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Microbial safety of milk and milk products sold in local shop in and around Thanjavur region

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Abstract

Milk is a nutrient rich source of proteins and milk products can serve as good source nutrients for the growth of microorganisms as milk. In the present study a total 40 samples, includes 20 ice-cream, 04 cheese, 06 butter, 04 Yoghurt and 06 milk shake samples were collected from local shop in and around around Thanjavur region and subjected for the isolation and identification of bacteria of Zoononotic importance. The isolated bacteria were confirmed by morphological, biochemical and PCR techniques. The results showed, that four samples were free from bacteria (10%), while 36 samples (90%) shown presence of bacteria. Ice cream samples were confirmed for presence of Staphylococcus spp. 08 (36%), E. coli 08 (36%) and Listeria spp.04 (18%), Butter samples shown presence of Staphylococcus spp. 02 (33%) and E. coli 02 (33%), Cheese samples shown presence of Staphylococcus spp. and E. coli (100%), Yoghurt samples were shown presence of Staphylococcus spp. 02 (50%) and milk shake sample were shown presence of mixed form of bacteria as Staphylococcus spp. The highest numbers of isolates detected were Staphylococcus spp. 018 (42%) and E. coli 018 (42%) followed by Listeria spp. 4 (9.5%) and Klebseilla 02 (2%). Polymerase chain reaction test confirmed the presence of 596 bp hlyA gene of Listeria monocytogenes and 500 bp coa gene of Staphylococcus aureus respectively. According to these study findings, the surveyed milk products were highly contaminated with different pathogenic bacteria and they have been associated with the onset of food borne infection in human beings.

Keywords: Milk products, microbial contamination, zoonotic bacteria

Introduction

Microbial contamination is a significant food safety problem because microbial contamination with food-borne pathogens leads to a wide range of health problems (Akanele et al., 2016) [1]. Besides health problems, it can also lead to economic losses due to spoilage and milk pdoucts recalls due to bacterial contamination. Milk-borne diseases are typically caused by bacteria, their metabolites, parasites, viruses and toxins. Contamination of milk products can occur mainly due to inadequate storage conditions and handling in manufactures and processing factories. During milk product preparations, various processes like use of contaminated water and improper storage after processing which can ultimately end in milk and milk product (Lavilla et al., 2013) [2]. The contaminating microbes mainly come from faeces, urine and hide of the animal. The food-borne pathogens associated with milk and milk products are Salmonella spp., Campylobacter, Yersinia enterocolitica, verotoxigenic Escherichia coli and Staphylococcus aureus (Asfaw et al., 2023) [3]. Reducing the microbial contamination of milk product will reduce the transmission of food-borne pathogens to consumers thereby reducing food-borne illness. Therefore, the objective of the study was to investigate the prevalence of food-borne pathogens in milk products samples sold in Thanjavur region. In addition, the antimicrobial sensitivity pattern of isolated food-borne pathogens was also studied.

Materials and Methods

Sample Collection A total of 40 milk product samples, includes 20 ice-cream, 04 cheese, 06 butter, 04 Yoghurt and 06 milk shake were collected from different regions of Thanjavur.

The samples of approximately 100 g were collected in sterile polythene bags and transported to the laboratory and processed within 2 h of sampling.

- Microbiological analysis: A portion of 10g of milk products samples were aseptically transferred to sterilized mortar containing 90 ml of phosphate buffered saline (PBS) and homogenized using sterile pestle. A volume of 1 ml of homogenate was added to 9 ml of selenite cysteine broth, Macconkey broth and Nutrient broth and incubated at 37°C overnight for selective enrichment of Salmonella, E. coli and S. aureus. Following incubation, enriched cultures were streaked on Xylose Lysine Dextrose (XLD) agar, Sorbital MacConkey Agar (SMA) and Mannital Salt Agar (MSA) for isolation of Salmonella, E. coli, E. coli O157:H7 and S. aureus. All the isolates were purified by sub culturing in nutrient broth and further streaking on nutrient agar.
- **Biochemical characterization:** The presumptive *Salmonella*, *E. coli* and *S. aureus* colonies were subjected to Gram's staining, catalase test, indole test, oxidase test, methyl red test, VP test, Simmon's citrate utilization tests and triple sugar iron utilization tests.
- **Antimicrobial susceptibility testing:** All the isolated *E*. coli, S. aureus and Salmonella were tested for antimicrobial susceptibility using disc diffusion methods (Azeez et al., 2023) [4]. The agents used in this study were procured from Himedia, which include Erythromycin (15 mcg), Streptomycin (10 mcg), Nalidixc acid (30 mcg), Gentamicin (120 mcg), Doxycycline (30 mcg), Tetracycline (30 mcg), and *Amoxycilin clavulinic* acid (20 and 10 mcg), Chlormphenicol (30 mcg), Trimethoprim (5 mcg), co-trimoxozole (25 mcg), norfloxacin (10 mcg), ampicillin (10 mcg), cefotaxime (30 mcg), cefpodoxine (10 mcg), oflaxacin (5 mcg), ciprofloxacin (5mcg) and ceftriazone (30 mcg). Pure bacterial cultures were enriched in brain-heart infusion broth at 37 °C for 6-8 h. The cultures were streaked on Mueller Hinton agar plates (Himedia, India) using a sterile cotton swab and the antibiotic discs were dispensed using a disc dispenser (Himedia, India) with sufficient space in between each disc to avoid

