Available Online at http://www.recentscientific.com

ISSN: 0976-3031

International Journal of Recent Scientific Research
Vol. 6, Issue, 2, pp.2827-2829, February, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

OCCURANCE OF *CLOSTRIDIUM* SPECIES IN DIFFERENT DAIRY PRODUCTS AND ITS ASSOCIATED HEALTH RISK

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ARTICLE INFO

Article History:

Received 2nd, January, 2015
Received in revised form 10th, January, 2015
Accepted 4th, February, 2015
Published online 28th, February, 2015

Key words:

Clostridium species, Raw milk, Cheese, Curd and Spoilages.

ABSTRACT

Clostridium species is a food contaminating anaerobic bacteria causes food poisoning and spoilages in milk and milk products. A total of 200 samples were studied for the presence of *Clostridium* species in raw milk, curd, cheese and paneer samples in which 62(31%) sample showed positive growth for *Clostridium* species. Out of these 62 *Clostridium* isolates 22(35.48%) were confirmed as *Clostridium perfringens*, 21(33.87%) *Clostridium sporogenes*, 12(19.35%) *Clostridium butyricum* and 7(11.29%) were confirmed as *Clostridium tyrobutyricum.* In the present investigation the milk and milk products showed the high occurrence of *Clostridium perfringens* which is 26% in raw milk. The study shows the existing contamination in dairy products which is the hazardous for the human health as the contamination of *Clostridium* species is responsible for the food poisoning and food spoilage in certain products.

INTRODUCTION

Milk is a highly nutritious food, ideal for microbial growth and the fresh milk easily deteriorates to become unsuitable for processing and human consumption (Fao, 2001). Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass (Coorevits *et al*., 2008). 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 (Bramley, 1990). *Clostridium* spp. is a *Gram* positive and rod-shaped microorganism. It is an obligate anaerobe, meaning that oxygen is poisonous to the cells. However, *Clostridium botulinum* tolerates traces of oxygen due to the enzyme called superoxide dismutase which is an important antioxidant defense in nearly all cells exposed to oxygen (Borah *et al.*, 2011). The species most frequently isolated from milk and spoiled cheeses are *Clostridium perfringens, Clostridium tyrobutyricum, Clostridium butyricum, Clostridium sporogenes* and *Clostridium beijerinckii* (Feligini *et al.*, 2014). *C. tyrobutyricum* is usually considered to be the primary cause of late blowing, *C. sporogenes C. beijerinckii*, and *C. butyricum* are also significant secondary causes of gas production in cheese (Bourhis *et al.*, 2007). *Clostridium* spores present in cheese originate from milk, which becomes contaminated during the milking process. Silage of poor microbiological quality has been identified as the main source of raw milk contamination with spores that are able to survive the pasteurization process (Garde *et al.*, 2011).

High bacterial counts are indicator of poor production hygiene or ineffective pasteurization of milk**.** Milk and milk derived products are among the most widely consumed food products worldwide. *Clostridium* spp. is a common food contaminating anaerobic bacteria. Because of the pathogenic properties, there is a need of studying the various characteristics of the species to get a better idea about the functioning of metabolic activity of cell and which would help in finding out best applicable ways to check its growth and reduce the chances of infection in human society.

METHODOLOGY

Collection of samples

A total of 200 different milk products (Raw milk, Curd, Cheese and Paneer) 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.

Isolation of bacteria from dairy products

Isolation of *Clostridium* species were performed by serial dilution of milk products up to 10^{-6} -10^{-8} fold and inoculated on Reinforced Clostridial Agar media (HIMEDIA-M154) with the

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composition of (g/100ml): Casein enzymic hydrolysate (1.0g), Yeast extract (0.3g), Beef extract (1.0g), Dextrose (0.5g), Sodium Chloride (0.5g), Sodium acetate (0.3g), Starch soluble (0.1g), L-Cystein hydrochloride (0.05g), Agar (1.35g) pH (6.8), which is a selective media for *Clostridium* species. These plates were incubated anaerobically at 37°C for 24 hrs.

