Research Article

Isolation and Identification of pathogenic *Listeria monocytogenes* from diarrhoeal cases in human infants and young animals

Mamta Negi[a], Deepak Bhiwa Rawool[a*], Jess Vergis[a], Pankaj Dhaka[a], Deepthi Vijay[a], Vysakh Mohan[a], Rahul Suryavanshi[a], Satya Veer Singh Malik[a], Ashok Kumar[a], Sukhadeo Baliram Barbuddhe[b], Pramod Wasudeo Ramteke[c]

[a]Division of Veterinary Public Health, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India, Pin- 243 122, [b]ICAR Research Complex for Goa, Ela, Old Goa, India, [c]Pin- 403 402, Sam Higginbottom Institute of Agriculture, Technology and Sciences Allahabad, Uttar Pradesh, India, Pin- 211007.
*Corresponding author: deepak.rawool@yahoo.com

ARTICLE HISTORY	ABSTRACT
	Studies addressing isolation of *Listeria monocytogenes* from febrile gastroenteritis cases are very sparse. In the present study sporadic febrile diarrheal cases in human infants (less than 5 years of age) and young animals (0 - 6 months) were initially analyzed for isolation of *L. monocytogenes*. The isolates recovered were further characterized for their virulence potential using *in vitro* and *in vivo* pathogenicity tests and serogrouping by multiplex serotyping PCR. A total of 315 diarrhoeal samples (165 human infants and 150 young animals) were collected and analyzed for isolation of *L. monocytogenes*. On microbiological and biochemical analysis, four *L. monocytogenes* isolates were identified, two each from human infants and piglet respectively. The overall isolation rate of *L. monocytogenes* was very low in human infants (1.2%) and piglets (1.3%); however all the isolates were found to be highly pathogenic when assessed using *in vitro* and *in vivo* assays. Further, serotyping revealed that all the four *L. monocytogenes* isolates, belonged to serogroup 4b, 4d, 4e, which is a matter of great concern as serovar 4b in particular, is generally associated with listerial foodborne outbreaks worldwide. To conclude, isolation of pathogenic *L. monocytogenes* from sporadic febrile diarrheal cases (human infants and piglets) appears to be the first report in India. Thus, from future human and animal health perspective, due consideration should be imposed for either isolation or detection of *L. monocytogenes* from febrile gastroenteritis cases.

INTRODUCTION

In developed countries, food-borne pathogens are responsible for causing millions of gastrointestinal cases each year (Iyer et al., 2013), while in developing countries, these pathogens are responsible for causing approximately 1.8 million death annually (WHO 2013). The overall incidence of *Listeria* species is rather low if compared to other foodborne pathogens such as *Salmonella* species, *Campylobacter* species, *Vibrio* species and *Shigella* species (Barbuddhe and Chakraborty, 2009). However, the case fatality rate associated with listerial cases is as high as 30% (Liu, 2006). Listeriosis caused by *Listeria monocytogenes*, is a severe foodborne disease characterized by bacteremia and meningoencephalitis in neonates, individuals with impaired cell-mediated immunity, elderly persons, and immunosuppressed patients and pregnant woman (Barbuddhe, et al., 2012).

Listeriosis in general is considered to be invasive in nature exhibiting neural, visceral and reproductive disorders particularly in various species of animals and humans (Barbuddhe and Chakraborty, 2009). Although, antecedent diarrhoea has been reported in invasive form of listeriosis (Schwartz et al, 1989), however in recent past it has been convincingly proven that *L. monocytogenes* can cause a non-invasive acute, self-limiting, febrile gastroenteritis in healthy individuals including immunocompromised hosts (Ooi and Lorber, 2005). After ingestion of *L. monocytogenes* through contaminated food, the intestinal tract serve as a major portal of entry, wherein *L. monocytogenes* penetrate the mucosal tissue either directly, via invasion of enterocytes, or indirectly, via active penetration of the Peyer's patches. Thus the onset of clinical symptoms generally occurs 24 h after ingestion of very high inoculum of *L. monocytogenes* and the symptoms usually lasts for 2 days (Barbuddhe and Chakraborty, 2009). The major clinical symptoms include diarrhoea, abdominal pain and cramps, headache, fever, muscle and joint pain. As the symptoms are often mild in nature, majority of listerial gastroenteritis either undergoes

