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RESEARCH ARTICLE

Molecular characterization and antibiogram of *Listeria monocytogenes* isolated from chicken and mutton of retail markets

Vinay Kumar B. N.[1], Nadeem Fairoze[1], Madhavaprasad C. B.[2], Nagappa Karabasanavar[2], Kotresh A. M.[3], Prakash Nadoor[4], Prashant S.[2], Shilpa A. G.[2], S. B. Barbuddhe[5], Nitin Kurkure[6], Sandeep Chaudhary[7]

[1] *Department of Livestock Product and Technology, Veterinary College, Hebbal, Bengaluru, Karnataka, India*
[2] *Department of Veterinary Public Health & Epidemiology, Veterinary College, Shivamogga, Karnataka, India*
[3] *Department of Veterinary Physiology & Biochemistry, Veterinary College, Shivamogga, Karnataka, India*
[4] *Department of Veterinary Pharmacology & Toxicology, Veterinary College, Shivamogga, Karnataka, India*
[5] *National Institute for Biotic Stress Management, Raipur, Chhattisgarh, India*
[6] *Department of Veterinary Pathology, Nagpur Veterinary College, Nagpur, Maharashtra, India*
[7] *Department of Veterinary Public Health & Epidemiology, Nagpur Veterinary College, Nagpur, Maharashtra, India*

ABSTRACT

Objective: To isolate *Listeria monocytogenes* from chicken and mutton meat sold at retail outlets and to characterize isolates for virulence determinants.

Methods: L*isteria monocytogenes* was isolated from chicken and mutton meat samples using ISO: 11290 method. Multiplex PCR was performed for the detection of virulence associated genes.

Results: Out of 198 retail meat samples (72 chicken and 126 mutton) analyzed, *Listeria monocytogenes* was isolated from 4.5% (8.3% chicken and 2.3% mutton) samples. All the 9 isolates (6 from chicken and 3 from mutton) belonged to 1/2a servar and carried virulence genes viz. haemolysin (hlyA), phosphatidylinositol phospholipase (plcA), actin polymerization protein (actA) and invasive associative protein p60 (Iap).

Conclusion: *L. monocytogenes* is an organism of food safety and public health significance, its recovery from the meat sold at retail outlets indicated breach in the quality assurance. *J Microbiol Infect Dis 2016;6(2): 65-68*

Key words: *Listeria monocytogenes*, chicken, mutton, virulence marker

Perakende pazarlarındaki tavuk ve koyunlardan izole edilen *Listeria monocytogenes*'lerin moleküler özellikleri ve antibiyogramları

ÖZET

Amaç: Bu çalışmada, perakende satılan tavuk ve koyun etlerindeki *Listeria monocytogenes*'lerin izole edilmesi ve virülans belirleyicileri için izolatların karakterize edilmesi amaçlandı.

Yöntem: *Listeria monocytogenes*, tavuk ve koyun etlerinden ISO: 11290 yöntem kullanılarak izole edildi. Virülans ile ilişkili genlerin saptanması işlemi multipleks PCR yöntemi ile yapıldı.

Sonuçlar: Çalışmada, 198 perakende et örneği (72 tavuk eti ve 126 koyun eti) analiz edildi. *Listeria monocytogenes* örneklerin % 4,5'inden (% 8,3 tavuk ve % 2,3 koyun eti) izole edildi. Dokuz izolatın (altısı tavuk ve üçü koyun etinden) tamamı serovar 1/2a ile ilişkili bulundu ve viz. hemolizin (hlyA), fosfatidilinozitol fosfolipaz (plcA), aktin polimerizasyon proteini (actA) ve invasyonla ilişkili protein p60 (Iap) virülans genlerini taşıyordu.