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.

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

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

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

Statistical analysis

The data recorded during the course of investigation was statistically analyzed by using χ^2 test and conclusion was drawn accordingly.

RESULTS

In the present study total 200 samples of different selected dairy product namely Raw milk (50), Curd (50), Cheese (50) and Paneer (50) were studied for the presence of *Clostridium* species. Out of these 200 samples, 62 (31%) showed positive growth for *Clostridium* species on Reinforced Clostridial Agar media (Table 1). Raw milk samples (51.61%) were found to be highly contaminated with *Clostridium* species as compared to Curd (22.58%), Paneer (14.51%) and Cheese (11.29%) tested and the difference was found to be statistically significant ($P<0.05$).

Table1 Incidence of *Clostridium* species in selected dairy samples

Total no. of sample	No. of isolates	Incidence (%)			
		Raw Milk	Curd	Cheese	Paneer
200	62 (31%)	32 (51.61%)	14 (22.58%)	07 (11.29%)	09 (14.51%)

Among the 200 samples of dairy products 62 (31%) *Clostridium* species were isolated. On the basis of cultural, morphological and biochemical tests (table 2) it was found that out of these 62 *Clostridium* isolates 22 (35.48%) were confirmed as *Clostridium perfringens* having an incidence of 26% in Raw milk, 8% in Curd, 4% in Cheese and 6% in Paneer. Twenty one (33.87%) was *Clostridium sporogenes* which shows the incidence of 22% in Raw milk, 14% Curd, 4% Cheese and 2% Paneer. Similarly the occurrence of 12 (19.35%) *Clostridium butyricum* and 7 (11.29%) *Clostridium tyrobutyricum* were observed with the incidence of 10% and 6% in Raw milk, 4% and 2% in Curd, 6% and 2% in Cheese and 4% each in Paneer samples respectively. Table 3 and fig. 1 shows the distribution of different *Clostridium* spp. in selected dairy products showing the high occurrence of *Clostridium perfringens* which is 26% in raw milk.

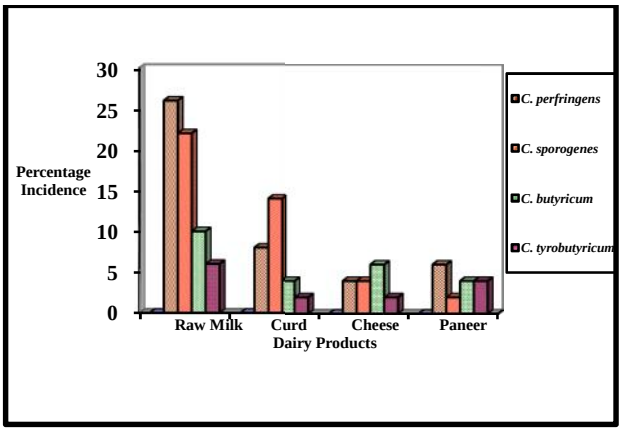

Fig. 1 Incidence of different *Clostridium* species in dairy products

DISCUSSION

This study has shown that milk handled by the milk marketing agents was poor quality considering the high percentage of *Clostridium* species in milk samples. Edema and Akingbade (2007) studied on total of forty-five samples of three most commonly consumed unsweetened evaporated milk for occurrence of spore-forming bacteria. All the samples had high numbers of spore-forming bacteria of the genera *Bacillus* spp. and *Clostridium* spp. Among the all isolates 20% isolates were *Clostridium* spp.

Feligini *et al.* (2014) reported the incidence 98.7% of *Clostridium perfringens* in milk and 100% in curd similarly they also found the presence of *Clostridium sporogenes* as 77.2% in milk and 86.1% in curd, they also retrieved the occurrence of *Clostridium beijerinckii* and *Clostridium tertium* 100% in milk and curd.

