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Screening of bacteria responsible for the spoilage of milk

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ABSTRACT

A total of 280 isolates were recovered from 100 milk samples. The samples were collected from the various dairies in Paonta Sahib. The isolates were characterized and identified on the basis of morphological characterization such as Gram staining reactions, colony characteristics and Biochemical test based on the Morphological and Biochemical characterization. It was observed that milk sample have the prevalence of *E.coli* sp. (32.14%), *Staphylococcus* sp. (21.4%), *Salmonella* sp. (21.4%), *Proteus* sp. (17.8%) and *Bacillus* sp. (7.14%) respectively. The Amoxycillin, Ciprofloxacin, Gentamicin, Ofloxacin, Ceftriaxome and Tetracycline were very sensitive to the *Salmonella*, *Staphylococcus* and *Proteus*. The Penicillin-G and Ampicillin were resistant to the *E. coli* and *Proteus*.

Keywords: Aqueous, pathogens, pasteurization, resistance, antimicrobial activity.

INTRODUCTION

Milk is an extremely nutritious food. It is an aqueous colloidal suspension of proteins, fats and carbohydrates that contains numerous vitamins and minerals. Many of the pathogenic bacteria encountered do not grow well in milk but remain viable for undesirable lengths of time [1]. Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of the specific production it is impossible to avoid contamination of milk with microorganisms therefore the microbial content of milk is a major feature in determining its quality [2]. Dairy products such as milk, butter, cream and cheese are all susceptible to microbial spoilage because of their chemical composition. Milk is an excellent growth medium for all of the common spoilage organisms, including molds and yeasts. Fresh, non pasteurized milk generally contains varying numbers of microorganisms, depending on the care employed in milking, cleaning, and handling of milk utensils. Raw milk held at refrigerator temperatures for several days invariably shows the presence of several or all bacteria of the following genera: *Enterococcus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Lactobacillus*, *Microbacterium*,

Propionibacterium, *Micrococcus*, coliforms, *Proteus*, *Pseudomonas*, *Bacillus*, and others. Those unable to grow at the usual low temperature of holding tend to be present in very low numbers. The spoilage of pasteurized milk is caused by the growth of heat-resistant streptococci utilizing lactose to produce lactic acid, which depresses the pH to a point (about pH 4.5) where curdling takes place [3]. The Gram-positive organisms can be present in raw milk, but they also may enter milk products at various points during production and processing. The quality of milk is determined by aspects of composition and hygiene. Due to its complex biochemical composition and high water activity milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms. Therefore in the processing of milk, some of them may produce undesirable effects and some micro-organisms produce food infections which can either carry the pathogens that will increase the likelihood of infection of the consumer's food. The contamination of milk and milk products is largely due to human factor and unhygienic conditions. Usually milk is contaminated with different kinds of microorganisms at milk collecting places. Milk is a major part of human food and plays a prominent role in the diet. Approximately 50% of the milk produced is consumed as fresh or boiled, one sixth as yoghurt or curd and remaining is utilized for manufacturing of indigenous varieties of milk products such as Ice cream, Butter, Khoa, Paneer, Rabri, Kheer, Burfi and Gulabjaman. Milk is such delicately flavoured, easily changed food that many preservative methods cannot be used without causing an undesirable change or at best making a different food product. After microorganisms have entered milk, it is difficult to remove them effectively. The process of centrifugation, as in clarifying or separating, will remove some micro organisms from milk.

EXPERIMENTAL SECTION

Milk samples were collected from the dairy shops present in the different area in a sterile container. The milk samples are serially diluted in distilled water on the very same day and then spread on the Nutrient agar plates and incubated at 37⁰C for 24 hrs. in incubator. Colonies of the microorganisms were counted with the help of Quebec Colony Counter. The various morphological characteristics of recovered isolates viz., colony morphological (colour, shaped, arrangement and Gram staining) were studied. The culture was characterized by biochemical tests i.e., **Indole Test, Methyl Red, Voges-Proskauer Test, Citrate Utilization Test, Carbohydrate Fermentation Test, Nitrate reduction test and Catalase Test** for identification of isolates [4]. The bacterial isolates were tested for the antibiotic susceptibility performed on Muller-Hinton agar by using Kirby-Bauer disc diffusion method.

