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Conventional and Molecular Detection of *Listeria monocytogenes* and its Antibiotic Sensitivity Profile from Cattle Sources of Aizawl, Mizoram (India)

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A B S T R A C T

The present study was conducted to study the prevalence of the food borne zoonotic pathogen of animal origin, *L. monocytogenes* by isolation and identification, molecular detection and antibiotic sensitivity pattern from different samples of cattle sources in Aizawl district of Mizoram. A total 200 numbers of sample including cattle faeces (50), raw milk (50) and milk products (100) were collected randomly from different unorganized shop and farms. The seasonal variation in the occurrence of *L. monocytogenes* was also studied. The *L. monocytogenes* was isolated by using two step enrichment method of culturing and identified based on cultural characteristics, gram staining, biochemical properties, tumbling motility and *in vitro* pathogenicity tests. The molecular detection of *L. monocytogenes* strains were done by PCR using published primers. The antibiotic sensitivity was studied against 12 numbers of commonly used antibiotics in animals and human. The prevalence of *L. monocytogenes* was recorded as 6.50 percent including 8.00 percent from cattle faeces, 6.00 percent from raw milk, 8.00 percent from lassi, dahi and ice-cream samples, respectively. The *L. monocytogenes* strains showed 100 percent sensitivity towards Penicillin, Ampicillin, Oxacillin, Cephotaxime/Clavulanic acid, Ciprofloxacin, Tetracycline and Trimethoprim/Sulphamethoxazole followed by Streptomycin (84.61%), Chloramphenicol (53.84%), Gentamicin (53.84%) and Ceftriaxone (46.15%).

Introduction

Listeriosis has been among important food borne zoonotic diseases since long, mostly due to its high mortality rate despite of being uncommon in human beings (Atil *et al.*, 2011). The severity of the disease has become significant as the causative organism *Listeria monocytogenes* is the most important species in the genus can be secreted through milk of both healthy and infected animals (Wagner *et al.*, 2000). This is still called an emerging pathogen as its transmission through contaminated food is recently recognized. *L. monocytogenes* is a gram positive, ubiquitous, non-spore forming organism that can survive a

wide range of pH from 4.0-9.6 and temperature from -1.5°C to 45°C (Lado and Yousef, 2007). Listeriosis is commonly characterized by meningoencephalitis, generalised septicaemia and abortion (Late pregnancy) in both human and animals. Young individuals along with immunocompromised ones are more susceptible than others. Way back in 1985, Listeriosis was declared as serious public health hazards (Rocourt and Catimel, 1985).

Listeria spp. have been reported as susceptible to antibiotics active against gram positive bacteria but in recent years like many other bacterial pathogens *Listeria* are developing resistance to many currently used antibiotics. Current choice of antibiotics for all forms of listeriosis is combination of Ampicillin and Gentamicin (Schlech and Acheson, 2000). The studies on *L. monocytogenes* in the perspective of foodborne pathogen are scanty in North-East Region states of India including Mizoram. Therefore keeping the above points in view, the present study was undertaken to isolate, identify and to study the prevalence and antimicrobial sensitivity pattern of *L. monocytogenes* from different samples of cattle source s in Aizawl district of Mizoram.

Materials and Methods

Study area

The present study on isolation and identification, molecular detection and antimicrobial sensitivity pattern of *L. monocytogenes* from different samples of cattle sources was carried out in Aizawl district of Mizoram. It is mainly a hilly state of North-eastern region. It extends from 21°56'N to 24°31'N, and 92°16'E to 93°26'E. It is the 2nd least populous state in the country and it is covers an area of approximately 21,087 square kilometres. About 91percent of the area in the state is forested.

Period of the study

The study was conducted for a period of one year from July, 2017 to June, 2018 and the study period was divided into two halves; Summer (March to September) and Winter (October to February).

Collection of Samples

A total of 200 numbers of faecal samples of cattle, raw milk and milk products were collected randomly from different unorganized cattle farms/ milk vendors/ shops periodically during the study period by following aseptic measures for detection of *L. monocytogenes* during the study period. Distributions of different samples collected are given in the Table 1.

Isolation and phenotypic characterization of *L. monocytogenes*

Enrichment of faecal sample

The USDA (USDA FSIS, 2002) method was employed for isolation of *Listeria spp*. from faecal samples of cattle by two step enrichment method. Primary enrichment of five grams of faecal sample was done in 45 ml 1/2 strength UVM-I broth containing selective supplements (HiMedia Pvt. Ltd., Mumbai)® and incubated for 24 hours at 30°C followed by secondary enrichment of 0.1 ml from the primary broth culture in 10 ml UVM-II broth containing selective supplements and incubated for 48 hours at 37°C.

