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Research Article

ISOLATION AND CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* FROM FOODS OF ANIMAL ORIGIN

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ABSTRACT

Listeria monocytogenes is the only pathogenic species affecting both humans and animals. There is a rise in listeriosis due to the consumption of food contaminated with *L. monocytogenes*. Herein, the objective was to study the occurrence of *L. monocytogenes* in meat and milk samples collected from different parts of Karnataka. A total of 458 samples comprising milk (n=424) and raw meat (n=34) were randomly collected from three districts. All the samples were processed for isolation as per IS 14988 (Part 2): 2020 protocol and characterized using biochemical characterization. The confirmation was performed using duplex PCR targeting the *prs* and *isp* genes. Finally, the confirmed isolates were subjected to serotyping employing multiplex-PCR. Of the 458 samples, eight meat samples (1.74%) revealed the presence of *L. monocytogenes*. All the recovered isolates belonged to the 1/2b, 3b and 7 serogroup. The study highlighted the presence of the 1/2b, 3b and 7 serogroup in animal foods and warranted more similar studies from different parts to understand the presence of various serotypes of *L. monocytogenes* prevailing in our country.

Keywords: Foods of animal origin, Isolation, *Listeria monocytogenes*, Serotyping

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Listeria monocytogenes is one of the major foodborne zoonotic pathogens culpable for causing life-threatening diseases called listeriosis. Globally, over the past few years, there has been an upsurge in *Listeria* food borne outbreaks due to the consumption of contaminated food products (Barbuddhe *et al.*, 2021). Presently, there are 27 different species of *Listeria*, out of which *L. monocytogenes* and *L. ivanovii* are pathogenic (Raufu *et al.*, 2022). However, *L. monocytogenes* is the only species that is capable of causing disease in both humans and animals. While *L. ivanovii* affects only animals. *L. monocytogenes* is currently classified into four evolutionary lineages, fourteen serotypes, and four PCR serogroups (Moura *et al.*, 2016; Yin *et al.*, 2019). *Listeria monocytogenes* has been categorized by World Health Organization as the major notable foodborne pathogen associated with high case-fatality and hospitalization (Barbuddhe *et al.*, 2021; Abdeen *et al.*, 2021). *L. monocytogenes* can penetrate both blood-brain and placental barriers, which increases the impact of disease in comparison to other foodborne pathogens (Farber and Peterkin, 1991). Clinical manifestations in human listeriosis may range from critical sepsis in immuno-compromised individuals; febrile gastroenteritis, meningoencephalitis in infants and adults; abortions, stillbirth in pregnant women, and septicemia in neonates and the elderly (Matle *et al.*, 2020).

Listeria monocytogenes can survive extreme conditions like low pH, high salt concentrations, and low temperature (Roberts *et al.*, 2020). *L. monocytogenes* is a robust pathogen found to be distributed in a variety of agroecosystems hence, facilitating transmission to humans (NicAogain and O'Byrne, 2016).

A thorough review predicting sporadic cases of human and animal listeriosis from the Indian perspective indicated that the available epidemiological data is insufficient to assess the extent of true infection in humans and animals; in addition, the disease is largely underreported as a result of the lack of appropriate diagnostic assays (Barbuddhe and Malik, 2009). Considering the insufficiency of data on the occurrence of *L. monocytogenes* in foods, the objective of the present study was to screen food samples from the state of Karnataka for the presence of *L. monocytogenes*.

MATERIAL AND METHODS

Sample collection: A total of 458 samples comprising milk (n=424) and raw meat (n=34) were randomly collected from milk parlours and slaughterhouses in three districts of Karnataka *viz*., Bengaluru (n=176), Mysore (n=210), and Belgavi (n=72). The samples were collected under sterile conditions and transported under cold conditions. The samples were stored at 4º C until use.

Isolation of *Listeria*: All the samples were processed for isolation as per IS 14988 (Part 2): 2020 protocol. Briefly, samples were subjected to two-step enrichment using half Fraser broth followed by full Fraser broth. Each of the collected samples (5 gm meat sample or 5 ml milk sample) was aseptically transferred into hlf Fraser broth (45 ml) and incubated overnight at 37°C followed by inoculating 1

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ml of enriched half Fraser to 9 ml of Fraser broth and incubating at 37°C for 18-24 hrs. A loopful of inoculum from enriched Fraser was further streaked on selective PALCAM plates, which were then incubated at 37°C for 48 hrs. The typical greenish-yellow glistening, iridescent, and pointed colonies of about 0.5 mm diameter surrounded by a diffuse black zone of aesculin hydrolysis were presumptively identified as *Listeria* spp. and were further processed for biochemical characterization and PCR confirmation.