unnoticed or are often unreported. Even today, the incidence data of listerial gastroenteritis is unavailable with the food surveillance agencies such as FoodNet, because during routine investigation of stool cultures, *L. monocytogenes* is never considered as a vital pathogen responsible for febrile gastroenteritis. As a result there are only few reports of outbreaks of febrile listerial gastroenteritis (Dalton et al., 1994; Salamania et al, 1996; Riedo et al, 1994; Aureli et al, 2000; Ooi and Lorber, 2005). These outbreaks are generally involved due to ingestion of high doses of *L. monocytogenes* by otherwise healthy individuals or young infants (Barbuddhe and Chakraborty, 2009).

The present study appears to be the first report in India on isolation of pathogenic *L. monocytogenes* from human infants and piglets suffering from diarrhoea. The

pathogenic potential of the recovered isolates was assessed using *in vitro* and *in vivo* tests. Also, serogroup identification of the isolates was carried using multiplex serotyping PCR.

MATERIALS AND METHODS
Bacteria
The standard culture of *Listeria monocytogenes* (MTCC 1143), was procured from Microbial Type Culture Collection Centre, Institute of Microbial Technology (IMTECH), Chandigarh, India. The standard *L. monocytogenes* (MTCC 1143) was assessed for its purity using recommended microbiological and biochemical tests. The standard strain was maintained in the laboratory by monthly sub culturing on Brain heart infusion slants.

Table 1: Details of the samples collected from human diarrhoeal cases

Age (Years)	Maharashtra (Nagpur)			Kerala (Ernakulam)			Uttar Pradesh (Bareilly)		
	M	F	T	M	F	T	M	F	T
0-1	49	20	69	12	13	25	10	9	19
1-2	2	-	2	4	2	6	6	4	10
2-3	2	-	2	3	2	5	-	2	2
3-4	1	2	3	-	-	-	5	3	8
4-5	-	4	4	2	-	2	7	1	8
Total		80			38			47	
							Total number of samples = 165		

M- Male; F- Female; T- Total

Samples
Diarrhoeal stool samples were collected from human infants and young animals of different species (canine, caprine, bovine, ovine, porcine and feline). The details of sample collected are presented in Table 1 and 2. A total of 315 stool samples were collected from 165 human infants and 150 young animals suffering from diarrheal problems.

The stool samples were collected aseptically using *Listeria* Transport swabs (HiMedia Labs, Mumbai, India). All the collected samples were immediately transported to the laboratory under chilled conditions and processed further for the isolation and identification studies.

Table 2: Details of the samples collected from animal diarrhoeal cases

Species	Maharashtra (Nagpur)			Kerala (Ernakulam)			Uttar Pradesh (Bareilly)		
	M	F	T	M	F	T	M	F	T
Caprine	1	5	6	2	4	6	16	10	26
Bovine	20	8	28	5	9	14	12	8	20
Canine	3	1	4	8	6	14	5	5	10
Feline	-	-	-	1	-	1	-	-	-
Porcine	8	5	13	4	4	8	-	-	-
Total		51			43			56	
							Total number of samples = 150		

M- Male; F- Female; T- Total

Isolation and Identification of L. monocytogenes
Isolation of *Listeria* spp. was attempted as per USDA–FSIS method (USDA, 2013). In brief the faecal swabs were aseptically inocualted in 10 ml of University of Vermont Medium (UVM)–1 supplemented with acriflavin and nalidixic acid, and incubated at 37°C for 18–24hours. Further, UVM-1 enriched inoculums (0.1ml) was inoculated in 10 ml of UVM2 and incubated again at 37°C for 24 hours. The enriched UVM-2 broth was then streaked on PALCAM agar (HiMedia Labs, Mumbai, India) plate for selective isolation of *Listeria* spp. colonies.

The typical grayish-green glistening pin point colonies of about 0.5 mm diameter surrounded by a diffuse black zone of aesculin hydrolysis were presumptively identified as *Listeria* spp. The presumed colonies of *Listeria* spp. (at least 3/plate) were further confirmed by biochemical tests such as Gram's staining, catalase reaction, tumbling motility at 20–25°C, methyl red-Voges Proskauer (MR-VP) reactions, nitrate reduction and fermentation of sugars (rhamnose, xylose, mannitol and α- methyl-D-mannopyranoside).

