

Prevalence of *Listeria monocytogenes* in Temple Milks Offered to the Devotees as Sacred Liquid in Tiruchirappalli, Tamilnadu, India

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Abstract Investigation was carried out to test the occurrence of *Listeria monocytogenes* in the temple milk. *L. monocytogenes* is a pathogen that prevails in food and environmental samples. It can survive the extreme storage conditions. The pathogen can cause listeriosis disease which affect immunocompromised persons. In India, milk is used for abhishekam (an abhishekam is a religious bathing ceremony of the deity). Such milks are collected during the rituals and are offered to the devotees as a theertham (a sacred liquid, offered at temples to the worshipers); people have the faith that consuming theertham cures all their ailments. Milk samples were collected in two spells from ten randomly selected temples, situated in Tiruchirappalli city, Tamilnadu, India and the samples were investigated for the presence of the bacterium using NF EN ISO 11290-2 method. The results indicated that, all the samples were contaminated by the pathogen and the number exceeds the recent safety limit of the Indian food safety and regulation standards.

Keywords *L. monocytogenes*, Safety of Milk, Temple Milk

1. Introduction

Milk contains pathogens like *Salmonella enterica*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Mycobacterium paratuberculosis*, *Brucella abortus*, *Coxiella burnetii*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Listeria monocytogenes* etc. Of the various milk pathogens, *L. monocytogenes* is one of the deadly organisms occurs largely in all types of environment, including foods grown in contaminated environment, poorly processed/stored food, milk and associated products. The organism causes listeriosis to people who consume contaminated food, provided they lack immune power. The risk of being affected by listeriosis is more among the immunocompromised persons like pregnant women & their fetuses, children, AIDS affected individuals, TB & cancer-patients, diabetics and elderly people[1].

The infection is exhibited in the form of fever, abortion, still birth, brain damage and death depending upon the individual's immune power and the nature of contamination. Average mortality rate caused by the pathogen is in adults 30-40%, neonates 50% and general hospitalization rate of the disease is 91%. Considering its pathogenesis and

mortality rate among the infected individuals, the Indian government has recently included the organism in the food safety and regulations lists, in 2011[2]. In the earlier study, conducted by us, in Trichy district of Tamilnadu, India *L. monocytogenes* was found 100 % in the unpasteurised milk sold locally[3]. The findings of the study gave us insight for the present proposed work, because in India particularly in Tamilnadu, cow's milk is preferred in fresh (without pasteurisation) largely for feeding the infants and performing temple rituals particularly for abhishekam ('abhishekam' is a religious bathing ceremony of idols in the temples); such milks are consumed raw as theertham (a sacred liquid offered to devotees in temples for consumption) by the peoples of all ages, and hence quality of such milks need to be explored for microbial safety, before it affects large sector of people.

2. Materials and Methods

2.1. Study Area and Sample Collection

The city, Tiruchirappalli, India houses with some marvellous temples and colonial churches, and also is a pilgrimage town. The major temples found in the city are the Rock Fort Temple, Sri Ranganathaswamy Temple, Tiruvanaikkaval Jambukeshwara Temple, Pallava Temple, Tirukkozhi Divya Desam, Sri Ranganatha Swami Temple, Samayapuram Mariamman Temple and Vayalur Skanda

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Temple.

Ten Milk samples were collected from ten randomly selected temples at two different periods (including replicates). The temples included for the study are temples of Lord Ganesh, Muruga, Anjneya, and Shakthi, located in BHEL, Milakuparrai, Airport, Palakarai, Karumandapam and Uraiur. Samples were taken directly from the idol during the sacred bathing ceremony. The samples were brought to the laboratory keeping in ice-box (4°C) and assessed for *L. monocytogenes* and pH.

2.2. Isolation and Identification of *L. monocytogenes*

Milk samples were initially homogenized for 3 min and the pH of the samples were measured using Elico LI 617[®] pH meter. About 25 ml of each samples were introduced separately in to the 500 ml conical flasks (Borosil) containing 225 ml of Buffered Peptone Water (pH7.2±0.2). After homogenization the samples were stored at room temperature for 1 h ± 5 min for enrichment of organisms which were previously under stressed condition. One ml of the sample from the enrichment buffer was poured into Petri plates in duplicates and the test organisms were allowed to culture on Polymixin Acryflavin Lithium chloride Ceftazidime Asculin Mannitol (PALCAM) (HIMEDIA[®]) agar and the plates were incubated at 37 °C for 48h. Black colour colonies with grey centre were counted and further streaked onto Trypticase Soy Agar with Yeast extract (TSA Y) (HIMEDIA[®]) as prescribed by NF EN ISO 11290-2[4].

2.2.1. Physiological and Biochemical Examination

Four to five suspected colonies from each TSA Y bacterial plate were picked, identified, sub cultured and purified. Gram staining and Motility tests were done. The presence of *L. monocytogenes* was confirmed through various biochemical tests such as Oxidase, Catalase, Indole, Methyl red, Voges-Proskauer, Urease production, Simmon's citrate agar, and various sugar fermentation (Mannitol, Rhamnose and Xylose) and CAMP[5] tests (Table 1).

Table 1. Biochemical characterization of *L. monocytogenes*

| Biochemical test | | Reaction |
|---------------------------|----------|----------|
| Oxidase | | - |
| Catalase | | + |
| Indole | | - |
| Methyl red | | + |
| Voges- Proskauer | | + |
| Urease production | | - |
| Citrate | | - |
| Carbohydrate fermentation | Mannitol | - |
| | Rhamnose | + |
| | Xylose | - |
| CAMP test | | + |
| Gram staining | | + |
| Motility | | Tumbling |

3. Results and Discussion

In the present study, milk samples, collected from ten temples were examined for the presence of *L. monocytogenes* (Table 2). All the samples were found to contain *L. monocytogenes* ranging between 85 to 123 CFU/ml (Figure1).

