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Original Article

Genetic diversity, virulence and distribution of antimicrobial resistance among *Listeria monocytogenes* isolated from milk, beef, and bovine farm environment

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Abstract

Background: *Listeria monocytogenes* is an opportunistic intracellular foodborne pathogen and is ubiquitous in nature. The occurrence of *L. monocytogenes* in animal production units coupled with their presence in milk, faeces, feed, water, sewage, and soil is a contributory factor for foodborne listeriosis in humans and animals. **Aims:** The study was aimed to characterize genotype and serogroup of *L. monocytogenes* recovered from different types of samples and also to study antimicrobial patterns by phenotypic and genotypic methods. **Methods:** Multiplex polymerase chain reaction (PCR) was used for the confirmation of *L. monocytogenes*, the identification of its serogroup and lineage, and the detection of virulence markers. Enterobacterial repetitive intergenic consensus (ERIC), and randomly amplified polymorphic DNA (RAPD)-PCR were used to characterize those isolates, and antimicrobial patterns were studied phenotypically by Kirby-Bauer method and genotypically by PCR. **Results:** Out of the screened 474 samples (274 milk and 50 each of soil, feed, sewage, and beef), ten *L. monocytogenes* isolates (milk=8, soil=1, and beef=1) were confirmed by PCR targeting the *hlyA* gene and found to belong to the 1/2a, 3a serogroup and fall under type II lineage. Virulence potential assessment revealed that all the ten isolates harbored the *iap* gene while the presence of *plcA* and *plcB* genes were noticed in seven and eight isolates respectively. Six isolates from milk were found to group in the same cluster by ERIC and RAPD fingerprinting, suggesting both methods to be efficient molecular typing tools for *L. monocytogenes*. Genotypic characterization of antimicrobial resistance (AMR) genes revealed that seven isolates were positive for *tetM*, five for *mefA*, four for *msrA*, and one for *lnuA* genes while none of the isolates showed *tetK*, *ermA*, *ermB*, and *lnuB* genes. **Conclusion:** The presence of *L. monocytogenes* in bovine farm environments coupled with virulence markers, and multidrug resistance from the study area suggest a possible transmission from the environment to humans and animals which needs to be monitored regularly to ensure food safety and the well-being of animals and humans.

Key words: Antimicrobial resistance, ERIC, *Listeria monocytogenes*, RAPD-PCR, Serogrouping

Introduction

The genus *Listeria* consists of seventeen species of which *Listeria monocytogenes* is an important zoonotic pathogen with the potential to cause serious infections in both humans and animals. Listeriosis is of prime importance in high risk groups such as pregnant females, neonates, old and immunocompromised persons with a fatality rate of 20-30%, the hospitalization rate of 91%, and neonatal death rate of 50% (Doijad *et al*., 2015). Over the years, considerable increases in the incidence of listeriosis are due to its ubiquitous distribution and its presence in various urban and natural environments, animals, and humans (Orsi *et al*., 2011).

Serotyping and lineage studies of *L. monocytogenes* help us to understand the circulating serotype and to identify the origin and virulence characteristics of the isolates. Of the 13 *L. monocytogenes* serotypes known, 1/2a, 1/2b, and 4b were more commonly isolated from food and human samples and considered virulent (Orsi *et al*., 2011). DNA hybridization studies have categorized *L. monocytogenes* into four different lineages I to IV (Ward *et al*., 2008). *Listeria monocytogenes* has several virulence factors including *prf*A, *plcA*, *hly*, *mpl*, *actA*,

iap, and *plcB* which are important for its pathogenicity and completing the cell infection cycle.

Genotyping methods such as pulsed-field gel electrophoresis (PFGE), randomly amplified polymorphic DNA (RAPD) and enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) are commonly employed to study the epidemiological and evolutionary relationships among *L. monocytogenes* isolates recovered from different sources. Although these methods are employed with varying success, ERIC and RAPD-PCR-based approaches are simple and cost effective and yield specific, unique and distinctive DNA fingerprints (Shakuntala *et al*., 2019).

In recent times, antimicrobial resistant strains of *L. monocytogenes* have been isolated from food, environment and sporadic cases of human listeriosis. Hence, there is a need to monitor the drug resistance patterns of *L. monocytogenes* and to reduce complications associated with listeriosis.

To the authors' knowledge, there were only few reports that elucidated the presence of *L. monocytogenes* from bovine farm environments in Tamil Nadu. Hence, the present investigation was designed to study the presence of *L. monocytogenes* in raw milk, soil, feed, sewage and beef samples collected from different bovine farms and slaughterhouse of Chennai, Tamil Nadu, in order to characterize the isolates genotypically and study their antimicrobial resistant patterns against commonly used antibiotics.

Materials and Methods

Sample collection

A total of 474 samples, milk (n=274) and fifty samples from each soil, feed and sewage were collected from bovine farms in and around Chennai, Tamil Nadu, India. Beef samples (n=50) were collected from retail outlets and slaughter houses located in Chennai, Tamil Nadu, India.