- overlapping. The agar plates were incubated at $37\,^{\circ}\text{C}$ for $16\text{-}18\,\text{h}$ and the zones of inhibition for each antibiotic were measured.
- Molecular diagnosis of the isolates: A genomic DNA bacteria kit was used to extract the DNA of the isolates. The results, in which the DNA is visible as bands under UV light, were initially identified by electrophoresis on 0.8% Agarose (Sambrook and Russell, 2001) [5].

Results and Discussion

In the present study, it was found that out of all the prevalence of food-borne pathogens viz, Ice cream samples were confirmed for presence of Staphylococcus spp. 08 (36%), E. coli 08 (36%) and Listeria spp.04 (18%), Butter samples shown presence of Staphylococcus spp. 02 (33%) and E.coli 02 (33%), Cheese samples shown presence of *Staphylococcus* spp. and E. coli (100%), Yoghurt samples were shown presence of Staphylococcus spp. 02 (50%) and milk shake sample were shown presence of mixed form of bacteria as Staphylococcus spp. The highest numbers of isolates detected were Staphylococcus spp. 018 (42%) and E. coli 018 (42%) followed by Listeria spp. 4 (9.5%) and Klebseilla 02 (2%) in Thanjavur region based on colony morphology, Gram's staining, biochemical characterization and molecular techniques. The isolated E. coli had pink colonies in SMA, Grams negative rod in Gram's staining, positive for Indole and Methyl red tests and negative for Voges-Proskaur tests and acidic butt and acidic slant in TSI utilization test. S. aureus isolated from samples had characteristic yellow colonies in MSA, Gram positive cocci, positive for methyl red, Voges-Proskaur and Citrate utilization tests and negative for Indole test. The antimicrobial susceptibility test of isolated organism was determined and the results revealed that all S. aureus, E.coli and Klebsella were sensitive to Amoxicillin, Cefotaxime, Gentamicin, Ofloxacin, Ceftriaxone Ciprofloxacin and resistant to Methicillin (100%), Vancomycin (100%), Trimethoprim (65%). Polymerase chain reaction test confirmed the presence of 596 bp hlyA gene of Listeria monocytogenes and 500 bp coa gene of Staphylococcus aureus respectively.

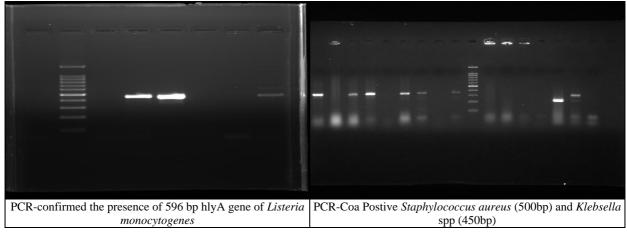


Fig 1: Confirmation of food borne pathogen by polymerase chain reaction

The prevalence of *S. aureus* is higher in icecream, butter, cheese, yohurt (36-50%) than other micro-organism (13-25%). Other studies from Ethiopia and Nepal estimated the prevalence of contamination of milk products with microorganisms as 52% and 41%, respectively (Berhe *et al.*, 2020; Rizal *et al.*, 2023) [6, 7]. The increasing of *S. aureus*, *E.*

coli, Listeria and Klebseilla contamination in milk product shop levels in the current study may be due the poor hygiene conditions practiced by middlemen and at collection centers during the handling of milk, as well as an insufficient cold chain that supports the exponential growth of previously introduced microorganisms at the milk producer level. The contamination of milk products with a high average may be due to milk product used in the absence of hygienic measures, unclean equipment used, and imperfect cooling store, resulting significant multiply of bacteria. The hazard analysis critical control point must be applied at the retail marketplaces to reduce the likelihood of infection by foodborne diseases (Bacigale *et al.*, 2023) [8]. Therefore, in milk products the bacterial contaminants can be controlled by using natural antibacterial agents, and irradiation, in addition to good hygiene management at the farm and milk plants (Yang *et al.*, 2023) [9].

Conclusion

This study was carried out to study the microbiological quality of dairy products. According to these study findings, the surveyed milk products were highly contaminated with different pathogenic bacteria. Further studies are needed not only to investigate other biological hazards in dairy products but also to evaluate sanitation conditions of marketplaces and plant milk, as well as the sources of products where natural milk is derived. Adoption of food safety management is a good strategic intervention that provides food safety for consumers and prevents the spread of food-borne diseases or outbreaks in human populations.

Conflict of Interest

Not available

Financial Support

Not available

Reference

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