InVitro Pathogenicity Testing of L. monocytogenes Isolates

The biochemically confirmed *L. monocytogenes* isolates were subjected to *in vitro* pathogenicity tests which includes hemolytic titre assay, Phosphatidylinositol phospholipase C (PI-PLC) activity on Agar *Listeria* according to Ottaviani and Agosti medium (ALOA) and a multiplex PCR targeting crucial virulence associated genes (*prf*A, *plc*A, *hly*, *act*A).

Table 3: Nucleotide sequences of the primer sets used in the study

Target gene	Primer sequence	Product size (bp)	Reference
*plc*A	F: 5'-CTGCTTGAGCGTTCATGTCTCATCCCCC-3' R: 5'-CATGGGTTTCACTCTCCTTCTAC-3'	1484	Notermans et al., 1991b
*prf*A	F: 5'CTGTTGGAGCTCTTCTTGGTGAAGCAATCG-3' R: 5'-AGCAACCTCGGTACCATATACTAACTC-3'	1060	Notermans et al., 1991b
hly	F: 5'-GCAGTTGCAAGCGCTTGGAGTGAA-3' R: 5'-GCAACGTATCCTCCAGAGTGATCG-3'	456	Paziak- Domanska *et al.*, 1999
*act*A	F: 5'-CGCCGCGGAAATTAAAAAAAGA-3' R: 5'- ACGAAGGAACCGGGCTGCTAG - 3'	839	Suarez and Vazquez- Boland, 2001

Hemolytic titre assay

The haemolytic titre of the *L. monocytogenes* isolates were measured as per the protocol described by Young et al. (1986). The hemolytic units were expressed as the reciprocal of highest dilution of toxin that is required to lyse 50% of erythrocytes compared to lysis obtained by distilled water.

PI-PLC Activity on ALOA Medium

ALOA medium (Hi-media Labs, Mumbai, India) was prepared as per manufacturer's instructions. On this medium all the *Listeria* spp. formed bluish green colonies due to the presence of a chromogenic compound X-glucosidase which detects β-glucosidase present in all *Listeria* spp. Pathogenic *Listeria* spp., can be distinguished from other *Listeria* spp. through the production of PI-PLC, which is detected by a substrate in the medium to form opaque halos around the colonies.

Multiplex PCR (mPCR) Targeting Virulence Associated Genes

The primers used for the multiplex PCR targeting virulence associated genes were procured from Sigma-Aldrich, USA. The details of primers are presented in Table 3. The chemical used in PCR master mix were procured from 3B Black Bio, Spain. The mPCR targeting virulence associated genes (*prf*A, *plc*A, *hly*, *act*A) was performed as per the method described by Rawool et al., (2007) with suitable modifications. In brief, the multiplex PCR assay was standardized in two sets, each set was performed in a 25µl reaction volume; containing 2.5µl of 10X PCR buffer (100mM Tris-HCl buffer, pH 8.3 containing 500 mM KCl, 15 mM MgCl2 and 0.01% gelatin), 3µl of 10 mM dNTP mix (a final concentration of 1 mM), 3µl of 25 mM MgCl2 (final concentration 7.5 mM) and 30µM of three primer sets (set 1: *plc*A, *act*A and *hly* and Set 2: *prf*A, *act*A, *hly*) @ 10 µM of each primer set, 3 units of Taq DNA polymerase (3B Black Bio, Spain), a single colony of *Listeria* as DNA template and sterilized nuclease free water to make up the reaction volume. The reaction was performed in Mastercycler Pro Thermocycler (Eppendorf, Germany). The cycling conditions for PCR included an initial denaturation at 95°C for 5 minutes followed by 35 cycles each of 15 seconds denaturation at 95°C, annealing at 60°C for 30 seconds and extension at 72°C for 1 minute 30 second, followed by a final extension of 10 minutes at 72°C and hold at 4°C. The resultant PCR products (10 µl) were analysed by submarine gel electrophoresis, using 1.5% agarose gel stained with ethidium bromide (0.5µg/ml) and visualized and documented by UV Gel documentation system (UVP Gel Seq Software, England).

InVivo Chick Embryo Inoculation Test

The pathogenicity of the reference strains as well as the test isolates of *Listeria monocytogenes* was assessed by chick embryo inoculation test as per the method described by Notermans et al. (1991a). The isolates causing embryo mortality after 24 h of inoculation up till 5 days were considered to be pathogenic.