Isolation and confirmation of *L. monocytogenes* by cultural and molecular methods

Food and Drug Administration-Bacteriological Analytical Manual (FDA-BAM) protocol was followed for the isolation and identification of *L. monocytogenes*. Presumptive colonies with grey greenish colors, black sunken centers and black halos on PALCAM agar were picked and further characterized by Grams staining, biochemical tests, and modified Christie, Atkinson, Munch and Peterson (CAMP) tests with *Staphylococcus aureus* (Seeliger and Jones, 1986). The presumptive isolates were confirmed by PCR targeting to have the *hly*A gene (Agersborg *et al*., 1997).

Serogrouping and lineage identification of L. monocytogenes

Multiplex PCR assays were performed to determine serogroups (1/2a, 1/2c, 3a, 3c, 1/2c, 3c, 1/2b, 3b, 4b, 4d, 4e, 4b, 4d, and 4e) (Doumith *et al*., 2004) and lineages (I, II and III) of *L. monocytogenes* (Rawool *et al*., 2016). The primers are listed in Table 1 and the cycling conditions followed Rawool *et al*. (2016) and Shakuntala *et al*. (2019) for serogroup and lineage identification.

Virulence gene profiling

The presence of three virulence genes namely *iap*, *plcA* and *plcB* among the *L. monocytogenes* were determined by PCR using standardized cycling conditions as mentioned in Shakuntala *et al*. (2019) with suitable primers (Table 1).

Genomic fingerprinting by ERIC- and RAPD-PCR

The primers and cycling conditions used for ERIC-PCR followed Shakuntala *et al*. (2019). For the RAPD-PCR assay, two primers for OPM-01 and UBC155 were used and the amplification conditions were met as mentioned by Chen *et al*. (2014). Fingerprints generated by both typing (ERIC and RAPD) methods were

Table 1: Primers used for detecting serogroup, lineage and virulence genes of *L. monocytogenes*

Target gene	Primer sequence (5´-3´)	Amplicon size (bp)	Serogroup/lineage/protein encoded	References
lmo0737	F: AGGGCTTCAAGGACTTACCC R: ACGATTTCTGCTTGCCATTC	691	1/2a, 1/2c, 3a, and 3c	
lmo1118	F: AGGGGTCTTAAATCCTGGAA R: CGGCTTGTTCGGCATACTTA	906	1/2c and 3c	Doumith *et al*. (2004)
ORF2819	F: AGCAAAATGCCAAAACTCGT R: CATCACTAAAGCCTCCCATTG	471	1/2b, 3b, 4b, 4d, and 4e	
ORF2110	F: AGTGGACAATTGATTGGTGAA R: CATCCATCCCTTACTTTGGAC	597	4b, 4d, and 4e	
L1	F: GGCGCATTCAAATCCAAGAG R: GTGGTTGCTTGGTACAATGAG	384	LMOF2365_RS13380 cell wall surface anchor family protein	
L2	F: CAGAAAATGGCTGGGGATTA R: GCGGAACATTGGTCTGAACT	476	lmo0525 hypothetical protein	Rawool *et al*. (2016)
L3	F: GTAAGCGAGCTTTAGGAGAGTT R: CGTATATGCCTAAACCTACACCA	261	LMO4A_RS05595 hypothetical protein	
hlyA	F: GCAGTTGCAAGCGCTTGGAGTGAA R: GCAACGTATCCTCCAGAGTGATCG	456	Listeriolysin O	Paziak-Domanska *et al*. (1999)
iapA	F: ACAAGCTGCACCTGTTGCAG R: TGACAGCGTGTGTAGTAGCA	131	Invasion associated protein	Furrer *et al*. (1991)
plcA	F: CTGCTTGAGCGTTCATGTCTCATCCCCC R: CATGGGTTTCACTCTCCTTCTAC	1484	Phosphatidylinositol-specific phospholipase C	Notermans *et al*. (1991)
plcB	F: GCAAGTGTTCTAGTCTTTCCGG R: ACCTGCCAAAGTTTGCTGTGA	795	Phosphatidylicholin-specific phospholipase C	Nishibori *et al*. (1995)

Table 2: Primers used for the identification of antimicrobial resistance genes in *L. monocytogenes*

Target gene	Primer sequence (5´-3´)	Amplicon size (bp)	References
tetM	F: GTGGACAAAGGTACAACGAG	450	
	R: CGGTAAAGTTCGTCACACAC		
tetK	F: CGATAGGAACAGCAGTATGG	614	
	R: TTAGCCCACCAGAAAACAAACC		
ermA	F: CTTCGATAGTTTATTAATATTAGT	645	Morvan *et al*. (2010)
	R: TCTAAAAAGCATGTAAAAGAA		
ermB	F: GAAAAGGTACTCAACCAAATA	636	
	R: AGTAACGGTACTTAAATTGTTTAC		
mefA	F: AGTATCATTAATCACTAGTGC	345	
	R: TTCTTCTGGTACTAAAAGTGG		
msrA	F: GCAAATGGTGTAGGTAAGACAACT	401	
	R: ATCATGTGATGTAAACAAAAT		
lnuA	F: GGTGGCTGGGGGGTAGATGTATTAACTGG	323	Lina *et al*. (1999)
	R: GCTTCTTTTGAAATACATGGTATTTTTCGATC		
*lnu*B	F: CCTACCTATTGTTTGTGGAA	405	Bozdogan *et al*. (1999)
	R: CCTACCTATTGTTTGTGGAA		

Table 3: Cycling conditions for the identification of antimicrobial resistance genes in *L. monocytogenes*

