RESEASRCH ARTICLE

Phenotypic and Molecular Characterization of *Listeria* Spp. from Raw Chicken Meat

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Abstract

The present study was aimed to find the prevalence of *Listeria monocytogenes* in raw chicken meat purchased across the Nagpur city. Cultural examination of 100 raw poultry meat samples revealed 35.0 % prevalence of *Listeria* spp. Among them, *L. grayi* was predominant (15 %) followed by *L. monocytogenes* (12 %), *L. welshimeri* (5 %), *L. innocua* (2 %) and *L. seeligeri* (1 %). A typical β-haemolysis clear zone was exhibited by 18 listeriae isolates. Of these, 12 isolates with characteristic biochemical pattern and showing haemolytic zone. All 12 *L. monocytogenes* isolates expressed the PI PLC activity. Multiplex PCR study of *Listeria* spp. revealed that four *L. monocytogenes* isolates were positive for *plc*A, *act*A, *hly*A and *iap* genes. Amplified products of three virulence-associated genes namely, *act*A, *hly*A and *iap* were detected in five *L. monocytogenes* and three *L. monocytogenes* isolates were positive for *hly*A and *iap* genes. *L. grayi* isolates revealed the amplified product of *iap* gene. Two isolate of *L. innocua* were positive for *act*A and *iap* genes, Five *L. welshimeri* isolates and one *L. seeligeri* isolate were positive for *iap* gene, lacking *plc*A, *act*A and *hly*A genes. As *act*A and *iap* genes were detected in a two isolate of *L. innocua.* It is clear that *act*A and *iap* genes are shared by *L. monocytogenes* and *L. innocua.* It is concluded that genes in three different combinations were recorded in *Listeria spp.* and that *prf*A and *hlyA* detected by PCR distinctively in *L. monocytogenes* suggest the usefulness of PCR amplification from various food samples.

Key words: β-haemolysis, *L. monocytogenes*, PCR, PI-PLC, SBA

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Introduction

Listeria monocytogenes is a food contaminant pathogen a cause of listeriosis worldwide. *Listeria* spp. are small Gram-positive rod (0.5-4 µm in diameter and 0.5-2 µm in length), non-spore-forming, facultative anaerobic, catalase-positive, and oxidase-negative organisms. Listeria has tumbling motility at 20-25˚C due to peritrichous flagella. To date, 20 species of bacteria belonging to the genus Listeria have been identified. Some of the notable species are *L. monocytogenes, L. innocua, L. seeligeri, L. welshimeri, L. ivanovii, L. grayi, L. marthii,* and *L. rocourtiae* (Leclercq *et al*., 2019). Among the different species, *L. monocytogenes* has the most significant impact on public health because of its ability to cause listeriosis (Kumar *et al*., 2016) both to human and animals. Surveillance and control of food-borne human pathogens, such as *Listeria monocytogenes,* is a critical aspect of modern food safety programs at food production facilities (Fox *et al*., 2015). *Listeria monocytogenes*, possesses a high mortality rate (approximately 20%) and is considered one of the most dangerous foodborne pathogens. Although the usual reservoirs for Listeria transmission have been extensively studied, little is known about the relationship between Listeria and live poultry production. There are at least six known species: *Listeria grayi, Listeria seeligeri, Listeria welshimeri, Listeria ivanovii, Listeria innocua*, and *L. monocytogenes.* Of the six species, *L. ivanovii* is pathogenic to animals and *L. monocytogenes* is the only species pathogenic to humans and animals. While Listeria commonly colonize multiple mammalian hosts, it remains unclear the exact relationship of Listeria with avian species (Rothrock *et al*., 2017). Determination of the pathogenic potential of *L. monocytogenes* is important from food safety and public health perspective (Jamshidi and Zeinali, 2019). Therefore, this research work was aimed to study identification, prevalence and virulence profiling of *Listeria Spp*. from poultry origin.

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Materials and Methods

Collection and Processing of Samples

A total of 100 samples comprising muscles of broiler chicken were collected from local poultry market in Nagpur (India)

aseptically in UV sterilized polyethylene bags and quickly transported to the laboratory under chilled condition and stored at 4°C and processed within 24 hrs. Isolation of listeriae was attempted from the collected poultry meat samples of muscles as per method ISO11290-1(2017).