Enrichment of milk and milk products

Food and Drug Administration (2015) testing methodology with slight modification was employed for isolation and identification of *L. monocytogenes* from cattle faeces, raw milk and milk products (lassi, dahi, ice-cream and rasmalai). Twenty five ml of sample was

mixed with 225 ml of UVM broth properly for 2 minutes and the mixture was incubated at 30 ± 2°C for 24 hours. For secondary enrichment, 0.1ml of the cultured UVM was transferred to 10 ml of Fraser broth (FB) and incubated at 37°C for 24 ± 2 hours.

Selective plating of *L. monocytogenes* (UVM-Broth and FB culture) in PALCAM, McBride and TSYEA agar

A drop of approximately 0.1ml of FB broth culture turning to black colour was streaked aseptically upon PALCAM and McBride agar plates and the plates were incubated at 37°C for 24–48 hours. The suspected colonies on PALCAM/ McBride agar plates were streaked with the help of a sterile loop on TSYEA plate and incubated at 37°C for 24 hours and subsequently tested for further biochemical and *in vitro* pathogenicity characteristics.

Morphological and biochemical characteristics of *L. monocytogenes*

The *L.monocytogenes* strains were phenotypically characterized by morphological characteristics, Gram staining reaction and biochemical characteristics (Catalase, Oxidase, Motility, Indole, Methyl Red, Voges-Proskauer, Citrate utilization, fermentation patterns of sugars like L-Rhamnose, D-Xylose and Mannitol *etc.*) (Quinn *et al.*, 1994).

In vitro pathogenicity test

Beta haemolysis test on five percent sheep blood agar

The suspected colonies on PALCAM/ McBride/ TSYEA agar plates were streaked on five percent Sheep Blood Agar (SBA) plates and the plates were incubated at 37°C for 24 hours. The *L. monocytogenes* positive SBA plate showed translucent colonies surrounded by a small zone of β-haemolysis after back light.

Christie, Atkins, Munch- Petersen (CAMP) Test

The presence of *in-vitro* pathogenicity of *L. monocytogenes* by CAMP test was as per the method of ISO (1996). The standard strains of *Rhodococcus equi* (MTCC 8144) and *Staphylococcus aureus* (MTCC 43300) were streaked on freshly prepared 5 percent SBA plates wide apart and parallel to each other. The test strains were streaked at 90° angle to *R. equi* and *S. aureus* with a distance of three mm apart from these strains streaking line.

The streaked plates were incubated for 24 hours at 37°C and examined for haemolytic zone from partial haemolysis to a wider zone of complete haemolysis. The isolates with CAMP- positivity against *S. aureus* were characterized as *L. monocytogenes* giving a spade shaped haemolytic zone formation.

Molecular detection of *L. monocytogenes*

Bacterial lysate preparation

All the culturally, phenotypically and biochemically positive *L. monocytogenes* isolates were processed for bacterial lysate preparation using boiling and snap chill method. A single colony of phenotypically confirmed strain was inoculated into one ml of LB broth and incubated at 37°C for 16-18 hours. After overnight incubation at 37°C, cells were pelleted by centrifugation at 8000 rpm for 10 minutes at 4°C. Then the pellet was washed three times with sterile normal saline solution (0.85%) and finally re-suspended in 500µl of nuclease free sterile distilled water. The cell suspension was heated in a boiling water bath for five minutes followed by immediate chilling. The cellular debris was sediment by centrifugation at 5000 rpm for

five minutes. The supernatant was used as template DNA for PCR assay.

Detection of species specific gene (*16S-rRNA*) of *L. monocytogenes* isolates by PCR

All the culturally, phenotypically and biochemically positive *L. monocytogenes* isolates were subjected for *16S-rRNA* species specific gene amplification by PCR using published primer and according to the methodology described by Jallewar *et al.*, (2007). The details about the primer sequence are given at Table 2. The PCR assay was carried out in 0.2 ml thin PCR tube. To detect species specific genes of *L. monocytogenes*, the PCR protocol was standardized by using standard *L. monocytogenes* (MTCC 1143) as positive control and sterile milli-Q water as negative control. The final composition for 25 µl reaction mixture is given at Table 3.

Amplification of DNA was performed in a Thermal cycler machine with a pre-heated lid. The detail of the cycling condition for the species specific gene was given in the Table 4. All the amplified PCR products were analyzed by agarose gel electrophoresis using one percent agarose gel in 1X TAE buffer (pH 8.0). About five µl of PCR product was mixed with 2 µl of 6 X gel loading dye and loaded into each well. DNA ladder (3000 bp) was used as reference to compare the size of amplified products. The gel was visualized under UV transilluminator (Alpha Imager) and documented by gel documentation system (Alpha Imager).