Virulence genes	Cycling conditions				
	Initial denaturation	Denaturation	Annealing	Extension	Final extension
tetK and *tetM*	94°C for 5 min	94°C for 60 s	62°C for 60 s	72°C for 60 s	72°C for 5 min
			Repeat for 30 cycles		
lnuA, *ermA*, *mefA*, *msrA*, *ermB*, *lnu*B	94°C for 5 min	98°C for 10 s	49-59°C for 40 s (59°C, *lnu*A; 56°C, *ermA*; 54°C, *msrA*, *ermB*, *lnuB*; 53°C, *mefA*)	72°C for 60 s	72°C for 7 min

analyzed by the Gel J (Spain) software using the unweighted pair group algorithm and dice correlation coefficient. The discriminatory power was assessed by Simpson's diversity index (DI).

Antimicrobial susceptibility

All the ten *L. monocytogenes* isolates were subjected to testing to determine their phenotypic antimicrobial resistance patterns against commonly used antibiotics (Hi Media, India) in human and veterinary therapeutics, namely, ampicillin (10 µg), ciprofloxacin (10 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), penicillin (10 IU), rifampicin (5 µg), tetracycline (30 µg), and vancomycin (30 µg) following the Kirby-Bauer method (as per CLSI guidelines) (Soni *et al*., 2013).

Genotypic characterization of antimicrobial resistance by PCR targeting found various antimicrobial resistant genes namely *tetM*, *tet*L for tetracycline, *lnuA*, *lnu*B for clindamycin, *mef*A, *msr*A for erythromycin and *ermA*, *ermB* for macrolides-lincosamides-streptogramin B complex called MLSB as shown in Table 2. The cycling conditions are illustrated in Table 3.

Multiple antibiotic resistance (MAR index)

Multiple antibiotic resistance index was calculated for all isolates following Krumperman's (1983) procedure using the formula a/b, where "a" represents the number of antibiotics to which the isolate is resistant and "b" the number of antibiotics to which the isolate was tested.

Results

Identification of *L. monocytogenes* in bovine farm environment

Out of the 474 samples collected and screened for *L. monocytogenes* from different bovine farms in Chennai, fifty samples showed the presence of characteristic colonies on PALCAM agar. However, upon further characterization, only ten isolates (eight from milk, one from soil and one from beef samples) were confirmed as *L. monocytogenes* by biochemical tests, CAMP test and PCR. The prevalence rates of *L. monocytogenes* from different types of samples are given in Table 4.

Table 4: Prevalence rates of *L. monocytogenes* from different types of samples

Type of sample	No. of samples collected	No. of samples positive by *hly*A	% of positivity of *L. monocytogenes*
Raw milk	274	8	2.9
Soil	50	1	2
Feed	50	0	0
Sewage	50	0	0
Beef	50	1	2
Total	474	10	2.1

Serogrouping and lineage identification of *L. monocytogenes*

All of the ten *L. monocytogenes* isolates obtained in the present study belong to a single serogroup namely, 1/2a and 3a (100%) and lineage II.

Virulence gene profiling of L. monocytogenes

All the *L. monocytogenes* isolates in the present study harbored *hlyA* (100%) and *iap* (100%) virulence markers, irrespective of their source, while seven (70%) and eight (80%) *L. monocytogenes* isolates harbored *plcA* and *plcB* genes, respectively.

Genome fingerprinting analysis

At 100% similarity, the ERIC-PCR typing differentiated 10 *L. monocytogenes* isolates into ten ERIC types, with a Simpson's DI of 1 (Fig. 1). At the dissimilarity coefficient of 70%, 1 cluster (I), clustering 7 isolates (M25, M26, M23, M5, M20, B10, M10) and three outliers were seen. At 100% similarity, the RAPD-PCR typing using UBC-155 primer differentiated ten *L. monocytogenes* isolates into 10 types, with a Simpson's DI of 1 (Fig. 2). At the dissimilarity coefficient of 70%, 2 clusters (I to II) and one outlier were seen, among which cluster II was the major one, clustering 7 isolates from milk (M25, M26, M23, M5, M20, M22, M10). At 100% similarity, the RAPD-PCR typing using OPM-01 primer differentiated all of the ten *L. monocytogenes* isolates into 10 types, with a Simpson's DI of 1 (Fig. 3). At a dissimilarity coefficient of 70%, 3 clusters (I to III) and one outlier were seen, among which cluster III was the major one, clustering 5 isolates (M25, M26, M22, M21, M10).