Hassan and Afify (2007) studied fifty random samples of Karish cheese and reported the bacteriological presence of *Clostridium perfringens* with the percentage incidence of 3 (6%). They also reported the highest frequency distribution of *Clostridium perfringens* was 47 (94%) which was in the range $<10^2$ *Clostridium perfringens*/g.

Garde *et al.,* (20011) was investigated the presence of lactate-fermenting Clostridial spores in 194 samples of raw bulk-tank ovine milk and in 57 Manchego cheeses with late blowing defect. Spores were detected in 97% of milk samples analysed, with a most probable number (MPN) of 14.5 spores mL^{-1} and significantly higher counts in summer milk. Out of 223 isolates from spoiled cheeses, 78.9%, 10.3%, 9.0% and 1.8% were identified as *Clostridium sporogenes, Clostridium beijerinckii, Clostridium tyrobutyricum* and *Clostridium butyricum*, respectively. Matteuzzi *et al.,* (1977) 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*.

CONCLUSION

In conclusion, the findings of this study highlight the poor microbiological quality of milk and milk products. This is most likely due to poor handling, the use of unsterile milk transport

Table 2 Identification of organisms on the basis of cultural, morphological and biochemical characteristics

Characteristics		*Clostridium* species			
		Clostridium perfringens	***Clostridium butyricum***	***Clostridium sporogenes***	***Clostridium tyrobutyricum***
Cultural characteristics	Colour	White cream	White to cream	Grey	Greyish white
	Shape	Irregular large	Circular flat	Circular to irregular	Circular
	Size (in diameter)	2-5mm	1-5mm	1.5-6mm	0.5-1.5mm
Morphology characteristics	Gram stain Reaction	+ve	+ve	+ve	+ve
	Cell shape	Rods	Rods	Rods	Rods
Biochemical Characteristics	Indole production	-ve	-ve	-ve	-ve
	MR	+ve	+ve	+ve	+ve
	VP	-ve	-ve	-ve	-ve
	Citrate utilization	-ve	-ve	-ve	-ve
	Motility	-ve	+/-ve	+ve/-ve	+ve/-ve
	Catalase	-ve	-ve	-ve	-ve
	Oxidase	-ve	-ve	-ve	-ve
	Starch hydrolysis	+ve/-ve	+ve	-ve	-ve
	Lipase	-ve	-ve	+ve	-ve
	Esculin hydrolysis	+ve/-ve	+ve	+ve	-ve
	Nitrate reduction	+ve/-ve	-ve	-ve	+ve/-ve
	H_2S production	+ve	-ve	+ve	-ve
Sugar fermentation	Arabinose	-ve	+ve	-ve	-ve
	Xylose	-ve	+ve	-ve	+ve/-ve
	Mannitol	-ve	-ve	-ve	-ve
	Raffinose	+ve/-ve	+ve	-ve	-ve
	Sucrose	+ve	+ve	-ve	-ve
	Lactose	+ve	+ve	-ve	-ve

[+ve = (90 – 95% of strains are positive), -ve = (90 – 95% of strains are negative), +ve/-ve = (11- 89% of strains are positive)].

Table 3 Incidence of different *Clostridium* species in dairy products

Organisms present	Raw Milk (50)	Curd (50)	Cheese (50)	Paneer (50)	Total Isolates
C. perfringens	13 (26%)	4 (8%)	2 (4%)	3 (6%)	22
C. sporogenes	11 (22%)	7 (14%)	2 (4%)	1(2%)	21
C. butyricum	5 (10%)	2 (4%)	3 (6%)	2 (4%)	12
C. tyrobutyricum	3 (6%)	1 (2%)	1 (2%)	2(4%)	07

equipment, and high ambient temperature prevailing in the study area. The presence of *Clostridium* species in milk and milk products indicate the potentially hazardous product which is likely to be posing a serious public health risk to consumers, particularly if the milk is not pasteurized or adequately boiled. These findings highlight the need to implement improved hygiene practices and to apply effective monitoring throughout the production to delivery chain. Moreover, further studies are needed to positively eliminate the occurrence of toxins produced by *Clostridium* species and other harmful microorganisms.

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