Biochemical Confirmation of *Listeria*: The presumed colonies of *Listeria* were tested by Grams's staining, commercial latex agglutination test (HiListeria Latex Test Kit LK07), and tested by HiMotility commercial Biochemical kit for *Listeria* (KBM003A).

Phosphatidylinositol-specific phospholipase C (PI-PLC) activity: The biochemically characterized *Listeria* isolates were then tested for their PI-PLC activity by stabbing on the ALOA medium (Ottaviani *et al.*, 1997). The production of PI-PLC by the pathogenic *Listeria* spp. was exhibited by halo formation around the inoculated site.

PCR Confirmation: The biochemically confirmed isolates were finally subjected to confirmation by duplex PCR targeting the *prs* (genus-specific) and *isp* (species-specific) genes, as described earlier (Rawool *et al.*, 2016). Briefly, the duplex PCR assay was performed to detect genus *Listeria* and species *L. monocytogenes* wherein a 25µL reaction volume was prepared; containing 2.5µL of 10X PCR buffer, 2µL of 10 mM dNTP mix, 2µL of 50 mM $MgCl_2$ and 1µM of each primer sets (*prs* and *isp*) at 10 µM for each primer set, 1 unit of Taq DNA polymerase, 3 µL DNA template and the reaction volume was made up by nuclease-free water. The cycling conditions included an initial denaturation at 95°C for 5 min followed by 40 cycles each of 30 s of denaturation at 95°C, 1 min annealing at 53°C and 2 min extension at 72°C, followed by 10 minutes of final extension at 72°C and hold at 4°C in a Thermal cycler (BioRad, C-10000 Touch Thermal cycler). The resultant amplified PCR products were resolved in 1% agarose gel containing ethidium bromide (10 g /ml) by electrophoresis using Tris-Acetate-EDTA as running buffer and visualized by gel documentation system (Eppendorf, BIOPRINT-CX4) employing Biovision software.

Serotyping PCR: The serotyping multiplex-PCR (mPCR) was performed as described earlier (Doumith *et al.*, 2004). In brief, 25 l reaction volume; containing 2.5 l PCR buffer, 1 mM dNTP mix, 25 mM $MgCl_2$, and 50 M of five primer sets (Table 1), 1 unit of Taq DNA polymerase, 50 ng of DNA template and nuclease-free water to make up the reaction volume. The cycling conditions for mPCR included an initial denaturation at 95°C for 5 min, followed by 40 cycles each of denaturation at 95°C for 30 s, annealing at 56°C for 1 min, and extension at 72° C for 2 min. At the end of all cycles, a final extension at 72°C for 10 min was performed in a Thermal cycler (BioRad, C-1000 Touch Thermal cycler). The resultant amplified PCR products were resolved in 1% agarose gel containing ethidium bromide (10 g /ml) by electrophoresis using Tris-Acetate-EDTA as running buffer and visualized by gel documentation system (Eppendorf, BIOPRINT-CX4) employing Biovision software.

RESULTS AND DISCUSSION

Following the IS 14988 (Part 2): 2020 protocol revealed the presence of *Listeria* spp. presumptive colonies on selective PALCAM agar in a total of 10 meat samples. All 10 suspected isolates were Gram-positive cocco-bacillary, and were positive for Latex Agglutination Test (LK-07 HiListeria Latex Test Kit (Himedia, India)) (Table 1). Out of 10, only 8 isolates showed biochemical characteristics of *L. monocytogenes* (Table 1). The suspected *L. monocytogenes* isolates (n=8) exhibited prominent PI-PLC activity (halo formation) on the ALOA medium within 24 h of inoculation while the other two suspects were negative for PI-PLC activity. Thus, on microbiological analysis of 458 samples, 8 (1.746%) samples turned out to be positive for *L. monocytogenes*. All eight isolates were recovered from beef samples collected from Belgavi. The standardized mPCR allowed amplification of both the *prs* and *isp* genes in all 8 isolates confirming their identification as *L. monocytogenes*. The results of serotyping mPCR were used to distinguish different serogroups of *L. monocytogenes*. All the eight isolates belonged to 1/2b, 3b, and 7 serogroup of *L. monocytogenes*.

