_1 revealed faecal streptococci. Of these, 13 (61.90 %) samples had a count of 10 per ml and in eight (38.1 %) samples the count was 10^2 per ml. Except one (4.76 %), all the samples collected from S_2 source revealed the presence of faecal streptococci. The count per ml in seven (33.33 %) samples was 10^2, in seven (33.33 %) samples it was 10^3, four (19.04 %) samples had a count of 10, while in two (9.53 %) samples it was 10^4. All the samples obtained from the S_3 sources had revealed the presence of faecal streptococci. Twelve (57.14 %) samples had a count of 10^3 per ml, six (28.57 %) had a count of 10^2 per ml and in three (14.24 %) samples the count was 10 per ml.

Analysis of variance of staphylococcal count indicated a highly significant difference ($P < 0.01$) in the count of samples from S_1, S_2 and S_3 sources. Critical difference test showed a highly significant ($P < 0.01$) difference in the count between the samples collected from S_1 and S_2; and S_1 and S_3 sources. The count in the samples from S_2 and S_3 sources did not show significant difference. Nineteen (90.47 %) samples from S_1 source showed the presence of staphylococci. Out of them, 13 (61.91 %) samples had a count of 10^2 per ml. Three samples (14.28 %) each had a count of 10^3 per ml and 10 per ml, respectively.

Twenty (95.23 %) samples from S_2 source yielded staphylococci and of this 16 (76.19 %) samples had a count of 10^3 per ml and four (19.04 %) samples had a count of 10^2 per ml. All the samples from S_3 source yielded staphylococci. In 14 (66.66 %) samples the count per ml was 10^3, in four (19.04 %) samples the count was 10^2 while in three (14.30 %) samples the count was 10^4

The analysis of variance indicated that the S. aureus count of samples obtained from S_1, S_2 and S_3 sources did not differ significantly. Only 14 (66.66 %) samples collected from S_1 source yielded S. aureus. Nine (42.86 %) samples had a count of 10 per ml and in five (23.80 %) samples it was 10^2 per ml. Of the samples from S_2 source, 13 (61.9 %) yielded S. aureus. Six (28.57 %) samples each had a count of 10 per ml and 10^2 per ml, respectively and in one (4.76 %) sample the count was 10^3 per ml. Only ten out of 21 (7.61 %) samples collected from S_3 source yielded S. aureus. In six (28.57 %) of these samples the count was 10 per ml and in four (19.04%) the count was 10^2 per ml.

Escherichia coli count of the samples from S_1, S_2 and S_3 sources showed a highly significant difference ($P < 0.01$). Critical difference test of the over all sample means revealed highly significant difference ($P < 0.01$) in the count of samples between S_1 and S_2., S_1 and S_3., and S_2 and S_3 sources. Sixteen samples (76.19 %)

collected from S_1 source yielded E. coli. Eight (38.095 %) samples each from S_1 source had a count of 10 per ml and 10^2 per ml, respectively. All the samples from S_2 source yielded E. coli. In 11 (52.38 %) samples the count was 10^3 per ml but in six (28.55 %) it was 10^4 per ml. The count in three (14.28 %) samples was 10^2 per ml and in one (4.76 %) sample it was 10 per ml. All the samples from S_3 source also yielded E. coli. Eleven (52.38 %) of 21 samples showed a count of 10^3 per ml and in 10 (47.62 %) samples the count was 10^2 per ml.

BACTERIAL COUNT IN POOLED MILK SAMPLES

Table 4 reveals the mean CFU per ml of pooled milk samples collected from the three sources and the analysis of variance of the count is shown in Table 5.

Analysis of variance of total viable count of pooled milk samples from S_1, S_2 and S_3 sources showed a highly significant diffference($P < 0.01$). Critical difference test (Table 6) of the overall samples means of the three sources revealed a highly significant difference ($P < 0.01$) in count between samples of S_1 and S_2., and also S_1 and S_3 but there was no significant difference between the counts in the samples of S_2 and S_3. All the samples collected from the S_1 source had a total viable count of 10^4 per ml.

Table 4. Mean bacterial counts of pooled samples collected from S_1, S_2 and S_3 sources

Bacterial counts	Mean colony forming unit per ml of samples from:		
	S_1	S_2	S_3
Total viable count	4.0×10^4	1.8×10^6	2.1×10^5
Coliforms count at 37°C	5.5×10^1	2.0×10^5	6.4×10^3
Coliforms count at 44°C	2.8×10^1	3.6×10^4	4.4×10^3
Faecal streptococcal count	2.0×10^2	4.8×10^3	2.9×10^3
Staphylococcal count	9.2×10^2	5.3×10^4	1.3×10^4
Staphylococcus aureus count	1.0×10^2	4.8×10^2	1.1×10^2
Escherichia coli count	2.7×10^2	8.9×10^4	1.9×10^3

Table 5. ANOVA of different bacterial counts of pooled samples from S_1, S_2 and S_3 sources

Degree of freedom		TVC	Coliforms at		Faecal Streptococci	Staphylococci	*S. aureus*	*E. coli*
			37°C	44°C				
Treatment	2	1.967	23.485	15.180	2.230	6.892	1.616	7.692
Error	18	0.287	0.463	0.967	0.285	0.708	1.375	0.478
F value		6.858**	50.674**	15.696**	7.812**	9.736**	1.175	16.105**

** P<0.01

Table 6. Critical difference test of different bacterial counts of pooled samples from S_1, S_2 and S_3 sources

Source of samples	Mean bacterial counts in milk (Mean ± SE in $\log_{10}$ CFU per ml)						
	T V C	Coliforms at		Faecal streptococci	Staphylococci	S. aureus	E. coli
		37°C	44°C				
S1	4.604 ± 0.086[a]	1.267 ± 0.291[a]	1.140 ± 0.279[a]	2.344 ± 0.136[a]	2.463 ± 0.480[a]	1.044 ± 0.495[a]	2.063 ± 0.279[a]
S2	5.656 ± 0.3061[b]	4.858 ± 0.298[b]	3.822 ± 0.563[b]	3.440 ± 0.207[b]	4.334 ± 0.226[b]	1.996 ± 0.457[a]	4.15 ± 0.279[b]
S3	5.242 ± 0.143[b]	3.691 ± 0.166[c]	3.536 ± 0.136[b]	3.126 ± 0.245[b]	3.971 ± 0.143[b]	1.629 ± 0.366[a]	3.063 ± 0.192[c]
Critical difference	0.6013	0.7637	1.1038	0.5992	0.9444	1.3162	0.7760

* Figures with the same superscript within the column do not differ significantly.

Of thesamples collected from the S_2 source, three (42.86%) had a count of 10^5, while two (28.57 %) samples each had a count of 10^4 per ml and 10^6 per ml, respectively. Five (71.4 %) samples collected from the S_3 source had a count of 10^5 per ml and in two (28.6 %) samples the count was 10^4 per ml.

A highly significant difference ($P < 0.01$) was observed in coliform count (37°C) of the samples collected from S_1, S_2 and S_3 sources. The means of the samples from each source were subjected to critical difference test. The count was significantly ($P < 0.01$) more in samples from S_2 and S_3 as compared to S_1 source. There was significant difference between the count of samples from S_2 and S_3 sources. All seven samples collected from S_1, yielded coliforms at 37°C, of which five (71.4 %) had a count of 10 per ml and in two (28.6 %) samples the count was 10^2 per ml. All the samples collected from S_2 revealed the presence of coliforms. Three (42.85 %) samples each showed a count of 10^4 per ml and 10^5 per ml, respectively, but in one (14.3 %) the count was 10^3 per ml. All the samples from S_3 source also yielded colifomrs at 37°C. Four of them (57.1 %) showed a count of 10^3 per ml. In two (28.6 %) samples the count was 10^4 per ml while in one (14.3 %) it was 10^2 per ml.

Analysis of variance of faecal coliform count of the samples from S_1, S_2 and S_3 sources revealed highly significant difference ($P < 0.01$). Critical differnce test of the sample means from the above sources revealed that samples form S_2 and S_3 sources had highly significant ($P < 0.01$) count as compared to the count of samples from S_1 source. No significant difference in the count of samples between S_2 and S_3 sources was observed. Six (85.5 %) out of seven samples collected from S_1 source yielded coliforms at 44°C of incubation and the count in these samples was 10 per ml. All the samples collected from S_2 source yielded faecal coliforms. Four (57.1 %) samples showed a count of 10^4 per ml. In the remaining three samples, one (14.3 %) each had a count of 10^5 per ml, 10^3 per ml and 10 per ml, respectively. All the samples collected from S_3 source revealed the presence of faecal coliforms. Five (71.4 %) samples showed a count of 10^3 per ml and in one (14.3 %) sample each the count was 10^4 per ml and 10^2 per ml, respectively.

Analysis of variance of the faecal streptococcal count of samples collected from S_1, S_2 and S_3 sources revealed a highly significant difference ($P < 0.01$). Critical difference test showed highly significant difference ($P < 0.01$) in the count of samples from S_2 and S_3 sources as compared to S_1, source. Highly significant difference ($P <$

0.01) was observed between the counts of S_1 and S_2 sources; and S_1 and S_3 sources but no significant difference was observed between the counts of samples from S_2 and S_3 sources. All the samples from S_1 source showed the presence of faecal streptococci. The count in five samples was 10^2 per ml and two samples had a count of 10 per ml.

All the samples from S_2 revealed the presence of faecal streptococci. Four (57.1 %) samples collected from S_2 source had faecal streptococcal count of 10^3 per ml. The count in two (28.6 %) samples was 10^2 per ml and in one (14.3 %) sample it was 10^4 per ml. All the seven samples collected from S_3 sources had faecal streptococci. In three (42.85 %) samples the count was 10^3 per ml while in other three samples the count was 10^2 per ml and one (14.3 %) sample revealed the count of 10^4 per ml.

Highly significant difference ($P < 0.01$) in staphylococcal count was observed in samples collected from S_1, S_2 and S_3 sources. Critical difference test revealed that samples from S_2 and S_3 had a significantly ($P< 0.01$) high count than the sample from S_1. There was no significant difference between the counts of samples from S_2 and S_3 sources. Five (71.4 %) of the seven samples collected from S_1 source had a count of 10^3 per ml but in

one (14.3 %) sample the count was 10^2 per ml. One (14.3 %) of the samples did not show the presence of staphylococci. All the seven samples collected from S_2 source revealed the presence of staphylococci. Of these samples four (57.14 %) revealed a count of 10^4 per ml. Two (28.57 %) samples had count of 10^3 per ml and one (14.3 %) sample had a count of 10^5 per ml and in the other sample it was 10^3 per ml. Of the seven samples collected from S_3 source, four (57.15 %) samples had a count of 10^3 per ml and in three (42.85 %) samples the count was 10^4 per ml.

Analysis of variance of the Staphylococcus aureus count of samples collected from S_1, S_2 and S_3 sources did not reveal significant difference. Of the seven samples from S_1 source, four (57.1%) revealed presence of S. aureus. In two samples (28.6 %) the count was 10^2 per ml and in other two the count was 10 per ml. Six (85.71 %) out of seven samples collected from S_2 source revealed S. aureus. Out of this two (28.57 %) samples had a count of 10^3 per ml. Two samples each had a count of 10^2 per ml and 10 per ml, respectively. Total six (87.71 %) samples from S_3 source revealed the presence of S. aureus. Four (57.11 %) samples had a count of 10^2 per ml and two (28.8 %) samples had a count of 10 per ml.

The Escherichia coli count of milk samples collected from S_1, S_2 and S_3 sources revealed a highly significant

difference ($P < 0.01$) between the count of samples collected from S_1, S_2 and S_3 source. Critical difference test of the mean count of samples revealed a highly significant ($P < 0.01$) difference in the count of samples between S_1 and S_2; S_1 and S_3; and S_2 and S_3. All the samples collected from S_1 showed the presence of E. coli. Of this, four (57.2%) samples had a count of 10^2 per ml but in three (42.8 %) samples the count was 10 per ml. All samples collected from S_2 showed the presence of E. coli. The count in five (71.4 %) samples of the S_2 source was 10^3 per ml respectively. One (14.13 %) sample each had a count of 10^4 per ml and 10^5 per ml, respectively. All the samples collected from S_3 source revealed the presence of E. coli. Five (71.4 %) samples had a count of 10^3 per ml and in two samples (28.6 %) the count was 10^2 per ml.

