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ANTIBIOTIC STUDY OF *LISTERIA MONOCYTOGENES* FROM DIFFERENT FOOD SOURCES OF ANDHRA PRADESH

D. Siva Mallika and K. Kasturi*

Department of Biotechnology, Acharya Nagarjuna University, Guntur.

***Corresponding Author: K. Kasturi**
Department of Biotechnology, Acharya Nagarjuna University, Guntur.

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ABSTRACT
The isolated Listeria monocytogenes (n= 128) isolated from food were evaluated for antimicrobial rresistance. All 128 L. monocytogenes isolates were more resistant to different antimicrobial combinations. But most of the isolates were commonly showed resistance to penicillin, ampicillin and chloramphenicol. All these isolates were screened for presence of resistance gene (pen A, amp C and cml A) and out of 128, 54 isolates showed the amplification of pen A (500 bp) gene, 21 isolates showed amp C (550 bp) and 13 showed cml A (698 bp). As India adopting the international food habits the incidence of Listeria sps has been increasing. Because of no proper diagnosis, now a day's antibiotic resistance increased.

KEYWORDS: Antibiotic resistance, *Listeria monocytogenes*, penicillin resistance.

INTRODUCTION
Listeria is widely present in nature, having been isolated from the different food sources likely, Dairy Products, Street vended food and Refrigerated ready to eat food from Andhra Pradesh.[1] Six *Listeria species* are known to exist in two closely related but distinct lines of descent[2,3] from a medical point of view; *L. monocytogenes* is the life-threatening *Listeria species*, causing a wide spectrum of clinical symptoms in humans, summarized as listeriosis. Clinical symptoms range from mild influenza-like illness to meningitis, frequently accompanied by septicemia, and meningoencephalitis.[1] Strains of about all *Listeria species* are expended through food, even from pasteurized milk and water, regularly in substantial sums, and pathogenic properties have been found in `apathogenic' listeria too.[4,5] Treatment to this opportunistic bacterium may become a problem, because of showing low virulence and are naturally resistant to numerous antibiotics. The antimicrobial susceptibilities of *Listeria spp.*, even for *L. monocytogenes*, little data is accessible on including natural susceptibility patterns. The aim of the present study was to access the susceptibility of Listeria monocytogenes to a wide range of antibiotics.

METHODOLOGY
Isolation of *Listeria monocytogenes* from different food sources was done USDA method which is a double enrichment method for selective isolation of *Listeria sps.* Pure colonies from the PALCAM® Agar were inoculated into BHI broth to access the antibiotic resistance. The isolates, which show at least three virulence genes, were subjected to antibiotic susceptibility by disc diffusion method.

Antibiotic Susceptibility
Fresh bacterial colonies of *L. Monocytogenes* were isolated and grown on BHI broth at 37^{o}C for 24 hours and each inoculum was applied on Muller Hinton (MH) agar and disc diffusion method was used for determination of antibiotic susceptibility. Standard discs were applied using a disc dispenser and the plates were incubated at 37°C for 24 h to 48 h. Then the size of inhibition zone was determined as previously described.[6] The Antibiotics tested were selected considering the most frequently used in treatment, those used to prevent and control of human infections.[7,8] List of antibiotics was given in table 1. Based on the results, isolates were subjected to DNA isolation by the snap chilled method and gone for amplification of Antibiotic resistance genes likely *pen A*, *amp C* and *cml A*. Primer sequences were given in table 2.

PCR Conditions for the Amplification of Antibiotic-Resistant Genes
The antimicrobial resistance present in *L. monocytogenes* encoding for penicillin-binding protein gene (pen A), β-lactamases- ampicillin resistant gene (amp C) and Chloramphenicol resistant protein (cml A). The DNA thermal cycler with preheated lid was used for amplification of target genes for all isolates was performed and the reaction mixture (50 µL total volume) consisted of 10.0 µl of 10X master mix PCR buffer