Isolation, Identification and Biochemical Characterization

Isolation of the *Listeria* was attempted from the samples by selective enrichment in University of Vermont Medium (UVM) and plating onto Dominguez-Rodriguez isolation agar (DRIA). The bacterial colonies were isolated and subjected to Gram's staining and requisite biochemical tests. *L. monocytogenes* suspected colonies were subjected to various biochemical tests as per Doijad *et al* .,2010. The isolates were confirmed up to species level on the basis of biochemical characters and *in-vitro* pathogenicity tests like, haemolysis on SBA, CAMP test and PI-PLC activity. Sheep blood agar media (5%) was applied to examine the haemolysis activities of *Listeria* spp. Christie, Atkins, Munch and Petersen (CAMP) test was applied to recognize diverse species. *S. aureus* and *Rhodococcus equi* were applied as indicator organisms.

Molecular Characterization

The DNA was extracted from suspected colonies and tissues using Himedia DNA extraction kits, and subjected to PCR using published primers. Standardization of PCR was done by using standard strain of *L. monocytogenes* 4b (MTCC 1143). The details of the primer sequence are shown in Table 1.

Results and Discussion

Isolation and Identification

A total of 100 poultry meat samples collected from retail outlets screened indicated an overall positivity for listeriae in 35 (35.0%) samples. Of these, 12 (12.0 %) isolates were confirmed as *L. monocytogenes*, 15 (15.0 %) as *L. grayi*, 2 (2.0%) as *L. innocua*, 5 (5.0 %) as *L. welshimeri* and 1 (1.0 %) as *L. seeligeri* . Similar to the present findings Nikas (2009) reported 35% prevalence of *Listeria* spp. in chicken samples from Mhow area of Madhya Pradesh. Centinkaya *et al*. (2004) and Loura *et al*. (2005) also recorded 31% prevalence of *Listeria* spp. from chicken samples, while Inoue *et al*. (2000) reported 37 % prevalence of *L. monocytogenes* in minced chicken samples from Japan. In Greece (Sakaridis *et al*., 2011) and Iran (Zeinali *et al*., 2017) scientists isolated *L. monocytogenes* from poultry meat with percentages of 38% and 18%, respectively.

All the biochemically confirmed *Listeria* isolates were streaked on SBA (5 %) and haemolysis was observed. A typical β-haemolysis with broad and clear zone was exhibited by 18 listeriae isolates. Of these, 12 isolates with characteristic biochemical pattern and showing characteristic enhancement of haemolytic zone with *S. aureus* on SBA were characterized as *L. monocytogenes* (Fig. 1) as documented by Kenar *et al*. (2006). Whereas, remaining non-haemolytic isolates towards *S. aureus* with characteristic biochemical pattern were identified as *L. seeligeri* (Doijad *et al*., 2010). All 12 *L. monocytogenes* isolates expressed the PI-PLC activity (Fig. 2).

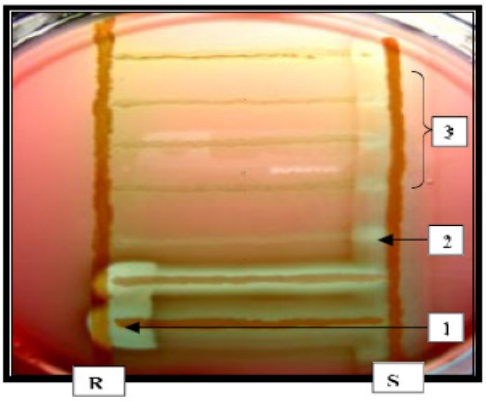

Fig. 1: CAMP TEST

PCR

PCR analysis of 12 haemolytic *L. monocytogenes* isolates recovered from raw poultry meat samples revealed the different genotypic patterns for four virulence-associated genes, namely *plc*A, *act*A, *hly*A and *iap* (Table 2). Multiplex PCR assay was standardized so as to amplify these four virulence

Table 1: Details of primers used for amplification of virulence marker associated genes of listeriae

