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Research Article

In Vitro Characterization of *Listeria monocytogenes* Isolates by Haemolysis, Camp, Piplc Assay with Protein Profiling and Antibiotic Resistance Recovered from Nagpur Region

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ABSTRACT

Listeria monocytogenes is one of the most virulent foodborne zoonotic pathogen with 20 to 30% of clinical infections resulting in death. The *L. monocytogenes* is ubiquitous in the nature and has been isolated from a variety of foods and environmental sources infecting human and animals revealing the pathogenicity of organism. Reports are very few regarding the pathogenesis of organism in Nagpur region, Maharashtra, India. So the present study was proposed to evaluate the pathogenicity of 12 *L.monocytogenes* isolates recovered from foods of animal origin (Chevon, pork, beef and carabeef) collected in and around Nagpur city. In vitro expression of pathogenisity was carried out by using viz., Haemolysis on Sheep blood agar (SBA), Christie Atkins Munch Petersen (CAMP) Test, Phosphatidylinositol–specific phospholipase–C (PI– PLC) assay along with protein profiling and subsequently studied for their pattern of antibiotic resistance. All 12 isolates of *L. monocytogenes* were found haemolytic in nature and showed PIPLC activity with variation in degree of production which designated them as pathogenic one. The protein profiles of all isolates exhibited minimum 25 to maximum 29 bands where protein bands of molecular weight from 56.237 kDa to 60.33 kDa and 29.226 kDa to 33.48 kDa shared by all isolates can be correlated with the haemolytic LLO (Listerolysin–O) and PI–PLC activity respectively. In antibiotic assay, most isolates showed multidrug resistance, besides no resistance was recorded towards Gentamycin, Norfloxacin, Ampicilin and Cefotaxime. The study revealed a high virulence nature of isolates of *L. monocytogenes* among food animals in and around Nagpur region. Haemolysin on SBA, PI–PLC activity along with exhibition of polypeptide in the range for these activities can be suggestive of protein profiling as technique for determination of pathogenicity.

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INTRODUCTION

Surveillance of food borne diseases is utmost priority in the public health agenda worldwide, as a leading cause of death from a food borne pathogen, *Listeria monocytogenes* continues to cause sporadic cases and outbreaks of illness. The organism is one of the most virulent food borne zoonotic pathogen having very high mortality rate (Schlech, 2010) and the reports are increased in prevailing years worldwide (EFSA, 2012). Recently major outbreaks are reported in American and European countries (Todd and Notermans, 2011), revealing to date huge public health significance. The organism is ubiquitous in nature and widespread in environment inevitably results in contamination of numerous food products. Many attempts has been made to isolate Listeria spp. from a variety of foods and environmental sources such as water, sludge, soil, plants, vegetation, food, infecting humans and animals (Liu, 2008; Dhama et al., 2013). However, the published information on the status of pathogenecity of *L. monocytogenes* from these foods is very scattered and unsystematic, both in the veterinary and public health sectors (Khan et al., 2013). Till date the cases of abortion, stillbirths, neonatal septicemia and encephalitis have been documented in human beings and domestic animals (Jamali and Radmehr, 2013).

To assess the virulence properties of a particular *L. monocytogenes* isolate, the mouse pathogenicity and chick embryo test were preferred, for this skilled personnel and animal ethics are major concerns. Therefore alternative methods ideally should be based on the phenotypic characterization by *in–vitro* methods as Haemolysis, CAMP and PIPLC assay

Hemolysis is an important characteristic which would seem to be directly related to the pathogenicity of *Listeria*,

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since non–haemolytic *Listeria* species can be considered as non–pathogenic (FAO/WHO/OIE, 2008) and the pathogenicity of the pathogen is highly correlated to the haemolytic factor (Yadav et al., 2010; Momtaz and Yadollahi, 2013). Considering haemolysis, only *L. monocytogenes* and *L. ivanovii* produced characteristic haemolysin like listeriolysin–O (LLO) and ivanolysin–O, respectively.