GRADING OF MILK

The individual and pooled milk samples were graded as very good, good, fair and poor based on the criteria described in IS: 1479 (part III) 1977. The percentage of samples falling into different grades are shown in Table 7. Among the samples collected from three sources, only one sample obtained from S_2 source fell into poor grade. Twenty individual and all the pooled samples collected from S_1 belonged to the very good grade, one individual sample from this source was graded as good.

Table 7. Grades of milk samples from S_1, S_2 and S_3 sources as per IS: 1479

Source of milk	Type of sample	Number of samples tested	Per cent of samples graded as:			
			Very good	Good	Fair	Poor
S_1	Individual	21	95.24	4.76	0.0	0.0
	Pooled	7	100.00	0.00	0.0	0.0
S_2	Individual	21	76.20	23.80	0.0	0.0
	Pooled	7	42.84	28.60	14.28	14.28
S_3	Individual	21	80.95	19.05	0.0	0.0
	Pooled	7	57.14	42.86	0.0	0.0

Of the individual samples collected from S_2 source 16 fell in to very good grade and 5 in good grade. The pooled samples of this source belonged to different grades. Three samples were graded as very good, two samples as good, one sample as fair and one sample was of poor grade.

Among the individual samples of the S_3 source 17 samples belonged to very good grade whereas four samples belonged to good grade. Four pooled samples from the above source belonged to very good and three sample fell into good grades.

STUDIES ON BACTERIAL ISOLATES

Isolation and identification of staphylococci:

Of the 84 raw milk samples examined, 53 showed the presence of staphylococci. From these samples 60 isolates were recovered for further characterisation. The test characteristics and reactions of the isolates are shown in Table 8. After the primary screening tests, 58 isolates which were gram positive cocci, forming irregular clusters, non-motile, fermented glucose and produced acid from glucose aerobically and catalase positive but oxidase negative, were selected for further study. The isolates produced acetoin and phosphatase and hydrolysed arginine.

Table 8. Characteristics of staphylococcal isolates from samples of S_1, S_2 and S_3 source

Tests	Number of isolates	
	Positive	Negative
Growth in air	60	0
Growth anaerobically	58	2
Motility test	0	60
Catalase test	60	0
Oxidase test	0	60
Oxidation and Fermentation	58	2
Acetion production (VP test)	58	0
Arginine hydrolysis	58	0
Phosphatase test	58	0
Acid from carbohydrates:		
Glucose : Aerobic	58	0
Anareobic	58	0
Mannitol: Aerobic	57	1
Anaerobic	55	3
Lactose	58	0
Maltose	58	0
Sucrose	58	0
Gelatin liquifaction	54	4
Coagulase test	54	4

They also produced acid from glucose both aerobically and anaerobically. One of the isolates did not produce acid from mannitol aerobically and three of them did not produce acid from mannitol anaerobically. All the isolates produced acid from lactose, maltose and sucrose aerobically. Fifty four isolates liquified gelatin and they coagulated rabbit plasma. Based on the results of the above tests 54 isolates were identified as Staphylococcus aureus.

Isolation and Identification of Escherichia coli

Out of the 84 raw milk samples examined 79 revealed the presence of colonies showing characteristics of Escherichia coli. From these samples 70 isolates were recovered. These isolates were subjected to various tests and the results of these tests are given in Table 9. The primary screening tests revealed that all the 70 isolates were gram negative, rods. The isolates produced acid from glucose aerobically and also attacked glucose by fermentation. All the isolates produced catalase but none produced oxidase. The isolates produced gas from glucose and were positive to methyl red test. All the isolates produced indole but none produced acetoin, and H_2S on TSI medium. Of the 70 isolates only 66 showed a positive reaction in L-lysine decarboxylase test but all of them were

Table 9. Characteristics of *Escherichia coli* isolates from of S_1, S_2 and S_3 source

Tests	Number of isolates	
	Positive	Negative
Growth in air	70	0
Growth anaerobically	70	0
Motility test	67	3
Catalase test	70	0
Oxidase test	0	70
Oxidation and Fermentation	70	0
Acid from glucose	70	0
Gas from glucose	70	0
Methyl red reaction	70	0
Acetion production (VP test)	0	70
Indole production	70	0
Lysine decarboxylase	66	4
H_2S on TSI reaction	0	70
Acid from carbohydrate:		
Lactose	70	0
Maltose	70	0
Sucrose	70	0
Trehalose	70	0
ONPG test	70	0

positive to ONPG test. None of them revealed a positive result in citrate utilisation test. All the isolates produced acid from lactose, maltose,sucrose and trehalose. Based on the results of the above tests, 66 isolates were identified as Escherichia coli.

STUDIES ON ANTIBIOGRAM OF ISOLATES

Antibiogram of Staphylococcus aureus:

The results of the antibiotic sensitivity test of the 54 S. aureus isolates are shown in Table 10 and Fig. 1. All the isolates were sensitive to cloxacillin and gentamicin. Out of the 54 isolates, 47 (87.03 %), 42 (77.80%) and 19 (35.20 %) were found sensitive to amoxycillin, chloramphenicol and pencillin-G, respectively. Six (11.22 %) isolates revealed intermediate sensitivity to amoxycillin while 12 (22.20 %) showed intermediate sensitivity to chloramphenicol. One (1.85 %) isolate was resistant to chloramphenicol and 35 (64.80 %) isolates were resistant to pencillin-G.

Antibiogram of Escherichia coli:

The 66 isolates of Escherichia coli were tested to detect their sensitivity pattern against five antibiotics, and the results of the test are shown in Table 11 and Fig. 2. None of the isolates revealed 100 per cent sensitivity

Table 10. Antibiogram of Staphyloccus aureus isolates from milk

Antibiotics	Percentage of isolates		
	Sensitive	Intermediate sensitive	Resistant
Cloxacillin	100.00	0.0	0.0
Gentamicin	100.00	0.0	0.0
Amoxycillin	87.03	11.12	1.85
Chloramphenicol	77.80	22.20	0.00
Penicillin - G	35.20	0.0	64.80

Fig.1 Antibiogram of Staphylococcus aureus Against Five Antibiotics

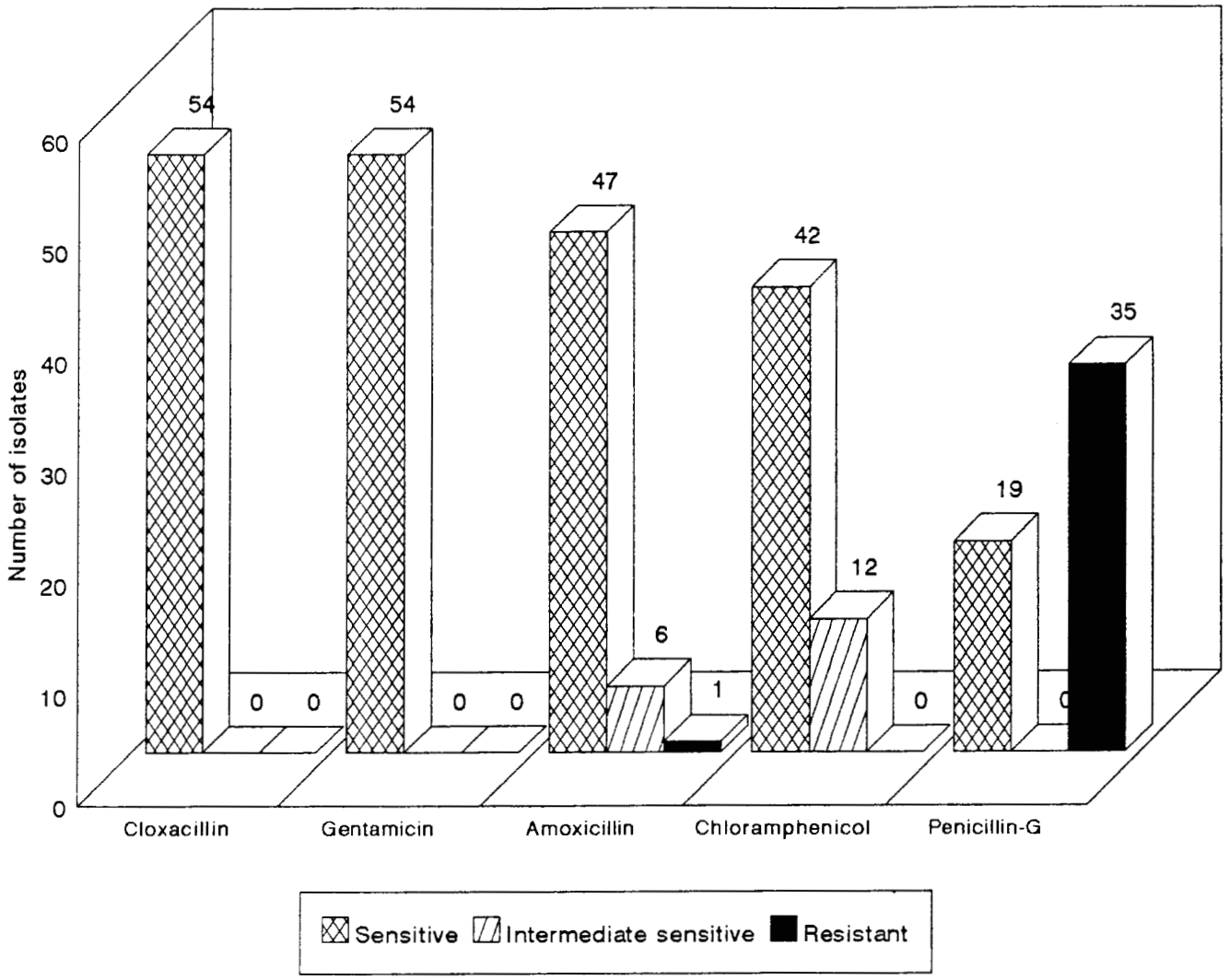
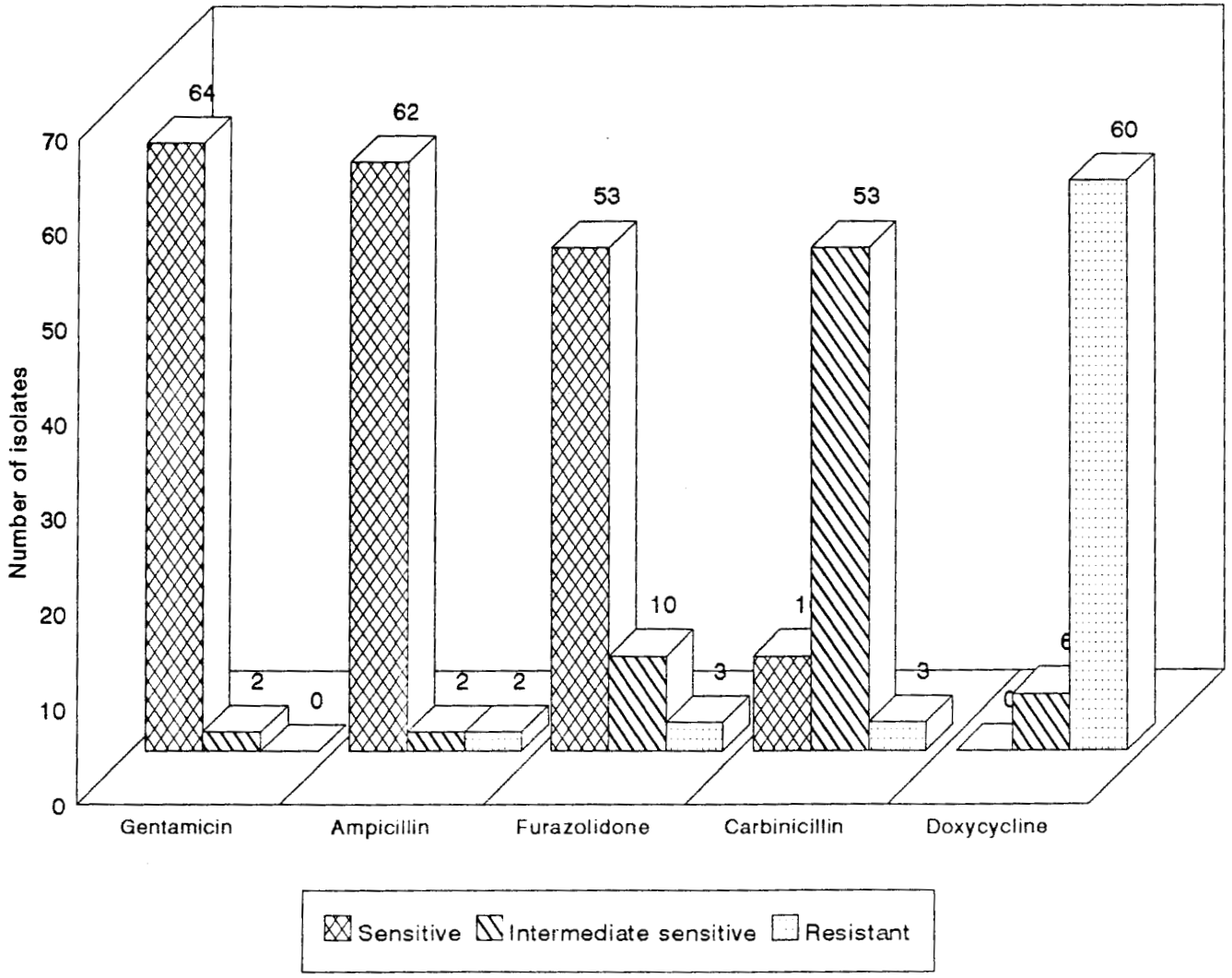