Human listeriosis is attributable to consuming contaminated foods (Stone *et al.*, 2020). The first known account of a human listeriosis epidemic was chronicled in 1980 resulting from the consumption of contaminated food (Schlech *et al.*, 1983). Meat and poultry items were incriminated in incidences of listeriosis (Yehia *et al.*, 2020). Listeriosis cases had been reported as sporadic as well as in epidemic forms (Barbuddhe *et al.*, 2012). From the Indian perspective, epidemiological data on human and animal listeriosis are insufficient to determine the extent of true infection; in addition, the disease is underreported

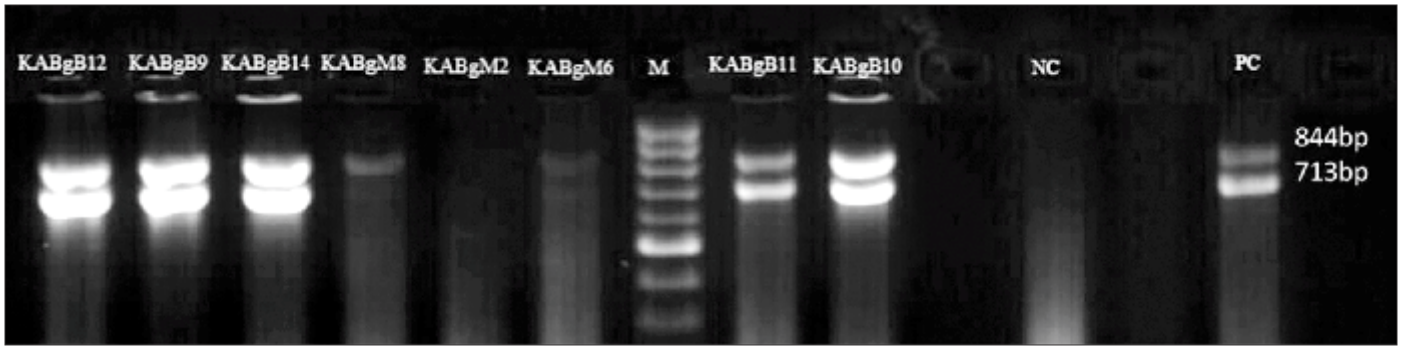

Fig. 1. Gel electrophoresis image of mPCR of representative samples for the detection of genus *Listeria* and species *L. monocytogenes*

Table 1. Biochemical characterization and PCR profile of *L. monocytogenes*

Isolate's ID	Source	Biochemical profile									PCR Profile Species specific genes		Latex Agglutination Test
		Grams staining	Catalase	motility @ 25°C	MR test	VP test	Nitrate reduction	Rhamnose	Xylose	-ethyl-D-mannopyranoside	*isp*	*prs*	
KBgB3	Beef	+	+	+	+	+	-	+	-	+	+	+	+
KBgB4	Beef	+	+	+	+	+	-	+	-	+	+	+	+
KBgB5	Beef	+	+	+	+	+	-	+	-	+	+	+	+
KBgB9	Beef	+	+	+	+	+	-	+	-	+	+	+	+
KBgB10	Beef	+	+	+	+	+	-	+	-	+	+	+	+
KBgB11	Beef	+	+	+	+	+	-	+	-	+	+	+	+
KBgB12	Beef	+	+	+	+	+	-	+	-	+	+	+	+
KBgB14	Beef	+	+	+	+	+	-	+	-	+	+	+	+

(Barbuddhe *et al.*, 2021).

The present study was performed to estimate the occurrence of *L. monocytogenes* in raw milk and meat collected from different parts of Karnataka. Upon microbiological screening and molecular confirmation, 8 beef samples (1.746%) were positive for *L. monocytogenes*. The isolation of *L. monocytogenes* in this study was very low; this was compounded by an already low occurrence of the organism in the country. This finding was consistent with that of a study, which reported 19.3% fish, 1.7% chicken and 1.3% pork samples were positive for *L. monocytogenes* in Punjab (Kaur *et al.*, 2017). Another study recorded the prevalence of *Listeria* as 1.82%, 3.21%, and 6.66% in chevon, mutton, and swab samples, respectively (Alka *et al.*, 2019). Papatzimos *et al.* (2022) recorded a 3·96% prevalence of *L. monocytogenes* in meat and meat products. Further, in a variety of meat and meat products, *L. monocytogenes* was reported including raw processed meat (19.5%), and raw intact meat (10.1%) (Matle *et al.*, 2020).