In CAMP test, haemolysin acts synergistically with the β– haemolysis of *S. aureus* on sheep erythrocytes and gives synergistic zone of hemolysis towards *S. aureus* which is due to either phosphatidylinositol specific or phosphatidyl–choline specific C from *L. monocytogenes* and sphingomyelinase from *Staphylococcus aureus* (Farber and Peterkin, 1991; McKellar, 1994). Evaluation of the pathogenicity of *Listeria* species by phosphatidylinositol specific phospholipase C (PI– PLC) based assay has been reported to be reliable indicator to discriminate pathogenic and non–pathogenic Listeria species (Notermans et al., 1991). Copious workers have been used CAMP test to charecterised *L. monocytogenes* isolates (Tasci et al., 2010; Mahmoodi, 2010; Gebretsadik et al., 2011). In recent years, many resistant strains have been detected of listeriosis (Charpentier and Courvalin, 1999) this may be due to indiscriminate use of antibiotics results in the inflation of multi–drug resistance in *L. monocytogenes* bacteria, so antibiogram studies can be very useful in deciding line of treatment.

Keeping things in mind, the published information on status of *L. monocytogenes* in meat and methods available for the detection of pathogenic *L. monocytogenes*, we investigated the pathogenecity of *L. monocytogenes* isolates recovered from various meat samples in Nagpur, India, by examining *in vitro* characterization of *L. monocytogenes* isolates via Haemolytic, CAMP and PIPLC assay along with protein profiles.

MATERIALS AND METHODS

Isolates

A total of 12 isolates of *Listeria monocytogenes* were isolated from meat samples and maintained in Division of Veterinary Public Health, Nagpur veterinary college, Nagpur Maharashtra and used for expression of *in–vitro* pathogenicity, protein profiling and antibiotic sensitivity tests.

Biologicals

The standard bacterial strains of *Listeria monocytogenes* (MTCC 1143), *Staphylococcus aureus* (MTCC 3160) and *Rhodococcus equi* (MTCC 1135) were obtained from Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. Chemicals/conjugates required for the study were procured from Sigma Aldrich (US) and Merk/Gene bank, India. The organisms were maintained on brain heart infusion agar slopes at 4°C and used for preparation of LLO.

Haemolysis on Sheep Blood Agar (SBA)

Detection of haemolysin production was performed on sheep blood agar (SBA) as per Cruickshank et al., (1975). Freshly grown isolates were streaked onto blood agar plates prepared using defibrinated sheep blood (5 per cent), incubated at 37^{0}C for 24 hrs and observed for the zone of haemolysis (Courtieu, 1991).

Christie Atkins Munch Petersen (CAMP) Test

The isolates were subjected to CAMP test as per the method of ISO (1996). Isolates of *Staphylococcus aureus* (MTCC 3160) and *Rhodococcus equi* (MTCC 1135) were freshly grown in Brain Heart Infusion (BHI) broth for 18–24 hrs and streaked onto freshly prepared SBA plates (7 %) in a manner of straight line with a distance of 3–4cm so that the streaks were wide apart and parallel to each other. Freshly grown isolates of *L. monocytogenes* were streaked in between two parallel streaks of *S. aureus* and *R. equi* at angle of 90^0 leaving a space of three mm against parallel streaks. The plates were incubated at 37^{0}C for 24 hrs and observed for enhanced zone from partial hemolysis to a wider zone of complete hemolysis.

Phosphatidylinositol–Specific Phospholipase–C (PI–PLC) Assay

The PI– PLC assay was performed according to the standard procedure described by Yadav et al. (2010). The isolates of *Listeria* were overgrown on SBA 5 per cent Figures at 37^{0}C. These isolates were freshly grown in BHI broth for 18–24 hrs and then streaked onto *L. mono* differential agar (Hi Media Ltd, Mumbai, India). The inoculated Figures were incubated at 37^{0}C in a humidified chamber for 24 hrs. Development of light blue colonies were considered positive for PI–PLC assay and accordingly designated as pathogenic.

Protein Profile Employing SDS– PAGE

The protein profiling studies of *L. monocytogenes* isolates was employed as per the standard procedure described by Lammeli, (1970). All isolates of *L. monocytogenes* were inoculated in 20 ml BHI broth with continuous aeration (shaker) at 37^{0}C for 12 hrs. The grown cultures were then centrifuged at 4^{0}C at 10,000 rpm for 5 min (REMI, India) to harvest the supernatants. The culture supernatants were then filtered through nitrocellulose acetate filters (0.45µ) to cell free culture supernatants. The cell free culture supernatants were processed for protein estimation as per Lowry et al., (1951) and 24 µl of each were loaded onto lane of SDS–PAGE.

