DETECTION AND CHARACTERIZATION OF *LISTERIA* SPECIES FROM BUFFALO MEAT

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ABSTRACT

The isolation of *Listeria* spp. from buffalo meat samples sold in retail meat market was evaluated. Isolation of the *Listeria* was attempted from the samples by selective enrichment in University of Vermont Medium (UVM) and plating onto PALCAM, Oxford and Dominguez-Rodriguez isolation agar (DRIA). The pathogenicity of the isolates was tested by Christie, Atkins, Munch Petersen (CAMP) test and sugar fermentation patterns were used for identification of the isolates. Out of 150 buffalo meat samples examined, 10 (6.7%) samples were found positive for *Listeria* species, of which 4 (2.7%) were positive for *L. monocytogenes,* 2 (1.3%) for *L. innocua,* 3 (2.0%) for *L. seeligeri* and 1 (0.7%) for *L. welshimeri*. PALCAM yielded a cent percent isolates where as recovery rate on DRIA and Oxford agar was 90.0 and 60.0 percent, respectively. The PCR assay targeting *iap* gene was used for species specific detection *L. monocytogenes* isolates up to $2x10^1$ CFU/ml.

Keywords: *Listeria*, buffalo meat, selective media, PCR

INTRODUCTION

India has made rapid strides in meat exports to about 50 countries of the world. Quality meat is produced adopting OIE guidelines and international quality standards. Out of total meat production of more than 6 million tones, the buffalo alone contributes about 1.47 million tones and India is 5th largest exporter of buffalo meat in the world. (Agnihotri, 2008). Meat, whether wholesome or unwholesome, fresh or spoiled, has been held responsible for a number of food borne infections in human beings. Listeriosis, caused by *Listeria* spp. is one of the important food-borne bacterial zoonotic infections worldwide. Among the different species, *Listeria monocytogens* is known to cause listeriosis in humans and in more than 40 species of animal and 22 species of birds (Gray and Killinger, 1966). Listeriosis is a relatively rare disease, but fatality rate ranges from 15.0 to 30.0 percent with the highest hospitalization rates (90.5%) amongst known food-borne pathogens (CDC, 2000). Keeping in view these facts, many countries have adopted "zero tolerance limit" for *L. monocytogenes* in ready-to-eat food products. A number of reports have indicated the occurrence of the organism in various meat and meat products with overall incidence rate varying from 0 to 92.0 percent (Farber and Peterkin, 1991). There is paucity of comprehensive information regarding occurrence of *Listeria* spp. in buffalo meat in India. Therefore, keeping in view the above facts, the magnitude of the problems and the gap in knowledge regarding these aspects, the present study was carried out with a view to isolate and identify *Listeria* spp. from different samples of buffalo meat.

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MATERIALS AND METHODS

Altogether 150 samples of buffalo meat collected from retail meat shops were studied.

Isolation and identification

The method used for isolation of *Listeria* spp. from meat samples was divided in two phases 1) enrichment of samples and 2) selective plating on the three different media.

Samples were processed by two-step enrichment in UVM broth (Donnelly and Baigent, 1986) and loopful of inoculum was streaked directly onto DRIA, PALCAM and Oxford agar separately. The isolates were confirmed on the basis of colony characters, biochemical tests (Cheesbrough, 1991; Cowan and Steel, 1993) and also tested for xylose, rhamnose and a′methyl D-mannopyranoside fermentation patterns as per the method of Cowan and Steel (1993) for characterization up to species level. The CAMP test was performed as per the method of Bureau of Indian Standards (1994).

Confirmation of *L. monocytogenes* by PCR

Listeria monocytogenes isolates were confirmed by PCR assay targeting *iap* gene (Bubert *et al.,* 1999) using primer sequence narrated in Table 1. The tenfold serial dilution was followed (Barros *et al.,* 2007). The standard strain of *L. monocytogenes* (MTCC1143) was obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India, was used as positive control during the assay.

Table 1. Primer sequence for detection of *L. monocytogenes.*

Species	Primer sequence (3' to 5')		Size	Reference
L. monocytogenes	Forward	TTA TAC GCG ACC GAA GCC AAC	660 bp	Bubert *et al.,* 1999
	Reverse	CAA ACT GCT AAC ACA GCT ACT A		

Table 2. Thermal cycling protocols for detection of *L. monocytogenes*.

Species	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension
L. *monocytogenes*	95°C, 5 minutes	95°C, 45 seconds	50°C, 1 minute	72°C, 1 minute	72°C, 10 minutes
		Repeated for 31 cycles			

RESULTS

Isolation and identification

Out of 150 buffalo meat samples examined, 10 (6.7%) samples were found positive for *Listeria* species, of which four (2.7%) were positive for *L. monocytogenes,* two (1.3%) for *L. innocua,* three (2.0%) for *L. seeligeri* and one (0.7%) for *L. welshimeri*.

Recovery of the *Listeria* spp. on different selective plating media

From 10 positive buffalo meat samples, PALCAM yielded a recovery rate of 100 percent of isolates whereas the recovery rates on DRIA and Oxford agar were 90.0 and 60.0 percent, respectively (Table 3).