The presence of *L. monocytogenes* in milk samples was not detected. The negative results in milk samples can be attributed to the good health of the animals, hygienic milk collection practices, and hygienic milk transportation systems.

The pathogenic and non-pathogenic *Listeria* strains can be distinguished using the ALOA medium [*L. monocytogenes* and *L. ivanovii*] (Ottaviani *et al.*, 1997). All the eight isolates were tested for PI-PLC activity, which revealed PI-PLC activity in 24h of inoculation indicating their pathogenicity. Other studies described PI-PLC activity on the ALOA medium (Ramanjeneya *et al.*, 2019; Negi *et al.*, 2015). The serogroup mPCR revealed that all 8 isolates as 1/2b, 3b and 7 serogroup of *L. monocytogenes*. Karthikeyan *et al.* (2014) reported *L. monocytogenes* isolated from milk and milk samples as serogroups 1/2a and 4b. However, Braga *et al.* (2017) found both 1/2b and 4b as the most frequently identified serotypes in food samples.

CONCLUSION

To conclude, the present study revealed no cultural positivity for *L. monocytogenes* in milk samples from different parts of Karnataka; however, positivity was observed in beef samples. In the absence of appropriate control measures, the presence of virulent strains of *L.*

monocytogenes in the raw milk and meat samples may pose a potential risk to consumers. The study highlighted the presence of the 1/2b, 3b, and 7 serogrouping animal foods and warrants more studies from different parts to understand the serotype diversity of *L. monocytogenes* prevailing in our country.

REFERENCES

Abdeen, E.E., Mousa, W.S., Harb, O.H., Fath-Elbab, G.A., Nooruzzaman, M., Gaber, A., Alsanie, W.F. and Abdeen, A. (2021). Prevalence, antibiogram and genetic characterization of *Listeria monocytogenes* from food products in Egypt. *Foods,* **10(6)**: 1381.

Alka, Singh, R., Kaur, S., and Bedi, J.S. (2019). *Listeria* contamination in chevon and mutton from retail meat shops and slaughter house environment of Punjab, India. *FEMS Microbiol. Lett*, **366(9)**: fnz111.

Barbuddhe, S.B. and Malik, S.V.S. (2009). Recent advances in the molecular diagnosis of listeriosis. In: ICAR sponsored winter school on "Molecular diagnostic techniques for zoonotic and foodborne infections", Feb.07-27, Division of Veterinary Public Health, Indian Veterinary Research Institute, Izatnagar. 191-199.

Barbuddhe, S.B., Malik, S.V.S., Kumar, A., Kalorey, D.R. and Chakraborty, T. (2012). Epidemiology and risk management of listeriosis in India. *Int. J. Food Microbiol.,* **154(3)**: 113-118.

Barbuddhe, S.B., Rawool, D.B., Doijad, S.P., Vergis, J., Malik, S.S. and Chakraborty, T. (2021). Ecology of *Listeria monocytogenes* and *Listeria* species in India: the occurrence, resistance to biocides, genomic landscape and biocontrol. *Environ. Microbiol*., **24(6)**: 2759-2780.

Braga, V., Vázquez, S., Vico, V., Pastorino, V., Mota, M.I., Legnani, M., Schelotto, F., Lancibidad, G. and Varela, G. (2017). Prevalence and serotype distribution of *Listeria monocytogenes* isolated from foods in Montevideo-Uruguay. *Braz. J. Microbiol.*, **48**: 689-694.

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C. and Martin, P. (2004). Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.*, **42(8)**: 3819-3822.

Farber, J.M. and Peterkin, P. (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Rev*., **55(3)**: 476-511.

IS 14988: Part 2: 2020 Microbiology of the Food Chain. Horizontal Method for Detection and Enumeration of *Listeria monocytogenes* and of *Listeria* spp. Part 2 Enumeration Method (First Revision). Bureau of Indian Standards.

Karthikeyan, R., Gunasekaran, P. and Rajendhran, J. (2014). Molecular Serotyping and Pathogenic Potential of *Listeria monocytogenes* Isolated from Milk and Milk Products in Tamil Nadu, India. *Foodborne Pathog. Dis.*, **12(6)**: 522-528.

Kaur, S., Singh, R., Sran, M.K. and Gill, J.P.S. (2017). Molecular characterization of *Listeria monocytogenes* in white meat samples from Punjab, India. *Indian J. Anim. Res*., **52**: 1635-1641.

