



*Original Research*

## Screening of Zoonotic *Listeria* spp. in Milk and Dairy Products by Conventional Method and PCR

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Rec. Date:	Jul 20, 2018 04:33
Accept Date:	Nov 02, 2018 00:28
DOI	<a href="https://doi.org/10.5455/ijlr.20180720043304">10.5455/ijlr.20180720043304</a>

### Abstract

The present study was conducted to study the prevalence of zoonotic *Listeria monocytogenes* in milk and dairy products. About 567 samples comprising of milk and dairy products were collected randomly from several retail outlets and supermarkets in different zones of Chennai, India. Out of 567 samples, 9 (1.58%) were positive for *Listeria* spp. by conventional culture method. The occurrence of *Listeria* spp. in heat and acid coagulated dairy products, frozen dairy products and other dairy products like butter and cheese was 2 (6.06%), 3 (9.37%) and 4 (8.69%) respectively, while *Listeria* spp. was not detected from other dairy products analysed. Further, confirmation by biochemical characterization revealed 7 isolates of *L. welshimeri*. Moreover, the polymerase chain reaction targeting the virulent *hlyA* gene of *Listeria monocytogenes* revealed that none of the samples screened were positive for *L. monocytogenes*.

**Key words:** Conventional Method, Dairy Products, *hlyA* gene, *Listeria* spp., *Listeria monocytogenes*, Milk

**How to cite:** Parseelan, A., & Sekar, M. (2019). Screening of Zoonotic *Listeria* spp. in Milk and Dairy Products by Conventional Method and PCR. International Journal of Livestock Research, 9(2), 78-83. doi: 10.5455/ijlr.20180720043304

### Introduction

India's dairy sector had shown remarkable development in the past decade and had become the largest producers of milk and value added milk products in the world. The annual growth rate of milk production in Tamil Nadu for the year 2016 – 2017 is 4.3 per cent sharing 4.6 per cent of the total milk production in the country BAHS (2017). The consumption of milk and milk products has increased in both rural and urban areas by 29 and 26 per cent, respectively Ministry of Agriculture and Farmers Welfare (2016). In recent years, a number of food-borne illness caused by the consumption of milk and dairy products have increased public health awareness and concern about food quality and safety. Milk and dairy products are rich in nutrients; hence it can be considered a potential source for the transmission of food-borne diseases



to human. There are many food-borne pathogens that may be transmitted through milk and dairy products such as *Staphylococcus aureus*, *E. coli*, *Salmonella* spp., *Listeria monocytogenes* etc. Various *Listeria* spp. like *L. welshimeri*, *Listeria monocytogenes*, *L. ivanovii* and *L. seeligeri* are commonly found in the dairy environment, farm and processing plants Sarfaz *et al.* (2017). Amongst the *Listeria* spp., *Listeria monocytogenes* is a zoonotic food-borne pathogen, which ranks third for its high mortality rate among the susceptible populations. Other species like *L. ivanovii* and *L. seeligeri* has also been reported by Gilot and Content (2002). However, in dairy sector, *L. monocytogenes* is of public health significance as it was related to post pasteurization contamination and considered a potential threat to consumer Olszewska *et al.* (2015). Owing to the capability of growth in refrigeration temperature and adverse environmental conditions such as high acidity and reduced water activity, *L. monocytogenes* becomes a potential threat in milk and dairy products.

Hence, the present study was carried out to detect the presence of pathogenic *Listeria* spp. especially *L. monocytogenes* in milk and dairy products in Chennai, India.

## Materials and Methods

### Bacterial Strains

Standard cultures of *Listeria monocytogenes* (MTCC 1143), *Staphylococcus aureus* (MTCC 3160) were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

### Collection of Samples

The study area for conducting this study was purposively selected based on the bifurcations of Chennai into three regions *viz.* Chennai north, Chennai central and Chennai south and each region constitutes five zones and thus the city is composed of fifteen zones. From each zone, the samples were collected by a simple random sampling procedure yielding a total of 567 samples (75 raw milk, 45 pasteurized milk, 447 dairy products) *viz.*, channa based sweets (85), khoa based sweets (99), fermented dairy products (73), concentrated or partially desiccated dairy products (37), heat and acid coagulated dairy products (33), frozen dairy products (32), chilled and flavoured dairy products (42) and other dairy products like butter, cheese (46). These samples were collected from local milk vendors, cooperative milk dairies, dairy/confectionery shops, supermarkets and sweet shops in sterilized milk collecting tubes and clean polyethylene bags aseptically at various locations of Chennai city and transported in insulated containers under refrigerated condition to the laboratory (Dept. of VPH&E, Madras Veterinary College, Chennai, India) and stored at 4°C and analyzed within 24 hr.

