

Prevalence of *Listeria monocytogenes* in Raw and Pasteurized Milk Samples from Tiruchengode (TN), India

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ABSTRACT: Listeriosis is an important food-borne disease caused by *Listeria monocytogenes* and is transmitted through contaminated raw and pasteurized milk. Hence, it is also listed as a pathogen in the Prevention of Food Adulteration (PFA) rules, 1956. There are a few reports on detection of this pathogen from milk samples in India. We report here the investigation of milk samples for detection of *L. monocytogenes*. Twelve (12) samples each of raw (R1 and R2) and branded (pasteurized, B1, B2 and B3) milk samples were collected from Tiruchengode located in Namakkal District of Tamil Nadu state for this purpose. There was a higher incidence of *Listeria* in branded (pasteurized) milk samples, 1 out of 4 and 2 out of 4 in (B1) and (B2), i.e. 25 and 50% prevalence, respectively. The pathogen was not detected in (B3) branded samples. In the case of raw milk samples there was 16.6% prevalence of *Listeria* in R1 and R2 samples (6 samples each). Five strains of *L. monocytogenes* strains were isolated from the five positive raw and branded samples and characterized. Antibiotic sensitivity results showed variation among the isolates. All the strains showed sensitivity to chloramphenicol, ciprofloxacin and penicillin-G.

KEYWORDS: Epidemiology, food poisoning, Listeriosis, *Listeria monocytogenes*, pasteurized milk.

I. INTRODUCTION

Listeriosis, a food infection usually in adults (over 55 years) and children (under 1 year) caused by *Listeria monocytogenes* gram positive, rod-shaped, facultative anaerobic, psychrotrophic, halotolerant bacterium, and comprises of eight species [1, 2]. It is widely distributed in the environment and occurs naturally in many raw foods and in various animals including rabbits, guinea pigs and humans [3, 4]. Since 1981 *L. monocytogenes* has been recognized as an important food borne pathogen. Listeriosis is the third leading cause of death due to food-borne bacterial pathogens, with fatality rates exceeding that of *Salmonella* and *Clostridium botulinum* [5]. The mortality due to Listeriosis is high typically 20-30% in pregnant women, immune compromised persons, foetuses in later stages and newborn [6, 7]. The other risk groups include persons with cancer, diabetes, kidney disease, AIDS persons associated with agricultural field [8] and fisheries [9]. The transmission of the *L. monocytogenes* may be through contamination from the farm, by the diseased /carrier animal (cow) or through raw milk or after the pasteurization process through the milk processing plant environment or by operator error [10, 11]. A person with listeriosis usually has fever, muscle aches and sometimes gastro-intestinal symptoms such as nausea and diarrhoea. If the infection spreads to the nervous system, symptoms such as head-ache, stiff neck, confusion, loss of balance or convulsions can occur. This can lead to meningitis, encephalitis and septicaemia [12]. According to FDA method the blood or spinal fluid sample is enriched for 24 to 48 hours and cultured on Blood agar, on which it give β -haemolysis. Further, gram staining, motility and biochemical test differentiates *L. monocytogenes* from haemolytic *Streptococci* (<http://virology->

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online.com/Bacteria/*Listeria* 5.htm). If the infection occurs during pregnancy, antibiotics given promptly to the pregnant women can prevent infection of the foetus or newborn. Babies with listeriosis receive the same antibiotics as adults such as Ampicillin in combination with an Aminoglycoside [13]. In rural India, most of the milk consumed and dairy products are not prepared under unhygienic conditions. Ignorant producers utilize milk that is most often not boiled enough or pasteurized. This too, obviously affects the health of the consumer [14, 15]. There are a few reports on the detection of *Listeria* spp. in food (milk and dairy products) and clinical samples in India [16, 17, 18]. We here report our findings on the prevalence of *L. monocytogenes* in pasteurized and raw milk samples from rural area of Tamil Nadu in India.

II. MATERIALS AND METHODS

Sampling

In this present investigation the Pasteurized and raw milk samples were collected in and around Tiruchengode, Namakkal district in Tamil Nadu. All the samples were collected from local milk vending shops and milk societies. Twelve sample each of Branded and raw milk samples (total 24) were collected. The raw milk samples were brought to the laboratory in sterile bottles and the pasteurised milk samples were collected in sealed packets. The samples were appropriately labelled and used immediately for testing.