Detection of antibiotic sensitivity and resistance pattern of *L. monocytogenes* strains

All the *L. monocytogenes* isolates were subjected to *in vitro* antibiotic sensitivity test by disc diffusion method (Bauer *et al.*, 1966) against a panel of 12 antibiotics namely Penicillin G, Ampicillin, Oxacillin, Streptomycin, Erythromycin, Cephotaxime/ Clavulanic acid, Ceftriaxone, Chloramphenicol, Ciprofloxacin, Gentamicin, Tetracycline and Trimethoprim/ Sulphamethoxazole as per Clinical and Laboratory Standard Institute (CLSI) guidelines (2014). The *L. monocytogenes* isolates were inoculated into Brain Heart Infusion (BHI) broth and incubated for 24 hours at 37°C. After that, 200 µl of each inoculum was taken on Muller Hinton agar plates and spread eventually with the help of sterile L-shaped spreader. Then the plates were allowed to dry and antibiotic discs were placed on media aseptically with the help of sterile forceps. Next, the plates were incubated at 37°C for 24-48 hours. After completion of incubation the diameter of zone of inhibition was compared with the standard known value against each specific antimicrobial agent from interpretation guide line (Hi-Media)®.

Results and Discussion

Isolation and identification of *L. monocytogenes*

Out of 200 different samples from cattle sources (cattle faeces, raw milk and milk products) of Aizawl, a total 29 (14.50%) samples were found to be positive for *Listeria spp.* by the cultural method in which isolates turned into black colour in different broth (FB and UVM) and also showed different characteristics of colonies on different agars such as green colonies with black haloes in PALCAM agar, dense white to iridescent white appearing as crushed glass in McBride agar and clean glass like colonies in TSYEA agar after 24-48 hours of incubation at 37°C. Based on the Gram staining reaction and different biochemical tests (catalase: positive:; oxidase: negative: tumbling motility; indole: negative; methyl red: positive; Voges-Proskauer: positive citrate: negative; L-

rhamnose: positive; D- Mannitol fermentation: negative; D- Xylose fermentation: negative, weak haemolysis on sheep blood agar and positive CAMP test against *Staphylococcus aureus* characteristics), 13 (6.50%) numbers of *L. monocytogenes* were identified and the findings were in accordance with Gupta and Sharma (2012) and Walse *et al.,* (2003) (Table 5, 6 and Figure 1–12).

The detection of *Listeria* spp. from food products is challenging due to the concurrence presence of other organisms within the food product. In this respect, the isolation method in respect to specific pathogen is critical and must allow recovery and detection of injured cells too. In food, detection of *Listeria* spp. is generally performed in a two-step cultural enrichment process and along with selective supplements like antibacterial and antifungal agents. The bacteriological culture methods commonly used for detection and identification of the bacteria include aesculin and ferric iron in enrichment or plating media, which results through the hydrolysing capacity of *Listeria* spp., in the formation of intense black colour (Fraser and Sperber, 1988). Results of *in vitro* pathogenicity tests showed that *Listeria* spp. brought about haemolysis on five per cent SBA similar to the earlier records of Blanco *et al.,* (2008). The Christie Atkins Munch-Petersen (CAMP) test is a unique confirmatory tool for identification of this food borne pathogen. The *Listeria* spp. isolates recovered during the study have shown the positive CAMP pattern against *S. aureus* (ISO, 1996).

Prevalence of *L. monocytogenes* in different samples of cattle sources (faeces, raw milk and milk products) from Aizawl, Mizoram

The prevalence of *L. monocytogenes* was recorded as 6.50 percent (13/200) *comprised* of 8.00 percent (4/50) strains from cattle faeces, 6.00 percent (3/50) from raw milk, 8.00 percent (2/25) from lassi, dahi and ice-cream samples, respectively (Table 7 and Figure 13). *Listeria monocytogenes* was detected from the raw milk and ready to eat refrigerated milk products produced locally from unpasteurized milk like dahi, lassi and ice-cream whereas the organism was not isolated from rasmalai which is a well-cooked milk product stored for a short duration of time in the sweet shops. The Higher prevalence rates of *L. monocytogenes* from faecal samples of ruminants were recorded by Lawan *et al.,* (2003) (10.00%) and Kalorey *et al.,* (2006) (16.00%) from Nigria and Nagpur (India), respectively. Waghmare, (2006) evaluated the incidence of *Listeria spp*. in raw milk from different markets of Mumbai city (India) and revealed prevalence of *Listeria spp*. and *L. monocytogenes* amongst the pasteurized milk samples with the incidence of 21.32 and 5.88 per cent in unpasteurized milk samples. Similarly, Chandio *et al.,* (2007) reported 6.00 per cent of *L. monocytogenes* in raw cow milk where as higher incidence of prevalence of *L. monocytogenes* (21. 70%) was reported by Sharma *et al.,* (2012) from 115 raw cow milk samples in Meerut and Babugarh Cantt, Hapur, India. In contrast, studies conducted at Coimbatore (Tamilnadu) and Mangalore, India reported that branded milks were more prone to *L. monocytogenes* than the local milk (Dhanashree *et al.,* 2003; Sheela and Muthukmar, 2011). However, Moharram *et al.,* (2007) reported 5.00 percent incidence of *L. monocytogenes* from non-branded ice-cream samples from different ice cream parlours of Mysore (India).