Sodium–Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS–PAGE)

In order to determine the molecular weight of purified protein, vertical polyacrylamide slab gel (12.5 %) and stacking gel (5 %) was casted in assembly (Banglore, Genei). An amount of approximately containing 7 µg proteins was mixed in 5x sample buffer. The mixture was boiled for three minutes and loaded after cooling in lane of each gel. Assembly was run at 40V (till stacking gel) and further at 90V (in separating gel). The dye front was noted for migration and completion of run. The protein molecular weight marker of medium range (Banglore, Genei) comprising of 97.4 kDa, 66 kDa, 43 kDa, 29 kDa, 20.1 kDa and 14.3 kDa was used as standard. The protein profiles were visualized by employing silver staining technique as per Rosenberg, (1996). The gels were photographed using gel documentation system (BioRad, US) and further calculations of molecular weights (MW) of the peptides

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were done by extrapolation of relative mobility of the unknown protein against that of standard molecular weight marker using quality– One software (BioRad, US).

Antibiotic Sensitivity Test

Antibiotic sensitivity of *L. monocytogenes* isolates to various antibiotics and chemotherapeutic agents was studied by agar disc diffusion method using single antibiotic disc (Bauer et al., 1966). The selection of antibiotic was based on the routinely used antibiotic in field *viz*., Ampicillin, Cephotaxime, Cloxacillin, Erythromycin, Gentamicin, Kanamycin, Nalidixic acid, Norfloxacin, Penicillin–G, Rifampicin, Oxytetracycline and Ceftriaxone (Table 12).

The isolates were freshly grown in BHI broth for 18–24 hrs and then spread evenly on BHI agar plates by using sterile cotton swabs. The antibiotic discs were then placed with the help of sterile forceps and plates were incubated at 37°C for 24 hrs. After incubation, zone of inhibition around each antibiotic disc was measured and each isolate was noted as a sensitive, moderately sensitive and resistant against respective antibiotic based on the size of inhibition zone according to manufactures instruction (Hi–Media Mumbai).

RESULTS

All 12 isolates of *L. monocytogenes* were found haemolytic with variation in degree of haemolysin production based on which differentiated into strong and weak haemolysin producing strain. However among 12 isolates, isolate p49 showed extensive haemolytic zone while remaining 11 isolates showed a mild zone of haemolysis (Figure1; Table 1).

Significant enhanced zone of haemolysis was produced by all 12 isolates of *L. monocytogenes* towards *S. aureus* (MTCC 3160) and subsequently no zone was recorded towards *R. equi* (MTCC1143). Similarly, the isolates showed cent percent positivity in case of PIPLC activity. However, the results are varied as strong PI–PLC producer (++) in case of isolates from cattle (C14) and other for goats (G21) were noted (Figure 2 and 3; Table 1).