Sonuç: *L. monocytogenes* gıda güvenliği ve halk sağlığı açısından önemli bir mikroorganizmadır. Bu çalışma, perakendede satılan etlerin kalite güvencesinin iyileştirilmesi gerektiğini göstermektedir. *J Microbiol Infect Dis 2016;6(2): 87-90*

Anahtar kelimeler: *Listeria monocytogenes*, tavuk, koyun eti, virülans belirleyiciler

Correspondence: Nagappa Karabasanavar, Department of Veterinary Public Health & Epidemiology, Veterinary College, Shivamogga, Karnataka, India Email: pub.nag@gmail.com
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INTRODUCTION

Members belonging to the genus Listeria are widespread in the environment. There are eight species of *Listeria viz., L. monocytogenes, L. innocua, L. seeligeri, L. welshimeri, L. ivanovii, L. grayi, L. marthii*, and *L. rocourtiae*; of which *L. monocytogenes* has emerged as an important food-borne and zoonotic pathogen of public health significance [1,2]. *Listeria monocytogenes* has been isolated from a wide variety of foods including meat. Chicken and mutton in particular have been incriminated as major vehicles of human transmission [3]. Hygienic and sanitary measures practiced across the production chain determine the level of Listeria contamination especially during slaughtering, evisceration and meat processing [4]. Apart from livestock and poultry, Listeria could also be carried as environmental contaminants over the carcass meat through fomites, personnel, utensils and the water used for meat processing [5-7]. Occurrence of virulent and clinically significant *L. monocytogenes* in the food chain constitutes potential risk to the public health [8]. The purpose of this study was to isolate and identify Listeria from chicken and mutton sold at retail outlets and characterizes isolates for virulence determinants.

METHODS

Samples

A total of 198 mutton (72 chicken and 126 mutton) samples were collected from urban retail markets (Table 1) located at Shivamogga (coordinates 13.9333° N, 75.5667° E) and Bengaluru (coordinates 12.9667° N, 77.5667° E) cities of the Karnataka state, India. Meat samples (250 grams) were collected into sterile polythene bags and brought to the laboratory under chilled conditions for analysis. Depending upon the number of animal species processed and sold, the retail outlets were categorized as either single species (selling chicken or mutton singly) and multiple species (chicken + mutton + goat meat) outlets.

Table 1. Occurrence of *Listeria monocytogenes* in fresh raw chicken and mutton meat sold at retail markets

Species meat	Nature of outlet	Samples analyzed	Samples positive for *L. monocytogenes*	Prevalence	Serotype
1 Chicken	*Single* species meat (chicken meat only)	36	2	5.5%	1/2a
	Multiple species meats (chicken+ mutton + goat meat)	36	4	11.1%	1/2a
2 Mutton	*Single* species meat (mutton only)	48	1	2.0%	1/2a
	Multiple species meats (chicken+ mutton + goat meat)	78	2	2.5%	1/2a
Total		198	9	4.5%	

Isolation of *Listeria monocytogenes*

The ISO: 11290 method was used for the isolation of *L. monocytogenes* from meat samples [9]. Briefly, primary enrichment of sample (25 g) was carried out in 225 mL of half Fraser Broth (hFB); following incubation at 30°C for 24 hours, secondary enrichment was undertaken in the full Fraser Broth (fFB) by transferring 0.1 mL of black colored hFB pre-enriched inoculum into 10 mL of fFB. Following incubation at 37°C for 24 hours, selective plating was carried out on Polymixin Acriflavin Lithium Chloride Ceftazidime Esculin Mannitol (PALCAM) agar. Following incubation at 37°C for 24 hours, grey-green and shiny colonies showing diffuse black shadow were suspected as Listeria species. The putative colonies were then re-streaked on the PALCAM agar to get isolates in the pure culture. After growing pure cultures on the Brain Heart Infusion (BHI) slant at 37 °C for 24 hours, a battery of tests i.e. Gram's staining, motility at 25°C, biochemical tests such as catalase, oxidase, methyl red and Voges-Proskauer test, sugars fermentation tests (glucose, mannitol, xylose, rhamnose, salicin, and esculine), beta haemolysis on sheep blood agar, hydrogen sulphide production and triple sugar iron agar were performed following standard methods [10]. The serovar of the *L. monocytogenes* was determined using a multiplex-PCR as described by Doumith et al. [11].

Detection of virulence genes

DNA from *L. monocytogenes* isolates was extracted using the method of Makino et al. [12] and multiplex polymerase chain reaction (mPCR) was performed

for the detection of virulence markers viz. (1). plcA gene that encodes 'phosphatidylinositol phospholipase C', (2). actA gene that encodes 'actin polymerization protein', (3). hlyA gene that encodes 'haemolysin' gene and (4). iap gene that encodes 'invasive associative protein p60' following the method of Rawool et al. [13].