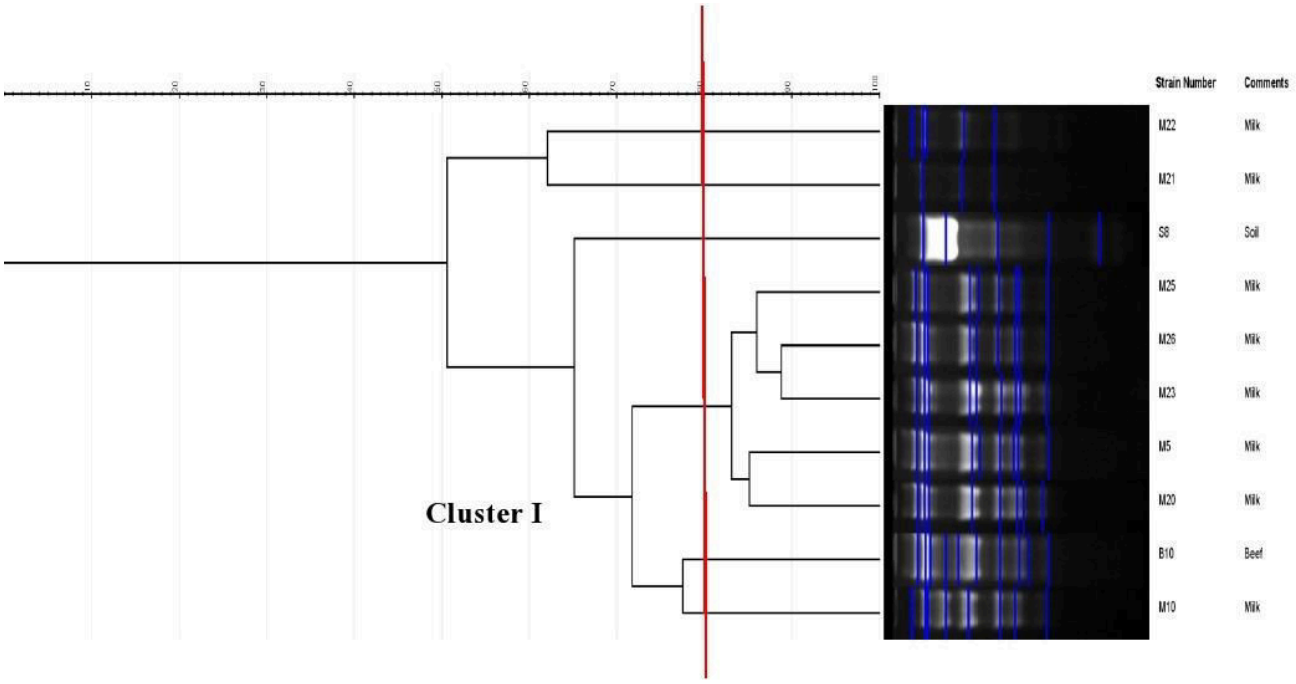

Fig. 1: ERIC fingerprint profiles of *L. monocytogenes* isolates

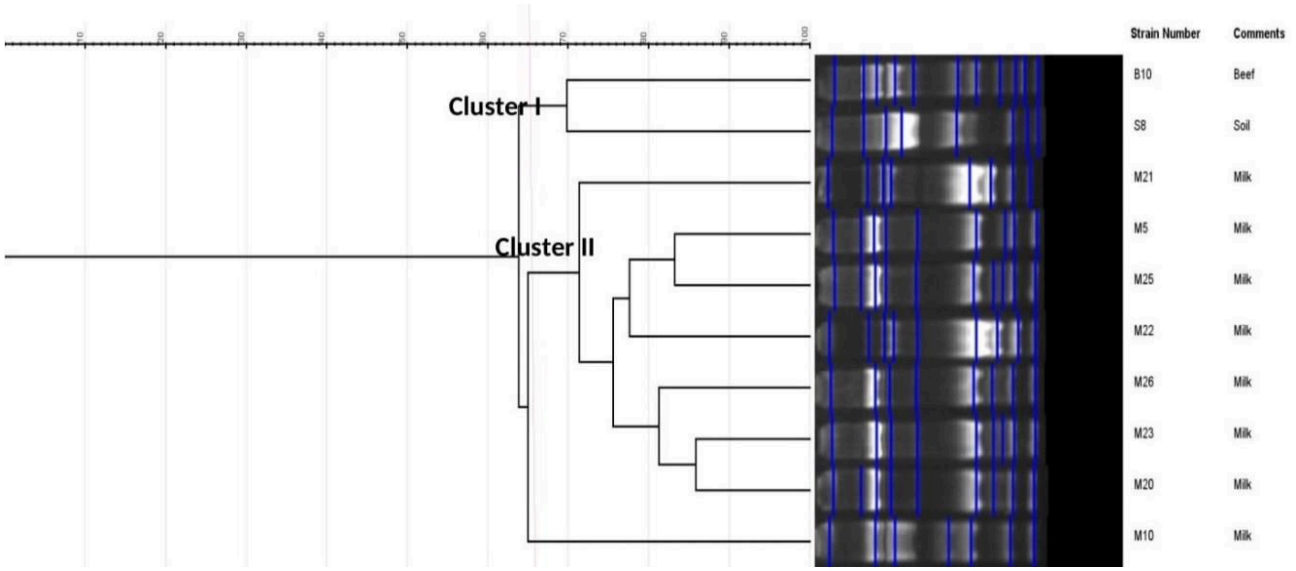

Fig. 2: RAPD (UBC-155) fingerprint profiles of *L. monocytogenes* isolates

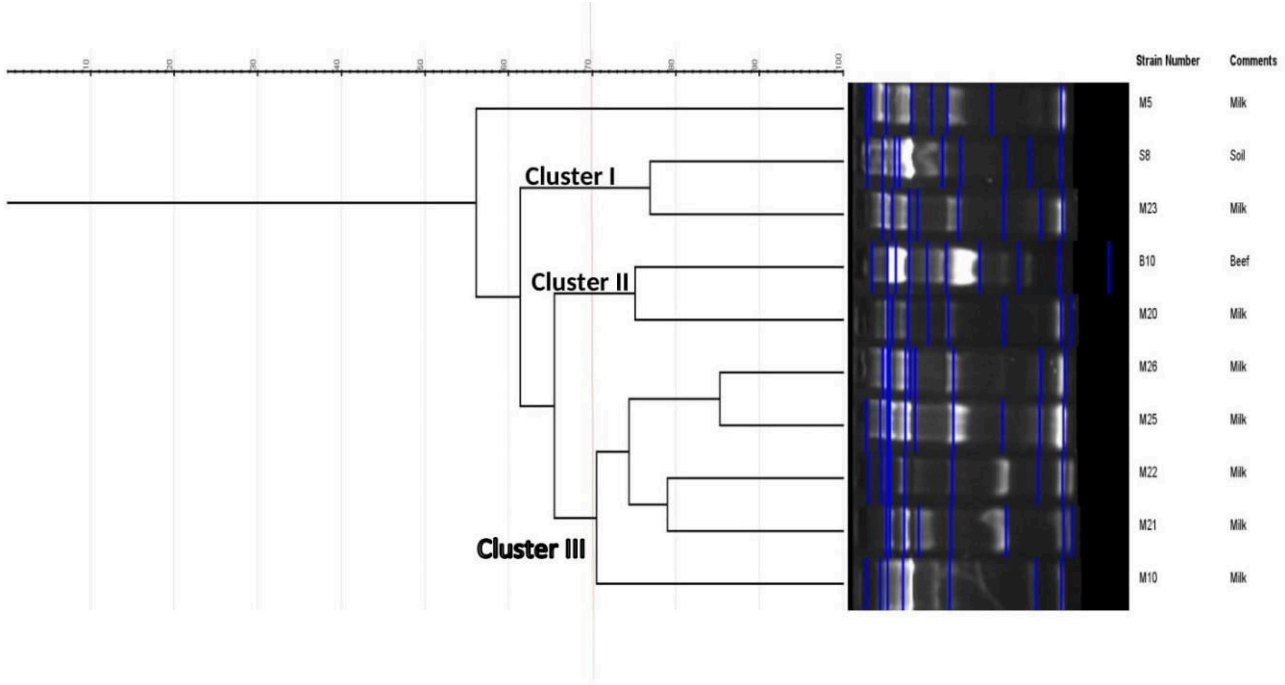

Fig. 3: RAPD (OPM-01) fingerprint profiles of *L. monocytogenes* isolates

Antimicrobial susceptibility

All ten *L. monocytogenes* isolates showed 100% resistance to penicillin and rifampicin antibiotics. Further analysis showed that two isolates (from milk) were resistant to four antibiotics, whereas other isolates were resistant to five and six antibiotics, respectively (Fig. 4) and none of the isolates was resistant to vancomycin. The MAR index calculated for all ten *L. monocytogenes* isolates in the present study ranges from 0.56 to 0.78 (Table 5).

