Isolation and Characterization of *Listeria monocytogenes* from Raw Milk in Durg District of Chhattisgarh

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**ABSTRACT**

The work was conducted to determine the total aerobic plate count of milk samples, isolation of the *Listeria* spp. and to determine their pathogenicity. Raw milk samples, collected from the dairies located in Durg district, showed mean APC of 24 x10^5 cfu/ml (6.380 log_{10} cfu/ml). Cultural examination of 100 raw milk samples revealed an overall 6% prevalence of *Listeria* spp. and only *L. monocytogenes* was isolated from milk. All the *Listeria* isolates exhibited a typical α-heamolysis with narrow zone on sheep blood agar. The haemolytic listerial isolates in CAMP test showed enhancement of haemolytic zone and developed purulent conjunctivitis in Anton's test. Similarly, *L. monocytogenes* isolate in embryonated chicken eggs showed severe haemorrhages in liver, heart and surface of embryo along with conspicuous thickened CAM.

**Keywords:** Characterization, Chhattisgarh, isolation, *L. monocytogenes*, raw milk

Listeriosis has been recognized as an emerging foodborne bacterial infection and a nagging public health hazard (Farber and Peterkin, 1991). Recently, special attention in the food industry has been directed towards *Listeria monocytogenes*. It is the only species of public health concern, which is pathogenic both for humans and animals. The important unique nature of *Listeria* spp. contributing to food-borne transmission are the capability to grow at a low temperature, survive osmotic stress and mild preservation treatment. Raw milk is one of the most common paths for transmission of *L. monocytogenes*. It is important to point out that healthy animals are often carriers of *L. monocytogenes* and as such can be source of contamination of the environment or milk. Currently there is limited information available on prevalence of *Listeria* spp. among foods in India. Looking to the potential health hazard of *Listeria* organism to domestic animal and human, present study was aimed to determine the prevalence and incidence of *Listeria* spp. in milk.

During the present study, a total of 100 raw milk samples were collected from outlets of various dairy farms located in Durg district of Chhattisgarh, for isolation of *Listeria* spp. and for total aerobic count. All the samples were collected aseptically and transported immediately to the laboratory under chilled condition, stored at 4°C and processed within 24 h.

The aerobic plate count (APC) of milk sample was determined, following the method described by International Commission on Microbiological Specifications for Foods (1978) with minor modifications. Ten-fold serial dilution of each sample was prepared in sterile normal saline solution (NSS) up to 10^-8 dilution. Inoculum from each dilution then spread on the surface of the plate count agar medium (Himedia, India) and incubated at 37°C for 24 h. The plate with colonies between 30-300 was counted and bacterial count was determined by multiplying the number of colonies with reciprocal of dilution factor.

Isolation of *Listeria* spp. from milk samples was attempted following the method described by Donnelly and Baigent (1986). Briefly, 1 ml of milk sample was inoculated into 10 ml of University of Vermont medium (UVM-I, containing 12 mg of acriflavin hydrochloride) and incubated at 30°C for 18-24 h. Thereafter, 0.1 ml enriched inoculum from UVM-I was transferred to 10 ml of UVM-II (containing 25 mg of acriflavin hydrochloride) and incubated at 37°C for 18-24 h. After incubation, 0.1 ml enriched inoculum from UVM-II was transferred to 10 ml of UVM-III (containing 25 mg of acriflavin hydrochloride) and incubated at 37°C for 18-24 h.

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hydrochloride) and incubated again at 30°C for 24-36 h. The enriched inoculum from UVM-II was then streaked directly on PALCAM agar, L. monocytogenes Differential (LMD) agar and Mac-Bride agar and incubated at 37°C for 48 h. The presumptively identified Listeria spp. on these media were subjected to staining, morphological characterization and were examined for characteristic tumbling motility at 20-25°C in brian heart infusion (BHI) broth. The Listeria isolates were further identified by catalase, oxidase, methyl red (MR), voges-proskauer (VP) and nitrate reduction tests. All the catalase-positive, oxidase-negative, MR and VP positive and nitrate negative Listeria isolates were also tested for mannitol, rhamnose, sucrose, xylose, and α-methyl D-mannopyranoside fermentation. The biochemically characterized Listeria isolates were then examined for the type and degree of hemolysis in CAMP test as well as on sheep blood agar (SBA) (Nikas, 2009). The pathogenicity of biochemically confirmed Listeria isolates was also assessed by Anton’s test and by inoculating in chicken embryo via allantoic cavity route following the method described by Nigam et al. (1998).