Primer	Primer Sequence		Size	Reference
plc A	F	5'-CTG CTT GAG CGT TCA TGT CTC ATC CCC C - 3'	1484 bp	
	R	5'- CAT GGG TTT CAC TCT CCT TCT AC - 3'		
*prf*A	F	'-CTGTTGGAGCTCTTCTTGGTGAAGCAATCG-3'	60 bp	
	R	5'-ACAACCTCGGTACCATATACTAACTC-3'		
act A	F	5'- CGC CGC GGA AAT TAA AAA AAG A - 3'	839 bp	Rawool *et al* .,2007
	R	5'- ACG AAG GAA CCG GGC TGC TAG - 3'		
*hly*A	F	5'- GCA GTT GCA AGC GCT TGG AGT GAA - 3'	456 bp	
	R	5'- GCA ACG TAT CCT CCA GAG TGA TCG - 3'		
iap	F	5'- ACA AGC TGC ACC TGT TGC AG - 3'	131 bp	
	R	5'- TGA CAG CGT GTG TAG TAG CA -3'		

Table 2: Frequency of virulence-associated genes in *Listeria* spp. isolates recovered from raw poultry meat samples by multiplex PCR

Sr. No.	Species	No. of isolates	Amplified PCR products of virulence-associated genes detected in *Listeria* spp. isolates			
			***plc*A (1484 bp)**	***act*A (839 bp)**	***hly*A (456 bp)**	***iap* (131 bp)**
01	*L. monocytogenes*	04	+	+	+	+
02	*L. monocytogenes*	05	-	+	+	+
03	*L. monocytogenes*	03	-	-	+	+
04	*L. grayi*	15	-	-	-	+
05	*L. innocua*	02	-	+	-	+
06	*L. welshimeri*	05	-	-	-	+
07	*L. seeligeri*	01	-	-	-	+

+ = Positive, - = Negative

associated genes of *L. monocytogenes* of the corresponding size of 1484 bp, 839 bp, 456 bp and 131 bp, respectively by employing the gene-specific primers (Fig. 3).

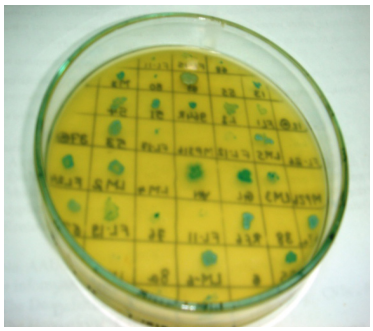

Fig. 2: PI-PLC activity

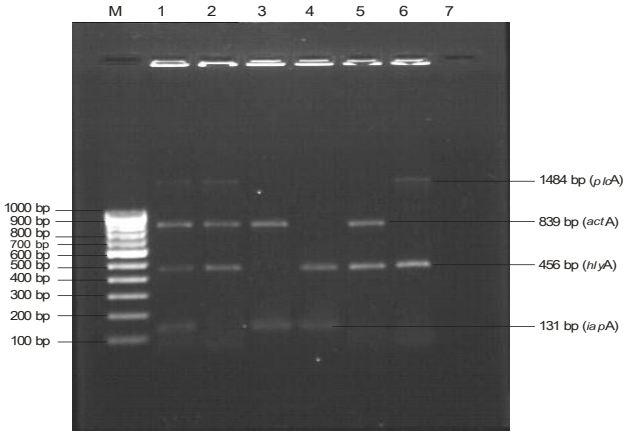

Fig. 3: Amplification of virulence associated genes of *Listeria* spp. by Multiplex PCR
Lane-M: 100 bp DNA Ladder, Lane 1: *L. monocytogenes* amplification of *plc*A (1484 bp), *act*A(839 bp), *hly*A (456 bp) and *iap* (131 bp) genes
Lane 2: *L. monocytogenes* amplification of *plc*A (1484 bp), *act*A(839 bp) and *hly*A (456 bp) genes
Lane 3: *L. innocua* amplification of *act*A(839 bp) and *iap* (131 bp) genes
Lane4: *L. monocytogenes* amplification of *hly*A (456bp) and *iap* (131 bp) genes
Lane 5: *L. monocytogenes* amplification of *act*A(839 bp) and *hly*A (456 bp) genes
Lane 6: *L. monocytogenes* amplification of *plc*A (1484 bp) and *hly*A (456 bp) genes
Lane 7: Negative Control

Multiplex PCR study of *Listeria* spp. recovered revealed that four *L. monocytogenes* isolates were positive for *plc*A, *act*A, *hly*A and *iap* genes. Amplified products of three virulence-associated genes namely, *act*A, *hly*A and *iap* were detected in five *L. monocytogenes* and three *L. monocytogenes* isolates were positive for *hly* A and *iap* genes.