The detection of AMR genes by PCR in this study revealed that seven isolates harbored *tetM*, five isolates carried *mefA*, 4 isolates harbored *msrA* and one isolate was positive for *lnuA*. None of the isolates showed *tetK*, *ermA*, *ermB*, and *lnuB* antimicrobial resistance genes.

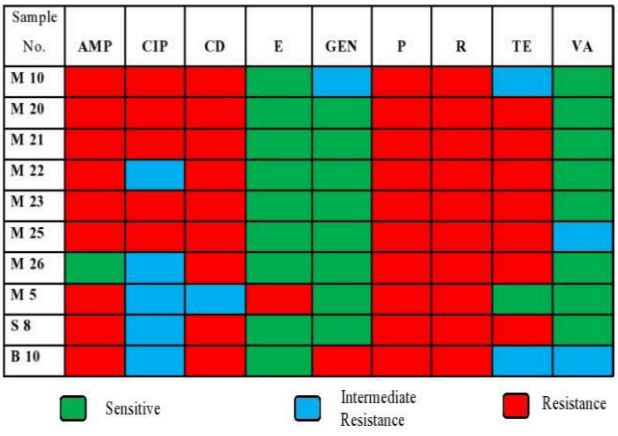

Fig. 4: Heat map of antibiotic resistance detected in *L. monocytogenes* isolates. AMP: Ampicillin, CIP: Ciprofloxacin, CD: Clindamycin, E: Erythromycin, GEN: Gentamicin, P: Penicillin, R: Rifampicin, TE: Tetracycline and VA: Vancomycin

Table 5: MAR indices of *L. monocytogenes* isolates obtained in the present study

Sample No.	MAR index	Antibiotics that are resistant
M 10	0.78	AMP, CIP, CD, GEN, P, RIF, TE
M 20	0.67	AMP, CIP, CD, P, RIF, TE
M 21	0.67	AMP, CIP, CD, P, RIF, TE
M 22	0.67	AMP, CIP, CD, P, RIF, TE
M 23	0.67	AMP, CIP, CD, P, RIF, TE
M 25	0.67	AMP, CIP, CD, P, RIF, TE
M 26	0.56	CIP, CD, P, RIF, TE
M 5	0.56	AMP, CIP, CD, E, P, RIF
S 8	0.67	AMP, CIP, CD, P, RIF, TE
B 10	0.67	AMP, CIP, CD, GEN, P, RIF