Table 11. Antibiogram of *Escherichia coli* isolates from milk

Antibiotics	Percentage of isolates		
	Sensitive	Intermediate sensitive	Resistant
Gentamicin	96.96	3.04	0.00
Ampicillin	93.92	3.04	3.04
Furazolidone	80.30	15.15	4.55
Carbenicillin	15.15	80.30	4.55
Doxycycline	0.00	9.10	90.90

Fig.2 Antibiogram of Escherichia coli Against Five Antibiotics



or resistance to any one of the antibiotics and no isolate was sensitive to doxycycline. Out of the 66 isolates 64 (96.96 %), 62 (93.92 %), 53 (80.30 %) and 10 (15.15 %) isolates were sensitive to gentamicin ampicillin, furazolidone and carbenicillin respectively. Two (3.04 %) isolates each revealed intermediate sensitivity to gentamicin and ampicillin, respectively, 10 (15.15 %) to furazolidone and 53 (80.30 %) to carbenicillin. Six (9.10 %) revealed intermediary sensitivity to doxycycline. None of the isolates were resistant to gentamicin. Two (3.04 %) isolates were resistant to ampicillin. Three (4.55 %) isolates each showed resistance to furazolidone and carbenicillin, respectively. Of the isolates 60 (90.90 %) isolates were resistant to doxycycline.

DISCUSSION

Milk, to be of good sanitary quality must be free of hazardous microorganisms or if present, should be at a safe low level. In general it is not feasible to examine individual animal's milk from the source for the presence of hazardous organisms. The practice which has been in effect for many years and continue to be followed, is to determine the sanitary quality of milk by looking the presence of certain indicator organisms and by standard plate counts.

Bacterial counts of individual milk samples:

Standard plate count is used as an indicator to assess the bacteriological quality of various foods. It reflects the handling history, state of decomposition or degree of freshness and in some instances indicates the sanitary quality of foods. The bacterial standards set forth by various authorities include standard plate count as one of the criteria to assess the quality of milk. The mean standard plate count of samples from S_1 source was one log less than the count observed in samples from S_2 and S_3 sources (Table 1). Milk samples collected from S_1 source had a highly significant ($P<0.01$) lower bacterial count as compared to the samples collected from S_2 and S_3 sources (Table 3). Further 95.2 per cent samples from S_1 fell into

very good grade as compared to 81 per cent and 76.2 per cent of the samples from S_3 and S_2 sources, respectively (Table 7). Only 4.76 per cent samples of S_1 source fell into good grade where as 23.8 per cent and 19.1 per cent samples from S_2 and S_3 sources, respectively, belonged to this grade. However, the over all bacterial quality of milk samples collected from the three sources were found to be acceptable as per IS. 1479 (Part III, 1977). The reason for lower bacterial count in milk sample of S_1 source might be the age of the milk. Time elapsed between the milking and collection of milk samples from the S_1 source was always less than S_2 and S_3 sources.

All the samples collected from S_1, S_2 and S_3 sources were found to be within the acceptable standard plate count limit specified for manufacturing grade milk in North America. Of the samples 18(85.7%), 10(47.6%) and 9(42.9%) from the S_1, S_2 and S_3 sources respectively, confirmed the standard of less than $1x10^5$ CFU per ml, prescribed for Grade I or Grade A raw milk intended for heat treatment before consumption. Only four (19.0%) milk samples from S_1 source confirmed the microbiological specification for the Military Federal Purcahse of fresh milk, while only one (4.8%) sample from the S_2 source satisfied this standard and none of the samples from S_3 source met the prescribed standard. Only two (9.5%) samples from S_1 source statisfied

the International Dairy Fedetration (IDF) specified standard for good hygeinic practices followed during production. None of the samples from the other sources met this criterion.

Coliform count of 12 (57.1%) and one (4.8%) samples from S_1 and S_3 sources, respectively confirmed the microbiological specification for fresh milk for the Military Federal Purchase. The presence of coliforms, particularly in high number, in samples from S_2 and S_3 sources indicate unhygienic milking and handling of milk in the farm or by the individual producers during its production. It may also be due to delay in prompt delivery of milk by the producers.

In the present investigation, the highest mean faceal streptococcal count was observed in the samples collected from S_2 source, followed by S_3 and S_1 sources, respectively (Table 1). Highly significant ($P<0.01$) difference in count (Table 3) in the samples from the above sources indicate that variation in the level of contamination of milk occurs at different levels of production. Faecal streptococci are the inhabitants of the intestine of man and animals and thus the presence of this organism in milk indicates faecal contamination and possible presence of enteric pathogens. The findings of this study invites the attention of dairymen engaged in milk production to take up adequate sanitary

practices in milk production, in order to ensure supply of better quality milk to the consumer.

A highly significant difference ($P < 0.01$) was observed in the samples collected from the three sources. The count in samples from S_1 source was significantly ($P < 0.01$) lower than that of the count observed in the samples from S_2 and S_3 sources, but there was no significant difference between the counts of S_2 and S_3 sources.

Staphylococcus aureus count of the samples collected from the three sources did not reveal any significant difference (Table 3). The highest count was observed in samples collected from the S_2 source, followed by S_1 and S_3 sources (Table 1). The organisms might have entered into the milk from the animal itself or from the milk handlers or from the environment. The presence of this organism in milk for human consumption is of great public health importance, since the growth of this organism in milk may produce enterotoxins and the consumption of such milk or its products lead to Staphylococcal food poisoning.

Escherichia coli count was highest in the samples obtaied from S_2 source and the least in samples of S_1 source (Table 1). Highly significant ($P<0.01$) difference in the count between samples from the source (Table 3) indicate

that the level of contamination of the organism vary in each source. The presence of this organism in milk is a clear indication of poor hygienic practices followed during the production and handling of milk, since this organsm is of intestinal origin in man and animals. Further, different types of Escherichia coli can cause various diseases in man and hence the finding is of great public health importance.

The results of different individual bacterial counts of samples collected from the three sources indicated that samples from S_2 source have the highest counts followed by S_3 and S_1 sources. The high counts observed in samples from individual animals and also from the society reception, S_2 and S_3 sources, increases the total bacterial population in the milk which will reduce the shelf life of milk as well as the bacteriological quality of product produced from such milk. Further the chances for the presence of pathogens in milk may also increase. Therefore it is essential to take necessary steps to improve the hygienic status of production, to reduce the bacterial load in milk and to improve its bacteriological quality.

Bacterial counts of pooled milk samples:

Total bacterial counts of pooled milks samples was highest in samples collected from S_2 source, followed by S_3 and S_1 sources (Table4). The count in the samples from S_1

source was low as compared to S_2 and S_3 sources and this difference in count was highly significant ($P<0.01$) (Table 6). As per Bureau of Indian Standards (BIS) recommendations, the standard plate count of all the samples collected from the S_1 source were found to be of very good grade (Table 7). In the samples from S_3 source, 57.14 per cent were categorised as very good grade and 42.86 per cent samples were graded as good. Of the counts of sample from S_2 source, 14.28 per cent each fell into fair and poor grade, respectively, but 42.84 per cent and 28.60 per cent samples were graded as very good and good respectively. All the samples from S_1 source met the standard plate count, of less than $1x10^5$ per ml, for Grade A or Grade I raw milk, but only 28.57 per cent samples each from the S_2 and S_3 sources met the above grade. All the samples from the S_1 and S_3 sources met the standard plate count of less than $3x10^6$ per ml, acceptable for manufacturing grade milk in North America, while only 71.42 per cent samples from the S_2 source satisfied the above criteria. In a study, Misra and Kuila (1989), reported that 11.3, 37.3, 36.7 and 14.7 per cent samples were categoriesed as good, fair, poor and very poor respectively. In a similar study Singh et al. (1994 a) observed that 28 per cent samples were of very good grade. Of the remaining samples 14, 21 and 37 per cent were graded as good, fair and poor grades, respectively. None of the

samples collected from S_1, S_2 and S_3 sources satisfied the standard of 10000 per ml, prescribed by the International Dairy Federation for raw milk produced under good hygienic practices or the standard of $2x10^4$ per ml, for fresh milk prescribed by Military Federal Purchase.

As compared to the count of S_1 source higher standard plate count in the milk produced in different farms had been reported (Jain and Saraswat, 1968, Milko et al., 1981, Yadava et al., 1983 and Reddy et al., 1994). The mean total count of the samples from the S_2 source are comparable to the count of city market milk as reported by Jain and Saraswat (1968); the milk samples from dairy farm, city market and rural collection centres reported by Vijai and Saraswat (1968) and the normal milk of individual quarters of cows as per finding of Reddy et al. (1994). A very high bacterial count in milk from rural collection centres to the extent of $1.1x10^7$ has been reported by Jain and Saraswat (1968) in the samples from milk supply scheme and $1.84x10^8$ per ml by Pandey and Mandal (1980). A few workers have also reported lower bacterial count in samples collected from different sources (Desai and Natarajan, 1981; Majewski and Rzaczynski, 1983 and Yadava et al., 1983). The count observed in samples from S_3 source corroborate with the counts recorded by a few workers like Majewski and Rzaczynski (1983) and Yadava et al. (1983). These findings

indicate that steps have to be taken to improve the hygienic status of milk production in all the three sources, particularly S_2.

None of the samples collected from the three sources satisfy coliform count specified in IS: 1479 (1977) or the microbiological specification for the count in fresh milk intended for Military Federal Purchase. The significant difference in coliform and thermotolerant coliform counts in the samples collected from the three sources are shown in Table 4. Though the lowest count was observed in samples from S_1 source, the highly significant ($P<0.01$) difference in count of samples from S_1, S_2 and S_3 sources revealed that the degree of contamination varied in each source or the hygienic status maintained during the production of milk was poor. Coliform count observed in the samples of S_1 source was lower than the $3.2x10^2$, and $2.4x10^3$ per ml recorded by Vijai and Saraswat (1968) and Rai et al. (1990) respectively. The mean count observed in S_3 source was similar to that observed in city market milk, a count of $2.2x10^3$ per ml by Vijai and Saraswat (1968) and Patel et al. (1993). In a similar study, a higher mean coliform count in samples collected from rural collection centre as compared to the present investigation was reported by Vijai and Saraswat (1968) and Hamama and El-Mouktafi (1990). The mean count observed in S_2 samples was similar to that reported by

Hamama and El-Mouktafi (1990) who had reported coliforms in regional collection centre sample as $1.8x10^5$ per ml. Many workers had reported lower coliform count as compared to the mean count observed in the S_2 source samples (Misra and Kuila, 1989; Rai et al., 1990, Patel et al., 1993 and Singh et al., 1994 a). The high coliform count in the samples of the present investigation indicate poor hygiene observed during milk production.

