LISTERIA SPECIES OF PUBLIC HEALTH SIGNIFICANCE AMONG BUFFALOES SLAUGHTERED IN NAGPUR CITY OF CENTRAL INDIA

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Abstract- Occurrence of *Listeria* spp. of public health significance including the pathogen *Listeria monocytogenes* was studied among the slaughtered buffaloes in the Nagpur City of central India. The overall prevalence of *Listeria* spp. was 11% with 6 per cent for *L. monocytogenes*, 3%for *L. seeligeri* and 2% for *L. welshimeri*. The antibiotic sensitivity of the isolates revealed Ampicillin, Gentamicin and Penicillin as the treatment of choice for most manifestations of listeriosis. Overall prevalence of 6% pathogenic, multidrug resistant *L. monocytogenes* among the slaughtered buffaloes could be a matter of concern from a public health point of view.

Key Words- *Listeria monocytogenes*, Buffaloes

I. INTRODUCTION

India has the largest livestock population in the world and ranks first in world buffalo population i.e. 94.1 million, 56.5% of world buffalo population. It is the 5th largest exporter of buffalo meat in the world. Though there was a growth of 7.5% of buffalo livestock during the previous five years, the slaughter rate for buffaloes has been reported to be 41% (FAO, 2010). According to the Agricultural Products Export Development Authority of India (APEDA, 2010) the export of buffalo meat is approximately 495,020 MT which is the highest among all animal products that are exported from India. Indian buffalo meat is witnessing strong demand in International markets due to its lean characteristic and its near organic nature.

Meat has been reported as the vector for a number of foodborne infections in humans. Amongst these, listeriosis is recognized as an important foodborne bacterial infection and a nagging public health hazard (Farber and Peterkin, 1991). Among the different species, *L. monocytogenes* is a pathogen in humans and animals. Listeriosis a serious invasive disease that leads to septicemia, abortion, stillbirth, meningitis and meningoencephalitis, especially in people with a compromised immune response (Churchill *et al*., 2005). A number of reports have indicated the occurrence of the organism in various meat and meat products with overall incidence rate varying from 0 to 92 percent (Farber and Peterkin, 1991). The pathogen is well known for its zoonotic nature, its potential of exchange among animals and humans has been demonstrated through molecular characterization studies using pulse field gel electrophoresis (PFGE) (Parihar *et al*., 2004). To ensure public safety, all strains of *L. monocytogenes* are regarded as potentially pathogenic. Listeriosis occurs in sporadic and epidemic forms throughout the world; has emerged to be more important but reported less frequently in developing countries (WHO, 1988), including India. The present study was done to provide an understanding of the prevalence and antibiotic resistance of *L. monocytogenes* and other *Listeria* species from meat and blood samples of buffaloes slaughtered in Nagpur region of central India.

II. MATERIALS AND METHODS

A total of 200 samples (100 each of meat and blood) were collected from 100 buffaloes slaughtered at Municipal corporation slaughterhouse, of Nagpur city located at Central India and processed for isolation and identification of *Listeria* species according to the United States Food and Drug Administration, (USDA) (Lee and McClain, 1986). A two step enrichment in University of Vermont 1 (UVM-I) and UVM-II followed by selective plating on Polymixin-Acriflavin-Lithium-chloride Ceftazidime Aesculin-Mannitol (PALCAM) agar was done to isolate strains. Typical greenish-yellow, glistening, iridescent and pointed colonies of about 0.5 mm

diameter surrounded by a diffuse black zone of aesculin hydrolysis were subjected further to cultural and biochemical characterization *viz*., characteristic tumbling motility at 25°C, catalase, oxidase, MR-VP , nitrate reduction, fermentation pattern of α-methyl D-mannopyranoside, L-rhamnose, D-xylose and CAMP test as per ISO (1996). The isolates of *Listeria* were subjected to antibiogram by agar disc diffusion method using single antibiotic disc against twelve antibiotics ie. ampicillin, cephotaxime, cloxacillin, erythromycin, gentamicin, kanamycin, nalidixic acid, norfloxacin, penicillin-G, rifampicin, tetracycline and trimethoprim.

