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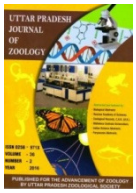

STUDY OF *Listeria monocytogenes* IN RAW MILK, PASTEURIZED MILK, CHEESE AND DAIRY WASTE

AVIJIT SARKAR[1*]

[1]Department of Dairy Technology, Faculty of Dairy Technology, West Bengal University of Animal and Fishery Sciences, Kolkata, India.

AUTHOR'S CONTRIBUTION

The sole author designed, analszed, interpreted and prepared the manuscript.

ARTICLE INFORMATION

Reviewers:

(1) Gisel Chenard Díaz, Federal University of Rio de Janeiro (UFRJ)/School of Chemistry (EQ)/GREENTEC Laboratory, Brazil.
(2) Prabha R, Dairy Science College, KVAFSU, Hebbal, Bengaluru-24, India.

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Short Research Article

ABSTRACT

Listeria monocytogenes has attracted worldwide attention as a food borne pathogen. Present study was conducted with an aim to get the best recovery of *Listeria monocytogenes* from milk, milk products and dairy waste employing various reported techniques and media. Maximum recovery of *L. monocytogenes* was obtained by using two stage enrichment techniques with 1:10 dilution with incubation period of 14 days with trypticase soy agar containing yeast extract, potassium thiocyanate and nalidixic acid. The percentage of positive samples for raw milk, pasteurized milk, cheese and dairy waste was 21.33 %, 0%,4%, and 78.66% respectively. Dairy waste gave maximum positive samples of *L. monocytogenes* .Dairy waste being the most potential source of *L. monocytogenes*, there is an utmost need to take care to avoid post contamination from dairy environment to prevent spread of the disease like 'listeriosis 'through milk and milk products.

Keywords: Listeria monocytogenes; enrichment; bio-chemical test; milk,cheese; dairy waste; pasteurization.

1. INTRODUCTION

Time and again *Listeria monocytogenes* has been proved to be highly pathogenic to human beings. An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk is reported by Dalton et al. [1]. Isolation of *Listeria monocytogenes* from raw milk is reported by Haekkinen et al. [2]. M.M.Mahmoodi [3] reported the occurrence of *Listeria monocytogenes* in raw milk and dairy products in Noorabad, Iran. There is research work regarding prevalence and fingerprinting of *Listeria monocytogenes* strains isolated from raw whole milk in farm bulk tanks and in dairy ptant receiving tanks by E.Waak [4]. S.V.Jayamanne and U. Samarajeewa [5] evaluated the resistance of *Listeria monocytogenes* in milk and milk products in Sri Lanka. Survival of *Listeria monocytoenes* during the manufacture and ripening of trappist cheese is reported by Kovincic et al. [6]. Research work is carried out regarding prevalence of and risk factors for *Listeria* species on dairy farms. By Vilar et al. [7].In view of all these critical observations, urgency was felt to conduct the

**Corresponding author: E-mail: sarkaravijit@rediffmail.com;*

study the incidence of this organism in raw milk, pasteurized milk ,cheese as well as dairy waste in and around Calcutta (India).

2. REVIEW OF LITERATURE

2.1 Source of *Listeria monocytogenes*

Listeria spp. had been isolated from all environment studies. The introduction of enrichment procedure had made it possible to isolate *L.monocytogenes* from a wide variety of sources including milk and milk products ,meat ,vegetable matter , straw ,maize,fodder,silage ,dairy waste ,sewage ,stream water , slaughter house waste (Watkins et al.; Doyle et al., Faber et al. [8].

2.2 Occurrance of *Listeria monocytogenes*

In 1983, there was an outbreak of Listeriosis in Massachusetts which was traced to the consumption of pasteurized milk. During this outbreak , Hayes et al. [9]. Examined 121 raw milk samples and 14 milk filters and recovered *L. monocytogenes* from 20% of milk samples and 14% of milk filters. Mexican style cheese was also documented as the vehicle of listeriosis outbreak by James et al. [10].Comi et al. [11] surveyed a total of 140 samples of 16 different Italian cheese which were analysed for the presence of *L.monocytogenes*.

2.3 Enrichment Technique and Methods for Isolation

Slade et al. [12] reported the suitability at two stage enrichment procedures for isolating *L.monocytogenes* from raw milk. Isolation of the organisms from milk was attempted using pre-enrichment broths at 1:5 and 1:10 dilutions of milk . Broths were incubated at 4 deg C for 0,7,14,21 and 28 days. Recoveries were compared by direct plating onto acriflavine nalidixic acid broth with and without acriflavine.