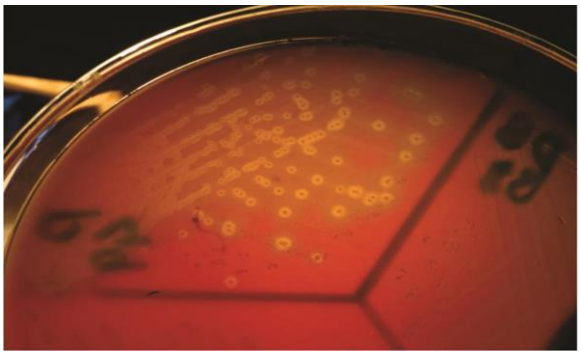

Figure 1: Haemolysin activity of *L. monocytogenes* isolates

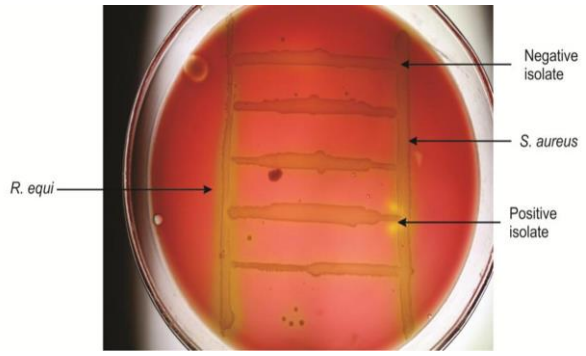

Figure 2: CAMP activity by *L. monocytogenes* isolates

Table 1: Details of pathogenicity profiles of 12 *L .monocytogenes* isolates

No.	Isolate No.	Source	Heamolysin on SBA	CAMP		PI–PLC Production assay	Isolates designated
				Staphylococcus aureus	*Rhodococcus equi*		
1	C12	Cattle	+	+	–	++	*L. monocytogenes*
2	C14	Cattle	+	+	–	+++	*L. monocytogenes*
3	G5	Goat	+	+	–	++	*L. monocytogenes*
4	G21	Goat	+	+	–	+++	*L. monocytogenes*
5	G31	Goat	+	+	–	++	*L. monocytogenes*
6	G32	Goat	+	+	–	++	*L. monocytogenes*
7	P13	Pig	+	+	–	++	*L. monocytogenes*
8	P24	Pig	+	+	–	+	*L. monocytogenes*
9	P28	Pig	+	+	–	+	*L. monocytogenes*
10	P36B	Pig	+	+	–	+	*L. monocytogenes*
11.	P38B	Pig	+	+	–	+	*L. monocytogenes*
12.	P49	Pig	++++	+	–	++	*L. monocytogenes*

The protein profiles of all isolates exhibited minimum 25 to maximum 29 bands ranging from lowest 6.090 kDa to 134.867 kDa (Figure 4a and 4b). The presence of protein bands of molecular weight from 56.237 kDa to 60.33 kDa shared by all isolates of *L. monocytogenes* (Table 2a and 2b) can be representing haemolysin LLO. Parallelly these isolates were found haemolytic on SBA and thereby the ability of isolate to invade the host cell confirming its pathogenicity. The isolates also exhibited and shared polypeptide in the range of 29.226 kDa to 33.48 kDa (Table 2a and 2b).

In antibiotic assay, all isolates of *L. monocytogenes* (100%) were found to be resistant to one or more antibiotics. Nalidixic acid, Penicilin and Cloxacillin

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exhibited 100% resistance followed by Kanamycin, Rifampicin, Ceftriaxone, Tetracycline and Erythromycin