III. RESULTS AND DISCUSSION

Of the 200 samples (100 each of meat and blood) subjected for isolation studies, 24 (24%) and 19 (19%) from meat and blood respectively exhibited typical colonies on PALCAM plates (Plate1). Further processing revealed Gram positive, coccobacillary morphology by 17 of meat (17%) and 11 of blood (11%). When these were subjected to tumbling motility at 25°C, 11 of meat (11%) and 4 of blood (4%) isolates were positive. These isolates were further subjected to catalase and oxidase tests wherein seven (7%) and four (4%) isolates from meat and blood respectively were catalase positive and oxidase negative. The results of sugars fermentation L-rhamnose (+ve), D-xylose (-ve) (+ve in case of two isolates from meat) and α-methyl d-mannoside (+ve), confirmed four (4%) isolates from meat and two (2%) isolates from blood as *L. monocytogenes*.

Plate1: Colonies of Listeria on PALCAM agar

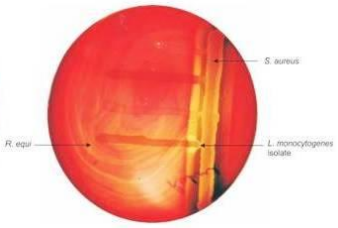

Plate 2: CAMP test

An isolate from meat and two isolates from blood were negative for L- rhamnose and α- methyl D-mannoside and positive for D-xylose fermentation were designated as *L. seeligeri.* Similarly two isolates from meat that were negative for L-rhamnose and α- methyl D-mannoside fermentation and fermented D- xylose were designated as *L. welshimeri*.

These 11 isolates (6 *L. monocytogenes*, 3 *L. seeligeri* and 2 *L. welshimeri*) were subjected to Christie Atkins and Munch-Petersen (CAMP) test for validation. The six isolates of *L. monocytogenes* including four from meat and two from blood showed zones of haemolysis with the standard strain of *S. aureus* (MTCC 3160) and accordingly were validated *as L. monocytogenes* (Plate 2).

The isolates designated as *L. seeligeri* revealed weak zone with *S. aureus* whereas *L. welshimeri* were CAMP negative. Thus a prevalence of *Listeria* species from slaughtered buffaloes in present study was reported to be 11%, revealing highest prevalence of *L. monocytogenes* six (6%), followed by *L. seeligeri* three (3%) and two (2%) for *L. welshimeri* (Fig 1).

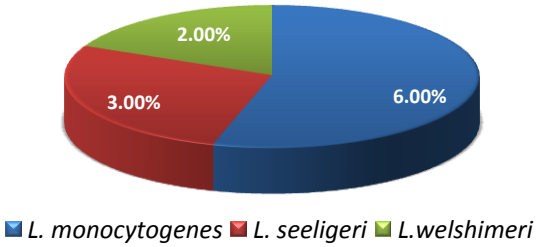

Figure 1. Overall prevalence of *Listeria* species among slaughtered buffaloes

Findings on overall prevalence of 11% *Listeria* spp. with 6%for *L. monocytogenes*, 3%for *L. seeligeri* and 2%for *L. welshimeri* among slaughtered buffaloes could be compared with the studies in India like findings of Brahmbhatt *et al.* (1994) who reported prevalence of *L. monocytogenes* in buffalo beef to the tune of 6%analysed from Gujarat region , Chaudhari *et al.* (2004) who reported 4.3% meat samples positive for *L. monocytogenes* obtained from buffaloes slaughtered at municipal slaughter house Bareilly (U.P). The study is also in accordance with Nayak *et al.* (2010) who analysed buffalo meat samples

obtained from retail meat market at Anand, Gujarat and recorded the prevalence of *L. monocytogenes* as 2.7 per cent. However, the findings of the present study deviate from those reported by Katre *et al.* (2009) and Zade and Karpe (2010). Katre *et al.* (2009) screened a total of 600 samples (100 each of buffalo beef, chicken, chevon, pork, fish and milk) from Nagpur region. The workers reported nine isolates (1.5%) to be *Listeria* species with no prevalence of *L. monocytogenes*. Similarly Zade and Karpe (2010) analyzed a total of 2054 samples of foods of animal origin collected from Vidarbha region of Maharashtra, India including mutton (50), chevon (200), pork (200), beef (200), fish (348), raw milk (600), pasteurized milk (155), chicken (250) and egg (51) and reported 0.6%overall prevalence of *Listeria* spp. with no samples positive for presence of *L. monocytogenes*. The variation in the prevalence specifically among the studies in this region can be attributed to the difference in the media employed in the present study. Earlier workers used combination of UVM-I and II as selective broth followed by DRIA as plating media.