Multiplex Serotyping PCR

The multiplex serotyping PCR was performed for serogroup identification as per the protocol described by Doumith et al. (2004). The multiplex serotyping PCR divides *L. monocytogenes* strains into four distinct serogroup: 1/2a, 1/2c, 3a, 3c; 1/2c, 3c; 1/2b, 3b, 4b, 4d, 4e and 4b, 4d, 4e. The primers used for multiplex PCR serotyping were synthesized from Eurofins Genomics (Bengaluru, India).

RESULTS

On microbiological analysis of 315 diarrhoeal stool samples (165 human infants and 150 young animals), 80 human and 76 animal faecal swabs revealed typical colonies with aesculin hydrolysis on PALCAM agar plates. Further, on morphological and biochemical characterization, 4 *L. monocytogenes* isolates were identified. Of the four *L. monocytogenes* isolates, two were isolated from human faecal swabs and two from porcine faecal swabs. The overall isolation rate of *L. monocytogenes* in human infants and young animals was found to be 1.2% and 1.3% respectively. None of the samples from bovine, caprine, canine and felines were found positive for *L. monocytogenes*.

All the 4 *L. monocytogenes* isolates recovered in the present study were assessed for their pathogenic potential using *in vitro* as well as *in vivo* tests. The isolates were subjected to *in vitro* pathogenicity tests viz., hemolytic titre assay, PI-PLC activity on ALOA medium and a multiplex

Table 4: Pathogenicity profile of *Listeria monocytogenes* isolates recovered from diarrheal cases from human infants and young animals.

			Pathogenicity profile of *Listeria monocytogenes* isolates							
			In vitro tests							*In Vivo* tests
Isolate ID	Place	Source of Isolate	Hemolytic Titre	PI-PLC on ALOA	PCR Profile of Virulence associated genes					Chick embryo lethality
					hly	*plcA*	*prfA*	*actA*		
PKLM1	Kerala	Porcine	32	+++	+	+	+	+		+
PKLM2	Kerala	Porcine	16	+++	+	+	+	+		+
HMHM32	Maharashtra	Human	16	+++	+	+	+	+		+
HGOM36	Goa	Human	8	++	+	+	+	+		+

PCR targeting virulence associated genes (*prfA*, *plcA*, *hly*, *actA*) as well as to *in vivo* chick embryo inoculation test.

On screening the 4 *L. monocytogenes* isolates (PKLM1, PKLM2, HMHM32 HGOM36) with hemolytic titer assay, of the two porcine isolates, one (PKLM1) was found to be highly hemolytic revealing a hemolytic unit of 32, whereas the other porcine isolate (PKLM2) was revealing a moderate (16) hemolytic unit. With regard to two human infant isolates, moderate (16) hemolytic unit was observed for one of the human isolate (HMHM32), while the other human isolate (HGOM36) revealed a hemolytic unit of 8. Similar results were also observed with regards to PI-PLC activity, as the porcine isolates (PKLM1 and PKLM2) and
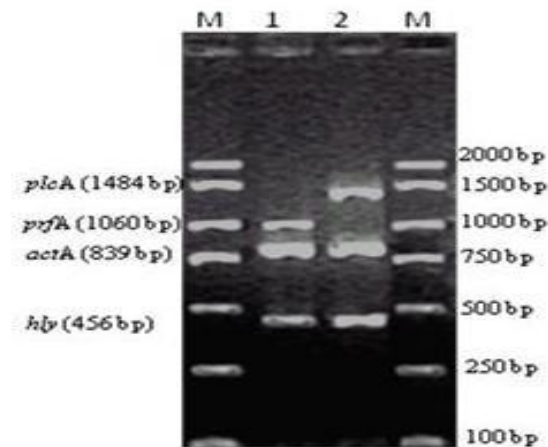