### Isolation and Identification of *Listeria* spp.

In brief, the samples were screened for the presence of *L. monocytogenes* using the standard procedure described in FDA- BAM by Hitchins *et al.* (2017). They were pre-enriched and enriched in Buffered *Listeria* Enriched Broth (BLEB) at 30°C for 4 and 44 hrs, respectively. During enrichment, *Listeria* selective supplement II was added prior to incubation. After incubation, a loopful of inoculum from buffered *Listeria* enrichment broth was streaked on sterile *Listeria* selective agar base as well as *Listeria* identification agar base (PALCAM Agar) and incubated at 37°C for 48 hr to observe the typical colonies of *Listeria* spp. On *Listeria* selective agar base, the characteristic colonies were small smooth and small colourless. On *Listeria* identification agar base (PALCAM agar), the characteristic colonies were grey green with black precipitate. Then, the colonies were examined for their morphology, gram staining, motility and further subjected to biochemical tests such as oxidase, catalase sugar fermentation and hemolysis for confirmation.

### Biochemical Characterization and Identification of Isolates

KB012A Hi*Listeria*™ identification kit procured from M/s. Himedia Laboratories, Mumbai, was used in this study as per manufacturer's instructions. It is a standardized colorimetric identification system, which utilizes the conventional biochemical tests and carbohydrate utilization tests. The biochemical tests performed with Himedia identification kit includes catalase, nitrate reduction, esculin hydrolysis, Voges Proskauer's reaction, methyl red and 7 different carbohydrates utilization test- xylose, lactose, glucose,  $\alpha$ -Methyl-D-Mannoside, rhamnose, sucrose and mannitol.

### Genotypic Characterization for the Detection of *L. monocytogenes*

DNA from the bacterial isolates was extracted using bio basic molecular biology kit (bio basic inc., Canada) as per manufacturer's instructions. Genomic DNA extracted from all the 9 *Listeria* isolates and was used as a template. PCR was carried out targeting the virulence factor Listeriolysin O (LLO) which encoded by *hlyA* gene which is specific for *L. monocytogenes* as described by Paziak-Domanska *et al.* (1999), Ueda *et al.* (2005), Aurora *et al.* (2007), Suriyapriya *et al.* (2016) and Sanlibaba *et al.* (2018).

The primer for amplification of *hlyA* gene which encodes LLO are *hlyA* (5'GAC TTT TCC AAT CCC GAA CGG AAT 3') and *hlyA* (5' TGC CGA TCA CTT AAG GGC CTT CAT 3') Paziak-Domanska *et al.* (1999). PCR was performed with initial denaturation at 95°C for 2 minutes followed by 30 cycles of denaturation for 15 sec at 95°C, annealing for 30 seconds at 60°C and initial extension for 90 second at 72°C and final extension for 10 minutes at 72°C. The PCR products were analysed by electrophoresis stained 2.5% agarose with 1% solution of ethidium bromide.

## Results and Discussion

### Distribution Pattern of *Listeria* spp. in Milk and Dairy Products

Out of 567 samples (milk and dairy products) collected, only 9 isolates were found positive for *Listeria* spp. All the 9 isolates were catalase positive and oxidase negative, which was then subjected individually to the three different sugar reduction tests using mannitol, rhamnose and xylose to identify the *Listeria* species. The results revealed that 7 (77.77%) isolates have reduced rhamnose and xylose but not mannitol. Hence, these isolates were identified as *L. welshimeri*. Remaining 2 (22.22%) isolates reduced only xylose but not other sugars, hence it may be either *L. ivanovii* or *L. seeligeri*. In the present study, *Listeria* spp. was not isolated from raw and pasteurized milk. Similarly, Sarker and Ahmed (2015) didn't report *Listeria* spp. in pasteurized milk samples analysed.