MAR: Multiple antibiotic resistance, M: Milk isolate, S: Soil isolate, B: Beef isolate, AMP: Ampicillin, CIP: Ciprofloxacin, CD: Clindamycin, E: Erythromycin, GEN: Gentamicin, P: Penicillin, R: Rifampicin, TE: Tetracycline and VA: Vancomycin

Discussion

Identification of *L. monocytogenes* in bovine farm environment

In the present study, the *L. monocytogenes* prevalence rate of 2.9% was obtained in milk samples which are similar to the findings of Sarangi and Panda 2012 (2.91%). On the contrary, higher and lower prevalence rates of 5.8% and 1.8% of *L. monocytogenes* were reported in milk by Soni *et al.* (2013) and Shakuntala *et al.* (2019), respectively. Zero prevalence of *L. monocytogenes* was also reported by Shantha and Gopal (2014) in milk samples.

The present study identified 2.0% occurrence of *L. monocytogenes* in soil samples which is almost similar to the findings of Fox *et al.* (2009) (3%). Compared to the present study, a very high (24%) prevalence of *L.*

monocytogenes in soil samples was found by Nightingale *et al*. (2004) while zero prevalence was recorded by Matto *et al*. (2018).

In the present study, none of the *L. monocytogenes* isolate was recovered from feed samples, a finding similar to those of Fox *et al*. (2009). On the contrary, Matto *et al*. (2018) recorded 2.6% prevalence of *L. monocytogenes* in feed samples from Uruguay.

The zero occurrence of *L. monocytogenes* in sewage samples in this study is similar to the findings of Enweani *et al*. (2003) and Biswas and Chandra (2011). In contrast, Sarangi and Panda (2012) recorded 10% occurrence in sewage samples while Taherkhani *et al*. (2013) reported 83.33% of *L. monocytogenes* from a South wastewater treatment plant in Iran.

In this study, beef has shown a 2% occurrence rate of *L. monocytogenes*, which is the same as the findings of Nayak *et al*. (2010) in India. Similar studies in India have recorded a higher occurrence than that of the present study; Doijad *et al*. (2010) reported 6.49%, Shakuntala *et al*. (2019) 8.9% and Biswas *et al*. (2008) reported a very low occurrence of 0.9% in beef samples.

The reasons for this variance in occurrence rates of *L. monocytogenes* between India and other countries might be due to different management practices of livestock in the farms such as feeding, housing and hygienic practices followed in the farm (Dhama *et al*., 2015).

Serogrouping and lineage identification of *L. monocytogenes*

Many previous studies reported the virulence of *L. monocytogenes* strains to be associated with serogroup and lineage (Su *et al*., 2016). Similar to the present study, Colaço (2011), Jamali *et al*. (2013), and Shakuntala *et al*. (2019) have identified 1/2a, 3a as predominant serovars among *L. monocytogenes* isolates recovered from varied food samples. Previous reports also recorded that 95% of human listeriosis cases were caused by 4b, 1/2a and 1/2b serogroups, among which 1/2a serovar was more frequently associated with listerial gastroenteritis outbreaks (Swaminathan and Gerner-Smidt, 2007). Our results are in accordance with the results of other researchers who have found that 1/2a and 3a were the most prevalent serovars in food and environmental samples.

Virulence gene profiling of L. monocytogenes

Differentiating between pathogenic and non-pathogenic strains of *L. monocytogenes* is necessary in order to evaluate the significance of this organism in food safety and public health risks (Soni *et al*., 2013). Shakuntala *et al*. (2019) recorded 98.4% frequency of *hlyA*, 85.7% of *iap*, 73% of *plcA* and 68.2% of *plcB* in the *L. monocytogenes* isolates tested in their studies.

Genome fingerprinting analysis

In the present work, in order to study genetic diversity and obtain a better epidemiological understanding of the recovered *L. monocytogenes* strains, they were characterized by employing RAPD-PCR (two primers) and ERIC-PCR. All our isolates were subjected to RAPD-PCR (two primers) and ERIC-PCR, and the DI/Discriminatory Index of both these fingerprinting techniques met the suggested 90% standard with good discriminatory potential (Hunter and Gaston, 1988). Six isolates from milk (M25, M26, M23, M5, M20 and M10) were grouped in the same cluster in both ERIC-PCR and RAPD-PCR fingerprinting. This suggests ERIC and RAPD-PCR to be efficient molecular typing tools for *L. monocytogenes*.

Antimicrobial susceptibility

The results of antimicrobial studies in the present research were in accordance with the work done by Soni *et al*. (2013) and Su *et al*. (2016) who also reported variable resistance among *L. monocytogenes* isolates to commonly used antibiotics. From the previous studies, multi drug resistant index values higher than 0.2 (Bilung *et al*., 2018) indicate that the isolates might have originated from high risk sources and have a high risk potential.

Similar to the present study, Odjadjare *et al*. (2010) found that none of the isolates in their studies was harbored with *ermA*, *ermB*. Su *et al*. (2016) recorded seven food isolates that belonged to 1/2a, 3a, exhibited tetracycline resistance (5.83%), and harbored the *tetM* gene. Our results have shown no correlation between phenotypic and genotypic methods of antimicrobial resistant patterns.