Detection of faecal streptococci in milk is an indication of poor hygienic practices followed during its production. In the present investigation, the highest faecal streptococcal count was seen in samples collected from S_2 source, followed by S_3 and S_1 sources (Table 4). The significant difference observed in the count is shown in Table 6. A reduction of one log in the count of samples collected from S_1 source, as against the samples from S_2 and S_3 sources clearly indicated unhygienic production and handling of raw milk in the later two sources as compared to the first source. The presence of streptococal species in milk had been reported by Shah et al. (1984) and Gill et al. (1994). A high faecal streptococcal count of $1.2x10^4$.ml, in milk samples, collected from regional collection centre was reported by Hamama and El-Mouktafi (1990). The observation in the study emphasise the need for an all round effort to be made by dairymen to improve the quality of raw milk.

Staphylococcal count of milk from the three sources and the significant difference in the count of samples from the S_2 and S_3 source as compared to the S_1 source, revealed that unhygienic practices are followed during production and handling of milk after its producion, in the former two sources. The incidence of staphylococci in 47.5 per cent of pooled raw milk samples collected from milk supply scheme was reported by Pandey and Mandal (1980). The count observed in the S_2 and S_3 sources of the present study was comparable with the observation of Rajmany et al. (1989) who had reported a mean staphylococcal count of 6.35×10^4 CFU per ml. The high counts of staphylococci could be attributed to contamination due to the unsanitary practices; unhygienic surroundings and manual contact during handling.

Mean Staphylococcus aureus count of samples collected from the three sources and their significant difference is shown in Tables 4 and 6. No significant difference in the count of samples was observed. This observation indicated that the count of this organism in samples from the various sources was comparable. The presence of Staphylococcus aureus in raw milk sample was reported by many workers. Pandey and Mandal (1980) reported that 15 per cent of pooled raw milk samples collected from milk supply scheme yielded Staphylococcus aureus, while Shah et al. (1984) observed that the incidence of Staphylococcus aureus in cow's milk

was 20 per cent. Contrary to the observation made in the present study, Rajmany et al. (1989) reported considerably high numbers of coagulase positive staphylococci in cent per cent of raw milk samples, with an average count of 3.15×10^4 CFU per ml. Hamama and El-Mouktafi (1990) also reported similar count of 4×10^4 Staphylococcus aureus in raw milk obtained from regional collection centers in Morocco. Contamination of milk, particularly with Staphylococcus aureus, may cause health hazard in consumers. These organisms find their way into milk from the nasal carriers or from milk handlers whose hands and arms are inflicted with boils and carbuncles. The organisms are also found on the skin, nose and other parts of animals and are associated with mastitis. Thus, there is a need for the attention of all those who are engaged in the production and distribution of milk to be directed towards the production of good quality milk.

Mean Escherichia coli count of pooled milk samples is given in Table 4 and their significant difference is shown in Table 6. The highest mean count 8.9×10^4 per ml of this organism was found in samples collected from S_2 source, followed by S_3 and S_1 sources. The difference in count from each source was highly significant ($P<0.01$). The observed two log difference in count between S_1 and S_2 sources and one log difference in count between S_2 and S_3 sources

clearly indicated that the level of contamination in each source was different. The highest level of contamination was observed in milk produced in the S_2 source. The presence of E. coli. in milk had been reported by many workers (Pandey and Mandal 1980, Morgan et al. 1989, Othenhajmer, 1989, and Popovic et al., 1991). E. coli is normally found in the intestinal tract of man and animals. Their presence in milk reflect the poor hygienic practices during production. Thus, priority should be set forth to improve the hygienic status of production to produce good quality milk.

The pooled milk samples from the S_1 source were collected immediately after pooling the milk brought by each milker into a milk can. In the other two sources the milk brought by the individual members were measured in the society and were then pooled in milk cans. A closer examination of the Table 1 and Table 4 revealed that the total bacterial load, coliform count at 37°C incubation and staphylococcal count of the samples from S_2 source were more by one log in the pooled milk samples as compared to the individual samples. This may be attributed to too much handling and changing of too many containers under unhygienic conditions. The increase in count in the samples from S_1 and S_3 sources was found only in Staphylococcus aureus count. These findings indicated the need for a better hygienic practice to be followed in these sources

during production. On the basis of the above finding it is suggested that to reduce the bacterial load in fresh milk, the milker should practice good personal hygiene, the handling of milk should be as minimum as possible and utensils used at every stage of production and storage should be clean and sterile.

STUDIES ON ISOLATION AND IDENTIFICATION OF BACTERIA

During production, milk get contaminated with bacteria of various kinds. Many of these are saprophytic organismsm, while some are pathogenic to man. Not only the number and kind of bacteria associated with milk differ, but also the ability of milk to support the growth of these organisms. Therefore, it is necessary to know which organisms are assocaited with milk in its natural state, for implemeting effective processing technique to control milk borne infections and intoxications. In the present investigation, an attempt has been made for the isolation and identification of Staphylococcus aureus and Escherichia coli.

Staphylococcus aureus:

Staphylococci get into the milk from the udder of animals suffering from clinical and subclinical mastitis caused by this organism or from external sources

(Prasad, 1986). Staphylococcus aureus is one of the organisms which multiply in food before they are able to cause infection or intoxication (WHO, 1989). Staphylococci are capable of growing and producing enterotoxin in raw milk and its production is much faster in milk with very low bacterial count. In poor quality milk, competition with other organisms and changes brought about by them inhibits toxin production by Staphylococci (Batish et al., 1981). The presence of Staphylococci in milk poses a high risk to the consumers.

Of the 60 suspected isolates selected, 54 were identified as coagulase positive staphylococci (Table 8). Many investigators have reported the isolation of Staphylococci from milk. Pandey and Mandal (1980) reported that the incidence of Staphylococcus aureus in the pooled raw milk samples was 15 per cent, while Shah et al. (1984) recorded that the incidence of Staphylococcus aureus was 20 per cent in the milk samples from the University Farm. In a study of 105 milk samples from three different sources, Yadava et al. (1985) reported that 19.14 per cent samples yielded Staphylococcus aureus. Gill et al. (1994) reported that 12.24 per cent bacterial isolates of cow's milk and 15.09 per cent isolates of buffaloe's milk were Staphylococcus aureus. The detection of Staphylococcus aureus in milk intended for human consumption is of great

public health significance, since milk support the growth of staphylococci and a good number of S. aureus are capable of causing food poisoning in man. Therefore, it is suggested that care should be taken to minimise the number of S. aureus in milk to a safe level by proper management of livestock for reducing clinical and subclinical mastitis, enforcing strict personal hygiene, proper cleaning of all utensils which are used for milking and storing of milk and by avoiding delay in processing after production.

Escherichia coli:

Contamination of milk with Escherichia coli capable of producing enterotoxin can take place at any stage from production through handling, transport and distribution. The organism can survive on fingertips and other surfaces for varying periods of time, and in some cases evenafter hand washing (WHO, 1989). Increasing number of serotypes of E. coli are now being associated with a variety of clinical syndromes. Most of these occurs in countries with relatively poor standard of hygiene.

In the present investigation, 70 suspected isolates were selected out of which 66 were identified as E. coli (Table 9). The recovery of this organism from milk should be viewed as of great public health significance, as many

pathogenic serotypes play important role in causation of disease in man. The reported recovery of E. coli from raw milk was 10 per cent (Pandey and Mandal, 1980) and 78.57 per cent (Singh et al., 1994 b). Yadava et al. (1985) reported that 70 per cent of milk samples from three different sources yielded E. coli. The isolation of the organism from cow's and buffaloe's milk was 10.20 per cent and 7.55 per cent, respectively (Gill et al, 1994). Since these organisms are primarily of intestinal origin the possibility of the presence of other intestinal pathogens cannot be ruled out. Therefore it is necessary to follow hygienic practices and sanitary measures for reducing their numbers to the lowest level, for insuring the supply of safe and wholesome milk to the consumer.

In the present study, the 54 Staphylococcus aureus isolates were tested against five chemotherapeutic agents. The isolates showed heterogenicity in their sensitivity to various agents tested. Cent per cent of the isolates were sensitive to cloxacillin and gentamicin, while the sensitivity to pencillin was the least. The per cent sensitivity to Amoxycillin and chloramphenicol was 87.03 and 77.80 respectively. Cent per cent of isolates revealed sensitivity to two antibiotics. The per cent of isolates sensitive to three, four and five antibiotics was 87.04, 68.52 and 29.63, respectively. The

observations clearly indicated that cloxacillin and gentamicin were the antibiotics of choice for the treatment of Staphylococcal infection from the source of milk, followed by amoxycillin, chloramphenicol and pencillin-G.

Cloxacillin was one of the most effective chemotherapeutic agents against which all S. aureus isolates were sensitive. The reported cloxacillin sensitivity of S. aureus was 92.13 per cent (Buragohain and Dutta, 1990) and 89.34 per cent (Bansal et al., 1990). As compared to the above workers observation, the high sensitivity observed in this study could be attributed to the judicious use of cloxacillin or it might not have been used for the treatment of animals from which the milk was produced.

Gentamicin was found highly effective to all S. aureus isolates. The per cent of S. aureus showing sensitivity of gentamicin was recorded as 95.42 (Buragohain and Dutta, 1990). Harne et al. (1990) reported sensitivity of staphylococcal isolates from clinical and subclinical mastitis as 50 per cent and 77.77 per cent respectively. The high susceptibility of isolates to gentamicin could be attributed to judicious use of this antibiotic to the treatment of animals.

The observed sensitivity of S. aureus to amoxycillin was 87.03 per cent, but a total resistance was shown only by 1.85 per cent isolates. Contrary to this finding singh et al. (1994 b). reported that S. aureus revealed maximum resistance against amoxycillin. The sensitivity of S. aureus to chloramphenicol was 77.80 per cent and none of the isolates revealed resistance to it. It may be noted that the reported percentage of susceptibility of S. aureus to chloramphenicol was 97.45 (Gupta et al., 1979), 94.44 (Rao et al., 1982) and 85.24 (Bansal et al., 1990). The percentage of sensitivity reported by the above workers are very high as compared to the observation in the study which may be due to frequent and indiscriminate use of this chemotherapeutic agent in the treatment of various ailments in animals. Th lowest percentage of sensitivity of S. aureus was encountered for penicillin-G (35.20 %) and 64.80 per cent of the isolates were resistant. Contrary to this observations, Gupta et al. (1979) recorded that 72.61 per cent of S. aureus were sensitive to pencillin-G while Buragohain and Dutta (1990) found 84.26 per cent of the isolates were sensitive. The high resistance observed in the study could be attributed to the indiscriminate and frequent use of this drug and may also be due to their ability to produce penicillinase.

Sixty six isolates of Escherichia coli from the individual and pooled milk samples were tested to detect their susceptibility to five antibiotics. Among the isolates 96.96 per cent were sensitive to gentamicin and none of the isolates showed total resistance to it. On the contrary, none of the isolates were found totally sensitive to doxycycline and 90.90 per cent isolates were resistant to it. Next to gentamicin the order of sequence of drug which revealed maximum sensitivity to the isolates were ampicillin, furazolidone and carbenicillin. The per cent of isolates showing sensitivity to any one chemotherapeutic agent was 96.97. Of the isolates 92.42, 76.76 and 7.58 per cent revealed sensitivity to any two, three or four antibiotics, respectively.

Gentamicin was found effective against 96.96 per cent of the isolates. None of isolates were totally resistant to this drug. Johnston et al. (1983) reported cent per cent sensitivity for the isolates from milk. The E. coli isolated from bovine udder were also 100 per cent sensitive to gentamicin (Mc Donald et al., 1977). The isolates from goat faecal sample were also cent per cent sensitive (Mir Shams U-Din, 1989). Following gentamicin, ampicillin was the most effective against E. coli. Of the isolates, 93.92 per cent revealed sensitivity while only 3.04 per cent of the isolates showed total resistance to it. On the contrary

to this finding, varying percentage of sensitivity to ampicillin was reported by many workers. Coates and Hoopes (1979) reported that 32 per cent of bovine isolates were sensitive to ampicillin while in the case of porcine isolates it was 42 per cent. The percentage of sensitivity of E. coli isolated from farm animals was 72.2 (Jackson, 1981), from neonatal calf diarrhoea was 69.71 per cent (Tripathy and Soni, 1981) and from milk products it was found to be 12 per cent (Kulshrestha, 1990).