Figure 1: Standardized multiplex PCR (mPCR) for detection of virulence associated genes: Lane M-1Kb plus DNA ladder, Lane 1: Set 2 revealing amplification of *prf*A, *act*A and *hly* genes, Lane 2: Set 1 revealing amplification of *plc*A, *act*A and *hly* genes

one human isolate (HMHM32) revealed prominent PI-PLC activity on ALOA medium within 24 hours of incubation, while one of the human isolate (HGOM36) showed moderate PI-PLC activity after 72 h of incubation (Table 4). On employing all the 4 *L. monocytogenes* isolates to the standardized multiplex PCR targeting virulence associated genes (Figure 1), all the 4 isolates revealed amplified products for all the virulence associated genes targeted in the present study (Table 4). *L. monocytogenes* MTCC 1143 was used as a positive control for all the tests. On comparing the results of *in vitro* pathogenicity tests with an

in vivo chick embryo inoculation test, a good correlation was observed. The two porcine *L. monocytogenes* isolates (PKLM1, PKLM2) and one human isolate (HMHM32) killed the chick embryos within 24-36 h post inoculation, while the remaining one *L. monocytogenes* isolate from human (HGOM36) killed the chick embryo within 48-72h post inoculation (Table 4).

The serogrouping of all the 4 *L. monocytogenes* isolates was carried out using multiplex serotyping PCR. On PCR analysis it was observed that all the isolates belonged to the serogroup 4b, 4d, and 4e. Among serogroup 4b, 4d and 4e, serovar 4b in particular have been widely reported to be associated with several listerial outbreaks.

DISCUSSION

L. monocytogenes has been associated with major food-borne outbreaks globally but yet there is insufficient data regarding isolation and identification of pathogenic *L. monocytogenes* from febrile listerial gastroenteritis. Recent investigations of food-borne outbreaks provided evidences that febrile gastroenteritis may indeed be the main clinical manifestation of infection caused by *L. monocytogenes* (Ooi and Lorber, 2005). Many patients experience antecedent diarrhoea in case of invasive listeriosis, however in recent past it was efficiently convinced that *L. monocytogenes* can cause an acute, self-limiting, febrile gastroenteritis in healthy individuals, which usually lasts for 2 days (Barbuddhe and Chakraborty, 2008). Over the last 20 years, so far only seven outbreaks of food-borne gastroenteritis due to *L. monocytogenes* infection have been reported (Schuppler and Loessner, 2010).

In India listeriosis is not a notifiable disease and also the epidemiological data available on listeriosis is not adequate for assessing the extent of infection in human beings and animals (Barbuddhe et al., 2012). The disease largely remains undiagnosed and under reported, largely due to the lack of a reliable, rapid and simple diagnostic test (Barbuddhe et al., 2012). Besides this, during routine stool sample examination *L. monocytogenes* is often ignored or not considered as a possible cause of febrile gastroenteritis. Therefore, the present study was undertaken to explore the isolation and identification of *L. monocytogenes* from spontaneous diarrheal cases in human infants and young animals.

In the present study, 4 *L. monocytogenes* isolates were isolated and identified. Of the 4 *L. monocytogenes* isolates, two were isolated from human infants and two from porcine respectively. Similar reports of isolation from febrile human listerial gastroenteritis cases have been reviewed (Ooi and Lorber, 2005; Schuppler and Loessner, 2010). Globally, there are no reports of isolation of *L. monocytogenes* from animal diarrheal cases. Thus, to the best of our knowledge, our finding appears to be the first report on isolation of *L. monocytogenes* from febrile diarrheal cases in piglets. Further, we were not successful in isolating *L. monocytogenes* from other species namely caprine, bovine, canine and feline, may be because the number of samples analyzed were very few or may be those species of animals were suffering from diarrhoea due to other etiological agents. Hence, before giving any recommendations, it would be wise to analyze more number of samples from above mentioned species having febrile gastroenteritis symptoms. The exact mechanism by which *L. monocytogenes* causes diarrhoea is yet unknown (Ooi and Lorber, 2005), however it has been reported that the organisms are well adapted to the conditions in the gastrointestinal tract and pursue different strategies to counteract changes in acidity, osmolarity, oxygen tension, or the challenging effects of antimicrobial peptides and bile. Besides this, *L. monocytogenes* are able to colonize and persist in the gallbladder for long-term resulting in chronic infections, which demonstrates their pathogenic ability to survive within the various microenvironments of the gastrointestinal tract (Schuppler and Loessner, 2010). Moreover, the adherence and invasive mechanism of *L. monocytogenes* responsible for causing febrile gastroenteritis has been extensively reviewed by Schuppler and Loessner (2010).