The seasonal distribution of *L. monocytogenes* revealed 4.95 and 8.08 percent of prevalence in summer and winter season, respectively. The Seasonal fluctuation of *L. monocytogenes* in the milk has been reported as 1.69 per cent in summer and 3.82 per cent in winter (Aurora *et al.,* 2006) (Table 8 and Figure 14).

Table.1 Distribution of different samples of cattle sources collected from Aizawl district of Mizoram

Sl. No	State	Type of sample	Number of samples	Seasonal distribution	
				Summer	Winter
1	Aizawl (Mizoram)	Cattle faeces	50	25	25
2		Raw cow milk	50	25	25
3		Lassi	25	13	12
4		Dahi	25	13	12
5		Ice-cream	25	12	13
6		Rasmalai	25	13	12
Total			**200**	**101**	**99**

Table.2 Oligonucleotide primers used for detection of species specific gene of *L. monocytogenes* by PCR

Target Genes	Primer Sequence (5'-3')	Base Pair (bp)	Reference
16Sr-RNA	F-GGACCGGGGCTAATACCGAATGATAA R- TTCATGTAGGCGAGTTGCAGCCTA	1200	Weidmann *et al.*,(1993)

Table.3 Composition of PCR reaction mixture for detection of species specific gene and virulence genes of *L. monocytogenes*

Sl. No.	Ingredients	Volume (µl)
1	PCR Master Mixture 2x	12.5
2	Forward primer	1
3	Reverse primer	1
4	Template	4
5	Milli-Q water	6.5
	Total	**25.0**

Table.4 Thermal cycling condition for detection of species specific (*16S-rRNA*) gene of *L. monocytogenes*

Sl. No	Stages	PCR for *16Sr-RNA* gene of *L. monocytogenes*
1	Initial denaturation	94°C for 4 min
2	Denaturation	94°C for 30 sec
3	Annealing	56.5°C 45 sec
4	Elongation	72°C for 30 sec
5	Final Extension for 1 cycle	72°C for 3 min
	No. of cycle	**35**

Table.5 Morphological and biochemical test results of *L. monocytogenes*

Sl. No.	Morphological/biochemical test	Positive characteristics
1	Gram staining	Positive
2	Catalase	Positive
3	Oxidase	Negative
4	Motility	Tumbling
5	Indole	Negative
6	Methyl Red	Positive
7	Voges-Proskauer	Positive
8	Citrate	Negative
9	L-Rhamnose fermentation	Positive
10	D- Mannitol fermentation	Negative
11	D- Xylose fermentation	Negative

Table.6 *Listeria monocytogenes* isolation by cultural method and confirmed by biochemical test collected from different source of cattle of Aizawl (Mizoram) district

Sl. No.	State	Type of sample	Number of samples analyzed	Number of samples positive for *Listeria spp.* by cultural method	Number of samples positive for *L. monocytogenes* after biochemical test
1	Aizawl (Mizoram)	Cattle faeces	50	7	4
2		Raw cow milk	50	9	3
3		Lassi	25	4	2
4		Dahi	25	4	2
5		Ice-cream	25	5	2
6		Rasmalai	25	-	-
Total			**200**	**29**	**13**

Table.7 Prevalence of *L. monocytogenes* in different samples of cattle source from Aizawl (Mizoram) district (n=200)

Sl. No.	State	Type of sample	Number of samples analyzed	Number of sample positive for *L. monocytogenes*	% prevalence of *L. monocytogenes*
1	Aizawl (Mizoram)	Cattle faeces	50	4	8.00
2		Raw milk	50	3	6.00
3		Lassi	25	2	8.00
4		Dahi	25	2	8.00
5		Ice-cream	25	2	8.00
6		Rasmalai	25	0	0.00
Total			**200**	**13**	**6.50**

Table.8 Season wise prevalence of *L. monocytogenes* isolated from different samples of cattle source from Aizawl (Mizoram) district