PCR assay for detection of *L. monocytogenes* in raw buffalo meat

All 4 (2.7%) *L. monocytogenes* isolates identified by biochemical tests were subjected to PCR, and all these isolates were successfully the amplified the desired amplicon of 660 bp. The PCR was performed from each diluted culture and showed the amplification up to as low as $2x10^1$ CFU/ml using primer pair of *iap* gene.

DISCUSSION

Out of 150 buffalo meat samples examined, 10 (6.7%) samples were positive for *Listeria* spp. The results in the present study are in close proximity to the findings of Barbuddhe (1996) and Barbudhe *et al.* (2002) who isolated *Listeria* spp. from 5.4 percent and 10.7 percent buffalo meat samples, respectively. However, Yucel *et al.* (2005) observed a higher prevalence of 10.7 percent in buffalo meat, which might have been due to differences in climatic conditions or sample size.

The recorded prevalence rate of *L. monocytogenes* in present study was 1.3 percent which is lower than earlier reports of Barbudhe *et al.* (2002), Chaudhari (1997) as well as Brahmbhatt and Anjaria (1993) who recorded 2.4, 3.0 and 5.5 percent prevalence, respectively. In contrast, Biswas *et al.* (2008) recorded a lower prevalence (0.9%) of the pathogen in the samples screened from buffalo meat packing plants where all the sanitary measures were observed to minimize the microbial contamination.

Lower prevalences of 1.3 percent, 2.0 percent and 0.7 percent of *L. innocua*, *L. seeligeri* and *L. welshimeri*, respectively, were observed in present study than in previous reports. Yucel *et al.*

Table 3. Recovery of *Listeria* spp. on different selective plating media.

Sr. no	*Listeria* spp.	No. of positive sample	PALCAM agar	DRI Agar	Oxford agar
1	*L. monocytogenes*	4	4	4	2
2	*L. innocua*	2	2	2	1
3	*L. seeligeri*	3	3	2	2
4	*L. welshimeri*	1	1	1	1
Total		**10**	**10 (100%)**	**9 (90.0%)**	**6 (60.0%)**

(2005) observed higher prevalence rates of 63.1 percent and 5.2 percent for *L. innocua* and *L. welshimeri,* respectively. Jalali and Abedi (2008) also reported 2.6 percent and 7.8 percent prevalences of *L. innocua* and *L.seeligeri* from fresh beef, respectively. This could be due to the lower overall prevalence of *Listeria* spp. in the present work.

Comparison of efficacy of different selective media

From 10 positive buffalo meat samples, PALCAM yielded a recovery rate of 100 cent percent of isolates whereas DRIA and Oxford agar yielded nine (90.0%) and six (60.0%), respectively. Scotter *et al.* (2001) observed more sensitivity of PALCAM (92.2%) than Oxford agar (91.1%) for the detection of *L. monocytogenes* from beef. Similarly, Gunasinghe *et al.* (1994) found recovery rates of 40.0 percent for *Listeria* spp. and 13.0 percent for *L. monocytogenes* in various meat products on PALCAM; the recovery rate was lower (23.0 percent and 7.7 percent, respectively) when Oxford medium was used. Capita *et al.* (2001) reported significantly higher results of isolation of *Listeria* spp. with PALCAM than modified Oxford medium (95.0% and 87.0%, respectively) and also a higher rate of *L. monocytogenes* with PALCAM (31.0%) when compared with the modified Oxford agar (27.0%). As many workers have tried these media for the isolation of *Listeria* spp., an attempt was not made to compare the efficacy of PALCAM with DRIA for the isolation of *Listeria* spp. Nevertheless, PALCAM agar was found to be superior for the recovery of the *Listeria* spp. including *L. monocytogenes* from buffalo meat samples.

PCR assay for confirmation of *L. monocytogenes*

A major 60-kDa extra-cellular protein *i.e.*p60, encoded by *iap* gene plays a vital role in intestinal invasion and *in vivo* survival and all the isolates of *L. monocytogenes* secrete a protein of 60 kDa as a major extracellular product (Kuhn and Goebel,1989) encoded by the *iap* gene. Primers targeting *iap* gene were used to amplify 660 bp amplicon for confirmation of *L. monocytogens*. In the present study, standard culture and all four *L. monocytogens* isolates identified by biochemical tests were successfully amplified desired amplicon of 660 bp which is in accordance with Zeng *et al.* (2006) who used *iap* gene as PCR - target for the species specific detection of *L. monocytogenes* from various samples including meat. It has been found that PCR amplification of *iap* gene is useful for the identification of *L. monocytogenes* to detect very low number of bacteria up to $2x10^1$ CFU/ml in the samples.

Thus it can be concluded from the study that 1.3 percent prevalence of *L. monocytogenes* was observed in raw buffalo meat samples. PALCAM agar was found to be superior to DRIA and Oxford agar for the recovery of the *Listeria* spp. Moreover, PCR assay targeting *iap* gene proved useful for species specific detection of *L. monocytogenes* up to level of $2x10^1$ CFU/ml in the meat samples.

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