PREVALENCE AND MOLECULAR CHARACTERIZATION OF LISTERIA SPP IN RAW MILK SAMPLES IN MHOW AND INDORE REGION OF MADHYA PRADESH

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ABSTRACT

Total 50 raw milk samples were screened for Listeria spp. contamination. The biochemically confirmed cultures were further examined for *in vitro* pathogenicity test and molecular characterization. *Listeria spp.* were obtained in 4 (8%) samples, two isolates were confirmed as *L. welshimeri* (50%) and two as *L. ivanovii* (50%). Only 2% of milk sample found to possess putative phosphoribosyl pyrophosphate synthetase (*PRS*) gene. Out of the two species of Listeria found in milk, only one species i.e. *L. ivanovii* was found to be hemolytic on sheep blood agar.

KEYWORDS: *Listeria*, Milk, PCR

INTRODUCTION

The genus Listeria comprises of seven species viz. *L. monocytogenes, L. ivanovii and L. seeligeri* which are hemolytic, whereas *L. innocua* and *L. welshimeri* are non haemolytic. Besides these two less frequently encountered non haemolytic species are *L. grayi* and *L. murrayi*. Milk and milk products appear to be particularly susceptible to contamination (Griffiths, 1989). All the seven recognized *Listeria spp.* has been reported from raw milk. Further, raw milk must be considered to be a potential source of dairy plant contamination, which can affect the entire production chain. Listeria monocytogenes is an important pathogen in medical and veterinary medicine, causing abortion and encephalitis in sheep and cattle, and a variety of diseases in other mammals, birds and fish (Kalorey *et al.*, 2008). In ruminants, the disease occurrence is associated with the feeding of silage. The objective of the present study was to evaluate prevalence and molecular characterization of Listeria spp in raw milk in Mhow and Indore region of Madhya Pradesh.

MATERIALS AND METHODS

Collection, Isolation and Identification

A total of 50 milk samples were collected from various organized (branded) and unorganized (unbranded) sectors supplying milk and milk products to consumers at Mhow, Indore of Madhya Pradesh and surrounding areas under aseptic conditions. Isolation of *Listeria spp.* from the samples was carried out using pre-enrichment, enrichment and selective media. Samples were subjected to two-step enrichment in University of Vermont broth (UVM broth) (Ralovich, 1989, Art and Andre, 1991). Further the identification was carried out as per the standard procedure (Cheesbrough, 1991, Cowan and Steel, 1993, Agarawal *et al.*, 2003).

In vitro pathogenicity and molecular characterization of Listeria isolates

All the Listeria isolates were tested for the type and the degree of hemolysis on SBA. Briefly, the isolates were streaked onto SBA plates and incubated at 37^{o}C in a humidified chamber for 24 hrs and examined for hemolytic zones around the colonies. Further all the Listeria isolates were streaked on L. mono differential agar (HiMedia Ltd, Mumbai, India) in order to assess Phosphatidylinositol specific phospholipase C (PI-PLC) activity. The inoculated plates were incubated in humidifying chamber at 37^{o}C for 24 h (Ralovich, 1989, Aurora *et al.*, 2008).

Polymerase chain reaction (PCR)

The Template DNA was prepared by Snap chill method (Rawool *et al.*, 2007). After that the PCR was standardized for detection of various genes. The positive control cultures were procured from ICAR research complex, Ela, old Goa. The multiplex-PCR serotyping (for lmo0737, ORF2819, ORF2110 and *PRS*) was standardized as per the methodology described by Doumith *et al.* (2004).

Detection of virulence associated genes

Multiplex PCR targeting virulence associated genes was subsequently carried out using the four isolates for simultaneous detection of four virulence marker genes, plcA, actA, hlyA and iap (Kaur *et al.*, 2010) with slight modification.

RESULTS AND DISCUSSION

On PALCAM agar *Listeria* colonies appeared grayish green surrounded with diffuse black zone of aesculin hydrolysis, while on Oxford agar, black colonies surrounded with diffuse black zone of aesculin hydrolysis were observed. On staining, the colonies suggestive of *Listeria* showed presence of Gram positive coccobacilli in pure culture. These rods exhibited characteristic tumbling motility at 20-25^{o}C temperature suggesting them to be 'presumptive *Listeria* isolates'.