The present study identified the occurrence of *L. monocytogenes* from a bovine farm environment with the most predominant serovar coupled with virulence and multiple antimicrobial resistance isolates. Our results suggest that ERIC-PCR and RAPD-PCR are less time consuming and more cost effective techniques to study the epidemiological and evolutionary relationship among *L. monocytogenes* isolates. Considering the zoonotic potential of this organism, strict hygienic measures and farm monitoring must be implemented to break the transmission to healthy animals in the farm and prevent the contamination of their environment.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Agersborg, A; Dahl, R and Martinez, I (1997). Sample preparation and DNA extraction procedures for polymerase chain reaction identification of *Listeria monocytogenes* in seafoods. Int. J. Food Microbiol., 35: 275-280.

Bilung, LM; Chai, LS; Tahar, AS; Ted, CK and Apun, K

(2018). Prevalence, genetic heterogeneity, and antibiotic resistance profile of *Listeria* spp. and *Listeria monocytogenes* at farm level: A highlight of ERIC- and BOX-PCR to reveal genetic diversity. Biomed. Res. Int., Article ID 3067494, 12 pages. https://doi.org/10.1155/2018/3067494.

Biswas, BK and Chandra, S (2011). Presence of *Listeria* spp. in ice cream and sewage water particularly *Listeria monocytogenes* and its pathogenicity. Int. J. Sci. Tech., 2: 36-39.

Biswas, AK; Kondaiah, N; Bheilegaonkar, KN; Anjaneyulu, AS; Mendiratta, SK; Jana, CH and Singh, RR (2008). Microbial profiles of frozen trimmings and silver sides prepared at Indian buffalo meat packing plants. Meat Sci., 80: 151-154.

Bozdogan, B; Berrezouga, L; Kuo, M; Yurek, D; Farley, K; Stockman, B and Leclercq, R (1999). A new resistance gene, *linB*, conferring resistance to lincosamides by nucleotidylation in *Enterococcus faecium* HM1025. *Antimicrob*. Agents Chemother., 43: 925-929.

Chen, M; Wu, Q; Zhang, J and Wang, J (2014). Prevalence and characterization of *Listeria monocytogenes* isolated from retail-level ready-to-eat foods in South China. Food Control. 38: 1-7.

Colaço, D (2011). Characterization of *Escherichia coli* and *Listeria* isolated from milk at different levels of collection and processing in Goa. Ph.D. Thesis, Goa University, Goa.

Dhama, K; Karthik, K; Tiwari, R; Shabbir, MZ; Barbuddhe, SB; Malik, SVS and Singh, RK (2015). Listeriosis in animals, its public health significance (food-borne zoonosis) and advances in diagnosis and control: a comprehensive review. Vet. Quart., 35: 211-235. doi: 10.1080/01652176.2015.1063023.

Doijad, SP; Barbuddhe, SB; Garg, S; Poharkar, KV; Kalorey, DR; Kurkure, NV; Rawool, DB and Chakraborty, T (2015). Biofilm-forming abilities of *Listeria monocytogenes* serotypes isolated from different sources. PLoS One. 10: e0137046. doi: 10.1371/journal pone.0137046.

Doijad, SP; Vaidya, V; Garg, S; Kalekar, S; Rodrigues, J; D'costa, D; Bhosle, S and Barbuddhe, SB (2010). Isolation and characterization of *Listeria* species from raw and processed meats. J. Vet. Public Health Sci., 8: 83-88.

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: 3819-3822. doi:10.1128/JCM.42.8.3819-3822.2004.

Enweani, IB; Esumeh, FI; Akpe, RA; Akharume, EE; Aghaiyo, ID; Igbinaduwa, IN and Igbinovia, AE (2003). Isolation of *Listeria* species from water bodies, sewage and soil samples. J. App. Basic Sci., 1(1-2): 87-90.

Fox, E; O'Mahony, T; Clancy, M; Dempsey, R; O'Brien, M and Jordan, K (2009). *Listeria monocytogenes* in the Irish dairy farm environment. J. Food Prot., 72: 1450-1456.

Furrer, B; Candrian, U; Hoefelein, C and Luethy, J (1991). Detection and identification of *Listeria monocytogenes* in cooked sausage products and in milk by *in vitro* amplification of haemolysin gene fragments. J. Appl. Bacteriol. 70: 372-379. doi:10.1111/j.1365-2672.1991.tb02951.x.

Hunter, PR and Gaston, MA (1988). Numerical index of the discriminatory ability of typing systems: An application of Simpson's index of diversity. J. Clin. Microbiol., 26: 2456-2466.

Jamali, H; Radmehr, B and Thong, KL (2013). Prevalence, characterisation, and antimicrobial resistance of *Listeria* species and *Listeria monocytogenes* isolates from raw milk in farm bulk tanks. Food Control. 34: 121-125.

Krumperman, PH (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Appl. Environ. Microbiol., 46: 165-170.

Lina, G; Quaglia, A; Reverdy, ME; Leclercq, R; Vandenesch, F and Etienne, J (1999). Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among *Staphylococci*. Antimicrob. Agents Chemother., 43: 1062-1066.