RESULT AND DISCUSSION

Out of 100 milk samples 280 bacteria were isolated. The *Bacillus*, *Staphylococcus*, *Proteus*, *Salmonella* and *E. coli* were the common pathogens found in the milk samples collected from dairies. In our study, ten different antibiotics were used for the determination of antimicrobial activity of bacteria causing contamination in milk. The Amoxycillin, Ciprofloxacin, Gentamicin, Ofloxacin, Ceftriaxome and Tetracycline were very sensitive to the *Salmonella*, *Staphylococcus* and *Proteus*. The Penicillin-G and Ampicillin were resistant to *E. coli* and *Proteus*. This study was carried out to investigate the quality of raw milk from the various dairies of Paonta Sahib. In our study, 280 bacteria were isolated from 100 milk samples. The prevalence of recovered isolates were i.e., *E.coli* sp. (32.14%), *Staphylococcus* sp. (21.4%), *Salmonella* sp. (21.4%), *Proteus* sp. (17.8%) and *Bacillus* sp. (7.14%). Our prevalence of bacteria isolated from raw milk as different as the prevalence reported by [5] of bacteria isolated from milk were *Staphylococcus aureus* (26.66%), *Streptococcus pyogenes* (13.33%), *Corynebacterium ovis* (6.66%), *E. coli* (16.66%), *Citrobacter Kasserii* (20%), *Klebsiella aergenes* (6.66%), *Pseudomonas aeruginosa*

(6.66%) and *Proteus mirabilis* (3.33%) and [6] isolated *Lactobacillus* sp. (40%), *Staphylococcus aureus* (20%) *Escherichia coli* (10%), *Bacillus* sp. (8%), *Pseudomonas fluorescens* (6%), *Salmonella* sp. (4%) and some other bacteria (8%). In our study, the prevalence of bacteria isolated from raw milk was (17.8%) but the prevalence reported by [7] was (11%). [8] Reported that Erythromycin and Tetracycline were effective antibiotics against *Staphylococcus aureus*. [9] reported the frequency of resistance to Ampicillin was 75.8% and 66.7% against *S. aureus* and *S. epidermidis* isolates, respectively. Resistance to Amikacin, Cefepime, Tobramycin or Chloramphenicol was rare. The isolates of *S. aureus* and *S. epidermidis* demonstrated high level of resistance to Ampicillin. [10] Demonstrated that the resistant strains may have been transferred to cow then to milk, which can be the reason of infection in human beings if we take raw milk. These results were consistent with a previous report forming hygienic conditions and careful handling of cow during milking. [5] Isolated the multiple resistances of the bacteria that were found in the tested antimicrobial agents. Penicillin, Cilindanycin and Ampicillin showed the highest resistance. *Staphylococcus aureus* showed high resistance to Chloramphenicol in the present study. The low resistance towards Chloramphenicol might be due to the uncommon use of this antibiotic in the local dairy farms, moreover Penicillin was reported as the least effective antimicrobial agent. Chloramphenicol showed the best antimicrobial effect *E. coli*, *Salmonella* sp. against the tested organisms followed by Gentamicin and Piperacillin. [11] Isolated *E. coli*, *Salmonella*, *Proteus*, *Staphylococcus aureus* and *Bacillus cereus*. In their study, he showed highly sensitivity against Norfloxacin and Ofloxacin (93.33% each), Amikacin, Ciprofloxacin, Gentamicin and Chloramphenicol (100% each), Ofloxacin (100%), Gentamicin (100%), Enrofloxacin (91.43%) and Amikacin (100%) respectively. Moreover, some isolates of showed multiple resistance against some antibiotics. If any outbreak occurs due to these bacteria after the consumption of milk products then we use these antibiotics Amoxycillin, Ciprofloxacin, Gentamicin, Ofloxacin, Ceftriaxome and Tetracycline very effective against the disease caused by these bacteria. If we maintain the proper hygienic conditions during the processing of milk then we can reduce the prevalence of these bacteria which spoil the milk products.

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