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Prevalence of *Listeria* spp. in Traditional Indian Dairy Products from Chennai Metropolis, Tamil Nadu

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Abstract

The present study was conducted between September, 2014 and June, 2015 to assess the prevalence of *Listeria monocytogenes* in Traditional Indian Dairy Products from Chennai, Tamil Nadu. Samples of Ghee, Rasagolla, Gulabjamun, Curd and Payasam were screened for the presence of *L. monocytogenes* using conventional culture method and validated by PCR. Among the 150 samples screened, 50 isolates from different dairy products (22 gulabjamun, 22 rasagolla and 06 curd samples) were presumptively identified as *Listeria* spp. Further confirmation by biochemical characterization and hemolysis on blood agar revealed that 34 isolates were *Listeria welshimeri*, 10 were *L. murrayi* and 06 were identified as *L. seeligiri*. The 34 isolates of *L. welshimeri* were present in 14 gulabjamun, 14 rasagolla and 06 curd samples. Similarly, 10 isolates of *L. murrayi* have been found in 04 gulabjamun and 06 rasagolla samples. *L. seeligiri* isolates were identified from 04 gulabjamun and 02 rasagolla samples. None of the *Listeria* species were found in ghee and payasam samples and interestingly, the results of both methods revealed that none of the traditional products screened were positive for *L. monocytogenes*

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1. Introduction

India is the leading and one of the most economic milk producers in the world. Of the total milk produced, 50% is being consumed by rural households and the rest is sold in the domestic market, wherein 50% as fluid milk, 35% as traditional products and 15% as other processed dairy products (Himabindu *et al.*, 2014). Vast majority of the population in the country have diverse food habits and consumption of dairy products is primarily skewed towards traditional ones. These products are generally classified into five different categories that includes Desiccated, Heat and acid coagulated, Fermented, Fat rich and Milk based puddings/desserts. The impact of

globalization and technological development has opened the new arena for marketing regional dairy products at both national and international level.

Various researchers have reported the incidence of food-borne pathogens like pathogenic *Escherichia coli*, *Salmonella* spp., *Aeromonas* and *Yersinia* in milk and dairy products (Laba et al., 2013; Yadav et al., 2014). *L. monocytogenes* is one such bacterial pathogen has been identified as a causative agent in series of outbreaks involving milk and dairy products (Shahbazi et al., 2013; Boujemaâ et al., 2013). This pathogen causes listeriosis in humans and the mortality rate is very high (30%). Pregnant women, infants, immunocompromised and the elderly people are at greatest risk for listeriosis (Boujemaâ et al., 2013).

However, no extensive study has been carried out in Chennai to assess the prevalence of *L. monocytogenes* in traditional Indian dairy products. Hence, there is a need to assess the prevalence of this pathogen in the study area that will through a light to identify the products that are vulnerable to serve as a source of this pathogen to the intended consumers. Further, the data obtained will also serve as a template for the standard setting bodies at national level and also to develop and implement control strategies to the vulnerable dairy products by food business operators. Keeping the above points in mind, the present study was designed to assess the prevalence of *L. monocytogenes* in traditional dairy products sold in Chennai.

2. Methodology

Indian traditional dairy products have been classified into five major categories viz., Fat rich, Heat and acid coagulated, heat desiccated, fermented products and dairy puddings & desserts. Prior to sample collection, a survey was conducted in Chennai, Tamil Nadu to identify the dairy product in each category that has been preferred by most of the consumers and thereby ghee, rasagolla, gulabjamun, curd and payasam were selected. The entire study was conducted during the period between September, 2014 and June, 2015.

A total of 150 samples comprising each 30 numbers of selected products that represent each category were screened for the presence of *L. monocytogenes* using the standard procedure described in Bacteriological Analytical Manual, 2011 with modifications. In brief, the samples were pre-enriched and enriched in Buffered Listeria Enriched Broth at 30°C for 4 and 44 hrs, respectively. During enrichment, Listeria Selective Supplement II was added prior to incubation and then streaked on Listeria Oxford Agar plates added with modified Listeria Moxalactam Supplement. The plates were incubated at 35°C for 48 hours. Characteristic colonies were picked and streaked on yeast extract added Tryptone Soya Agar plates for purification and incubated at 35°C for 48 hrs. 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.

For the further confirmation of isolates, PCR was carried out targeting *hlyA* gene which is specific for *L. monocytogenes* (Ueda et al., 2005). The PCR protocol was standardized by optimizing the annealing temperature, primer concentration, MgCl₂ concentration, template volume and cyclic conditions.

3. Results

Of the 150 samples of dairy products screened, 58 showed distinctive *listeria* colony features on Modified Oxford Agar. Upon purification on yeast extract added Tryptone Soy Agar, all the 58 isolates have again showed characteristic morphology of *Listeria* colonies (small, regular, smooth and dense/iridescent white in colour). Similarly, all isolates were positive in gram staining and appeared as short rod shaped bacteria. However, only 50 purified isolates that were incubated at 30°C for 24 hrs have shown typical tumbling motility pattern upon hanging drop preparation. These fifty presumptive *Listeria* colonies were preserved as glycerol stocks for further biochemical confirmation.

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