Sl. No.	State	Type of sample	Number of sample tested in winter	Number of sample tested in winter	Seasonal distribution of *L. monocytogenes*	
					Prevalence in summer	Prevalence in winter
1	Aizawl (Mizoram)	Cattle faeces	25	25	1 (4.00%)	3 (12.00%)
2		Raw milk	25	25	1 (4.00%)	2 (8.00%)
3		Lassi	13	12	1 (7.69%)	1 (8.33%)
4		Dahi	13	12	1 (7.69%)	1 (8.33%)
5		Ice-cream	12	13	1 (8.33%)	1 (7.69%)
6		Rasmalai	13	12	-	-
Total			**101**	**99**	**5 (4.95%)**	**8 (8.08%)**

Table.9 Antibiotic Sensitivity and Resistance pattern of *L. monocytogenes* isolated from different samples of cattle source of Aizawl (Mizoram) district

Sl. No.	Antimicrobial agent	No. of isolates	*L. monocytogenes* isolated from cattle faeces					
			Sensitive (%)		Intermediate (%)		Resistance (%)	
1	Penicillin G (P)	13	13	100	-	-	-	-
2	Ampicillin (AMP)	13	13	100	-	-	-	-
3	Oxacillin (OX)	13	13	100	-	-	-	-
4	Streptomycin (HLS)	13	11	84.61	2	15.38	-	-
5	Erythromycin (E)	13	-	-	1	7.69	12	92.30
6	Cephotaxime / Clavulanic acid (CEC)	13	13	100	-	-	-	-
7	Ceftriaxone (CTR)	13	6	46.15	-	-	7	53.84
8	Chloramphenicol (C)	13	7	53.84	2	15.38	4	30.76
9	Ciprofloxacin (CIP)	13	13	100	-	-	-	-
10	Gentamicin (GEN)	13	7	53.84	-	-	6	46.15
11	Tetracycline (TE)	13	13	100	-	-	-	-
12	Trimethoprim/Sulpha methoxazole (COT)	13	13	100	-	-	-	-

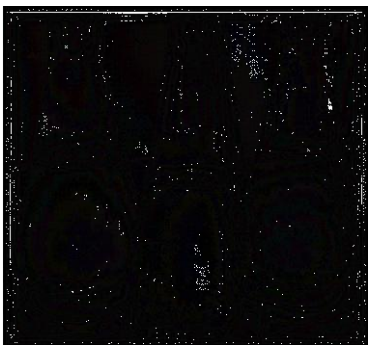

Figure 1: Milk products with UVM -I

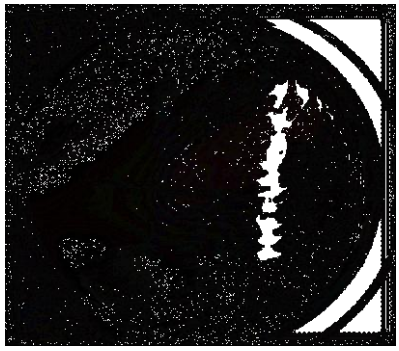

Figure 2: *L. monocytogenes* on PALCAM agar

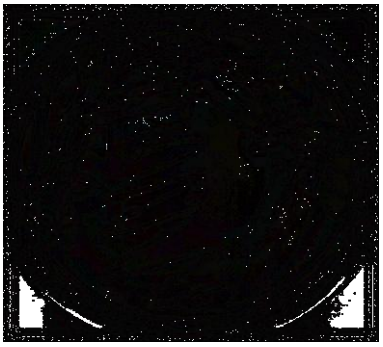

Figure 3: *L. monocytogenes* on McBride agar

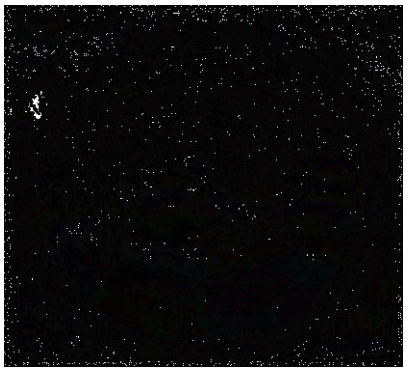

Figure 4: *L. monocytogenes* on TSYEA agar

Figure 5: *L. monocytogenes* showing gram staining positive

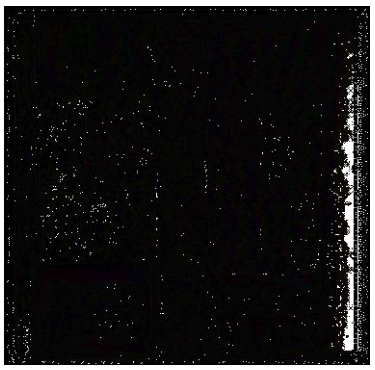

Figure 6: *L. monocytogenes* showing oxidase negative and Catalase positive

Figure 7: *L. monocytogenes* showing umbrella shaped growth in Listeria motility

Figur e 8: IMVIC test showing MR and VP +ve for *L. monocytogenes*

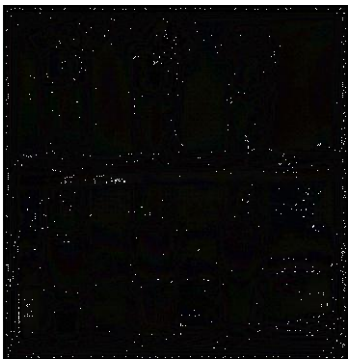

Figure 9: Sugar fermentation tests for *L. monocytogenes* (Rhamnose +ve, Xylose and Mannitol – ve)

