



RESEARCH ARTICLE

OCCURRENCE OF *LISTERIA MONOCYTOGENES* IN MILK AND MILK PRODUCTS

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ABSTRACT

The objective of the present study was to obtain information on the prevalence of *L.monocytogenes* in milk and milk products available in and around Chidambaram Taluk and to create awareness for the public about this dreadful pathogen. Out of the 35 samples examined 4 samples (11.42%) showed positive for the presence of *L.monocytogenes*. The 4 isolates recovered from the samples were identified based on morphological, cultural and biochemical characteristics. *L.monocytogenes* contamination was recorded in raw milk, yoghurt and milk kova samples.

Key words: Milk and Milk products, *Listeria monocytogenes*.

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INTRODUCTION

Listeriamonocytogenes is a food borne pathogen widely distributed in food stuffs such as vegetables, fruits, dairy products and processed food item. *Listeria* is a Gram positive, facultative anaerobic, non-spore forming, rod shaped, catalase positive, oxidase negative, motile and intracellular bacterium with a low G+C, which measures 0.5 µm in diameter and 1-2 µm in length. Gram staining showed that the cells can be found in chains or as single rods. Growth of the organism on bacteriological media is enhanced by the presence of glucose or other fermentable sugars, but is also dependent on the atmosphere and temperature in which they are grown (Seeliger and Jones, 1986). It has a growth temperature range of approximately 1°C-45°C, making it as a psychrotroph and a mesophile (Elzen and Snijders 1993). At 20-25°C peritrichous flagella are formed and cause the organism as motile (Norrungand Gerner Smidt, 1993). Additionally, its ability to not only survive but to grow as a psychrotroph at 4°C makes this pathogen unique from other commonly found food borne pathogens. For many years the genus *Listeria* contained only one species, *L.monocytogenes*, at present however, there are six recognized species including *L.monocytogenes*, *L.innocua*, *L.welshimeri*, *L.seeligeri*, *L.ivanovii*, and *L.grayi* (Rocourt, 1999). The strains of *L.monocytogenes* are pathogenic to humans and animals, while *L.ivanovii* are pathogenic only to animals (Elzen and Snijders, 1993). *L.Monocytogenesis* is a bacterial pathogen responsible for human listeriosis, which causes fever,

muscle aches and sometimes gastrointestinal infection and the symptom such as headache, stiff neck, confusion, nausea or diarrhea, loss of balance or convulsions can occur (Mead et al., 1999). Sometimes the infection may spread to nervous system also, the disease has a high fatality rate in the susceptible population (Garrido et al., 2008). Transmission is through eating contaminated food, particularly milk products (EFSA, 2009). Consumption of raw and partially cooked contaminated food leads to listeriosis, among the immune compromised people's elderly persons, pregnant women, neonates (Farber et al., 1991). Listeriosis may cause serious condition such as septicemia, meningitis and encephalitis infection that may result in spontaneous abortion (2nd -3rd trimesters) or stillbirth in pregnant woman. As these pathogens are capable of surviving even under refrigerated condition, posing threat to the food industries and there by the consumers (Ryser et al., 1991). The WHO has decided to notify the organism as a server threat to food and consumer safety (Broschet et al., 1996). The ubiquitous nature of the bacteria inevitably result in contamination of many food products (Farber and Peterkin, 1991).

Milk is the fluid secreted by mammals for the nourishment of their young ones. The principle components of milk are water, fat protein and lactose higher water activity moderate pH (6.4-6.6). The milk is an excellent culture medium for microorganisms, including pathogens (Adams and Moss 2008). The presence of pathogenic bacteria in raw milk have been a major factor for public health concern; the main sources of contamination are the dairy cattle: foodhandler, dairy equipments and consumption raw milk are reconsidered to be the main cause of several outbreaks of *Listeria monocytogenes*, *Salmonella sp.*, *Escherichia coli*. Based on the problems

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discussed above, and to reveal the hidden threat of *L.monocytogenes* in milk and milk products the present study was performed.

MATERIALS AND METHODS

Collection of Samples

To study the presence of *L.monocytogenes* in milk and milk products, the samples were collected from the shops around Chidambaram taluk, Tamilnadu, India. The study was conducted in the Laboratory of Department of Microbiology, Faculty of Science, Annamalai University. A total of 35 milk products were purchased and the samples were stored in the refrigerator for further use.

Isolation Methods

About 25 gm of samples were homogenized in a blender and transferred to 225 ml of peptone broth. The inoculated peptone broth was incubated at 30°C for 24-48 hrs. After enrichment, 1ml of the sample was pour plated on to Listeria selective agar and the plates were incubated at 35°C to 37°C for 24 -48 hrs. The typical colonies formed after incubation were presumed to be *Listeria* spp and they were subcultured on to nutrient agar for further identification.

Identification methods

The isolates thus obtained from milk products samples were further identified based on their morphological, cultural, and biochemical characteristics (Aneja, 2003).