The occurrence of *Listeria* spp. in heat and acid coagulated dairy products, frozen dairy products and other dairy products like butter and cheese was 2 (6.06%), 3 (9.37%), 4 (8.69%) respectively, while *Listeria* spp. was not detected from other dairy products analysed. Among these isolates, *L. welshimeri* was found in 2 heat and acid coagulated products, 2 frozen dairy products and 1 other dairy products like butter and cheese. Whereas, *L. ivanovii* and *L. seeligeri* has been found in 1 frozen dairy product and 3 other dairy products like butter and cheese. Mary and Shrinithiviahshini (2017) done a study at Tiruchirappalli, Tamil Nadu, analysed 415 milk and dairy products to determine the incidence of *Listeria monocytogenes* and reported *L. monocytogenes* in branded milk (65.9%), cheese (62.5%), ice-cream (49.2%), milk powder (26.6%), milk sweets (20%), ghee and paneer (13.3%) and yoghurt (6.6%). Whereas, in the present study, *L. monocytogenes* was not isolated from milk and dairy products analyzed. The incidence of *Listeria* spp. in frozen dairy products could be due to the lack of hygienic practices during processing and handling of products. This may be attributed due to contamination of raw milk, use of low quality of ingredients and polluted water supplies. The incidence of *Listeria* spp. in cheese may be attributed due to its composition, pH, moisture, salt, storage conditions, starter cultures etc. Kovincic *et al.* (1991).

Among the zones by conventional culture method, Chennai south showed higher incidence of *Listeria* spp. 6 (4.02%), followed by Chennai north 3 (2.05%). Among the 6 isolates of Chennai south, 3 frozen dairy products, 2 other dairy products like butter and cheese and 1 heat and acid coagulated dairy product were found contaminated by *Listeria* spp. Whereas, among the three isolates of Chennai north 2 heat and acid coagulated dairy product and 1 other dairy products like butter and cheese has been contaminated by *Listeria* spp. From the present study, it is to be noted that the microbial quality of dairy product depends upon storage, handling, manufacturing practices etc.

PCR targeting virulent *hlyA* gene was performed for all the 9 presumptive isolates and the results revealed that none of the isolates were *L. monocytogenes* (Table 1). Various other studies by Dhanashree *et al.* (2003), Navratilova *et al.* (2004), Mahmodi (2010) and Dharmendra *et al.* (2013) also reported the absence

of *L. monocytogenes* in the dairy products analyzed by them. Suriyapriya *et al.* (2016) also reported the absence of *L. monocytogenes* in traditional milk products screened in Chennai, Tamil Nadu. All the above studies were in accordance with the results of the present study.

**Table 1:** Distribution of *Listeria* spp. in different regions of Chennai

Zones	Culture Positive		PCR Positive		Test
	No. of Isolates	Per cent Isolation	No. of Isolates	Per cent Isolation	Chi-square Test
Chennai north	3	2.05	0	0	3.03 <sup>NS</sup>
Chennai central	0	0	0	0	Nil
Chennai south	6	4.02	0	0	Nil

Generally, in milk and dairy products, growth of *L. monocytogenes* may be influenced by a variety of factors, which can be affected by temperature, water activity, pH, and lactic acid levels, salt and the presence of preservatives. Absence of *L. monocytogenes* in the milk products might be attributed to the various processing methods like heat and acid coagulation, heat desiccation, and fermentation etc.,

### Conclusion

Various studies in India suggest the possibility of pathogenic *Listeria* contamination in food products especially in ready-eat dairy products. Hence, dairy products can be a threat to consumers especially to the new born, elderly and immuno compromised. Hence, all sectors of food production chain should improve hygienic measures, regular monitoring and strict approaches to food safety such as HACCP principles should be implemented as consumption of ready-to-eat food products has increased recently. Epidemiological studies should be carried out for better understanding about the pathogen.

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