Figure 10: ***L. monocytogenes*** **showing weak ? -haemolysis on SBA**

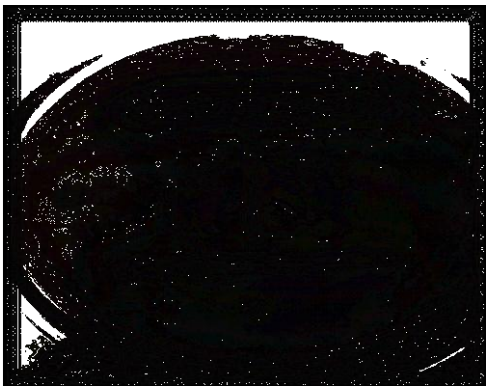

Figure 11: ***L. monocytogenes*** **showing positivity in CAMP test**

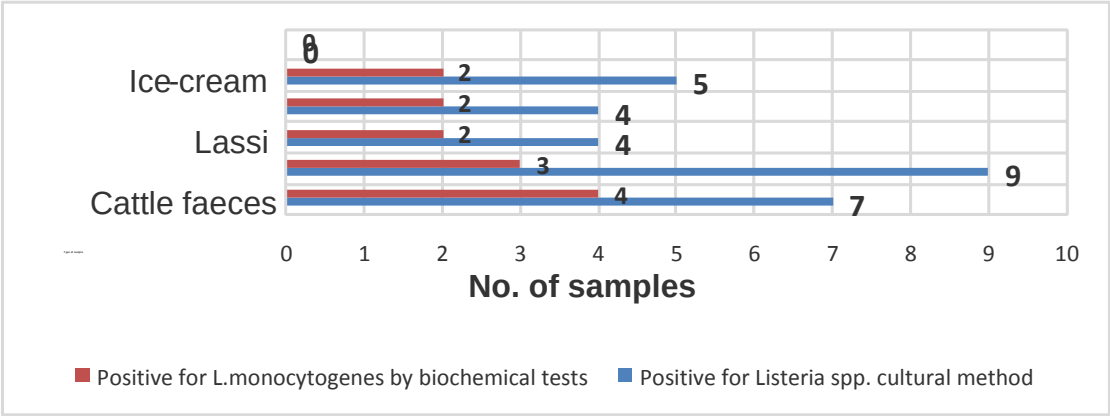

Figure 12: Detection of ***Listeria monocytogenes*** **from different samples of cattle source from Aizawl (Mizoram) district by cultural method (n=200)**

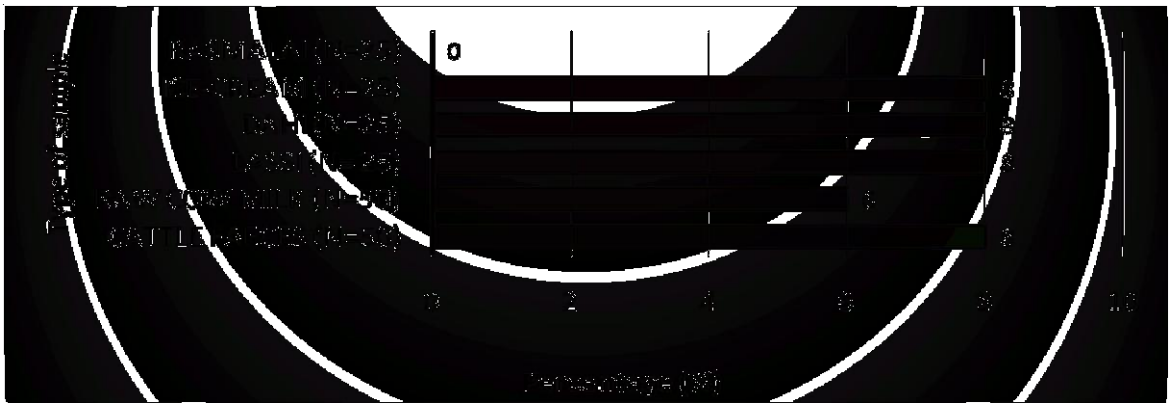

Figure -13 Prevalence of ***L. monocytogenes*** **in different samples of cattle source from Aizawl (Mizoram) district (n=200)**