Matto, C; Varela, G; Braga, V; Vico, V; Gianneechini, RE and Rivero, R (2018). Detection of *Listeria* spp. in cattle and environment of pasture-based dairy farms. Pesqui. Vet. Bras., 38: 1736-1741.

Morvan, A; Moubareck, C; Leclerc, A; Hervé-Bazin, M; Bremont, S; Lecuit, M; Courvalin, P and Le Monnier, A (2010). Antimicrobial resistance of *Listeria monocytogenes* strains isolated from humans in France. Antimicrob. Agents Chemother., 54: 2728-2731.

Nayak, JB; Brahmbhatt, MN; Savalia, CV; Bhong, CD; Roy, A and Kalyani, IH (2010). Detection and characterization of *Listeria* species from buffalo meat. Buffalo Bull. 29: 83-94.

Nightingale, KK; Schukken, YH; Nightingale, CR; Fortes, ED; Ho, AJ; Her, Z; Grohn, YT; McDonough, PL and Wiedmann, M (2004). Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. Appl. Environ. Microbiol., 70: 4458-4467.

Nishibori, T; Cooray, K; Xiong, H; Kawamura, I; Fujita, M and Mitsuyama, M (1995). Correlation between the presence of virulence associated genes as determined by PCR and actual virulence to mice in various strains of *Listeria* spp. Microbiol. Immunol., 39: 343-349. doi:10.1111/j.1348-0421.1995.tb02211.x.

Notermans, SH; Dufrenne, J; Leimeister-Wächter, M; Domann, E and Chakraborty, T (1991). Phophatidylinositol-specific phospholipase C activity as a marker to distinguish between pathogenic and nonpathogenic *Listeria* species. Appl. Environ. Microb., 57: 2666-2670.

Odjadjare, EEO and Okoh, AI (2010). Prevalence and distribution of *Listeria* pathogens in the final effluents of a rural wastewater treatment facility in the Eastern Cape Province of South Africa. World J. Microbiol. Biotechnol., 26: 297-307.

Orsi, RH; Den Bakker, HC and Wiedmann, M (2011). *Listeria monocytogenes* lineages: genomics, evolution, ecology, and phenotypic characteristics. Int. J. Med. Microbiol., 301: 79-96.

Paziak-Domanska, B; Boguslawska, E; Wieckowska-Szakiel, M; Kotlowski, R; Rozalska, B; Chmiela, M; Kur, J; Dabrrowski, W and Rudnicka, W (1999). Evaluation of the API test, phosphatidylinositol-specific Phospholipase C activity and PCR method in identification of *Listeria monocytogenes* in meat foods. FEMS Microbiol. Lett., 171: 209-214.

Rawool, DB; Doijad, SP; Poharkar, KV; Negi, M; Kale, SB; Malika, SVS; Kurkure, NV; Chakraborty, T and Barbuddhe, SB (2016). A multiplex PCR for detection of *Listeria monocytogenes* and its lineages. J. Microb. Methods. 130: 144-147.

Sarangi, LN and Panda, HK (2012). Isolation, characterization and antibiotic sensitivity test of pathogenic *Listeria* species in livestock, poultry and farm environment of Odisha. Indian J. Anim. Res., 46: 242-247.

Seeliger, HPR and Jones, D (1986). Genus *Listeria*. In:

Kandler, O and Weiss, N (Eds.), *Regular, non-sporing Gram-positive rods. Bergey's manual of systematic bacteriology*. (2nd Edn.), Baltomore, Williams and Wilkins. PP: 1235-1245.

Shakuntala, I; Das, S; Ghatak, S; Milton, AAP; Rajkumari, S; Puro, K; Pegu, RK; Duarah, A; Barbuddhe, SB and Sen, A (2019). Prevalence, characterization, and genetic diversity of *Listeria monocytogenes* isolated from foods of animal origin in North East India. Food Biotechnol., 33: 237-250. doi: 10.1080/08905436.2019.1617167.

Shantha, S and Gopal, S (2014). Prevalence of *Listeria* species in environment and milk samples. Adv. Anim. Vet. Sci., 2: 1-4.

Soni, DK; Singh, RK; Singh, DV and Dubey, SK (2013). Characterization of *Listeria monocytogenes* isolated from Ganges water, human clinical and milk samples at Varanasi, India. Infect. Genet. Evol., 14: 83-91. doi: 10.1016/j.meegid.2012.09.019.

Su, X; Zhang, S; Shi, W; Yang, X; Li, Y; Pan, H; Kuang, D; Xu, X; Shi, X and Meng, J (2016). Molecular characterization and antimicrobial susceptibility of *Listeria monocytogenes* isolated from foods and humans. Food Control. 70: 96-102.

Swaminathan, B and Gerner-Smidt, P (2007). The epidemiology of human listeriosis. Microb. Infect., 9: 1236-1243.

Taherkhani, A; Attar, HM; Moazzam, MA; Mirzaee, SA and Jalali, M (2013). Prevalence of *Listeria monocytogenes* in the river receiving the effluent of municipal wastewater treatment plant. Int. J. Environ. Health Eng., 2: 49.

Ward, TJ; Ducey, TF; Usgaard, T; Dunn, KA and Bielawski, JP (2008). Multilocus genotyping assays for single nucleotide polymorphism-based subtyping of *Listeria monocytogenes* isolates. Appl. Environ. Microbiol., 74: 7629-7642.