Highest degree of resistance by *Listeria* isolates was observed against nalidixic acid, kanamycin and trimethoprim (100% each), followed by cloxacillin (36.36%), rifampicin (27.27%), penicillin-G and norfloxacin (18.18% each), gentamicin, tetracycline and erythromycin (9.09% each). *Listeria* isolates were moderately sensitive to cephotaxime (45.45%) followed by cloxacillin and tetracycline (36.35%), erythromycin (18.18%), norfloxacin and rifampicin (9.09% each) ; whereas, highest degree of sensitivity was observed towards ampicillin (100%); followed by gentamicin (90.90%), penicillin-G (81.81%), erythromycin and norfloxacin (72.72% each), rifampicin (63.63%), tetracycline and cephotaxime (54.54 each %) and cloxacillin (27.27%) (Table 1). In the present study six *L. monocytogenes* isolates revealed resistance towards kanamycin, nalidixic acid and trimethoprim (each); followed by cloxacillin and norfloxacin (33.33% each); penicillin-G and rifampicin (16.66%). The moderate degree of sensitivity was revealed towards cephotaxime, cloxacillin and tetracycline (33.33%) each and erythromycin (16.66%). Highest degree of sensitivity was recorded towards ampicillin (100%); followed by gentamicin (90.90%); Erythromycin, Penicillin-G and rifampicin (83.33%) each; cephotaxime, norfloxacin, and tetracycline (66.66%) each and cloxacillin (33.33%) (Fig 2).

Table 1. Overall antibiotic sensitivity pattern of isolates of *Listeria*

Sr. No.	Antibiotics	No. of Isolates			Percentage		
		R	MS	S	R	MS	S
1	Ampicillin	00	00	11	00	00	100
2	Cephotaxime	00	05	06	00	45.45	54.54
3	Cloxacillin	04	04	03	36.36	36.36	27.27
4	Erythromycin	01	02	08	9.09	18.18	72.72
5	Gentamicin	01	00	10	9.09	00	90.90
6	Kanamycin	11	00	00	100	00	00
7	Nalidixic acid	11	00	00	100	00	00
8	Norfloxacin	02	01	08	18.18	9.09	72.72
9	Penicillin-G	02	00	09	18.18	00	81.81
10	Rifampicin	03	01	07	27.27	9.09	63.63
11	Tetracycline	01	04	06	9.09	36.36	54.54
12	Trimethoprim	11	00	00	100	00	00

(S: Sensitive, MS: Moderately Sensitive, R: Resistant)

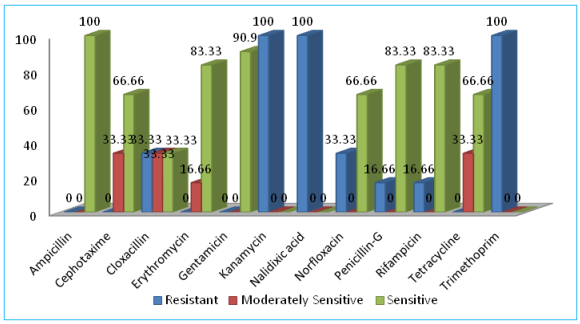

Fig 2.Antibiotic sensitivity pattern of isolates of *Listeria monocytogenes*

Ampicillin, gentamicin and penicillin remain the treatment of choice for most manifestations of listeriosis. In general, most *L. monocytogenes* isolated in this study were susceptible to the antibiotics used in veterinary and human listeriosis treatment.

Considering that *L. monocytogenes* is slowly becoming antibiotic resistant by acquisition of known antibiotic resistance genes from Gram positive bacteria, a continued surveillance of emerging antimicrobial resistance of this pathogen is important to ensure effective treatment of listeriosis. The data can be used to improve

background data on antibiotic resistance of strains isolated from foods of animal origin and for epidemiological and public health studies with respect to *L. monocytogenes*. The further investigation can be projected for evaluation of drug resistance plasmid among the *Listeria* and distribution of these strains among human and animal population by dendrogram study.

IV. CONCLUSION

In present investigation prevalence of 6% pathogenic, multidrug resistant *L. monocytogenes* among the slaughtered buffaloes is a matter of concern from public health point of view.

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