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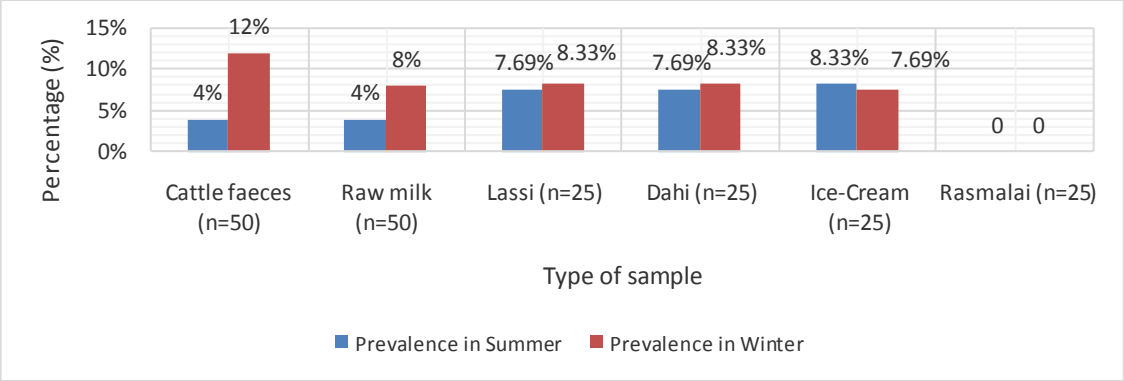

Figure 14: Season wise prevalence of *L. monocytogenes* isolated from different samples of cattle source from Aizawl (Mizoram) district (n=200)

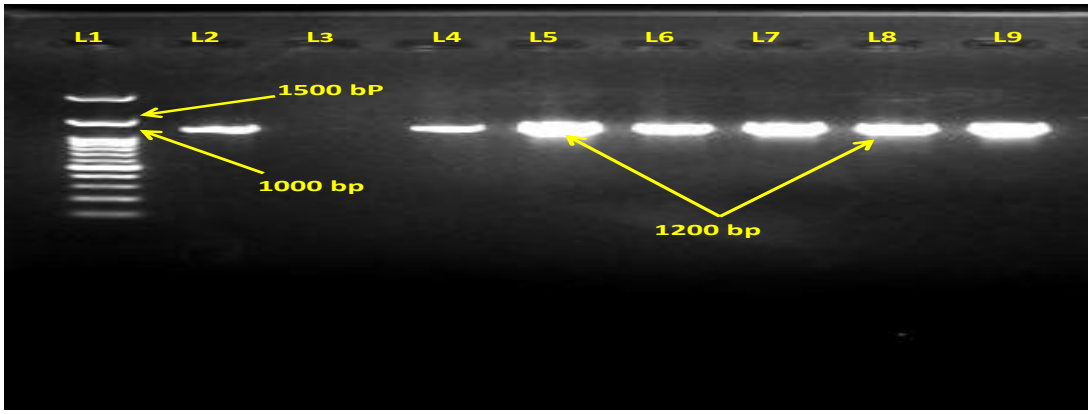

Figure-15: Agarose gel electrophoresis showing the PCR amplicons of *16S-rRNA* gene (1200bp) obtained from *L. monocytogenes* strains; L1: 3000 bp DNA ladder; L2: Positive control; L3: Negative control; L4 to L9: Representative samples

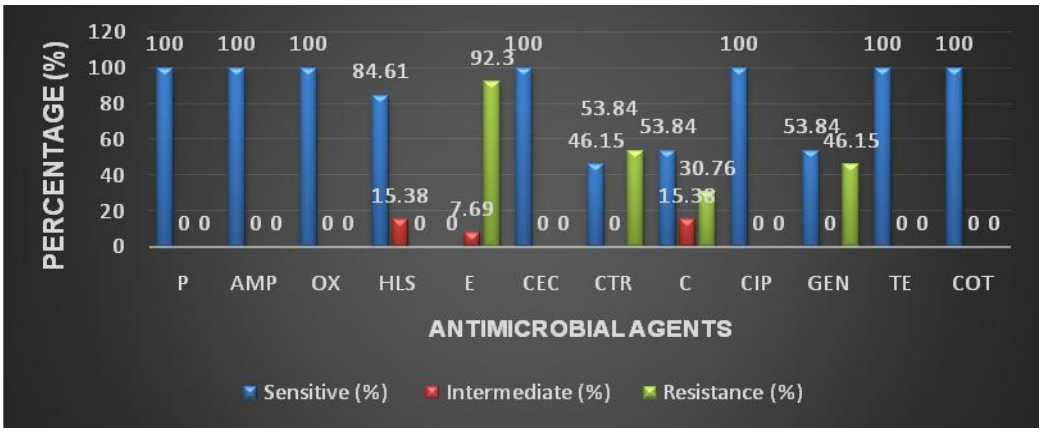

Figure 16: Antibiotic sensitivity and resistance pattern of *L. monocytogenes* isolated from different samples of cattle source of Aizawl (Mizoram) district