Microbiological methods

Isolation: Primary isolation was carried out by adding 15.0ml of samples into the 115.0ml of *Listeria* pre-enrichment broth (Trypticase Soy Broth with 0.6 % Yeast extract –TSYEB) in a 250ml sterile Erlenmeyer flask, incubated at room temperature ($28 \pm 2^{\circ}\text{C}$) for 24 hours [19]. From this pre-enrichment broth, 1.0ml of sample was transferred in to 10.0ml Fraser Secondary Enrichment Broth (FSEB) and incubated at 37°C for 24 hours. Isolation was done by streaking the enriched sample from FSEB on to Modified McBride *Listeria* agar (MMA), incubated at 37°C for 24-48 hours.

Identification: The isolates from the selective medium (Modified McBride *Listeria* Agar) were characterized by routine microbiological methods for gram staining, motility, catalase and oxidase tests, etc. Various selective and differential media such as Trypticase Soy Yeast Extract Agar (TSYEB), Blood Agar (BA) and Muller-Hinton Agar (MHA) were used to confirm growth and colony characteristics of *L. monocytogenes*. Biochemical tests such as IMVIC tests, urea utilization, nitrate reduction, fermentation of sugars, growth on Triple sugar Iron (TSI) agar, etc. were performed.

Special Test – CAMP Test: CAMP (Christie, Atkins and Munch Peterson) test was done to study the interaction between *Staphylococcus aureus* and *L. monocytogenes* were used for this test. *S. aureus* was streaked on the centre of the agar plate as straight line and *L. monocytogenes* was streaked as perpendicular to the central staphylococcal inoculum, starting about 5 mm from the central streak and extending towards the periphery of the agar incubated the plates at 37°C for 24 hours [20].

Antibiotic Sensitivity Testing: Kirby-Bauer disc diffusion method was used for antibiotic sensitivity testing [21] using Muller – Hinton Agar medium. Active culture was prepared by inoculating 5-6 colonies into Trypticase Soy Broth, incubated at $35-37^{\circ}\text{C}$ for 2-5 hours. A sterile cotton swab was used to spread the inoculums, antibiotic discs were placed and zone of inhibition were measured after incubation at 37°C for 16-18 hours.

III. RESULTS AND DISCUSSION

According to Tirumalai [22] human listeriosis, which is now prevalent in Indian sub-continent has originated from the West. Most of the cases of listeriosis are due to consumption of ready-to-eat (RTE) frozen foods and raw milk. Hence, various food regulatory agencies have made it mandatory to have zero tolerance for the presence of *L. monocytogenes* in RTE foods [7].

Isolation and identification: The isolation, identification, prevalence and anti-biogram of *L. monocytogenes* from twenty four (24) pasteurized and raw milk samples collected from different places of Namakkal District in Tamil Nadu. The samples were collected during the period of December 2005 to February 2006. In all five (5) isolates were

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obtained which were gram-positive, coccobacilli arranged singly or in pairs or V-shaped. Motility was observed at 25°C, and non-motile at 37°C.

The colonies appeared dense white to iridescent white with crushed glass appearance on Trypticase Soy Yeast Extract Agar (TSYEB) and Modified McBride Agar (MMA) medium; as white coloured colonies on Muller Hinton Agar (MHA); showed β -haemolysis on Blood Agar (BA) and CAMP test (Figure 1). In CAMP test the hemolysis is due to synergistic action of *S. aureus* and *L. monocytogenes*. The usefulness of this test for identification of *L. monocytogenes* has been reviewed by McKeller [23]. The conclusions drawn are that it can be used only as a presumptive test and cannot confirm the virulence of the isolate.