The complete sensitivity of the isolates to furazolidone was 80.30 per cent while only 4.55 per cent of the isolates revealed total resistance. Jackson (1981) reported that 89.11 of the E. coli isolated from farm animals were sensitive to furazolidone. Only 15.15 per cent of the isolates revealed complete sensitivity to carbenicillin while 4.55 per cent of the isolates revealed total resistance to it and 80.30 per cent of isolates showed intermediary sensitivity. The percentage of isolates sensitive to this chemotherapeutic agent from bovine udder infection was 92 per cent (Mc Donald et al., 1977). Bovine and porcine isolates revealed a sensitivity of 31 and 47 per cent, respectively (Coates and Hoopes, 1979), which was very high compared to the present observation. None of the isolates were found to be completely sensitive to doxycycline.

The presence of E. coli in raw milk is of public health importance. The organisms present special problems in human health and veterinary medicine because it may consist of varying numbers of different strains, many of which may be resistant to a number of antibiotics. Moreover, antibiotic resistance can be transfered between strains and even between two species.

The observed high proportion of drug resistant E. coli in raw milk may be due to increased and indiscriminate use of these antibiotics for treating various diseases in animals.

Summary

SUMMARY

In this investigation an effort has been made to study the bacteriological quality of raw milk obtained from University Livestock farm, Mannuthy (S_1), Ollukkara Ksheera Vyavasaya Co-operative Society (S_2) and Panannchery Ksheera Utpathaka Sahakarana Sangham (S_3) sources. A total of 21 individual and seven pooled samples were collected from each source, over a period of five months. The individual and pooled samples were subjected to different bacterial counts and also for the isolation and identification of Staphylococcus aureus and Escherichia coli. The isolates were tested for their sensitivity to various chemotherapeutic agents.

The total viable count of each sample was estimated. The average count for individual samples from S_1, S_2 and S_3 sources were 7.5×10^4, 1.4×10^5 and 2×10^5, per ml respectively. The counts showed highly significant difference ($P < 0.01$). Critical difference test revealed that the count from S_1 source was significantly ($P < 0.01$) lower as compared to S_2 and S_3 sources. No significant difference between the counts of S_2 and S_3 sources was observed.

The average coliform count at 37°C of samples from S_1, S_2 and S_3 sources were 2.4 x 10, 4.8 x 10^4 and 3.8 x 10^3 CFU per ml respectively. The count of individual samples showed highly significant difference ($P < 0.01$). Critical difference test revealed highly significant difference in the count of samples between S_1 and S_2 , S_1 and S_3, and S_2 and S_3 respectively. Thermotolerant coliform count of individual samples was estimated. The mean count of the samples from S_1, S_2 and S_3 sources was 2.2 x 10, 2.1 x 10^4 and 2.4 x 10^3per ml respectively. A highly significant difference ($P < 0.01$) in the count was observed. Critical difference test showed a highly significant ($P < 0.01$) lower count in samples from the S_1 source than that of the count in samples from S_2 and S_3 sources. No significant difference were observed in the count of samples from the S_2 and S_3 sources.

Faecal streptococcal count of each individual sample was evaluated. The average count per ml of the S_1, S_2 and S_3 samples was 1.5 x 10^2, 2.1 x 10^3 and 1.7 x 10^3 per ml, respectively. The count of the sample from the S_1, S_2 and S_3 sources showed highly significant diffference. Critical difference test of the count revealed a significantly lower count in the samples of S_1 source with that of the count in samples of S_2 and S_3 sources. There was no significant difference between the count of S_2 and S_3 sources.

All the individual samples were also examined for staphylococcal count. The mean count per ml of samples was 5.7×10^2, 2.8×10^3 and 6.8×10^3 per ml from the S_1, S_2 and S_3 sources, respectively. Highly significant difference ($P < 0.01$) in the count of samples from the three sources was observed. Critical difference test revealed significantly ($P < 0.01$) lower count in samples of the S_1 source with that of S_2 and S_3 sources. There was no significant difference in the count of S_2 and S_3 sources.

Staphylococcus aureus count of individual samples was estimated . The mean count of samples from the S_1, S_2 and S_3 sources was 8.5×10, 1.8×10^2 and 7.1×10 per ml respectively. The count of samples from three sources did not differ significantly.

Escherichia coli count of individual samples was estimated. The average count of the samples from S_1 source was 2.0×10^2 per ml. The corresponding count of the samples from S_2 source was 1.2×10^4per ml and in S_3 source it was 1.5×10^3 per ml. The counts revealed highly significant difference ($P < 0.01$). Critical difference test of the count showed a highly significant difference ($P < 0.01$) in the count of samples between S_1 and S_2; S_1 and S_3; and S_2 and S_3 sources.

Total viable count of the pooled samples was also estimated. The highest mean count was seen in samples from S_2 source, 1.8 x 10^6per ml. The lowest average count, 4.0 x 10^4 per ml was found in samples from S_1 source and it was 2.1 x 10^5 per ml in samples from S_3 source. The count of the samples from the three sources revealed a highly significant difference ($P < 0.01$). On critical difference test, the count of samples from S_1 source was significantly ($P < 0.01$) lower as compared to the count of samples for S_2 and S_3 sources. The test did not reveal any significant difference between the count of S_2 and S_3 sources.

Coliforms counts at 37°C of incubation for the samples from S_1, S_2 and S_3 sources was estimated. The mean count from S_1, S_2 and S_3 sources were 5.5 x 10, 2.0 x 10^5 and 6.4 x 10^3per ml respectively. The mean count coliform at 44°C of incubation of the samples from S_1 source was 2.8 x 10per ml. The corresponding count from S_2 and S_3 sources was 3.6 x 10^4per ml and 4.4 x 10^3 per ml, respectively. A highly significant difference ($P < 0.01$) in count at 37°C of incubation was seen. Critical difference test revealed that the count differed significantly ($P < 0.01$) between S_1 and S_2; S_1 and S_3; and S_2 and S_3 sources. A highly significant difference ($P < 0.01$) in faecal coliform count of samples from the three

sources was observed. Critical difference test revealed that the count of the samples from S_1 source was significantly lower with that of the count in samples with that of the count in samples from S_2 and S_3 sources.

Faecal streptococcal count of all samples was estimated. The average count of samples from S_1 source was 2.0×10^2 per ml. The mean count in samples from S_2 and S_3 sources was 4.8×10^3 and 2.9×10^3 per ml respectively. The count of samples from the three sources revealed a highly significant difference ($P < 0.01$). The count of samples from S_1 source was significantly lower than that of the samples from S_2 and S_3 sources. The count of samples from S_2 and S_3 sources did not differ significantly.

The mean staphylococcal count in the samples from S_1, S_2 and S_3 sources was 9.2×10^2, 5.3×10^4 and 1.3×10^4 per ml respectively. Staphylococcal count of the samples from the three sources differed significantly ($P < 0.01$). Critical difference test of the count revealed that the count was significantly ($P < 0.01$) lower in the samples of S_1 source as compared to the samples from S_2 and S_3 sources. No significant difference was noticed in the count of samples from S_2 and S_3 sources.

All samples were tested for the presence of Staphylococcus aureus. The mean count of samples from S_1 sources was 1 x 10^2 per ml. The average count of samples from S_2 and S_3 sources was 4.8 x 10^2 per ml and 1.1 x 10^2 per ml respectively. Analysis of variance of the count did not reveal significant difference.

Escherichia coli count of each sample was estimated. The mean count of samples from S_1, S_2 and S_3 sources was 2.7 x 10^2, 8.9 x 10^4 and 1.9 x 10^3per ml respectively. Analysis of variance of the count revealed highly significant difference ($P < 0.01$) in the count of the samples of S_1, S_2 and S_3 sources. Critical difference test of the data showed a significantly ($P < 0.01$) lower count in samples of S_1 source as compared to the count of samples from S_2 and S_3 sources. Significant difference ($P < 0.01$) in the count of samples between S_2 and S_3 sources was also observed.

The overall bacterial load of the samples from the S_2 source was very high and least bacterial load was found in samples from the S_1 source. All the individual samples obtained from the three sources belonged to very good and good grades according to the standard prescribed by IS: 1479 (1977). Of the samples collected from the S_1 source, 95.24 per cent samples were graded as very good

and only 4.76 per cent of samples graded as good. The per cent of samples graded as very good and good from S_2 source was 76.2 and 23.8, respectively. It was observed that 80.95 per cent of samples from the S_3 source satisfied the criteria for very good grade and the remaining 19.05 per cent samples met the count of good grade.

Based on the standard plate count prescribed by IS: 1479 (1977) the pooled samples received from the three source were graded as very good, good, fair and poor. Cent per cent of samples from the S_1 source satisfied the criteria specified for very good grade milk. The per cent of samples from the S_2 source graded as very good, good, fair and poor was 42.84, 28.60, 14.28 and 14.28, respectively.

Of the samples from S_3 source, 57.14 per cent belonged to very good grade and 42.86 per cent were catogorised as good grade.

All samples were tested for the isolation of S. aureus and E. coli. A total of 54 isolates were identified as S. aureus and 66 isolates were identified as E. coli.

The effect of cloxacillin, gentamicin, amoxycillin, chloramphenicol and pencillin-G on S. aureus isolates was

tested. All the isolates were sensitive to cloxacillin and gentamicin, but only 35.20 per cent isolates were found sensitive to pencillin-G. The per cent of isolates susceptible against amoxycillin was 87.03 and it was 77.80 per cent to chloramphenicol. The per cent of isolates revealed sensitivity to any one, two, three, four and five chemotherapeutic agent was 100, 100, 87.04, 68.52 and 29.63 respectively.

The invitro sensitivity of E. coli isolates was tested against gentamicin, ampicillin , furazolidone, carbenicillin and doxycycline. Gentamicin was the most effective chemotherapeutic agent against which 96.96 per cent isolates were sensitive, but none of the isolates was found sensitive to doxycycline. Of the isolates 90.90 per cent were resistant to doxycycline but none of the ioslates revealed resistance against gentamicin . The per cent of the isolates sensitive to ampicillin, furazolidone and carbenicillin was 93.92, 80.30 and 15.15 respectively. The per cent of isolates revealing sensitivity to any one , two, three or four chemotherapeutic agent was 96.97, 92.42, 76.76 and 7.58 respectively.

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ASSESSMENT OF BACTERIOLOGICAL QUALITY OF RAW MILK IN TRICHUR AND ITS PUBLIC HEALTH IMPORTANCE

By

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ABSTRACT OF A THESIS

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ABSTRACT

In the present study an effort has been made to assess the bacteriological quality of raw milk obtained from three different sources in Trichur. A total of 21 individual and seven pooled samples were collected from each source (S_1, S_2 and S_3), over a period of five months. The samples were subjected to different bacterial counts and also for the isolation and identification of S. aureus and E. coli. The isolates were tested for their sensitivity to various chemotherapeutic agents.

The average total viable count of individual milk samples from S_1, S_2 and S_3 were 7.5×10^4, 1.4×10^5 and 2×10^5 CFU per ml respectively. Significant difference ($P<0.01$) between the counts from S_1 and S_2; and S_1 and S_3 was noticed. The average coliform count for S_1 was 2.4 x 10, for S_2 was 4.8×10^4 and for S_3 was 3.8×10^3 CFU per ml . There was significant difference ($P<0.01$) between the counts from S_1 and S_2; S_1 and S_3; and S_2 and S_3. The average counts for thermotolerant coliforms in samples from S_1, S_2 and S_3 were 2.2 x 10, 2.4×10^4 and 2.4×10^3 CFU per ml . The counts from S_1 and S_2; and S_1 and S_3 differed significantly ($P<0.01$).