RESULT

Totally 35 milk and milk product samples were collected from shops in and around Chidambaram taluk (Table-1).

Table 1. List of samples collected for the study

S.No	Milk samples	No.of samples examined
1.	Raw milk	5
2.	Pasteurized milk	5
3.	Cheese	5
4.	Yogurt	5
5.	Butter	5
6.	Milk paneer	5
7.	Milk Kova	5
Total		35

Table 2. Occurrence of *L. monocytogenes* in milk and milk Products

S.No	Milk samples	No.of samples examined	Positive samples	Percent age(%)
1.	Raw milk	5	2	25
2.	Pasteurized milk	5	-	-
3.	Cheese	5	-	-
4.	Yogurt	5	-	-
5.	Butter	5	1	20
6.	Milk paneer	5	-	-
7.	Milk kova	5	1	20
Total		35	4	11.42

Out of 35 samples examined the pathogenic microorganism *L.monocytogenes* was isolated from 4 samples of milk and milk products. The 4 isolates recovered from the samples were identified based on morphological, cultural and biochemical

characteristics. About 11.42% of milk products were found to be contaminated with *Listeria monocytogenes*. In our study, raw milk (25%), yoghurt (20%) and milk kova (20%) samples contained *L.monocytogenes* (Table-2).

DISCUSSION

Numerous sporadic and epidemic outbreaks worldwide involving *Listeria* in contaminated foods have been reported. Foods include ready to eat foods (RTE) unpasteurized dairy products, meat products, seafoods have been common foods implicated (Schlech, 2000). Farber and Peterkin (1991) stated that cow milk has been implicated in food-borne fatal listeriosis. The ability of *L.monocytogenes* strains to proliferate in raw milk at ambient and under refrigerated conditions is well documented by many authors (Donnelly and Briggs, 1986; Lovett *et al.*, 1987; Rosenow and Marth, 1987; Slade *et al.*, 1988). When compared with other bacteria *Listeria* shows resistance to heat, especially in proteinaceous foods. A study carried out by Gunasena *et al.*, (1995) on the occurrence of *L.monocytogenes* in market samples of different food items indicated that 38% of the samples contained *L.monocytogenes*, of them 49% of vegetables 34% of chickens and 26% of dairy products were contaminated with *L.monocytogenes*. In our study, 11.42% of milk products were found to be contaminated with *Listeria monocytogenes*. The isolation of *L.monocytogenes* from foods require the use of selective agents and enrichment procedures allow the multiplication of *L.monocytogenes*.

In the present study, peptone broth was used as enrichment media and *Listeria* selective agar was used to select *Listeria* spp., from food samples. Typical *Listeria* sp. colonies on the selective agar plates were sub cultured on the Nutrient agar plates and further identification done by performing series tests to confirm morphological, physiological and biochemical characteristics. In the present study, out of 35 samples examined the pathogenic microorganism *L.monocytogenes* was isolated from 4 samples of milk and milk products. About 11.42% of samples showed positive for the presence of *Listeria*. Jayamanne and Samarjewa *et al.* (2001) reported that, out of 265 milk samples examined 39 samples (15%) contained virulent *L.monocytogenes*. Cow milk (29%), goat milk (27%), pasteurized milk (17%) and cheese (33%) samples contained virulent strains of *L.monocytogenes*. Sterilized milk, UHT milk, curd and yogurt did not contain *L.monocytogenes*. In our study, raw milk (25%), yoghurt (20%) and milk kova (20%). Samples contain *L.monocytogenes*. Yakuha *et al.*, (2012) Screened 192 raw milk samples collected from lactating cows in small scale dairy farms within sokoto metropolis to investigate the prevalence of *L.monocytogenes* in the milk. About 76 (39.58%) samples were positive for the *Listeria* sp. based on biochemical investigations.

Mansouri – Najand *et al.*, (2015) reported that out of 100 raw milk samples collected from dairy farms only 5 isolates (5%) were detected as *L.monocytogenes*. Seyoumet *et al.*, (2015) documented the presence of *L.monocytogenes* in 443 milk and milk products from highlands of Ethiopia. Out of the 443 milk and milk products examined, 28.4% product were contaminated with *Listeria* sp. Cheese was found to be highly contaminated at 60%, followed by pasteurized milk samples (40%), raw milk (18.9%) and yoghurt (5%). Herein, the presence of *L.monocytogenes* in milk and milk products might be due to environment inadequate cooling, unhygienic handling of food

by food handlers, post process contamination, improper packing and storage. The main reason for *L.monocytogenes* in foods due to its ubiquitous nature, resistance towards heat and cool environment etc.

Conclusion

Among the food borne diseases, the least notifiable disease is listeriosis, it is considered as most dreadful disease with high fatality rate. The pathogen *L.monocytogenes* can survive at extreme environments, and presents high risk to human health. The present study revealed the presence of *L.monocytogenes* in milk and milk products emphasizes the fact that proper handling, adequate cooling, proper storage condition are must in controlling the pathogen.

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