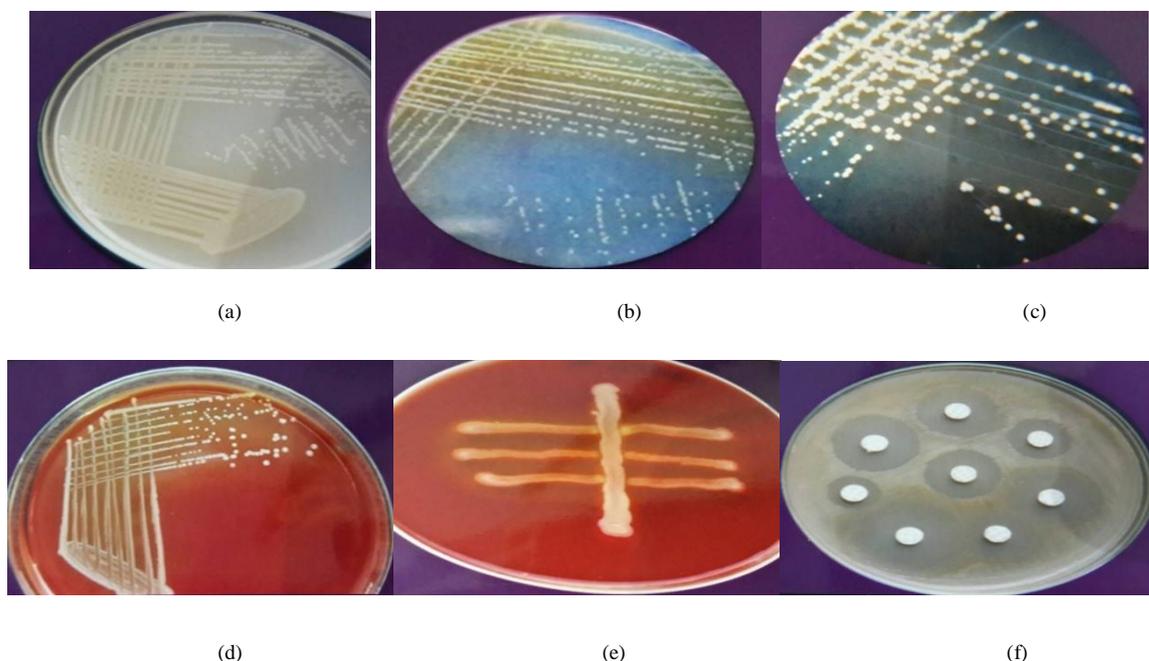


Figure 1. The colony morphology of *Listeria monocytogenes* (VLM3) on various media (a) On TSYEA, (b) MMA, (c) MHA, (d) BA, (e) CAMP test, and (f) Antibiogram

In this current study, twenty four milk samples (pasteurized and raw milk samples) were tested for presence of *L. monocytogenes*. Among the 24 samples 20.8% were positive for *L. monocytogenes*. The positive was high in branded milk sample 2 (B2) was 50% followed by branded milk sample 1 (B1), raw milk sample 1 (R1) and raw milk samples 2 (R2) were 25% and 16.6% (R1 & R2) respectively. Remaining one branded milk sample 3 (B3) showed negative results for *L. monocytogenes* (Figure 2 and Table 1). Dhanashree *et al.* [18] have reported incidence of *Listeria* in various food sample, lowest (0%) in cheese, ice-cream, pasteurized milk, chicken, mutton, cabbage, 2.9% in raw milk sample and highest in coriander (30%). *L. innocua* was present in raw milk. They reported the absence of *L. monocytogenes* in dairy products. The prevalence of *L. monocytogenes* in frozen dairy product such as ice cream, cheese, etc. is relatively less as reported by Farber and Peterkin [12]. Marica Cristina *et al.*, [24] who analysed 103 samples for the presence of *L. monocytogenes* and 11 samples were contaminated with *L. monocytogenes*.

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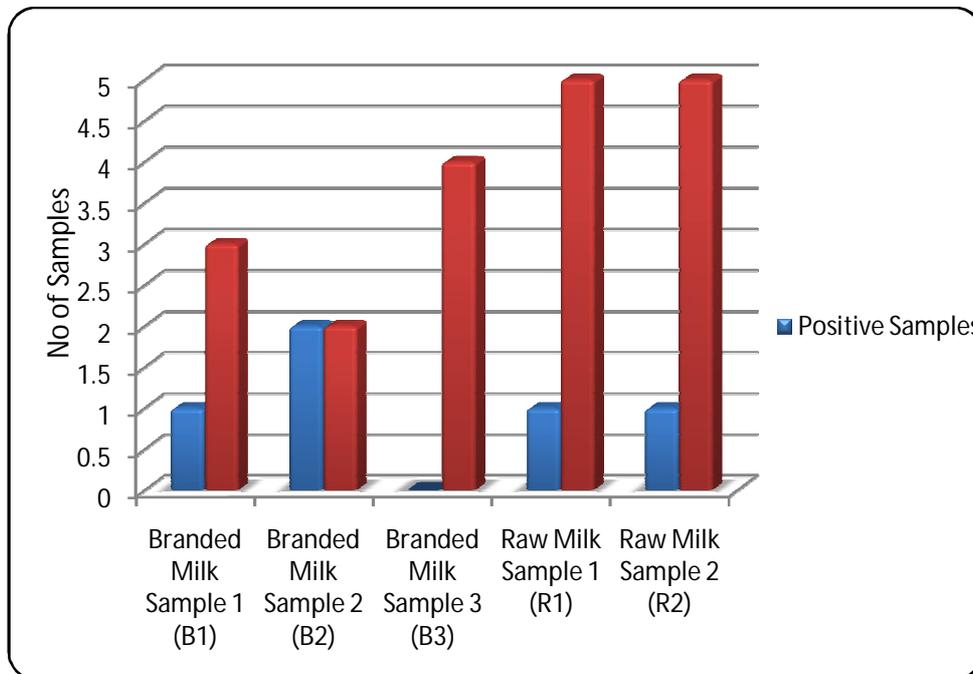