3. MATERIALS AND METHODS

3.1 Materials

Seventy five samples of raw milk were collected from commercial dairy and khatals. Seventy five pasteurized milk samples were collected from Dairy Plants. Seventy five branded cheese samples were collected from retail outlets (consisting of three different types). Forty and thirty five dairy waste samples were collected from two commercial dairies .All the materials were collected in and around Calcutta (India).

3.2 Methods

3.2.1 Isolation procedure

Two types of dilutions (1:10 and 1:5) were prepared for purpose of isolation [12]. For the first type of dilution, 20 ml (of raw milk, pasteurized milk, dairy waste) or 20 g (of cheese) was diluted in i)180 ml of tryptose broth containing 0.5 % yeast extract with 40 µg/ml potassium tellurite and for second type, 80 ml of tryptose broth containing 0.5 % yeast extract with 40 µg/ml potassium tellurite [13]. It was followed by pre-enrichment at 4 deg C (cold enrichment) and examined upto 28 days at 7 days interval [12].

Pre-enriched samples were directly plated and simultaneously were taken for selective enrichment. Plating was done from selective enriched samples too.

Direct plating and Plating from selective enrichment was done onto i) trypticase soy blood agar containing yeast extract , ii)tryptose blood agar, iii) trypticase soy agar containing yeast extract, nalidixic acid and potassium thiocyanate, iv) trypticase soy agar containing yeast extract and nalidixic acid .Plates were incubated and were viewed through Henry's Technique [14].

3.2.2 Primary test

Colonies were confirmed by using

i) Gram staining [15].
ii) Catalase test [16].
iii) Motility test [17].
iv) Haemolysis test [18].

3.2.3 Confirmatory test

For the sake of confirmation, isolates were characterized by distinctive bio-chemical test (fermentation test) [19]followed by antibiotic sensitivity tests [19].

4. RESULTS AND DISCUSSION

Maximum recovery of *L. monocytogenes* was obtained by using two stage enrichment technique with 1:10 dilution. Dilution 1:10 was found superior to 1:5 dilution.The observation is in conformity with observations made by other scientists [9]. The most effective incubation period , required for two stage enrichment was found to be 14 days and the media , trypticase soy agar containing yeast extract ,potassium thiocyanate and nalidixic acid , was found to be the best medium in the investigation. This is in conformity with the observations of other scientists [15].

All the seventy eight isolates obtained from a total of three hundred samples (75 samples from each type of source) were gram positive bacilli and their catalase test reactions were also positive. The organism showed motility at 22 deg C but not at 35 deg C. Zone beta haemolysis was observed for 90% of the *L. monocytogenes* isolates in case of haemolysis test.

The sugar fermentation results for *L. monocytogenes* varied from one sugar to another. The results for glucose, trehalose, salicin,lactose and maltose fermentation were 100% positive and those of galactose,raffinose, mannitol were negative. The isolates that fermented rahamnose, sucrose, sorbitol, xylose and melezitose were 90%, 62%,13%,10% and 85 % respectively.

A study of antibiotic sensitivity for seventy eight isolates of *L. monocytogenes* showed that there was maximum sensitivity for neomycin and maximum resistance was for polymyxin-B while maximum intermediate zones were observed for streptomycin. According to Fuzi [20], *Listeria* was very sensitive to neomycin.

The percentage of positive samples for raw milk, pasteurized milk, cheese and dairy waste were 21.33 %, 0%, 4%, and 78.66% respectively. Lovett et al. [21] found 25 (93%) isolated strains out of 27 samples while analyzing raw milk from 3 areas of United States. Becker et al. [22] detected *L. monocytogenes* in 9 cheese samples (65%) made from raw milk but the organism was not detected in any of the 36 cheese samples made from pasteurized milk. There are reports of wide distribution of *L. monocytogenes* in the environment especially in sewage sludge, vegetable matter and soil Watkin et al. [8].

5. CONCLUSION

It was evident from present investigation that dairy waste gave maximum positive samples of *L. monocytogenes* .Therefore, it may be suggested that contamination of milk and milk products through dairy waste has to be avoided with utmost care to prevent spread of the disease, 'listeriosis 'through milk and milk products. Simultaneously, preventive measures need to in place through Standard Operating Procedure to avoid contamination of milk and dairy products with *L. monocytogenes* in all the production stages, starting from collection of raw milk from farm, subsequent processing stage and complete supply chain leading to arrival at consumer's end.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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