Indian Journal of Science and Technology Vol. 3 No. 1 (Jan 2010) ISSN: 0974- 6846

# Microbial quality of raw milk samples collected from different villages of Coimbatore District, Tamilnadu, South India

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## Abstract

A study was undertaken to investigate the microbiological quality of raw milk samples from some of the villages and the surrounding areas of Coimbatore district. Among the 80 raw milk samples used for the study, bacteriological identification revealed a definite dominance of *Lactobacillus* sp. Besides it, the other genera *Staphylococcus*, *Escherichia*, *Bacillus, Salmonella* and *Pseudomonas* were isolated on selective agar and broth.

**Keywords:** Milk quality, public health, microorganism, dairy, India

## Introduction

Milk is a compulsory part of daily diet for the expectant mothers as well as growing children (Javaid *et al*., 2009). Milk being nutritious food for human beings, also serves as a good medium for the growth of many microorganisms, especially *Lactobacillus, Streptococcus, Staphylococcus* and *Micrococcus* sp. Bacterial contamination of raw milk can originate from different sources such as, air, milking equipment, feed, soil, faeces and grass (Torkar & Teger, 2008). The number and types of microorganisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health.

It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk (Torkar & Teger, 2008). Rinsing of milking machine and milking equipment with unclear water may also be one of the reasons for the presence of a higher number of microorganisms including pathogens in raw milk (Bramley & McKinnon,1990). The presence of these pathogenic bacteria in milk often emerge as a major public health concern, especially for those individuals who still drink raw milk. Keeping fresh milk at an elevated temperature together with unhygienic practices in the milking process may also result in microbiologically inferior quality. Tamilnadu, the southernmost state of India is one of the top ten milk producing states in the country with an annual milk production of 4.75 million tonnes (Serma Saravana Pandian *et al*., 2008). This study investigates the physical, and microbiological quality and safety of locally produced raw milk.

## Materials and methods

The study was conducted at the Department of Microbiology, RVS College of Arts and Science, Sulur, Coimbatore during the period from May to Nov. 2009.

### *Sources, collection and transportation of samples*

A total number of 80 raw milk samples (100 ml) were collected in the morning from diverse locations of Coimbatore and surrounding villages. After collection, the samples were transported to the laboratory on ice maintaining sterile condition.

### *Isolation of microorganisms from milk samples*

Ten fold serial dilutions of samples were made up to $10^{-6}$ in nutrient broth (Becton Dickinson Ltd, USA & BBL®) and Mac Conkey broth (Fluka Biochemika, Spain). Samples were plated in duplicate using pour plate technique. 0.5 ml of the diluted sample was delivered by pipette into 19.5 ml of enriched agar. Plates were inverted in an incubator at $37^0$C for 24-48 h. Total viable counts (aerobic mesophiles) were carried out on nutrient agar (Fluka Biochemika, Spain), plate count agar (Oxoid, England), trypticase soy agar and soybean casein digest agar (Becton Dickinson Ltd, USA). The number of colony forming units (CFU) per milliliter were counted and recorded after appropriate incubation periods on plates with a visible colony range of 20-100.

Quantitative analysis for the presence or absence of specific microorganisms was done by plating on selective media. *Salmonella* colonies on mannitol salt agar (Becton Dickinson Ltd, USA) were purified on Mac Conkey agar or *Salmonella Shigella* agar (SS-agar) (Fluka Biochemika India). Coliform count was done on Mac Conkey agar and eosin methylene Blue agar (International Diagnostic Group Plc, UK). Counts were also made on mannitol salt agar (Baird Parker Medium, Merck, Germany) and Cetrimide agar (Merck, Germany). Confirmatory biochemical and serological tests were performed on purified colonies. Carbohydrate studies and Indole test were done in peptone water (International Diagnostic Group Plc, UK).

### *Characterization of isolates from milk products*

At intervals, colonies on the incubated plates were picked and purified by repeated sub-culturing by streaking on the desired media with a sterile wire loop. The strategy consisted of picking 1 colony to represent

Indian Journal of Science and Technology Vol. 3 No. 1 (Jan 2010) ISSN: 0974- 6846

every visibly different morphology on each plate. A maximum of 5 colonies were obtained per samples, which were examined microscopically for Gram's reaction and colony morphology (shape, colour, texture, size) using 24 h old cultures. Motility and classical biochemical tests were performed. Appropriate positive and negative controls were used to make a distinction between positive and "false-positive" reactions.