L. grayi isolates revealed the amplified product of *iap* gene. Two isolates of *L. innocua* were positive for *act*A and *iap* genes, Five *L. welshimeri* isolates and one *L. seeligeri* isolate were positive for *iap* gene, lacking *plc*A, *act*A and *hly*A genes, while *act*A and *iap* genes were detected in a two isolate of *L. innocua.* Based on results it is clear that *act*A and *iap* genes are shared by *L. monocytogenes* and *L. innocua.* It is reported that *plcA* and *hlyA* are detected by PCR distinctively in *L. monocytogenes* suggesting the usefulness of PCR amplification of these genes for the identification of *L. monocytogenes* from various food samples.

L. monocytogenes is the main cause of foodborne listeriosis in humans. Rarely, foodborne infections were reported *by L. ivanovii* and *L. seeligeri*. Strains of *L. monocytogenes* have different pathogenic potential, as some strains are very virulent, whereas others are noninfectious agents (Liu *et al*., 2007). Wide range of bird species is affected by listeriosis, but most infections do not cause clinical signs. Sporadic cases in backyard chickens may cause encephalitis or septicemia and sudden death. Rawool *et al.* (2007) initially developed the multiplex PCR assay for detection of four virulence marker genes, namely *plcA, hlyA, actA* and *iap* of *L. monocytogenes* and later added *prf*A. They finally concluded that the additional band was due to interaction between primers of *plc*A and *prf*A genes. It has been documented that detection of only one virulence associated gene by PCR is not always sufficient to identify *L. monocytogenes*, and that PCR amplification of genes other than *hly*A and *plc*A may not be adequate for the detection of virulent strains of *L. monocytogenes* as well. Expression of virulence marker genes by *in-vitro* assay may be of pivotal importance in differentiation of pathogenic *L. monocytogenes* from other species. This approach has overcome the problems by saving the time, labour and the costly biologicals too.

Jaradat *et al*. (2002) concluded that the *L. monocytogenes* isolates showed 92-95 % genetic homogeneity on PCR analysis of virulence genes (*inl*A, *inl*B, *act*A, *hly*A, *plc*A, and *plc*B), in spite of their origins from two different geographic locations and environments. Zeinali *et al.* (2017) examined the chicken meat sold at different supermarkets by collecting 200 random fresh chicken carcasses and subjected them to isolation of *Listeria* spp. 40% of the samples did reveal *Listeria* spp. of which 18% were attributed to *Listeria monocytogenes*. This was further evidenced by use of multiplex PCR assay.

The high frequencies of virulence genes in the bacterial strains enable adhesion, invasion, and epithelial damage to the human digestive system (Momtazand Yadollahi, 2013). Moreover, these results indicate that these isolates possess the properties of virulent strain and their sequences may be further investigated to explore the differences between pathogenic and less pathogenic strains (Abdellrazeq *et al*., 2014). Thus, the presence of these genes proposes the incidence of a critical public health risk correlated with the consumption of examined food samples contaminated with *L. monocytogenes*.

Conclusion

From the food safety perspective, the presence of *L. monocytogenes* in the poultry meat and products is a multifaceted potential hazard. The current work describes that, Listeria is considered one of the major bacterial foodborne pathogens, but it is often not considered epidemiologically important in poultry production. Listeria prevalence and pathogenicity can be studied, there is an opportunity to better assess the potential public health effects of Listeria from the poultry industry and develop better management practices to overcome these effects.

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References

Abdellrazeq, G., Kamar, A., & ElHoushy, S. (2014). Molecular Characterization of *Listeria* species Isolated from Frozen Fish. *Alexandria Journal of Veterinary Science, 40,* 1. https://doi.org/10.5455/ajvs.45443.

Centinkaya, F., Cibik, R., Soyutemiz, G.E., & Ozakin, C. (2004). Prevalence of *Listeria* species in chicken at the retail level. *The Indian Veterinary Journal, 81,* 1313-1316.

Doijad, S.P., Vaidyal, V., Garg, S., Kalekar, S., Rodrigues, J., D'Costa, D., Bhosle, S.N., & Barhuddhe, S.B. (2010). solation and characterization of *Listeria* species from raw and processed meats. *Journal of Veterinary Public Health, 8(2).* 83-88.