Listeria is a widely distributed bacterium in nature and commonly found in soil, sewage, dust, water and causes listeriosis in humans and animals (Norton *et al.*, 2001). Of the various milk pathogens, *L. monocytogenes* is one of the deadly organisms which occurs largely in all types of environment, including foods grown in contaminated environment, poorly processed/stored food, milk and associated products (Priyanka and Alka, 2008). The study of incidence of *Listeria* spp. in cattle faeces, milk and milk products in their selling units provide information about the carrier status in cattle and contamination status of the milk and milk products.

The milk producing and processing environment and handling practices may vary place to place and production practices.

There are chances of increase in cross contamination as 47 per cent of surface of hand of the food handlers and 16 per cent on the processing tables were found to carry *L. monocytogenes* (Kerr *et al.,* 1993; Jayasekaran *et al.,* 1996). The presence of *Listeria* spp. particularly *L. monocytogenes* in ready to eat milk products like dahi, lassi, ice cream and raw milk could be a major food safety issue for consumers as *L. monocytogenes* should be absent in RTE foods (US-FDA) (Fusch *et al.,* 1992).

Detection of species specific gene (*16S-rRNA*) of *L. monocytogenes* in different samples of cattle source

The culturally, phenotypically and biochemically positive 13 numbers of *L. monocytogenes* isolates were subjected for *16S-rRNA* species specific gene amplification using the standardized PCR protocol by using published primer. All the 13 numbers of *L. monocytogenes* strains isolated from Aizawl districts of Mizoram were positive for *16S-rRNA* gene (Figure 15).

Antibiotic sensitivity pattern of *L. monocytogenes*

All the 13 *L. monocytogenes* strains showed 100 percent sensitivity towards Penicillin, Ampicillin, Oxacillin, Cephotaxime/ Clavulanic acid, Ciprofloxacin, Tetracycline and Trimethoprim/ Sulphamethoxazole followed by Streptomycin (84.61%), Chloramphenicol (53.84%), Gentamicin (53.84%) and Ceftriaxone (46.15%). Conversely the *L. monocytogenes* strains showed highest resistance to Erythromycin (92.30%), Ceftriaxone (53.84%), Gentamicin (46.15%) and Chloramphenicol (30.76%), respectively (Table 9 and Figure 16).

There is growing concern of bacterial adaptation and evolution resulting in the emergence of antimicrobial resistant bacteria pathogens since last 50 years. The prevalence of antimicrobial resistance among food borne pathogens has increased during recent decades (Akbar and Anal, 2014). The frequent and unnecessary use of antimicrobial agents in food animals for therapeutic and prophylactic purposes in animals are contributing to create resistant strains. Animal origin foods are the major sources of transmission of antimicrobial resistant organisms to human. The antimicrobial resistant bacteria from food animals may colonize the human population via food chain, contact through occupational exposure or waste run off from animal production facilities. Resistant bacteria may readily transferred from food animals to human beings as the similar kind of antimicrobial agents are used in human practice also, therefore the detection of antimicrobial resistance pattern is a matter of public health significance. Sharma *et al.,* (2012) detected 80-90 percent resistance of *L. monocytogenes* strains from raw milk of Meerut and Babugarh Cantt, Hapur (India) to Nalidixic acid, Amoxycillin + Sulbactum, Vancomycin,

Kanamycin, Cloxacillin, and Erythromicin whereas many were susceptible to the Ampicillin, Ofloxacin, Tetracycline, Streptomycin, Sulphafurazole, Oxacilin and Ciprofloxacin.

The findings of Sharma *et al.,* (2017) is alarming as they recently isolated Multi Drug Resistant (MDR) strains of *L. monocytogenes* from raw milk in Rajasthan and emphasized on the need of awareness among consumers.

Implementation of food safety regulations at different levels of milk production has come up as a great public health issue.

The present study detected the *L. monocytogenes*, a major zoonotic pathogen causing fatal infections in human by conventional and molecular detection methods in different samples of cattle sources namely faeces, raw milk and milk products in the study area indicating the public health significance of the pathogen.

The presence of the organism in cattle faeces indicated the carrier status and the presence in raw milk and refrigerated milk products produced locally and sold in local markets under unhygienic condition is alarming public health threat to the consumers.

The well-cooked milk product (Rasmalai) which is stored for a short period of time has been found to be free from *L. monocytogenes.*

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