*Identification of isolates from milk products*

Identification was based on growth on selective agar and broth, colony morphology, Gram's reaction, biochemical test results and criteria for disregarding negative cultures. Results were analyzed using Cowan and Steel manual, and other methods for the identification of medical bacteria (Barrow & Feltham, 1993; De silva *et al*., 2001; Ellis & Goodacre, 2006).

## Results and Discussion

Pathogenic bacteria in milk have been a major factor for public health concern since the early days of the dairy industry. Many diseases are transmissible via milk products. Traditionally raw or unpasteurised milk has been a major vehicle for transmission of pathogens. The health of dairy herd and milking conditions basically determine the milk quality. Another source of contamination by microorganisms is unclean teats. The use of unclean milking and transport equipments also contributed to the poor hygienic quality (Parekh & Subhash, 2008).

A range of physical parameters were studied after collecting the sampling of milk from different villages and surrounding areas in Coimbatore. Table 1 shows the colour and the pH of the samples analysed in the test which had a pH range between 6.5 and 6.9. Out of 80 samples analysed, 45 samples were found yellowish white, 15 samples were white, 10 samples were light yellowish white and remaining 10 samples were deep yellowish white in colour. These findings agreed with the reports of Judkins & Mack (1955), who reported that normal milk has a yellowish white colour due to the presence of fat, casein and the presence of small amount of colouring matter. These differences in colour may be due to the differences in nature of feed consumption or the breed of cow or the fat and solid contents of the milk (Khan *et al*., 2008).

The study indicated that the dominant microbial flora associated with raw milk samples in and around Coimbatore Dt. were in the order of *Lactobacillus* sp. > *Staphylococcus aureus* > *Escherichia coli* > *Bacillus* sp. > *Pseudomonas fluorescens* > *Salmonella* sp. > among the isolated pathogens. The presence of those bacteria in milk suggested contamination from various sources, such as animal, human, environment, utensils and others (Murphy and Boor, 2000). The high numbers of the isolated microorganisms not only contaminate the milk but also multiply and grow in it. This might be due to the fact that milk is a good nutritive medium for the growth of microorganisms, especially with poor sanitary procedures (Saeed *et al*., 2009) and lack of the cooling facilities (Murphy and Boor, 2000).

*Table 1. Physical parameters of raw milk samples*

| Locations | Colour | pH |
|---|---|---|
| Sundurapuram | Yellowish white | 6.5 |
| Kuruchi | White | 6.8 |
| Pothanor | Yellowish white | 6.9 |
| LIC Colony | Deep yellowish | 6.7 |
| G.K.Square | Light yellowish | 6.5 |
| Iyer hospital | White | 6.5 |
| KG Chavadi, Navakkarai, Pichanoor, Ayyampathi, Muruganpathi, Kumuttipathi Rottikavundanoor | yellowish white | 6.8 |
| Chinnampathi | Deep yellowish | 6.8 |
| Kaliyapuram | Yellowish white | 6.8 |
| Ettimadai | White | 6.9 |
| Veerappanoor | Light yellowish | 6.5 |
| Puliakulum | Yellowish white | 6.5 |

Among the food poisoning organisms, *S. aureus* and *E. coli* were isolated. This could be due to water used in the processing, unhygienic hawking habits, storage environment and not necessarily failure of Good Manufacturing Pratices (GMP). *S. aureus* has been linked to gastroenteritis by producing enterotoxins, boils, skin infections, pneumonia, deep abscesses and meningitis in debilitated persons. (Okpalugo *et al*., 2008). High microbial counts and the occurrence of pathogens are likely to affect the keeping quality and safety of raw milk as well as products derived from it. The achievement of hygiene in dairy farm directly influences the production oriented economic results and health safety perspectives in human beings. It is therefore critically important to ensure high quality raw milk production from healthy animals under good hygienic conditions and to apply control measures to protect human health. Therefore, it is recommended that training and guidance should be given to farms owners and their workers responsible for milking. Meanwhile, information on health hazards associated with contaminated raw milk should be extended to the public, so that consumption of untreated/improperly treated raw milk could be avoided.