Matle, I., Mbatha, K.R. and Madoroba, E. (2020). A review of *Listeria monocytogenes* from meat and meat products: Epidemiology, virulence factors, antimicrobial resistance and diagnosis. *J. Vet. Res.*, **87(1)**: 1-20.

Moura, A., Criscuolo, A., Pouseele, H., Maury, M.M., Leclercq, A., Tarr, C., Björkman, J.T., Dallman, T., Reimer, A., Enouf, V. and Larsonneur, E. (2016). Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. *Nat. Microbiol.*, **2(2)**: 1-10.

Negi, M., Vergis, J., Vijay, D., Dhaka, P., Malik, S.V.S., Kumar, A., Poharkar, K.V., Doijad, S.P., Barbuddhe, S.B., Ramteke, P.W. and Rawool, D.B. (2015). Genetic diversity, virulence potential and antimicrobial susceptibility of *Listeria monocytogenes* recovered from different sources in India. *Pathog. Dis.*, **73(9)**: 093.

NicAogain, K. and O'Byrne, C.P. (2016). The role of stress and stress adaptations in determining the fate of the bacterial pathogen *Listeria monocytogenes* in the food chain. *Front. Microbiol.*, **7**: 1865.

Ottaviani, F., Ottaviani, M. and Agosti, M. (1997). Esperienza su un agar selettivo e differentialeper *Listeria monocytogenes*. *Ind. Aliment*, **36**: 1-3.

Papatzimos, G., Kotzamanidis, C., Kyritsi, M., Malissiova, E., Economou, V., Giantzi, V., Zdragas, A., Hadjichristodoulou, C. and Sergelidis, D. (2022). Prevalence and characteristics of *Listeria monocytogenes* in meat, meat products, food handlers and the environment of the meat processing and the retail facilities of a company in Northern Greece. *Lett. Appl. Microbiol.*, **74(3)**: 367-376.

Ramanjeneya, S., Sahoo, S.C., Pathak, R., Kumar, M., Vergis, J., Malik, S.V.S., Barbuddhe, S.B. and Rawool, D.B. (2019). Virulence potential, biofilm formation, and antibiotic susceptibility of *Listeria monocytogenes* isolated from cattle housed in a particular gaushala (cattle shelter) and organized farm. *Foodborne Pathog. Dis.*, **16(3)**: 214-220.

Raufu, I.A., Moura, A., Vales, G., Ahmed, O.A., Aremu, A., Thouvenot, P., Tessaud-Rita, N., Bracq-Dieye, H., Krishnamurthy, R., Leclercq, A. and Lecuit, M. (2022). *Listeria ilorinensis* sp. nov., isolated from cow milk cheese in Nigeria. *Int. J. Syst. Evol. Microbiol.* **72(6)**: 005437.

Rawool, D.B., Doijad, S.P., Poharkar, K.V., Negi, M., Kale, S.B., Malik, S.V.S., Kurkure, N.V., Chakraborty, T. and Barbuddhe, S.B. (2016). A multiplex PCR for detection of *Listeria monocytogenes* and its lineages. *J. Microbiol. Methods.* **130**: 144-147.

Roberts, B.N., Chakravarty, D., Gardner, J.C., Ricke, S.C. and Donaldson, J.R. (2020). *Listeria monocytogenes* response to anaerobic environments. *Pathogens.* **9(3)**: 210.

Schlech III, W.F., Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, E.V., Wort, A.J., Hightower, A.W., Johnson, S.E., King, S.H., Nicholls, E.S. and Broome, C.V. (1983). Epidemic listeriosis-evidence for transmission by food. *N. Engl. J. Med.* **308(4)**: 203-206.

Stone, E., Lhomet, A., Neve, H., Grant, I.R., Campbell, K. and McAuliffe, O. (2020). Isolation and Characterization of *Listeria monocytogenes* phage vB_LmoH_P61, a phage with biocontrol potential on different food matrices. *Front. Sustain. Food Syst.*, **4**: 521645205.

Yehia, H.M., Elkhadragy, M.F., Aljahani, A.H. and Alarjani, K.M. (2020). Prevalence and antibiotic resistance of *Listeria monocytogenes* in camel meat. *Biosci. Rep.*, **40(6)**: p.BSR20201062.

Yin, Y., Yao, H., Doijad, S., Kong, S., Shen, Y., Cai, X., Tan, W., Wang, Y., Feng, Y., Ling, Z. and Wang, G. (2019). A hybrid sub-lineage of *Listeria monocytogenes* comprising hypervirulent isolates. *Nat. Commun.* **10(1)**: 1-16.