The average faecal streptococcal counts for the sample from S_1, S_2 and S_3 were 1.5 x 10^2, 2.1 x 10^3 and 1.7 x 10^3 CFU per ml . Significant difference ($P<0.01$) between the counts from S_1 and S_2, and S_1 and S_3 was noticed. The staphylococcal counts in samples from S_1, S_2 and S_3 averaged 5.7 x 10^2, 2.8 x 10^3 and 6.8 x 10^3 CFU per ml respectively. Significant differences ($P<0.01$) between the counts from S_1 and S_2, and S_1 and S_3 were noticed. The average S. aureus count in samples from S_1 was 8.5 x 10, from S_2 it was 1.8 x 10^2 and from S_3, 7.1 x 10 CFU per ml. The average E. coli counts in samples from S_1, S_2 and S_3 were 2 x 10^2, 1.2 x 10^4 and 1.5 x 10^3 CFU per ml respectively. The counts in samples from S_1 and S_2; S_1 and S_3; and S_2 and S_3 differed significantly ($P<0.01$).

The average total viable count in pooled milk samples from S_1, S_2 and S_3 were 4 x 10^4, 1.8 x 10^6 and 2.1 x 10^5 CFU per ml respectively. Significant difference ($P<0.01$) between the counts from S_1 and S_2 and S_1 and S_3 was noticed. The average coliform counts at 37°C of incubation in the pooled samples from S_1, S_2 and S_3 were 5.5 x 10, 2 x 10^5 and 6.4 x 10^3 CFU per ml respectively. The counts from S_1 and S_2 , S_1 and S_3; and S_2 and S_3 were found significantly different ($P<0.01$). The average thermotolerant count in samples from S_1, S_2 and S_3 were 2.8 x 10, 3.6 x 10^4 and 4.4 x 10^3 CFU per ml respectively. Significant difference

($P<0.01$) in the counts of S_1 and S_2; and S_1 and S_3 was noticed. The average faecal streptococcal count in samples from S_1, S_2 and S_3 were 2×10^2, 4.8×10^3 and 2.9×10^3 CFU per ml respectively. Significantly different ($P<0.01$) counts were noticed between S_1 and S_2; and S_1 and S_3. The average staphylococcal count in samples from S_1 was 9.2×10^2 from S_2 was 5.3×10^4 and from S_3 was 1.3×10^4 CFU per ml. The counts in samples from S_1 and S_2; and S_1 and S_3 were significantly different ($P<0.01$). The S. aureus counts in milk samples from S_1, S_2 and S_3 averaged 1×10^2, 4.8×10^2 and 1.1×10^2 CFU per ml respectively. The average E. coli count in samples from S_1, S_2 and S_3 were 2.7×10^2, 8.9×10^4 and 1.9×10^3 CFU per ml respectively. Significant difference ($P<0.01$) between the counts of samples from S_1 and S_2; S_1 and S_3; and S_2 and S_3 was observed.

All the individual samples from S_1 were either of very good or good grades (95.24 and 4.76%) respectively. All the pooled milk samples from this source was of very good grade. Most of the individual samples from S_2 were of very good or good grades (76.20 and 23.80%) respectively, but the pooled milk samples from S_2 were of very good, good, fair and poor grades (42.84, 28.60, 14.28 and 14.28%) respectively. Among the individual samples from S_3 source all were of either very good or good grades (80.95 and 19.05%) respectively.

Pooled milk samples from the same source had very good and good grade (57.14 and 42.86%) respectively. None of the samples from this source were of fair or poor grades.

Of the 60 suspected colonies isolated, 54 were identified as S. aureus. Antibiogram of S. aureus isolates showed highest sensitivity to cloxacillin (100%) and gentamicin (100%) followed by amoxycillin (87.03%), chloramphenicol (77.80%) and penicillin-G (35.20%). Of the 70 suspected colonies isolated 66 were identified as E. coli. The E. coli. isolates were most sensitive to gentamicin (96.96%) followed by ampicillin (93.92%), furazolidone (80.30%) and carbenicillin (15.15%). Doxycycline was least effective drug with no sensitivity and high resistance (90.90%).

Appendices

APPENDICES

Appendix - I

1. Plate count Agar

Ingredients	: gm
Tryptone	: 5
Yeast extract	: 2.5
Dextose	: 1
Agar	: 15

Final pH (at 25°C) 7.0±0.2

Suspend 23.5 gm in 1000 ml distilled water. Boil to dissolve the medium completely. Autoclave at 15 lbs pressure for 15 minutes.

2. Violet Red Bile Agar (VRBA)

Ingredients	: gms
Yeast extract	: 3
Peptone	; 7
Bile salt mixture	: 1.5
Lactose	: 10
Sodium chloride	: 5
Agar	: 15
Neutral red	: 0.03
Crystal violet	: 0.002

Final pH (at 25°C) 7.4±0.2. Suspend 41.5 gm in 1000 ml distilled water. Bring to the boil to dissolve completely. Autoclave at 121°C (15 lbs) for 15 minutes.

3. K. F. Streptococcal Agar

Ingredients	: gm
Proteose peptone	: 10
Yeast extract	: 10
Sodium chloride	: 5
Sodium glycerophosphate	: 10
Maltose	: 20
Lactose	: 1
Sodium Azide	: 0.4
Agar	: 20

Final pH (at 25°C) 7.2±0.2

Suspend 76.4 grams in 1000 ml distilled water. Boil to dissolve the medium completely. Sterlize by autoclaving at 15 lbs pressure (121°C) for 10 minutes.

4. Mannitol Salt Agar

Ingredients	: gms
Beef extract	: 1
Proteose peptone	: 10
Sodium chloride	: 75
D-mannitol	: 10
Phenol Red	: 0.025
Agar	: 15

Final pH at (25°C) 7.4±0.2

Suspend 111 grams in 1000 ml distilled water boil to dissolve the medium completely. Sterlize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

5. Tellurite Polymyxin Egg Yolk Agar

Basal medium

Ingredients	Grams
Tryptone	: 10
Yeast extract	: 5
Mannitol	: 5
Sodium chloride	: 20
Lithium chloride	: 2
Agar	: 18
Distilled water	: 900 ml

Suspend ingredients in distilled water and heat to boiling with frequent agitation to dissolve ingredients. Adjust to 900 ml with ditilled water. Adjust the pH to 7.2±0.2. Dispense into flask and autoclave for 15 minutes at 121°C.

Enrichment:

Egg yolk emulsion (30 % v/v) in physiological saline prepared by soaking fresh eggs for about one minute in a 1: 1000 dilution of saturated mercuric chloride solution. Crack eggs aseptically and separate yolk and white suspend egg yolk in 0.85 per cent sodium chloride solution (30 % v/v) and blend in high speed blender for about 5 seconds.

Complete medium:

Cool molten basal medium to 50-55°C in water bath. Add 100 ml enrichment to 900 ml basal medium, followed by 0.4 ml of one per cent filter sterlized solution of polymixin - B to a final concentration of 40 mg/ml and 10 ml of sterile one per cent solution of potassium tellurite.

6. EMB Agar(Eosin Methylene Blue Agar)

Peptone	: 10
Lactose	: 5
Sucrose	: 5
Dipotassium phosphate	: 2
Agar	: 13.5
Eosin y	: 0.4
Methylene blue	: 0.065

Suspend 36 gms in 1000 ml distilled water. Heat to boiling for 1 minute. Dispense amd sterilize for 15 minutes at 15 lbs pressure (121°C).

7. Hugh Leifson Medium

Ingredients	gms
Peptone	: 2.0
Sodium chloride	: 5
Dipotassium phosphate	: 0.3
Agar	: 2
Bromothymol Blue	: 0.05
Glucose	: 10
Final P^H (at 25°c)	7.1± 0.2

Suspend 19.3 grams in 1000 ml distilled water. Boil to dissolve the medium completely. Dispense in tubes in duplicable for aerobic and anaerobic fermentation. Sterilize by autoclaving at 15 lbs pressure (12°c) for 15 minutes.

8. MR-VP Medium

Ingredients	gms
Buffered peptone	: 7.0
Dextrose	: 5.0
Dipotassium phosphate	: 5.0

P^H(at 25°c) 6.9±0.2

Suspend 17 grams 1000 ml of distilled water. Distribute in test tubes and sterilze by autoclaving at 15 lbs pressure (121°c) for 15 minutes.

9. Peptone water

Ingredients	gms
Peptone	10
Nacl	5

Dissolve this in 1000 ml of water by heating Adjust P^H to 7.2 to 7.4 and sterilize at 115°c for 20 min.

10. Arginine Dihydrolase Broth

Ingredients	gms
Peptone	1
Sodium Chloride	5
Dipotassium phosphate	0.3
L-arginine	10
Agar	3
Bromo cresol purpol	0.016
Final pH (at 25°C)	7.2 ± 0.2

Suspend 19.3 g in 1000 ml of distilled water. Heat to dissolve completely and distribute in tubes. Sterilize by autoclaving at 10 lbs pressure (115°C) for 15 minutes.

Disolve the ingredients by heating in the water bath. Adjust to pH 8.0 to 8.4 with 10 N NaOH and boil for 10 minutes. Filter, adjust the pH to 7.2 to 7.4 and sterilize at 115°C for 20 minutes.

11. Phenolphthalein Phosphate Agar

Ingredients	gms
Peptone	: 5
Beef Extract	: 3
Sodium chloride	: 5
Agar	: 15
Sodium Phenolpthalein Phosphate	: 0.012

Final P^H (at 25°c) 7.4 ± 0.2

Suspend 28 grams in 1000 ml distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs (121°c) for 15 minutes.

12. Nutrient Gelatin agar

Ingredients	gms
Gelatin	4
Distilled water	50
Nutrient agar	1000 ml

Soak the gelatin in the water and, when throughly soften add to the melted nutrient agar. Mix and sterilize at 115°C for 10 minutes.

13. Nutrient broth

Ingredients	gms
Beef extract	10
Peptone	10
Sodium Chloride	5
Water	1000

Dissolve the ingredients by heating in the water bath. Adjust to pH 8.0 to 8.4 with 10 N NaOH and boil for 10 minutes. Filter, adjust the pH to 7.2 to 7.4 and sterilize at 115°C for 20 minutes.

14. Triple Sugar Iron Agar

Ingredients		gms
Beef Extract	:	3
Yeast Extract	:	3
Peptone	:	20
Lactose	:	10

Sucrose	:	10
D-glucose Monohydrate	:	1.0
Iron (II) Sulphate	:	0.2
Sodium Chloride	:	5
Sodium Thiosulphate	:	0.3
Phenol Red	:	0.024
Agar	:	13

Suspend 65.5 gm in 1000 ml distilled water. Heat to dissolve the medium completely. Mix well, distribute in tubes and sterilize at 15 lbs pressure (121°c) for 15 minutes. Allow the medium to set in sloped form with a butt about 2.5 cm long.

15. Moeller Decarboxylase Broth Base

Ingredients		gms
Peptone	:	5
Beef Extract	:	5
Dextrose	:	0.5
Brom cresol purple	:	0.01
Cresol red	:	0.005
Pyridoxal	:	0.005

Final P^H (at 25°c) 6.0 $\pm$ 0.2

Suspend 10. 5 grams in 1000 ml. distilled. Water add 10 gm of L-Lysine. Heat to dissolve completely. Dispense in tubes and sterilize at 15 lbs pressure (121°c) for 10 minutes.

16. ONPG Broth

Ingredients

ONPG	: 6 gm
0.01M-Na_2HPO_4	: 1000 ml

Dissolve at room temperature the ONPG (0-nitrophenyl B-D- galactopyranoside) in phosphate solution at P^H 7.5, sterilize by filteration.

ONPG Solution	: 250 ml
Peptone water	: 750 ml

Aseptically add ONPG solution to peptone water previously sterilized.

17. Nutrient Agar

Ingredients	: gm
Peptone	: 5
Sodium chloride	: 5
Beef extract	: 1.5
Yeast extract	: 1.5
Agar	: 10

Final pH (at 25°C) 7.4±0.2

Suspend 13 gm powder in 1000 ml distilled water mix thoroughly. Heat to boil with frequent agitation to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes.