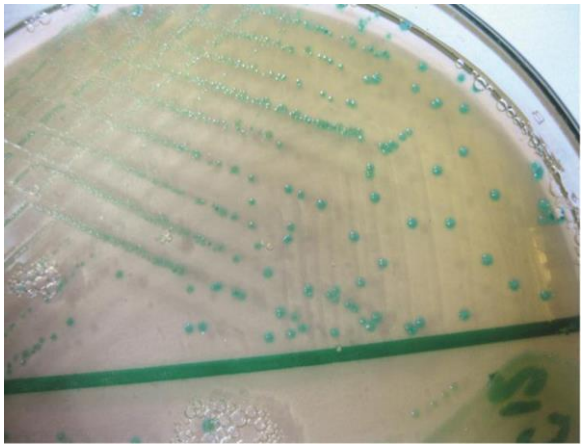

Figure 3: PI–PLC activity by L. monocytogenes isolates

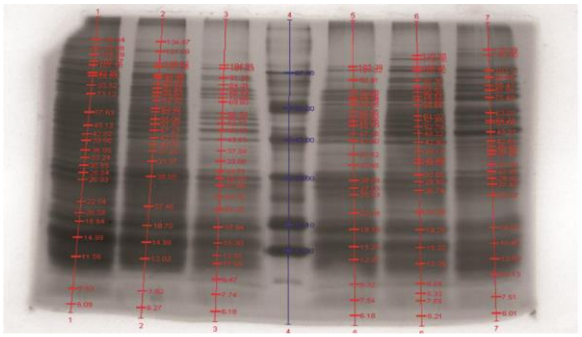

Figure 4a: Protein profile of six *L. monocytogenes* isolates

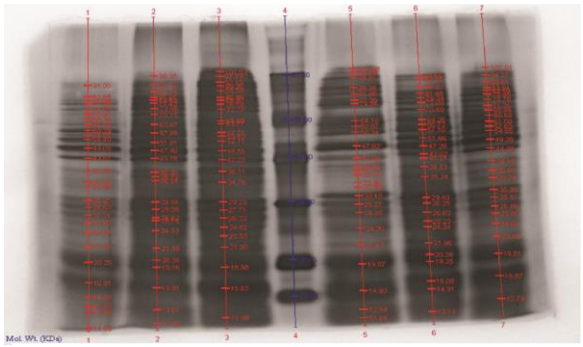

Figure 4b: Protein profile of six *L. monocytogenes* isolates

Table 2a: Protein profiles of six *L. monocytogenes* isolates

Isolate No. Isolate	C12 *L. monocytogenes*	G5 *L. monocytogenes*	G21 *L. monocytogenes*	Standard protein marker	P13 *L. monocytogenes*	P24 *L. monocytogenes*	P28 *L. monocytogenes*
Source	Meat	Meat	Meat		Meat	Meat	Meat
Mol. Wt (kDa)	134.442	134.867	104.913	97.4	105.388	120.38	129.388
	122.083	121.387	101.326	66	101.315	115.159	121.799
	114.288	106.016	91.284	43	90.909	105.908	102.112
	105.374	102.359	84.306	29	79.98	101.815	94.681
	101.182	93.057	80.219	20.1	75.761	91.358	86.474
	92.348	90.299	76.329	14.3	73.193	85.27	81.813
	89.578	85.025	72.99		69.675	80.375	75.478
	80.522	80.462	69.797		63.653	76.512	63.069
	73.12	76.527	60.333		59.925	73.555	56.789
	57.629	73.518	56.77		56.077	69.675	55.063
	48.122	69.922	52.77		52.793	61.02	48.371
	42.816	63.812	48.165		47.359	57.447	42.812
	39.857	60.752	42.526		43	52.793	40.445
	36.054	55.065	37.835		37.419	48.224	38.757
	33.244	51.467	33.978		33.48	42.801	37.139
	30.652	47.226	30.513		28.695	39.194	33.304
	28.541	42.648	28.477		27.026	35.89	31.019
	26.93	39.735	26.998		25.634	34.584	28.917
	22.54	37.196	24.701		22.185	30.658	27.494
	20.584	33.371	22.36		19.178	28.594	25.216
	18.642	28.562	18.94		15.254	26.742	19.033
	14.995	22.459	15.304		12.866	22.579	15.427
	11.561	18.701	12.965		9.316	19.291	12.504
	7.525	14.992	11.654		7.542	15.254	10.134
	6.09	12.018	9.473		6.177	12.348	7.507
		7.816	7.745			9.537	6.012
		6.266	6.184			8.333	
						7.586	
						6.213	
Total Bands	25	27	27	6	25	29	26

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was in 91.66, 66.66, 50, 33.33 and 16.66% of strain respectively. No resistance was recorded towards Gentamycin, Norfloxacin, Ampicilin and Cefotaxime. Highest sensitivity of 91.66% was found against Gentamycin (Table 3).

DISCUSSION

Multiple virulence factors such as hemolysin, CAMP, PIPLC assay are important in the pathogenesis of *L. monocytogenes* infections (Rawool et al., 2007) and indicate the potential of virulent strains to invade host cells and cause listeriosis (Ryser and Marth, 2007).

