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Incidence of spore forming *Clostridium sporogenes* in different dairy products and their industrial and public health significance

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Abstract

Clostridium sporogenes is a food contaminating anaerobic bacteria causes late bowing effect in cheeses and spoilages in other dairy products. A total of 150 samples were studied for the presence of *Clostridium sporogenes* in raw milk, curd and cheese samples. Out of 150 sample 11(22%) *Clostridium sporogenes* was found in raw milk, 7(14%) in curd and 2(4%) in cheese samples. The study shows the existing contamination in dairy products which is the hazardous for the human health as the contamination of *C. sporogenes* is responsible for the food poisoning and food spoilage in certain products.

Keywords: *Clostridium sporogenes*, Raw milk, Cheese, Curd and Spoilages.

1. Introduction

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 micro-organisms therefore the microbial content of milk is a major feature in determining its quality [6]. Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass [3]. The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health [6]. Rinsing water for milking machine and milking equipment washing also involve some of the reasons for the presence of a higher number of micro-organisms including pathogens in raw milk [1]. An analysis of raw milk samples and pasteurized milk, after processing and before packaging demonstrated an association between the properties of the raw milk used and the pasteurized milk produced [4].

Clostridium sporogenes is anaerobic Gram-positive straight rods (bacilli) which can survive in foods and dairy products that are incorrectly or minimally processed. *Clostridium sporogenes* is commonly found as a spoilage organism in canned foods and dairy products [7].

The role of *Clostridium* species in the late blowing of cheese has been emphasised, with *C. tyrobutyricum* a primary cause, and *C. sporogenes*, *C. beijerinckii*, *C. butyricum* reported to be possible secondary causes [2]. The species *Clostridium butyricum* and *Clostridium sporogenes* are the most important anaerobic bacteria being involved in the spoiling of cheese. Before the processing of eventually contaminated milk, proofing for the presence of these spore forming bacteria is necessary to guarantee a satisfying quality of the product [8]. The late blowing defect caused by *Clostridium* spp. in Gouda cheese results in economic loss for the cheese industry. Attempts have been made to prevent the presence of clostridia spores in raw milk and to make conditions in cheese unfavourable for the spores to germinate and grow [10].

The output of dairy and dairy products from India is increasing day by day in their international market. Considering its economic potential, extensive and intensive exploitation of milk can both contribute to the nutrient requirements of the Indian public and increase the income of farmers. In view of the growing public awareness about food safety and quality, knowledge of the microbial and chemical composition of milk is of great significance for further development of its hygienic processing into high quality consumer products. Until now, information on such aspects is scant and scattered. Thus this study was carried out to investigate the microbiological quality and safety of local milk and milk products.

2. Methodology

1) Collection of samples

A total of 150 different milk products (Raw milk, Curd and Cheese) were collected in the local markets of Allahabad (India) region. Fifty each products sample was collected in sterile cap vials and stored in refrigerator until used.

2) Isolation of bacteria from milk and milk products

Isolation of *Clostridium sporogenes* were performed by serial dilution of milk products up to 10^{-8} fold and inoculated on Reinforced Clostridial Agar media (HIMEDIA-M154) with the composition of (g/100ml): Casein enzymic hydrolysate (1.0 g), Yeast extract (0.3 g), Beef extract (1.0 g), Dextrose (0.5 g), Sodium Chloride (0.5 g), Sodium acetate (0.3 g), Starch soluble (0.1 g), L-Cystein hydrochloride (0.05 g), Agar (1.35 g) pH (6.8), which is a selective media for *Clostridium* species. These plates were incubated an-aerobically at 37 °C for 24-48 hrs.

3. Identification of the isolates

The isolates were identified on the basis of cultural, morphological and biochemical characteristics given in Bergey's Manual of Systematic Bacteriology [5].

3.1. Cultural Characteristics: The isolates were identified on the basis of different colony characteristic like colour, texture, margin, and size of colony on the culture plate.

3.2. Gram staining: Gram staining of isolated bacteria was done to observe the morphological characteristics.

3.3. Biochemical Tests: Different biochemical tests Methyl Red test, Voges- Proskauer test, Oxidase test, Indole test, Catalase test, Motility test, Starch hydrolysis test, Esculin Hydrolysis test and Lipase test were also performed for the final identification of *Clostridium sporogenes*.

4. Results and Discussion

A total of 150 samples were studied for the presence of *Clostridium sporogenes* in raw milk, curd and cheese samples. Bacterial colonies were obtained after inoculating the contaminated dairy products on Reinforced Clostridial Agar media. Large (2-5 mm in diameter), irregularly circular, smooth, greyish, translucent with a fibrillar edge that may spread colonies were found on Reinforced Clostridial agar plates. Different biochemical tests were performed for the final identification of *Clostridium sporogenes* (Table 1).

Table 1: Biochemical characteristics of the isolates

Biochemical tests and staining	Results
Gram's reaction	Positive
Endospore staining	Positive
Methyl Red Test	Positive
Voges- Proskauer Test	Negative
Oxidase Test	Negative
Indole Test	Negative
Catalase Test	Negative
Motility Test	Positive
Nitrate Test	Negative
Starch hydrolysis Test	Negative
Lipase test	Positive
Esculin Hydrolysis Test	Positive

A total of 150 samples were studied for the presence of *Clostridium sporogenes* in raw milk, curd and cheese samples. The present study predicts that the incidence was found 11(22%) in raw milk, 7(14%) in curd and 2(4%) in cheese samples (Table 2). The study shows the existing contamination in dairy products which is the alarming situation for the human health as the contamination of *C. sporogenes* is responsible for the food poisoning and food spoilage in certain products.

Table 2: Incidence of *Clostridium sporogenes* in dairy products

Total no. of sample	No. of <i>Clostridium sporogenes</i> isolates	Incidence (%) of <i>Clostridium sporogenes</i> in dairy products		
		Raw Milk	Curd	Cheese
150	20 (13.33%)	11 (22%)	7 (14%)	2 (4%)

The similar results was reported by Feligini *et al.* (2014) [13], they found that the presence of *Clostridium sporogenes* as 77.2% in milk and 86.1% in curd samples. Reindl *et al.* (2014) [14], also found that 38.3% *Clostridium sporogenes* in milk samples. Garde *et al.*, (2011) [11] reported that 78.9% cheese samples spoiled by *Clostridium sporogenes*.

Matteuzzi *et al.*, (1977) [12] also reported that out of 40 isolates from Grana cheeses with late blowing defect, 47.5% were identified as *Clostridium tyrobutyricum*, 45% as *Clostridium sporogenes*, and 7.5% as *Clostridium butyricum*. Torre *et al.*, (2004) [9] reported that the presence of *Clostridium sporogenes* in different food samples.

Increasing of high bacterial counts in dairy product samples are indicator of poor production hygiene or ineffective pasteurization of milk. The late blowing defect caused by *Clostridium* spp. in cheese results in economic loss for the cheese industry. Attempts have been made to prevent the presence of clostridia spores in raw milk and to make conditions in cheese unfavourable for the spores to germinate and grow.

5. Conclusion

The result of the present study is in direct favour of indicating the presence of bacterial strains including *Clostridium sporogenes* and also may be other bacterial strains which may cause food poisoning and other public health problems. Therefore, this data indicates that necessary to reducing the levels of Clostridia in milk other dairy products. It will also a strict need for hygienic practices with aseptic preservation techniques to handle the milk and milk products as they are much essential for the human health.

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