The occurrence of listeriae in nature is ubiquitous and therefore the possibility of cross contamination during collection of samples cannot be ruled out. Also *L. monocytogenes* exists and multiplies as a saprophytic organism in the soil, plants, sewage as well as river water (Anonymous, 1991; Farber and Peterkin, 1991). Besides, large number of *Listeria* isolates of various kinds (haemolytic and pathogenic, haemolytic and non-pathogenic as well as nonhemolytic and non-pathogenic), types (typical and atypical) and origin (clinical, food, environment, feed etc.) exits in clinical as well as surrounding environmental sources (Shoukat et al., 2013). Therefore, in order to link up the isolated *L. monocytogenes* as a possible cause of diarrhoea, they were assessed for their pathogenic potential. Usually the pathogenicity of *L. monocytogenes* depends on its ability to survive and multiply in the macrophages and other host cells (Vazquez-Boland et al., 2001). After internalization, virulent *L. monocytogenes* produces listeriolysin O (LLO) and PI-PLC which cause the lysis of phagosomal membrane and thus it comes in the cytosol from where it spreads from cell-to-cell with the aid of actin filaments (Vazquez-Boland, et al., 2001). Therefore, hemolysis is an important characteristic that seems to be directly related to the pathogenicity of listeriae since non-hemolytic *Listeria* spp. are considered as non-pathogenic (Courteiu, 1991). In view of above LLO being an important virulent marker, we assessed the hemolytic (LLO) titre of the recovered *L. monocytogenes* isolates. The hemolytic titre assay revealed that of the 4 *L. monocytogenes* isolates, three isolates (PKLM1, PKLM2 and HMHM32) were exhibiting high to moderate hemolytic titers except for one *L. monocytogenes* isolate (HGOM36) which suggests that these *L. monocytogenes* isolates may have the potential of causing febrile gastroenteritis. However, it is advisable to perform an alternative *in vitro* test such as detecting PI-PLC activity on ALOA medium. This method besides detecting PI-PLC activity also allows discrimination between the pathogenic species of *Listeria* from the non-pathogenic listeriae (Aurora et al., 2008). In the present investigation almost similar observation were seen for PI-PLC activity on ALOA medium as that observed for hemolytic titre for the recovered *L. monocytogenes* isolates. Similar results were also reported by several researchers (Shakuntala et al., 2006; Rawool et al., 2007; Kaur et al., 2009; Shoukat et al., 2012).

Studies addressing PCR based detection of a single virulence associated gene is neither sufficient to identify the isolate nor to reveal its true pathogenic potential (Nishibori et al., 1991; Shakuntala et al., 2006; Rawool et al., 2007a). Also detection of *hly* and *plcA* gene by PCR in *L. monocytogenes* isolates is not sufficient to elucidate the true pathogenic potential, because both the gene are regulated by a key regulator gene i.e., *prfA* (Shakuntala et al., 2006; Kaur et al., 2007; Rawool et al., 2007b; Aurora et al., 2008). In addition, other genes such as *actA*, internalins and several other virulent associated genes do play an essential role in pathogenesis of this bug. Practically, it is not possible to study all and hence in the present study four important virulence associated genes viz., *prfA*, *plcA*, *actA*, and *hly* were attempted in multiplex PCR format. In the present investigation all the four isolates of *L. monocytogenes* revealed amplification of all the four virulence associated genes, suggesting that the recovered isolates could be potentially pathogenic in nature. Similar observation has been reported, wherein *L. monocytogenes* isolates harboring *prfA*, *plcA* and *hly* are generally pathogenic *in vivo* model of infections (Shakuntala et al., 2006; Kaur et al., 2007; Rawool et al., 2007a; Aurora et al., 2008; Shoukat et al., 2012).

Virulence of *Listeria* spp. by *in vivo* method is often assessed by lethality in adult mice by intraperitonal route (Chakraborty and Goebel, 1988) and/or by chick embryo inoculation by chorio-allantoic membrane (CAM) route (Notermans et al., 1991a). However, the mouse inoculation assay is objectionable from an ethical point of view; the virulence potential of recovered isolates was studied by chick embryo inoculation test. It was observed that all the four *L. monocytogenes* isolates were found to be pathogenic, as death was evidenced in all the inoculated chick embryos. The results are in completed agreement with earlier studies (Shakuntala et al., 2006; Kaur et al., 2007; Rawool et al., 2007a; Aurora et al., 2008; Shoukat et al., 2013).