In the present study, the highest APC value recorded was 37 x10^5 cfu/ml (6.568 log_{10} cfu/ml), whereas lowest value was 14 x10^5 cfu/ml (6.146 log_{10} cfu/ml), with mean value of 24x10^5 cfu/ml (6.380 log_{10} cfu/ml). These findings are in agreement to the findings of Al-Qadri et al. (2008) who reported aerobic plate count of 6.66 log_{10} cfu/ml in milk. Wide variations in the APC values may occur due to differences in sampling methods, sampling sites, handling, and modes of evaluation, climatic conditions and lack of cleanliness on the farms (Nikas, 2009).

Listeria colonies appeared grayish green surrounded by diffused black zone on PALCAM agar, azure blue with opacity around it on LMD agar and grayish translucent on Mac-Bride agar. Similar observations have been reported by other investigators (Kalorey, 2006; Yadav, 2008 and Nikas, 2009).

Screening of milk samples for the presence of Listeria indicated an overall positivity of 6% and all isolates were identified as Listeria monocytogenes. Kenar et al. (2006) and NATP-CGP Project Report (2002-2004) reported 7% and 4.7% positivity, respectively in raw bovine milk samples. However several investigators had reported higher values up to 21.73% in milk samples of cow and buffalo (Bhilegaonkar et al., 1997; Aurora et al., 2006 and Sharma et al., 2012).

All the isolates appeared as coco-bacilli having positive catalase and negative oxidase activity with characteristic tumbling motility at 25°C. The isolates were positive for MR test and VP test and found negative for nitrate reduction test. They were considered as ‘presumptive Listeria isolates’. These isolates produced acid from α-methyl D-mannopyranoside and sucrose, and failed to produce acid from mannitol and xylose, and thus confirmed as L. monocytogenes. The findings of present study are in agreement with the several earlier reports (Gunjal et al., 2006; Yadav, 2008 and Nikas, 2009). A typical α-haemolysis with narrow zone was exhibited by all biochemically confirmed Listeria isolates. Haemolysis is an important characteristic, which is directly related to the pathogenicity of Listeria and attributed to the production of virulent factor listeriolysin, where as non-hemolytic Listeria spp. were practically considered as non-pathogenic. Listeria isolates showed characteristic enhancement of haemolytic zone in CAMP test. Kenar et al. (2006) also characterized all biochemically confirmed Listeria isolates by CAMP test with S. aureus and R. equi and reported positive reaction. The virulent strains of L. monocytogenes are strongly haemolytic against sheep erythrocytes due to extra cellular 58-kDa protein, listeriolysin O (LLO) secreted by isolates. The characteristic enhancement of the α-heamolytic zone towards S. aureus was due to the synergism between α-toxin produced by S. aureus and LLO, confirming L. monocytogenes (Farber and Peterkin, 1991).

All the biochemically confirmed Listeria isolates in chicken embryos showed stunting as well as haemorrhages. The liver, heart and muscle of embryos showed congestion when compared with control. The chorio-allantoic membrane showed conspicuous thickening and inoculated embryos died after 42 h post inoculation. Similar lesions in chicken embryos were also reported by Nikas et al. (1998). The findings of Anton’s test showed development of kerato conjunctivitis within 24-36 h. Conjunctivitis in rabbit due to inoculation of Listeria isolate was also reported by Kenar et al. (2006).

On the basis of present study, it was concluded that the raw milk samples collected from the outlets of various dairy farms located in Durg district of Chhattisgarh were highly contaminated and had high bacterial load. The presence of higher bacterial count in raw milk samples indicates that the milk was of poor quality with lower shelf life, which might be due to contamination or adulteration of milk either during its production, storage, handling or distribution. In addition to the above, present study also revealed the presence of Listeria spp. in 6% of total raw milk samples and all isolates were identified as Listeria monocytogenes with all pathogenicity/virulence attributes, which may be a potential threat to public health in this region of Chhattisgarh.

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**References**