Appendix - II

1. Total bacterial counts of milk samples from three sources.

Individual samples (CFU per ml)			Pooled samples (CFU per ml)		
S1	S2	S3	S1	S2	S3
1.03×10^4	2.5×10^5	8.3×10^5	4.00×10^4	5.95×10^5	4.70×10^4
5.4×10^4	2.35×10^5	2.6×10^5	4.70×10^4	4.40×10^4	1.05×10^5
2.47×10^4	2.7×10^5	3.45×10^4	2.17×10^4	3.04×10^5	2.41×10^5
2.80×10^4	1.26×10^5	3.17×10^5	8.75×10^4	2.33×10^5	6.35×10^5
7.05×10^3	5.15×10^4	1.10×10^5	2.92×10^4	3.30×10^6	2.59×10^5
3.35×10^3	5.1×10^4	1.06×10^5	2.35×10^4	7.75×10^6	8.70×10^4
2.75×10^4	2.70×10^4	5.7×10^4	6.85×10^4	8.20×10^4	2.92×10^5
6.8×10^4	2.71×10^4	5.7×10^4			
2.41×10^4	3.04×10^4	2.98×10^5			
3.10×10^4	1.49×10^5	9.85×10^4			
2.70×10^4	7.6×10^4	8.05×10^4			
7.9×10^4	1.49×10^5	1.31×10^5			
2.91×10^4	2.83×10^5	1.14×10^5			
2.78×10^4	3.05×10^4	1.63×10^5			
2.28×10^4	3.05×10^4	8.75×10^4			
1.88×10^4	1.15×10^5	2.88×10^4			
2.37×10^4	1.71×10^4	9.00×10^4			
2.17×10^4	2.95×10^5	1.48×10^5			
1.60×10^5	3.51×10^5	1.50×10^5			
2.73×10^5	2.67×10^4	8.5×10^4			
1.74×10^5	3.06×10^5	2.08×10^5			

2. Coliform counts (at 37°C) of milk samples from three sources

Individual samples (CFU per ml)			Pooled samples (CFU per ml)		
S1	S2	S3	S1	S2	S3
0	1.65×10^4	0	2.00×10^1	1.39×10^4	8.05×10^2
0	4.9×10^3	7.55×10^3	1.10×10^4	5.60×10^3	1.09×10^4
0	2.20×10^4	1.45×10^2	1.10×10^2	2.50×10^4	2.85×10^3
0	2.40×10^3	3.25×10^3	2.50×10^1	3.65×10^5	6.10×10^3
0	8.45×10^3	4.65×10^3	1.10×10^1	4.15×10^5	8.25×10^3
0	5.35×10^3	7.05×10^3	3.00×10^1	5.15×10^5	4.80×10^3
1.0×10^1	7.25×10^3	6.4×10^3	8.00×10^1	6.70×10^4	1.14×10^4
0	6.95×10^3	1.10×10^4			
0	8.05×10^3	4.3×10^3			
4.0×10^1	4.15×10^4	2.3×10^3			
4.0×10^1	5.65×10^4	5.75×10^3			
5.0×10^1	9.0×10^4	9.5×10^2			
0.5×10^1	1.46×10^5	2.1×10^3			
3.0×10^1	6.05×10^4	4.1×10^3			
0.5×10^1	3.75×10^3	2.1×10^3			
5.0×10^1	3.45×10^4	5.05×10^3			
0.5×10^1	1.55×10^4	2.15×10^3			
1.5×10^1	1.56×10^5	2.35×10^3			
5.0×10^1	1.64×10^5	3.95×10^3			
4.0×10^1	1.55×10^4	1.10×10^3			
1.65×10^2	1.42×10^5	2.75×10^3			

3. Coliform counts (at 44°C) of milk samples from three sources.

Individual samples (CFU per ml)			Pooled samples (CFU per ml)		
S1	S2	S3	S1	S2	S3
0	3.05×10^3	0	0.50×10^1	0.50×10^1	8.50×10^2
0	3.9×10^3	2.35×10^3	3.10×10^1	1.50×10^3	1.01×10^4
0	0	1.00×10^1	3.00×10^1	1.44×10^4	2.50×10^3
0	2.0×10^2	2.85×10^3	5.00×10^1	1.44×10^4	5.95×10^3
0	1.35×10^3	1.04×10^3	0	1.00×10^5	4.40×10^3
0	1.00×10^3	7.45×10^2	1.00×10^1	8.10×10^4	1.40×10^3
0.5×10^1	0	2.9×10^3	7.00×10^1	4.05×10^4	5.25×10^3
0	8.25×10^3	5.15×10^3			
0	3.4×10^3	1.95×10^3			
0.5×10^1	0	2.1×10^3			
3.0×10^1	2.5×10^4	1.14×10^4			
4.0×10^1	1.8×10^4	2.9×10^3			
0	9.35×10^4	6.5×10^2			
0	2.7×10^3	3.10×10^3			
0	2.5×10^3	8.00×10^2			
7.0×10^1	0	3.10×10^3			
1.0×10^1	5.5×10^2	1.55×10^3			
0.5×10^1	1.11×10^5	2.15×10^3			
5.0×10^1	1.00×10^5	2.35×10^3			
0	1.63×10^4	5.00×10^2			
2.55×10^2	5.35×10^4	1.95×10^3			

4. Faecal streptococcal counts of milk samples from three sources

Individual samples (CFU per ml)			Pooled samples (CFU per ml)		
S1	S2	S3	S1	S2	S3
0.5×10^1	2.76×10^3	7.50×10^2	7.50×10^1	4.25×10^2	1.45×10^2
4.35×10^2	3.0×10^1	8.00×10^1	4.35×10^2	3.45×10^3	1.06×10^4
5.7×10^1	5.0×10^1	1.34×10^3	2.15×10^2	1.86×10^3	4.50×10^2
0.5×10^1	7.5×10^2	7.25×10^2	2.85×10^2	7.50×10^2	4.35×10^2
3.5×10^1	3.0×10^3	7.30×10^2	6.10×10^1	6.70×10^3	3.15×10^3
2.5×10^1	1.65×10^3	1.05×10^3	1.10×10^2	6.20×10^3	2.45×10^3
2.7×10^2	3.65×10^2	1.7×10^3	1.90×10^2	1.41×10^4	3.25×10^3
2.0×10^1	8.0×10^1	3.25×10^3			
9.0×10^1	3.45×10^2	5.00×10^2			
4.0×10^1	8.4×10^2	4.00×10^1			
4.15×10^2	0	3.95×10^2			
6.0×10^1	1.39×10^3	6.00×10^1			
3.85×10^2	1.50×10^3	2.2×10^3			
1.8×10^2	5.4×10^2	1.3×10^3			
8.05×10^2	1.35×10^2	2.4×10^3			
8.5×10^1	2.45×10^3	2.1×10^3			
1.00×10^2	8.5×10^1	6.05×10^3			
1.25×10^2	1.22×10^4	7.7×10^3			
5.5×10^1	4.35×10^3	1.65×10^3			
5.5×10^1	2.9×10^2	4.00×10^2			
7.0×10^1	1.19×10^4	1.50×10^3			

5. Staphylococcal counts of milk samples from three sources

Individual samples (CFU per ml)			Pooled samples (CFU per ml)		
S1	S2	S3	S1	S2	S3
5.5×10^2	3.65×10^3	1.5×10^3	1.35×10^2	2.85×10^3	6.50×10^3
7.0×10^1	1.5×10^3	2.0×10^3	1.05×10^3	2.56×10^4	4.40×10^4
7.3×10^2	3.6×10^2	4.1×10^3	1.50×10^3	1.81×10^4	2.40×10^3
7.5×10^1	3.15×10^2	3.35×10^3	1.13×10^3	1.20×10^4	1.05×10^4
7.3×10^2	3.5×10^3	1.35×10^3	0	1.25×10^3	6.80×10^3
7.5×10^1	1.5×10^3	1.08×10^4	1.19×10^3	3.95×10^4	9.90×10^3
5.1×10^2	9.5×10^2	4.05×10^2	1.45×10^3	2.75×10^5	1.35×10^4
0	3.2×10^3	7.35×10^4			
0	1.85×10^3	2.05×10^3			
7.25×10^2	6.1×10^3	1.4×10^2			
4.05×10^2	2.1×10^3	2.85×10^3			
5.0×10^2	2.45×10^3	1.14×10^4			
1.13×10^3	6.4×10^3	1.4×10^2			
6.85×10^2	5.3×10^2	3.70×10^3			
4.0×10^2	6.15×10^3	1.40×10^2			
7.2×10^2	5.7×10^3	6.50×10^3			
9.0×10^2	2.4×10^3	4.25×10^3			
1.24×10^3	8.4×10^3	1.00×10^3			
1.34×10^3	1.3×10^3	5.5×10^3			
2.9×10^2	0	4.00×10^3			
9.65×10^2	1.35×10^3	3.15×10^3			

6. Staphylococcus aureus counts of milk samples from three sources

Individual samples (CFU per ml)			Pooled samples (CFU per ml)		
S1	S2	S3	S1	S2	S3
7.5×10^1	0.8×10^2	0	7.50×10^1	1.20×10^2	1.95×10^2
3.3×10^2	0	0	2.15×10^2	1.09×10^3	0
7.0×10^1	9.5×10^1	3.15×10^2	2.50×10^1	5.65×10^2	2.35×10^2
4.0×10^1	1.01×10^3	1.1×10^2	3.90×10^2	1.45×10^3	1.50×10^1
1.4×10^2	9.6×10^2	2.0×10^1	0	2.00×10^1	2.50×10^1
2.5×10^1	3.3×10^2	0	0	0	1.50×10^2
7.5×10^1	6.0×10^1	5.5×10^1	0	8.50×10^1	1.85×10^2
0	0	0			
0	3.8×10^2	2.0×10^1			
5.05×10^2	6.0×10^2	0			
4.0×10^1	0	0			
1.05×10^2	0	6.85×10^2			
1.5×10^1	1.2×10^2	1.0×10^1			
7.0×10^1	2.0×10^1	7.0×10^1			
6.0×10^1	3.0×10^1	0			
0	0	0			
0	8.0×10^1	9.5×10^1			
0	5.5×10^1	0			
0	0	0			
0	0	0			
2.4×10^2	0	1.10×10^2			

7. *Escherichia coli* counts of milk samples from three sources

Individual samples (CFU per ml)			Pooled samples (CFU per ml)		
S1	S2	S3	S1	S2	S3
5.2×10^2	4.65×10^3	4.0×10^3	7.15×10^2	6.80×10^3	1.70×10^2
4.45×10^2	6.6×10^3	1.95×10^3	3.90×10^2	8.35×10^3	1.55×10^3
2.3×10^2	4.9×10^3	1.80×10^3	3.20×10^2	5.55×10^5	4.50×10^2
5.9×10^2	8.45×10^3	2.65×10^3	1.00×10^1	7.10×10^3	7.15×10^3
6.0×10^2	7.6×10^3	1.55×10^3	3.50×10^1	3.75×10^4	1.45×10^3
2.5×10^1	4.95×10^3	1.40×10^3	2.00×10^1	7.65×10^3	1.45×10^3
6.5×10^1	3.5×10^4	6.0×10^2	4.00×10^2	2.00×10^3	1.55×10^3
2.0×10^1	1.00×10^1	4.50×10^2			
5.05×10^2	1.75×10^2	1.85×10^3			
2.0×10^1	1.05×10^3	3.0×10^2			
0	3.05×10^4	1.45×10^3			
3.5×10^1	2.85×10^3	7.15×10^3			
6.0×10^1	1.85×10^3	9.0×10^2			
0	7.45×10^3	1.3×10^3			
0.5×10^1	7.8×10^2	5.0×10^2			
5.0×10^1	1.3×10^4	2.0×10^3			
0	2.65×10^3	2.0×10^2			
0	7.55×10^4	4.5×10^2			
9.8×10^2	1.1×10^4	1.0×10^2			
1.52×10^2	4.0×10^2	5.0×10^2			
0	2.25×10^4	4.0×10^2			