Serotyping performed using molecular methods is an effective and an alternative choice to traditional seroagglutination methods, which are both time-consuming and expensive, with lower reproducibility of results (Zhang et al., 2004). In order to explore the serogroup of the recovered *L. monocytogenes* isolates, multiplex serotyping PCR was performed as described by

Doumith et al., (2004). In the present study all the test isolates belonged to the serogroup 4b, 4d and 4e. In this serogroup, serovar 4b in particular has been widely reported in several outbreaks of foodborne listeriosis (Ward et al., 2010; Liu, 2006). Our results are also in agreement with the reports of febrile gastroenteritis outbreak in northern Italy which have revealed the presence of serotype 4b (Aureli et al.,1997).

To conclude, isolation of pathogenic *L. monocytogenes* from sporadic gastroenteritis cases suffering from diarrhoea appears to be the first report in India. Thus, from future human and animal health perspective, isolation or detection of *L. monocytogenes* must be considered while analyzing samples of febrile gastroenteritis. Besides this, the incidence rate and factors that govern the onset of this non-invasive form are yet unexplored, and therefore to have better understanding of febrile non-invasive gastroenteritis, proper risk assessment studies are required to address this key issue.

REFERENCES

Anonymous (1991) *Listeria monocytogenes* Recommendation by the National Advisory Committee on microbiological criteria for foods. Intl. J. Food Microbiol. 14: 185.

Aureli P, Fiorucci GC, Caroli D, Marchiaro G, Novara O, Leone L, Salmaso S (2000). An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria monocytogenes*. N. Eng. J. Med. 342(17):1236 – 1241.

Aurora R, Prakash A, Prakash S, Rawool DB, Barbuddhe SB (2008). Comparison of PI-PLC based assays and PCR along with in vivo pathogenicity tests for rapid detection of pathogenic *Listeria monocytogenes*. Food Cont. 19: 641 – 647.

Barbuddhe SB, Chakraborty T (2009). *Listeria* as an enteroinvasive gastrointestinal pathogen. Curr. Top. Microbiol. Immunol. 337: 173 – 195.

Barbuddhe SB, Malik SVS, Kumar AJ, Kalorey DR, Chakraborty T (2012). Epidemiology and risk management of listeriosis in India. Intl. J. Food Microbiol. 154: 113 – 118.

Barbuddhe SB, Hain T, Chakraborty T (2008). The Genus *Listeria*. In:Practical Handbook of Microbiology, CRC Press, Boca Raton. 533 – 562.

Chakraborty J, Goebel W (1988). Recent developments in the study of virulence in *Listeria monocytogenes*. Curr. Top. Microbiol. Immunol. 138: 41 - 58.

Courteiu AL (1991). Latest news on Listeriosis. Comp. Immunol. Microbiol. Infect. Dis. 14: 1 - 7.

Dalton CB, Austin CC, Sobel J, Hayes PS, Bibb WF, Graves LM, Swaminathan B, Procter ME, Griffin PM (1997) An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. N. Eng. J. Med.336: 100 – 105.

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

Farber JM, Peterkin PI (1991). *Listeria monocytogenes*, a food borne pathogen. Microbiol. Rev. 55: 476 - 511.

Iyer A, Kumosani T, Yaghmoor S, Barbour E, Azhar E, Harakeh, S (2013). *Escherichia coli* and *Salmonella* spp. in meat in Jeddah, Saudi Arabia. J. Infect. Dev. Count. 7(11): 812 – 818.

Kaur S, Malik SVS, Bhilegaonkar KN, Vaidya VM, Barbuddhe SB (2009). Use of a phospholipase C assay, *in vivo* pathogenicity assays and PCR in assessing the virulence of *Listeria* spp. Vet. J. 184:366 - 370.

Liu D (2006). Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. J. Med. Microbiol. 55(6): 645 – 659.

Nishibori T, Cooray K, Xiang H, Kawmuru I, Fujita M, Mitsuyama M (1995). Correlation between the presence of virulence associated genes as determined by PCR and actual virulence in mice in various strains of *Listeria* spp. Microbiol. Immunol. 39: 343 - 349.