Table 2b: Protein profiles of six *L. monocytogenes* isolates

Isolate No.	C14	G31	G32	Standard marker	P36B	P38B	P49
Isolate	*L. monocytogenes*	*L. monocytogenes*	*L. monocytogenes*		*L. monocytogenes*	*L. monocytogenes*	*L. monocytogenes*
Source	Meat	Meat	Meat		Blood	Blood	Meat
Mol. Wt (kDa)	91.092	98.351	101.034	97.4	101.984	94.547	101.011
	82.553	91.023	97.187	66	98.439	92.229	94.266
	79.983	85.736	92.177	43	94.014	86.218	88.613
	77.765	81.614	89.295	29	86.361	81.461	83.908
	74.031	79.628	85.593	20.1	81.897	78.343	82.097
	70.973	77.417	80.894	14.3	77.39	75.079	79.453
	68.042	73.955	78.921		74.965	70.079	75.783
	64.676	70.152	76.184		64.162	70.685	71.759
	60.293	63.971	72.786		60.069	64.258	68.945
	57.269	57.978	65.897		56.237	60.719	65.681
	54.397	51.814	63.766		47.918	57.105	61.081
	49.077	47.402	57.507		42.094	51.96	57.634
	43.865	43.163	55.507		39.071	47.279	54.646
	38.993	38.381	52.106		37.709	43.02	49.364
	35.453	36.789	46.552		36.265	41.511	44.377
	33.984	35.512	42.231		33.901	38.523	39.182
	30.251	29.557	38.112		32.487	35.244	36.028
	28.632	28.082	34.761		30.154	29.396	33.738
	27.21	26.618	29.226		28.266	28.25	30.351
	25.924	24.532	27.709		26.853	26.629	28.506
	24.449	21.984	26.328		24.299	25.229	26.9
	22.364	20.365	24.824		21.874	24.338	25.654
	20.25	19.16	23.527		19.074	21.96	24.018
	16.81	15.906	21.901		14.901	20.383	22.075
	14.759	13.01	18.975		12.545	19.247	19.609
	13.55	11.464	15.628		11.64	16.075	15.87
	12.863		11.902			14.913	12.794
	11.086					12.133	
Total Bands	28	26	27		26	28	27

Table 3: Antibiotic sensitivity of 12 *L. monocytogenes* isolates

Sr. No.	Antibiotics	No. of Isolates (12)			Percentage		
		Resistant	Moderately Sensitive	Sensitive	Resistant	Moderately Sensitive	Sensitive
1	Nalidixic acid	12	0	0	100	0	0
2	Penicillin	12	0	0	100	0	0
3	Kanamicin	11	1	0	91.66	8.33	0
4	Cloxacillin	12	0	0	100	0	0
5	Ceftriaxone	6	6	0	50	50	0
6	Rifampicin	8	0	4	66.66	0	33.33
7	Ampicillin	0	6	6	0	50	50
8	Cephotaxime	0	12	0	0	100	0
9	Erythromycin	2	6	4	16.66	50	33.33
10	Gentamicin	0	1	11	0	8.33	91.66
11	Norfloxacin	0	5	7	0	41.66	58.33
12	Oxytetracycline	4	2	6	33.33	16.66	50

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The *L. monocytogenes* phospholipases are vital determinants of pathogenicity (Marquis et al., 1995) expressed solely by pathogenic spp. of Listeria which is haemolytic i.e., *L. monocytogenes* (Liu, 2004). For that reason, these assays remain a reliable indicator to demonstrate and differentiate the pathogenicity of *L. monocytogenes* (Notermans et al., 1991). β–haemolytic activity of *L. monocytogenes* isolates is associated with putative CAMP like factor (Koprnspan et al., 2014). The cent percent haemolytic activity showed by *L. monocytogenes* isolates has been reported by several researchers (Bhanu Rekha et al., 2006; Molla et al., 2004; Yucel et al., 2004; Osman et al., 2014) the same as in the present investigation and those isolates were further designated as the pathogenic one (Barbuddhe et al., 2000, Lacier and Centorbi, 2002; Chaudhari et al., 2004; Becker et al., 2006; Bhanu Rekha et al., 2006; Jallewar et al., 2007; Elezebeth et al., 2007) reveals the strong activity of LLO. Variation in haemolytic activity attributed to composition of medium, and/or a loss of ability to produce hemolysin may have occurred over successive passages through culture media which impaired the identification of these cultures as *L. monocytogenes* (Nunes and Hofer, 1994).

CAMP test is a diagnostic tool that reliably and quickly provides presumptive identification of *L. monocytogenes* (Savini et al., 2014). In CAMP assay, enhanced zone of haemolysis showed by all 12 isolates towards *S. aureus* designates them as pathogenic one seeing that synergistic effect of Listeriolysin–O (LLO) and Streptolysin–O (SLO) has directly implicated the virulence of LLO (Buchanan et al., 1989; McKellar, 1994; Barbuddhe et al., 2000, Molla et al., 2004; Becker et al., 2006; Bhanu Rekha et al., 2006; Abd EI–MaIek et al., 2010; Nayak et al., 2010; Gebretsadik et al., 2011). Further cent per cent PI–PLC activity of all 12 isolates adds to the pathogenic potential (Paziak–Domanska et al., 1999; Yadav et al., 2010; Momtaz and Yadollahi, 2013) consequently it is acted as a one of the best characterized virulence factor (Moreno et al., 2014; Park et al., 2014).