On biochemical identification the bacterium was found to be catalase-positive, oxidase-negative, MR and VP positive and Indole reduction-negative. Further these isolates were studied for fermentation of sugars viz. D-xylose, sucrose, α-methyl D-mannopyranoside and mannitol for characterization up to species level. The isolates which produced acid from D-xylose, but failed to produce acid from mannitol, sucrose and α–methyl D-mannopyranoside were identified as *L. ivanovii*. The isolates which produced acid from D-xylose and α-metyl D-mannopyranoside, but failed to produce acid from sucrose and mannitol were identified as *L. welshimeri.*

On screening of 50 raw milk samples, *Listeria* spp. were obtained in 4 (8%) samples, two isolates were confirmed as *L. welshimeri* (50%) and two as *L. ivanovii* (50%).Out of the two species of Listeria found in milk, only one isolate of one species i.e. *L. ivanovii* was found to be hemolytic on sheep blood agar. On L. mono differential agar, all the 4 biochemically confirmed *Listeria* isolates exhibited typical blue color colonies without any halo surrounding colonies indicating the non pathogenicity.

The four sets of primers employed in multiplex PCR serotyping targeting the four genes of *Listeria* spp. revealed the amplified PCR products of 691, 471, 591 and 370 bp size corresponding to Imo0737, ORF2819, ORF2110 and *PRS* genes, respectively in positive controls. Each primer set was found to be specific for the corresponding gene amplifying the DNA fragments. But out of total 4 isolates, only one (25%) isolate of *Listeria ivanovii* shown the amplification of 370 bp gene product corresponding to *PRS* gene. However, none of the presumptive or genetically confirmed listerial isolates revealed presence of any of virulent gene.

The biochemical findings of present study are in agreement with that of Mahmoodi (2010). Hemolysis is an important characteristic, which is directly related to the pathogenicity of listeriae since non-hemolytic *Listeria spp*. is practically considered as non-pathogenic (Seeliger, 1981). A typical β-hemolysis exhibited by isolate of *L. ivanovii* in this study was also reported by Kalorey *et al.* (2008) and Yadav (2008).

On biochemical characterization Listeria spp. was obtained in 8% of samples, these results are in close accordance with those of Sun-Young Beak *et al.* (2000) who isolated Listeria in 7.9% milk samples, in Korea.

On molecular characterization, out of 50 samples, only one isolate (2%) was confirmed as positive for *PRS* gene of *Listeria*. On contrary, Jami et al. (2010) recorded 34% prevalence of *Listeria spp.*

from 100 bulk tank milk samples and positivity for putative phosphoribosyl pyrophosphate synthetase (prs) gene. The record of low prevalence rate in this study indicates that there is low contamination level of these bacteria in raw milk samples, in this area may be due to high environmental temperature.

REFERENCES :

Agarawal, R K., K.N. Bhilegaonkar, D. K. Singh, A. Kumar and R.S. Rathore (2003) Laboratory Manual for the Isolation and Identification of Food borne Pathogens. 1st edn. Jai Ambey Pvt. Ltd., Bareilly, P-99.

Art, D. and P. Andre (1991). Zentralbl Bakteriol, 275: 79-84.

Aurora, R., A. Prakash, S. Prakash, D.B. Rawool and S.B. Barbuddhe (2008). Food control, 19: 641-647.

Cheesebrough, M. (1991). Medical laboratory manual for tropical countries. Volume II: Microbiology. Tropical health technology, 14 Bevills close, Doddington, Cambrideshire-PE150TT.

Cowan, S.T. and K.J. Steel (1993). Manual for the identification of Medical bacteria. 2nd edn. Cambridge university press. London, U.K., pp 114-116.

Doumith M, C. Buchrieser, P. Glaser, C. Jacquet, P. Martin (2004). J Clin Microbiol., 42: 3819-3822.

Griffiths, M.W. (1989). J. Sci. Food Agric., 47: 133.

Jami, S., A. Jamshidi and S. Khanzadi (2010). Iran. World Applied Sciences Journal, 10: 249-253.

Kalorey, D.R., S.R. Warke, N.V. Kurkure, D.B. Rawool and S.B. Barbuddhe (2008). Food control, 19: 109-112.

Kaur, S., S.V. Malik, K.N. Bhilegaonkar, V.M. Vaidya and S.B. Barbuddhe (2010). Vet. J., 184: 366-370.

Mahmoodi, M.M. (2010). Journal of Animal and Veterinary Advances, 9: 16-19.

Ralovich, B. (1989). Inter. J. Food Microbiol., 8: 269-272.

Seeliger, H.P.R. (1981).. Zbl Bakt. Microbiol. Hyg. Abt. I. Orig. A., 249: 487-493.

Sun-Young Beak, Soon-Young Lim, Dong-Ha Lee, HeeMin Kyung and Chang- Min Kim (2000). J. Food Prot., 63: 186-189.

Yadav, M.M. (2008). Isolation, identification and molecular characterization of Listeria spp. from animals and serodetection of antilisterial antibodies. PhD Thesis submitted to Anand Agriculture University, Anand, Gujarat.

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