ISO 11290-1 (2017) International Standard : Microbiology of food chain horizontal method for the detection and enumeration of Listeria monocytogenes and Listeria spp. Part 1 .Second Edition .1-44.

Fox, E.M., Wall, P.G., & Fanning, S. (2015). Control of *Listeria* species food safety at a poultry food production facility. *Food Microbiology, 51,* 81-85.

Inoue, S., Nakama, A., Arai, Y., Kokubo, Y., Maruyama, T., Saito, A., Yoshida, T., Terao, M., Yamamoto, S., & Kumagai, S. (2000). Prevalence and contamination levels of *Listeria monocytogenes* in retail foods in Japan. *International Journal of Food Microbiology, 59*(1-2), 73-77.

Jamshidi, A., & Zeinali, T. (2019). Significance and characteristics of *Listeria monocytogenes* in poultry products. *International Journal of Food Science*, 2019,1- 7. DOI: 10.1155/2019/7835253

Jaradat, Z.W., Schutze, G.E., & Bhunia., A.K. (2002). Genetic homogeneity among *Listeria monocytogenes* strains from infected patients and meat products from two geographic locations determined by phenotyping, ribotyping and PCR analysis of virulence genes. *International Journal of Food Microbiology, 76*(1-2), 1-10.

Kenar, B., Dilek, H., & Akkaya, L. (2006). *Listeria* spp. in dairy farms in the western part of central Anatolia. *The Indian Veterinary Journal, 83,* 4-6.

Kumar, B.N.V., Fairoze, N., Madhavaprasad, C.B., Karabasanavar, N., Kotresh, A.M., Nadoor, P., Prashant, S., Shilpa, A.G., Barbuddhe, S.B., Kurkure, N., & Chaudhary, S. (2016). Molecular characterization and antibiogram of *Listeria monocytogenes* isolated from chicken and mutton of retail markets. *Journal of Microbiology and Infectious Diseases, 6*(2), 65-68.

Leclercq, A., Moura, A., Vales, G., Tessaud-Rita, N., Aguilhon, C., & Lecuit. M. (2019). *Listeria thailandensis* sp. nov. *International Journal of Systematic and Evolutionary Microbiology, 69*(1), 74-81.

Liu, D., Lawrence, M. L., Ainsworth, A.J., & Austin, F.W. (2007). Toward an improved laboratory definition of *Listeria monocytogenes* virulence. *International Journal of Food Microbiology, 118*(2), 101-115.

Loura, C.A.C., Almeidaand, R.C.C., & Almeida, P.F. (2005). The incidence and level of *Listeria* spp. and *Listeria monocytogenes* contamination in processed poultry at poultry processing plant. *Journal of Food Safety, 25*(1), 19-29.

Momtaz, H., & Yadollahi, S. (2013). Molecular characterization of *Listeria monocytogenes* isolated from fresh seafood samples in Iran. *Diagnostic Pathology*, 8, 149.

Nikas, Y. (2009). Isolation and molecular characterization of *Listeria* spp. from raw chicken and pork. *M.V.Sc. Thesis.* Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, MP, India.

Rawool, D.B., Malik, S.V.S., Barbuddhe, S.B., Shakuntala I., & Aurora, R. (2007). A multiplex PCR for detection of virulence associated genes in *Listeria monocytogenes*. *International Journal of Food Safety, 9,* 56-62.

Rothrock, M.J. Jr., Davis, M.L., Locatelli, A., Bodie, A., McIntosh, T.G., Donaldson, J.R., & Ricke, S.C. (2017). Listeria occurrence in poultry flocks: Detection and potential implications. *Frontiers in Veterinary Science, 4,* 125.

Sakaridis, I., Soultos, N., Iossifidou, E., Papa, A., Ambrosiadis, I., & Koidis, P. (2011). Prevalence and antimicrobial resistance of *Listeria monocytogenes* isolated in chicken slaughterhouses in northern Greece. *Journal of Food Protection 74:* 1017–1021.

Zeinali, T., Jamshidi, A., Bassami M., & Rad, M. (2017). Isolation and identification of *Listeria* spp. in chicken carcasses marketed in Northeast of Iran. *International Food Research Journal, 24*(2), 881-887.