Variation in haemolysis and PIPLC activities was observed in study as one isolate (p49) found to be extensive haemolytic and others remains a mild haemolytic, similarly in PIPLC assay two isolates showed a strong acitivities (C14 and G21) while others showed mild activities, the disparity in the performance might be due no coordination of genes encoding for these activities or lack of virulence determinants due to mutation in the strain of *L. monocytogenes* (Notermans et al., 1991; Shakuntala et al., 2006; Kaur et al., 2010). Overall, in the present investigation all (12) isolates of *L. monocytogenes* isolated from food animals turn pathogenic in all tests employed, indicating their high virulence activity. To date, CAMP remains a reliable indicator for diagnosis of pathogenesity of organism (Savini et al., 2013) and may be performed directly to exclude or confirm *L. monocytogenes* (Bhat et al., 2013; Savini et al., 2014).

SDS–extracted patterns in protein profiling are strictly specific for species except a few major bands appear to be common to all listeria species (Tabouret et al., 1992). The presence of protein bands from 56.237 kDa to 60.33 kDa and consequently haemolytic activity indicated the production of β–Listeriolysin (55 – 60 kDa), which are also probably responsible for the positive CAMP phenomenon and this protein band (50–60 kDa) may eventually emerge as significant factor of Listerial pathogenicity as described by Parrisius et al. (1986). Also, all isolates exhibited and shared polypeptide in the range of 29.226 kDa to 33.48 kDa which can be representing PI–PLC as reported by Notermans et al. (1991) since these isolates were showed PI–PLC activity on L. Mono differentiating agar.

In present study, 100% resistance was showed against Nalidixic acid and Penicilin, the results are in accordance with Yucel et al. (2005); Ennaji et al. (2008); Ruiz–Bolivar et al. (2011) and Gunjal (2006); Pozark and Murano (2002) respectively. Whereas, Rahimi et al. (2010) recorded 96.4 % resistance for the Penicilin and Lotfolahi et al. (2011) revealed 77.77% of resistance towards Penicilin. Similarly, Kanamycin showed 91.66% resistance which has also noticed by Ruiz–Bolivar et al. (2011). In contrast, Rodas–Suaerez et al. (2006); Nwachuwu et al. (2010) and Morvan et al. (2010) reported 22.5, 40.91 and zero percent resistant for penicillin respectively. Nwachukwu et al. (2010) reported 22.22% resistance for Rifampicin while in present study 66.66%. In case of Tetracycline, 16.66% resistant was noticed which varies from the result of Rodas–Suarez et al. (2006) and Gunjal (2006) who reported 2.3 and 8.16 % resistance respectively. Variation in the geographical region and differences in therapeutic practices can think to be major contributing factors.

Overall 91.66 per cent of sensitivity was observed for Gentamicin, the same was observed by Wong et al. (1990), Walsh et al. (2001), Ennaji Hayat et al. (2008), Morobe et al. (2009) and Vasu et al. (2014) as 99.4, 99, 100, 84.21and 100% , respectively. These deviations in the antibiotic resistance pattern attributed to the fact that antibiotic resistance is encoded by the plasmid which has capability to transfer from one strain to another (Mayer, 1988). However, the presence of multi–drug resistance among virulent strains of *L. monocytogenes* in the region is pointing to increase in potential threat from public health point of view (Prazak et al., 2002).

CONCLUSION

The observation on exhibition of polypeptide in the range of 50–60 kDa indicative of LLO and its parallel expression as production of haemolysin on SBA as well as exhibition of protein band in the range of 29–33 kDa along with *in–vitro* demonstration of PI–PLC activity on agar can be suggestive of protein profiling as technique for determination of pathogenicity. Bearing of the proteins with common molecular weight among these isolates as well as *L. monocytogenes* isolates from the earlier studies can be exploited for study of epidemiological marker for the region specific isolates. Further, large number of samples will be needed to draw any conclusion pertaining to 'conserved protein profile' specific to the region. However, subsequent exhaustive studies are required in this direction to build up the remark on this matter.

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