Appendix - III

1. Characteristics of Staphylococcus aureus isolates

Sl. No.	Isolate No	Grams reaction	Motility	Growth		Catalase Test	Oxidase test	Fermentaton of glucose	VP reaction	Arginine Hydrolysis	Acid from							Sucrose	Gelatin liquifaction	Coagulase Test
				aerobically	anaerobically						Phosphatase Test	Glucose		Lactose	Maltose	Mannitol				
												aerobically	anaerobically			aerobically	anaerobically			
1	F_5S_1	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
2	F_6S_1	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
3	F_6S_2	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
4	F_8S_1	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
5	F_8S_2	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
6	F_9S_1	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
7	F_9S_2	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
8	$F_{12}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
9	$F_{13}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
10	$F_{14}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+

Sl. No.	Isolate No	Grams reaction	Motility	Growth		Catalase Test	Oxidase test	Fermentaton of glucose	VP reaction	Arginine Hydrolysis	Acid from							Sucrose	Gelatin liquifaction	Coagulase Test
				aerobically	anaerobically						Phosphatase Test	Glucose		Lactose	Maltose	Mannitol				
												aerobically	anaerobically			aerobically	anaerobically			
11	$F_{15}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
12	$F_{18}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
13	$F_{19}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
14	$F_{19}S_2$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
15	$F_{27}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
16	P_4S_2	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
17	P_4S_3	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
18	P_5S_1	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
19	P_6S_1	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
20	P_9S_1	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
21	$P_{11}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
22	$P_{12}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+

Sl. No.	Isolate No	Grams reaction	Motility	Growth		Catalase Test	Oxidase test	Fermentaton of glucose	VP reaction	Arginine Hydrolysis	Acid from									
				aerobically	anaerobically						Phosphatase Test	Glucose		Lactose	Maltose	Mannitol		Sucrose	Gelatin liquifaction	Coagulase Test
												aerobically	anaerobically			aerobically	anaerobically			
23	$P_{12}S_2$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
24	$P_{15}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
25	$P_{15}S_2$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
26	$P_{18}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
27	$P_{20}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
28	$P_{22}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
29	$P_{24}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
30	$P_{27}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
31	$P_{28}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
32	$P_{28}S_2$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
33	M_4S_1	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
34	M_5S_1	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
35	M_6S_1	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+

Sl. No.	Isolate No	Grams reaction	Motility	Growth		Catalase Test	Oxidase test	Fermentaton of glucose	VP reaction	Arginine Hydrolysis	Acid from							Sucrose	Gelatin liquifaction	Coagulase Test
				aerobically	anaerobically						Phosphatase Test	Glucose		Lactose	Maltose	Mannitol				
												aerobically	anaerobically			aerobically	anaerobically			
36	M_7S_1	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
37	M_9S_1	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
38	M_9S_2	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
39	$M_{11}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
40	$M_{12}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
41	$M_{13}S_2$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
42	$M_{16}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
43	$M_{17}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
44	$M_{20}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
45	$M_{20}S_2$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
46	$M_{22}S_2$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
47	$M_{23}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+

Sl. No.	Isolate No	Grams reaction	Motility	Growth		Catalase Test	Oxidase test	Fermentaton of glucose	VP reaction	Arginine Hydrolysis	Acid from							Sucrose	Gelatin liquifaction	Coagulase Test
				aerobically	anaerobically						Phosphatase Test	Glucose		Lactose	Maltose	Mannitol				
												aerobically	anaerobically			aerobically	anaerobically			
48	$M_{23}S_2$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
49	$M_{23}S_3$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
50	$M_{28}S_1$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
51	$M_{28}S_2$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
52	$M_{28}S_3$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
53	$M_{28}S_4$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
54	$M_{28}S_5$	S +ve	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+

2. Chracteristics of Escherachia coli isolates

Sl. No.	Isolate No	Grams reaction	Motility	Growth		Glucose Fermentation	Catalase Test	Oxidase test	MR reaction	VP reaction	Indole Production	Acid from		Acid from							
				aerobically	anaerobically							H_2S Production in TSI	Glucose aerobically	Glucose anaerobically	Gas from glucose	Lactose	Maltose	Mannitol	Trehalose	ONPG Test	Lysine-Decarboxylase
1	F_5E_1	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
2	F_5E_2	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
3	F_6E_1	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
4	F_8E_1	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
5	$F_{10}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
6	$F_{11}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
7	$F_{11}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
8	$F_{12}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
9	$F_{12}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
10	$F_{16}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
11	$F_{17}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
12	$F_{25}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+

Sl. No.	Isolate No	Grams reaction	Motility	Growth		Glucose Fermentation	Catalase Test	Oxidase test	MR reaction	VP reaction	Indole Production	Acid from		Acid from							
				aerobically	anaerobically							H_2S Production in TSI	Glucose aerobically	Glucose anaerobically	Gas from glucose	Lactose	Maltose	Mannitol	Trehalose	ONPG Test	Lysine-Decarboxylase
13	$F_{25}E_3$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
14	$F_{26}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
15	$F_{28}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
16	$F_{28}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
17	P_1E_1	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
18	P_4E_1	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
19	P_4E_2	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
20	P_5E_1	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
21	P_6E_1	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
22	P_7E_1	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
23	P_8E_1	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
24	P_9E_1	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
25	$P_{13}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+

Sl. No.	Isolate No	Grams reaction	Motility	Growth		Glucose Fermentation	Catalase Test	Oxidase test	MR reaction	VP reaction	Indole Production	Acid from			Acid from							
				aerobically	anaerobically							H_2S Production in TSI	Glucose aerobically	Glucose anaerobically	Gas from glucose	Lactose	Maltose	Mannitol	Trehalose	ONPG Test	Lysine-Decarboxylase
26	$P_{14}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
27	$P_{14}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
28	$P_{15}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
29	$P_{19}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
30	$P_{22}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
31	$P_{23}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
32	$P_{24}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
33	$P_{24}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
34	$P_{25}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
35	$P_{26}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
36	$P_{27}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
37	$P_{28}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
38	$P_{28}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+

Sl. No.	Isolate No	Grams reaction	Motility	Growth		Glucose Fermentation	Catalase Test	Oxidase test	MR reaction	VP reaction	Indole Production	Acid from			Acid from							
				aerobically	anaerobically							H_2S Production in TSI	Glucose aerobically	Glucose anaerobically	Gas from glucose	Lactose	Maltose	Mannitol	Trehalose	ONPG Test	Lysine-Decarboxylase
39	M_5E_1	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
40	M_5E_2	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
41	M_6E_1	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
42	M_6E_2	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
43	M_7E_1	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
44	M_9E_2	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
45	$M_{11}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
46	$M_{12}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
47	$M_{13}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
48	$M_{14}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
49	$M_{15}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
50	$M_{17}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
51	$M_{19}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
52	$M_{19}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+

Sl. No.	Isolate No	Grams reaction	Motility	Growth		Glucose Fermentation	Catalase Test	Oxidase test	MR reaction	VP reaction	Indole Production	Acid from			Acid from							
				aerobically	anaerobically							H_2S Production in TSI	Glucose aerobically	Glucose anaerobically	Gas from glucose	Lactose	Maltose	Mannitol	Trehalose	ONPG Test	Lysine Decarboxylase
53	$M_{20}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
54	$M_{21}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
55	$M_{22}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
56	$M_{23}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
57	$M_{23}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
58	$M_{24}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
59	$M_{24}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
60	$M_{25}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
61	$M_{25}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
62	$M_{25}E_3$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
63	$M_{26}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
64	$M_{26}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
65	$M_{28}E_1$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+
66	$M_{28}E_2$	-ve	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+

Appendix - IV

1. Zone of inhibition size for Staphylococcus aureus isolates

Sl. No.	Amoxic-illin	Chloram-phenicol	Cloxa-cllin	Genta-micin	Penci-llin - G
1	35	17	32	23	28
2	33	18	32	17	28
3	35	18	30	17	28
4	34	18	32	18	29
5	31	17	31	17	25
6	23	18	30	19	17
7	23	17	31	21	18
8	35	18	30	19	17
9	36	18	31	20	30
10	22	18	30	21	16
11	35	17	32	19	17
12	35	17	32	20	16
13	24	18	33	21	17
14	24	17	32	21	16
15	35	18	30	20	17
16	31	18	31	19	16
17	30	18	30	19	17
18	35	18	31	20	16
19	34	18	30	17	17
20	35	23	33	19	17
21	35	17	33	20	28
22	36	18	32	19	30
23	35	18	30	20	39
24	36	18	31	20	28
25	36	18	32	20	17
26	34	18	31	20	32
27	31	24	32	19	36
28	33	17	30	20	30

Sl. No.	Amoxic-illin	Chloram-phenicol	Cloxa-cllin	Genta-micin	Penci-llin - G
29	37	17	30	20	39
30	36	18	31	19	30
31	25	18	30	24	18
32	34	18	31	19	30
33	34	18	32	20	30
34	34	18	33	17	36
35	35	18	34	21	16
36	34	18	33	19	39
37	35	19	31	17	19
38	31	18	30	19	20
39	29	19	31	20	20
40	34	17	30	20	16
41	35	19	31	20	19
42	36	19	30	17	20
43	29	15	33	22	17
44	36	17	34	19	30
45	37	18	35	21	31
46	34	18	31	18	19
47	35	19	31	17	20
48	30	18	30	23	35
49	35	18	31	19	35
50	31	19	30	18	19
51	34	18	30	17	36
52	36	19	30	19	19
53	20	18	29	20	20
54	30	18	30	20	39

2. Size of zone of inhibition for Escherichia coli isolates

Sl. No.	Ampi-cillin	Carbeni-cillin	Doxycy-cline	Furazo-lidone	Genta-micin
1	19	18	11	20	15
2	19	18	11	21	15
3	20	22	12	20	16
4	18	21	11	22	24
5	19	19	12	20	21
6	20	20	11	17	15
7	20	20	12	18	15
8	21	19	10	18	25
9	19	20	11	18	17
10	20	18	12	18	18
11	23	23	11	18	22
12	19	20	11	16	17
13	19	21	11	17	23
14	21	21	12	16	20
15	18	19	13	16	19
16	22	19	11	18	19
17	20	19	11	16	17
18	19	18	10	17	16
19	18	19	10	18	16
20	19	19	11	18	21
21	19	20	11	16	17
22	23	21	11	18	15
23	13	21	12	18	22
24	18	21	12	19	21
25	19	23	13	20	18
26	23	24	11	21	20
27	19	17	11	14	15
28	18	24	11	21	15
29	12	18	9	19	17
30	20	23	11	16	20

Sl. No.	Ampi-cillin	Carbeni-cillin	Doxycy-cline	Furazo-lidone	Genta-micin
31	20	22	11	17	22
32	21	23	11	16	18
33	17	25	11	18	16
34	19	19	12	17	19
35	16	19	11	15	21
36	19	21	11	17	17
37	20	22	12	20	17
38	20	19	12	17	16
39	21	19	11	17	17
40	18	20	10	18	17
41	19	19	11	18	16
42	17	18	11	17	17
43	19	19	12	19	17
44	19	18	11	19	21
45	20	23	12	16	24
46	18	19	12	18	15
47	19	19	11	23	16
48	18	19	10	19	22
49	22	18	10	18	17
50	19	22	11	17	17
51	20	25	10	17	17
52	11	19	12	17	17
53	11	16	9	16	14
54	19	20	10	17	17
55	18	21	13	19	22
56	18	20	11	20	18
57	14	19	14	20	18
58	19	22	11	17	21
59	20	22	11	17	15
60	20	19	12	19	19
61	19	20	11	20	20

Sl. No.	Ampi-cillin	Carbeni-cillin	Doxycy-cline	Furazo-lidone	Genta-micin
62	25	17	12	21	15
63	18	19	12	16	18
64	19	21	13	17	17
65	17	23	11	14	17
66	15	20	14	14	14

3. Standard Zone size of inhibition given by Hi-media

Antibiotic discs	Potency in µg	Zone of inhibition (mm)		
		Resistant	Intermediately Sensitive	Sensitive
Amoxicillin	10	20	21-28	29
Ampicillin	10	11	12-13	14
Carbinicillin	100	17	18-22	23
Chloramphenicol	30	12	13-17	19
Cloxacillin	5	9	10-13	14
Doxycycline	30	12	13-14	16
Furazolidone	100	14	15-16	17
Gentamicin	10	12	13-14	15
Pencillin	10*	28	-	29

* International units.