*Table 2. Microbial examinations of raw milk samples*

| Microorganisms | No. of colonies appeared |
|---|---|
| *Lactobacillus* sp. | 26-40 |
| *Staphylococcus aureus* | 12-20 |
| *Escherichia coli* | 8-10 |
| *Bacillus* sp. | 5-8 |
| *Pseudomonas fluorescens* | 2-6 |
| *Salmonella* sp. | 2-4 |
| Other bacterial strains | 1-2 |

Indian Journal of Science and Technology Vol. 3 No. 1 (Jan 2010) ISSN: 0974- 6846

**Acknowledgement**

The author is grateful to University Grant Commission (UGC) for the financial support given to the present study under the Major Research Project programme entitled "A Study of Ethno-veterinary Medicinal Plants and invitro antimicrobial activities against Bovine Mastitis isolated bacterial pathogens" [Sanction No. F. No. 35-121 / 2008 (SR) dt.20 March 2009]. The author is thankful to the management of RVS educational trust for their encouragement and support.

**Reference**

1. Barrow GI and Feltham KA (1993) Cowan and Steel Manual for the Identification of Medical Bacteria. 3rd ed., Cambridge.
2. Bramley AJ and McKinnon CH (1990) The Microbiology of Raw Milk. In: Dairy microbiology, I. Robinson RK (Ed.), London, NY, Elsevier Applied Sci. pp: 171.
3. De Silva ZN, Cunha AS, Lins MC, Carneiro L, Almeida AC and Queuro ML (2001) Isolation and serological identification of enteropathogenic *Escherichia coli* in pasteurized milk in Brazil. *Rev. Saude publica*. 35 (4), 375-379.
4. Ellis DI and Goodacre R (2006) Detection, identification, and enumeration methods for spoilage yeasts. In: Food Spoilage organisms. Blackburn C de W (ed.), CRC Press LLC. pp:28-54.
5. Judkins HF and Mack MJ (1955) The principle of dairying. 3rd Rev. John Wiley & Sons, Inc. NY. pp:31.
6. Javaid SB, Gadahi JA, Khaskeli M, Bhutto MB, Kumbher S and Panhwar AH (2009) Physical and chemical quality of market milk sold at Tandojam, Pakistan. *Pak. Vet. J.* 29(1), 27-31.
7. Torkar KG and Teger SG (2008) The Microbiological quality of raw milk after introducing the two day's milk collecting system. *Acta Agri. Slovenica*. 92(1), 61-74.
8. Khan MTG, Zinnah MA, Siddique MP, Rashid MHA, Islam MA and Choudhury KA (2008) Physical and microbial qualities of raw milk collected from Bangladesh agricultural university dairy farm and the surrounding villages. *Bangl. J. Vet. Med.* 6(2), 217-221.
9. Murphy SC and Boor KJ (2000) Trouble shooting sources and causes of high bacteria counts in raw milk. *Dairy Food Environ. Sanitation.* 20(8), 606-611.
10. Parekh TS and Subhash R (2008) Molecular and bacteriological examination of milk from different milch animals with special references to coliforms. *Curr. Res. Bacteriol*. 1(2), 56-63.
11. Okpalugo J, Ibrahim K, Izebe KS and Inyang US (2008) Aspects of microbial quality of some milk products in Abuja, Nigeria. *Trop. J. Pharma. Res.* 7(4), 1169-1177.
12. Saeed AEA, Zubeir EM and Owni OAO (2009) Antimicrobial resistance of bacteria associated with raw milk contaminated by chemical preservatives. *World J. Dairy Food Sci.* 4(1), 65-69.
13. Serma Saravana Pandian A, Selvakumar KN and Prabu M (2008) Segmenting the milk production in the state of Tamil Nadu (India) into homogenous milk zones: A multi dimensional scaling approach. *Indian J. Sci Technol*. 1 (6),1-2. Domain: http://www.indjst.org