RESULTS

Of the total 198 meat samples analyzed, *L. monocytogenes* was detected in 9 samples (prevalence of 4.5% in meat samples, Table 1). Market retail outlets that sold single species meat showed comparatively lower rate of contamination with *L. monocytogenes* i.e. 5.5% chicken and 2.0% mutton. However, retail outlets selling multiple species meat (chicken + mutton + goat meat) showed higher rate of contamination i.e. 11.1% of chicken and 2.5% of mutton were found contaminated with *L. monocytogenes* (Table 1). Culturally and biochemically confirmed *L. monocytogenes* isolates were identified as 1/a serovar and all the isolates showed amplicons of size 1,484 bp (plcA gene), 839 bp (actA gene), 456 bp (hlyA gene) and 131 bp (iap gene) as shown in Figure 1.

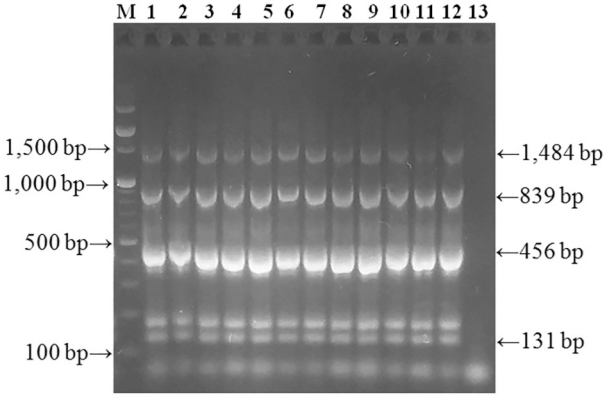

Figure 1. Multiplex PCR amplification of hlyA, plcA, actA and iap genes from *L. monocytogenes* isolated from raw chicken and mutton samples.

Lane M: 100 bp ladder; Lanes 1-3: *L. monocytogenes* MTCC pure culture; Lanes 4-9: *L.monocytogenes* isolated from chicken meat; Lanes 10-12: *L. monocytogenes* isolated from chicken mutton; Lane 13: No Template Control

DISCUSSION

L. monocytogenes has been isolated from a wide variety of meats; its prevalence of 4.5% observed in this study in fresh raw meat samples sold at retail outlets was comparatively lower than the reports of other investigators [14,15]. In a similar study, Barbuddhe et al. [16] reported 8-14% of occurrence of *L. monocytogenes* in meat samples collected from retail outlets. Studies conducted in different regions on fresh chicken meat have shown higher level of occurrence of *L. monocytogenes* at retail outlets viz. 10-12.5% in Pakistan [6]; 50% in Brazil [l5]; 69% in Turkey [17]; 65.5% in Turkey [18]; and 16.9% in Iran [19]. Similarly, varying level of *L. monocytogenes* contamination of mutton (2-7.4%) has been reported in India [7,15,20]. Nevertheless, contamination of mutton was lower than that of chicken meat. However, retail outlets selling multiple species meat showed higher level of contamination. Such a variation in the prevalence of *L. monocytogenes* in fresh raw meats could be attributed to differences in the degree of contamination that occurs during the slaughtering, processing, and handling of the carcass meat. Further, quality of the water used, processing environment and equipment hygiene also influence the level of contamination. Multiplex PCR has been widely used for the molecular characterization of Listeria isolates by several researchers [13,21,22]. It offers the advantage of simultaneous identification as well as molecular characterization of the *L. monocytogenes* isolates recovered from the meats and helps in the detection of virulence determinants of Listeria species [13,23].

CONCLUSION

L. monocytogenes was detected in 4.5% of meat samples sold at retail outlets. All the 9 isolates belonged to serovar 1/2a and possessed virulence determinants. Keeping in view the food safety and public health implications of *L. monocytogenes* in meat, adherence to good agricultural, animal husbandry and manufacturing practices is advocated.

Conflict of interest statement

Authors do not have any conflict of interest for this article.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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