Notermans S, Dufrenne J, Chakraborty T, Steinmeyer S, Terplant G (1991a). The chick embryo test agrees with the mouse bio-assay for assessment of the pathogenicity of *Listeria* species. Lett. Appl. Microbiol. 13: 161-164.

Notermans SHW, Dufrenne J, Leimeister-Wachter M, Domann E, Chakraborty T (1991b). Phosphatidylinositol-specific phospholipase C activity as a marker to distinguish between pathogenic and non-pathogenic *Listeria* species. Appl. Environ. Microbiol., 57: 2666 - 2670.

Ooi ST, Lorber B (2005).Gastroenteritis due to *Listeria monocytogenes*. Clinical Infectious Diseases. 40 (9): 1327 – 1332.

Rawool DB, Malik SVS, Barbuddhe SB, Shakuntala I, Aurora R (2007a). A multiplex PCR for detection of virulence associated genes in *Listeria monocytogenes*. Intl. J. Food Safety. 9: 56 - 62.

Rawool DB, Malik SVS, Shakuntala I, Sahare AM, Barbuddhe SB (2007b) Detection of multiple virulence associated genes in pathogenic *Listeria monocytogenes* from bovines with mastitis. Intl. J. Food Microbiol. 113: 201 - 207.

Riedo FX, Pinner RW, Tosca ML, Graves ML, Reeves MW, Weaver RE, Plikaytis BD, Broome CV (1994). A point-source foodborne listeriosis outbreak: documented incubation period and possible mild illness. J. Infect. Dis. 170: 693 – 696.

Salamina G, Dalle DE, Niccolini A, Poda G, Cesaroni D, Bucci M, Fini R, Maldini M, Schuchat A, Swaminathan B, Bibb W, Rocourt J, Binkin N, Salmaso S (1996) A foodborne outbreak of gastroenteritis involving *Listeria monocytogenes*. Epidemiol. Infect. 117:429 – 436.

Schwartz B, Hexter D, Broome CV, Hightower AW, Hirschhorn, RB, Porter JD, Hayes PS, Bibb WF, Lorber B, Faris DG (1989). Investigation of an outbreak of listeriosis: new hypotheses for the etiology of epidemic *Listeria monocytogenes* infections. J. Infect. Dis. 159:680 – 685.

Shakuntala I, Malik SVS, Barbuddhe SB, Rawool DB (2006).Isolation of *Listeria monocytogenes* from buffaloes with reproductive disorders and its confirmation by polymerase chain reaction. Vet. Microbiol. 117: 229 - 234.

Shoukat S, Malik SVS, Rawool DB, Kumar A, Kumar S, Shrivastava S, Barbuddhe SB , Das DP, Das S (2013).A Study on Detection of Pathogenic Listeria monocytogenes in Ovine's of Kashmir Region Having Abortion or History of Abortion. Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. DOI 10.1007/s40011 – 013 – 0228 - 0.

Schuppler M, Loessner MJ (2010).The Opportunistic Pathogen *Listeria monocytogenes*: Pathogenicity and Interaction with the Mucosal Immune System. Intl. J. Inflamm..doi:10.4061/2010/704321.

Todd J, Ward, Usgaard T, Evans P (2010). A Targeted Multilocus Genotyping Assay for Lineage, Serogroup, and Epidemic Clone Typing of *Listeria monocytogenes*. Appl. Environ. Microbiol. 76: 6680 – 6684.

USDA (2013). Laboratory Guidebook: Chapter MLG 8.09-Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry and Egg Products, and Environmental Samples. USDA

Vazquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Domı́nguez-Bernal G,Goebel W, Gonza´lez-Zorn B, Wehland J, Kreft J (2001). *Listeria* pathogenesis and molecular virulence determinants. Clin. Microbiol. Rev. 14: 584 – 640.

WHO. (2013). General information related to microbiological risks in food. Food saftey. December 14, 2013, from http://www.who.int/foodsafety/micro/general/en/.

Young JD, Leong LG, DiNome MA, Cohn ZA (1986) A semiautomated hemolysis microassay for membrane lytic proteins. Anal. Biochem. 154: 649 - 654.

Zhang W, Hughes A, Wilt G, Knabel SJ (2004). The BAX PCR assay for screening *Listeria monocytogenes* targets a partial putative gene lmo2234. J. Food Prot. 67:1507 – 1511.