Figure 2 Prevalence of *L. monocytogenes* in different pasteurized and raw milk samples.

Another research outcome stated by Bhilegaonkar *et al.*, [25] have reported isolation of sixteen (16) *Listeria* isolates from 105 milk samples. The Listeriosis prevalence was higher in salted and dried fishes; it was more than 50% as stated by Jayasekaran *et al.*, [9].

Table 1: Prevalence of *Listeria monocytogenes* in different pasteurized and raw milk samples.

S. No.	Milk sample varieties	Samples		Positive		Negative	
		No	%	No	%	No	%
1	Branded milk sample 1 (B1)	4	16.6	1	25	3	75
2	Branded milk sample 2 (B2)	4	16.6	2	50	2	50
3	Branded milk sample 3 (B3)	4	16.6	-	-	4	100
4	Raw milk sample 1 (R1)	6	25	1	16.6	5	83.3
5	Raw milk sample 2 (R2)	6	25	1	16.6	5	83.3
	Total	24	100	5	20.8	19	79

Lone Gram [26] reported *L. monocytogenes* can survive many adverse conditions like smoking, salting, drying, freezing and pasteurization. Thus, the detection of *L. monocytogenes* in food has become important for avoiding food poisoning.

It is important to note that the antibiotic sensitivity pattern showed that all the strains were sensitive to most of the commonly used chemotherapeutic agents (Table 2).

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Table 2: Antibiotic sensitivity pattern of *L. monocytogenes* strain isolated from milk samples.

S. No.	Name of the Antibiotic	Zone of inhibition in mm				
		Strain designation				
		VLM 1	VLM 2	VLM 3	VLM 4	VLM 5
1	Chloromphenicol	22 (S)	28 (S)	19 (S)	19 (S)	18 (S)
2	Co-trimoxazole	-	18 (S)	10 (R)	23 (S)	18 (S)
3	Ciprofloxacin	20 (R)	28 (S)	25 (S)	24 (S)	24 (S)
4	Gentamycin	14 (R)	21 (S)	13 (S)	13 (S)	13 (S)
5	Ofloxacin	16 (S)	21 (S)	19 (S)	17 (S)	12 (R)
6	Penicillin-G	26 (R)	29 (S)	18 (R)	20 (R)	22 (R)
7	Streptomycin	18 (S)	22 (S)	15 (S)	17 (S)	12 (R)
8	Norfloxacin	13 (R)	20 (S)	14 (R)	19 (S)	18 (S)

However, strains VLM1 and VLM 3 were moderately sensitive to norfloxacin. Chloramphenicol, ciprofloxacin and Penicillin-G were highly effective in inhibiting the growth of the isolates. In a recent report from Ethiopia multidrug resistant listeria species have been reported in ready-to-eat food sampled from Gondar town [27].

IV. CONCLUSION

WHO (2004) has recommended the mandatory testing of RTE foods for the presence of *L. monocytogenes*, as it is among the leading cause of death due to food poisoning, especially in developing countries. This pathogen was found in raw as well as pasteurized milk samples collected from Tiruchengode, Tamil Nadu. All the strains isolated were sensitive to the commonly used chemotherapeutic agents. Proper food handling and preservation procedures should be enforced for avoiding listeriosis in rural areas. Thus, this study would be useful from epidemiological point of view.

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