Table 2. *L. monocytogenes* in Templemilk collected in two different spells

| Sample sites | No. of Samples / period | Contamination positive (+)/negative(-) | | Contamination/ % |
|--------------|-------------------------|--|---------|------------------|
| | | Period1 | Period2 | |
| Temple 1 | 2 | + | + | 100 |
| Temple 2 | 2 | + | + | 100 |
| Temple 3 | 2 | + | + | 100 |
| Temple 4 | 2 | + | + | 100 |
| Temple 5 | 2 | + | + | 100 |
| Temple 6 | 2 | + | + | 100 |
| Temple 7 | 2 | + | + | 100 |
| Temple 8 | 2 | + | + | 100 |
| Temple 9 | 2 | + | + | 100 |
| Temple 10 | 2 | + | + | 100 |
| Total | 20 | + | + | 100 |

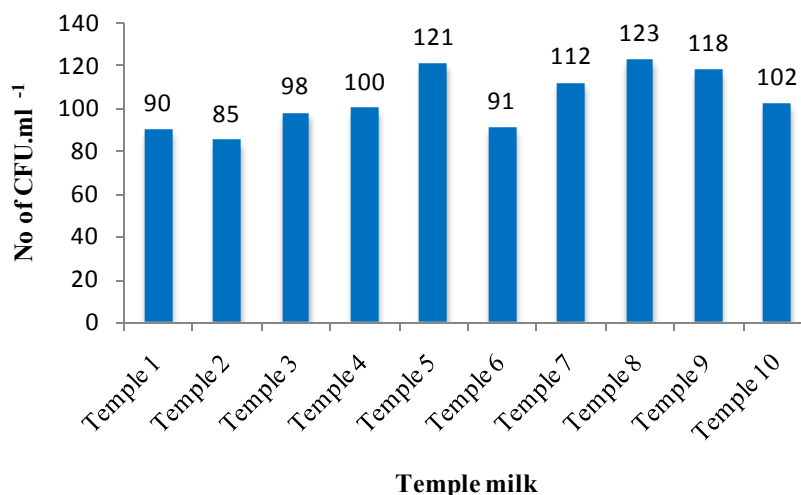


Figure 1. The average occurrence of *L. monocytogenes* in the temple milks collected in two different periods

Table 3. Mean and standard deviation of pH observed in the milk samples

| Samples | Period 1 | Period 2 | Mean | SD |
|-----------|----------|----------|------|------|
| Temple 1 | 7.35 | 7.18 | 7.26 | 0.12 |
| Temple 2 | 6.65 | 6.61 | 6.63 | 0.02 |
| Temple 3 | 7.43 | 7.29 | 7.36 | 0.09 |
| Temple 4 | 7.56 | 7.68 | 7.62 | 0.08 |
| Temple 5 | 8.14 | 8.03 | 8.08 | 0.07 |
| Temple 6 | 6.76 | 6.52 | 6.64 | 0.17 |
| Temple 7 | 8.01 | 7.91 | 7.96 | 0.07 |
| Temple 8 | 8.34 | 8.17 | 8.25 | 0.12 |
| Temple 9 | 7.93 | 8.05 | 7.99 | 0.08 |
| Temple 10 | 7.89 | 7.45 | 7.67 | 0.31 |

In India reasonable amount of work has been done only in milk and milk products, meat and sea foods. The Seasonal fluctuation of *L. monocytogenes* in the milk has been reported as 1.69% in summer and 3.82 % in winter[6]. A Study conducted at Coimbatore, Tamilnadu, India reported that, branded milks are more prone to *L. monocytogenes* than the local milk[7]. A Similar study carried out at Mangalore has reported branded milks which are sold in the Dairy/ Milk Depots are free from *L. monocytogenes*[8]. In contrast in our previous study[3] we have reported incidence of the *L. monocytogenes* in both local and branded milks; the frequency was 100% in local unpasteurized milks and 60% in the branded pasteurized milks available at Tiruchirappalli, Tamilnadu, India. Our Present finding also gives solid evidence for 100 % contamination of locally available unpasteurized milks because in temples only the unpasteurized milks are preferred for sacred bathing ceremony.

The pH analyses of the samples gave alkaline pH (Table 3), whereas in normal milk it is between 6.5 and 6.8[9]. Reason for the alkaline pH is unknown, whether it was due to the admixture of substances previously used in the sacred bathing ceremony or not is needed to be explored further. Since it is beyond the scope of the present work it was not studied further. However it has been reported that water, starch, detergent, glucose, formalin, soda, and urea are added in milk for adulteration[10]. Therefore the alkaline pH found in this study could also be due to the alkalinity of the water or soda added for the adulteration. In addition organisms such as *Salmonella*, *Yersinia*, *L. monocytogenes* are reported to withstand pH >8.5[11]. Research in this angle is being done by us and the result of the study would give evidence for such pH changes and the pathogen associated to.

4. Conclusions

Severity of the pathogen in causing listeriosis perse is unknown by the public and the pathogenesis is normally misdiagnosed as streptococcal infection by the most of the physicians in the country and this is mainly due to the lack of much information about the organism as a food borne pathogen. Because in India the incidence of Listeriosis was

less common in earlier days and came to light only after 1995. This is also one of the reason for the failure to act with the prudence in diagnosing the pathogenesis. By seeing the results of the present work it has been confirmed that the raw milk used in the abishekam are severely contaminated with the deadly pathogen and the load is above the safety limit i.e 100 CFU .ml⁻¹. Since the temple milks are consumed raw, the study report would create awareness among the public and reveal the situation for making stringent regulations and guidelines for taking preventive measures to safe guard the lives of common public.

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