(Emerald Amp® GT PCR master mix which contains dNTPs, Tag Polymerase and PCR Buffer), and 1 µmol l-1 forward and reverse primers of each, 5 µl of cell lysate and sterilized milliQ water to make up the reaction volume. Samples were subjected to PCR amplification. Pre-incubation was at 94°C for 4 min. Thirty PCR cycles were run under the following conditions: denaturation at 94°C for 45 sec, primer annealing at an optimum temperature for 45 sec, and DNA extension at 72°C for 45 sec in each cycle. After the last cycle, PCR tubes were incubated for 7 min at 72°C and then at 4°C. The amplified product was further confirmed by running agarose gel electrophoresis.[9]

RESULTS

Antimicrobial susceptibility test using Disc Diffusion method Isolates of *Listeria monocytogenes* (n=128) from different food samples obtained in different places of Andhra Pradesh. 67 percent of isolates showed resistant to penicillin, ampicillin, and chloramphenicol [Photo 1] whereas the standard MTCC 1145 L. monoctyogenes was sensitive. 89 isolates showed complete resistance to penicillin and 54, 62 isolates resistance to ampicillin and chloramphenicol respectively. Isolates from refrigerated food were resistant to cephalosporins.

The occurrence of Antibiotic Resistant genes

The prevalence of antimicrobial resistance genes in *L. monocytogenes* isolated from different food sources was determined with primers shown in Table 2. PCR amplicons sequences were blasted in NCBI database and confirmed. Ten of 128 *L. monocytogenes* contains three antibiotic resistant genes. High frequency of pen A (42%) followed by amp C (16%) and cml A (10%) was found in *Listeria monocytogenes*. 89 isolates showed resistance to penicillin but only 24 isolates possess pen A genes, 62 isolates had shown resistance to chloramphenicol in disc diffusion method but only 13 isolates having the resistant gene and 54 isolates from different food samples had resistance to ampicillin and only 21 having the gene.

Table 1: Antibiogram against of *Listeria monocytogenes* isolates from different food sources.

Antibiotic Class	Antimicrobial agent	Disc content	No. of isolates that were susceptible (N= 128)		
			R	I	S
NAM Synthesis inhibitor	Fosfomycin (FO)	50 mcg	43	62	23
Glycopeptide	Vancomycin (VA)	30 mcg	58	67	3
1st Generation Penams	Penicillin G (P)	10 UI (6 mcg)	89	31	8
2nd Generation Penams	Cloxacillin (COX)	30 mcg	54	42	32
3rd Generation Penams	Amoxicillin (AMX)	30 mcg	39	24	65
	Ampicillin (AMP)	25 mcg	54	46	28
4th Generation Penams	Ticarcillin (TI)	75 mcg	39	46	43
	Piperacillin (PI)	75 mcg	35	48	45
1st Generation Cephems	Cefazolin (CZ)	30 mcg	52	44	32
	Cefelexin (CN)	30 mcg	49	45	34
2nd Generation Cephems	Cefaclor (CF)	30 mcg	56	42	30
	Cefotetan (CTN)	30 mcg	53	41	34
3rd Generation Cephems	Cefexime (CFM)	10 mcg	58	24	34
	Cefotaxime (CTX)	10 mcg	42	51	35
4th Generation Cephems	Cefepime (CPM)	50 mcg	37	62	29
aminoglycoside antibiotic	Gentamicin (GEN)	30 mcg	48	34	46
Protein synthesis inhibitors	Chloramphenicol (C)	25 mcg	62	39	27
Combinational	Amoxyclav (AMC) (Amoxicillin/ Clavulanic acid)	50/10 mcg	35	32	61

R: Resistant; I: Intermediate; S: Suspetable.

Table 2: Primers used for detection of genes encoding resistance to different antimicrobials in *L.monocytogenes* isolates.

Gene	Primer	Nucleotide sequence (5'–3')	(bp)	Reference
cmlA	Cml A- F	CCGCCACGGTGTTGTTGTTATC	698	Gebreyes and Altier, 2002
	Cml A -R	CACCTTGCCTGCCCATCATTAG		
penA	PenA- F	ATCGAACAGGCGACGATGTC	500	Antignac et al., 2001
	PenA -R	GATTAAGACGGTGTTTTACGG		
ampC	AmpC-F	TTCTATCAAMACTGGCARCC	550	Lanz et al., 2003
	AmpC-R	CCYTTTTATGTACCCAYGA		

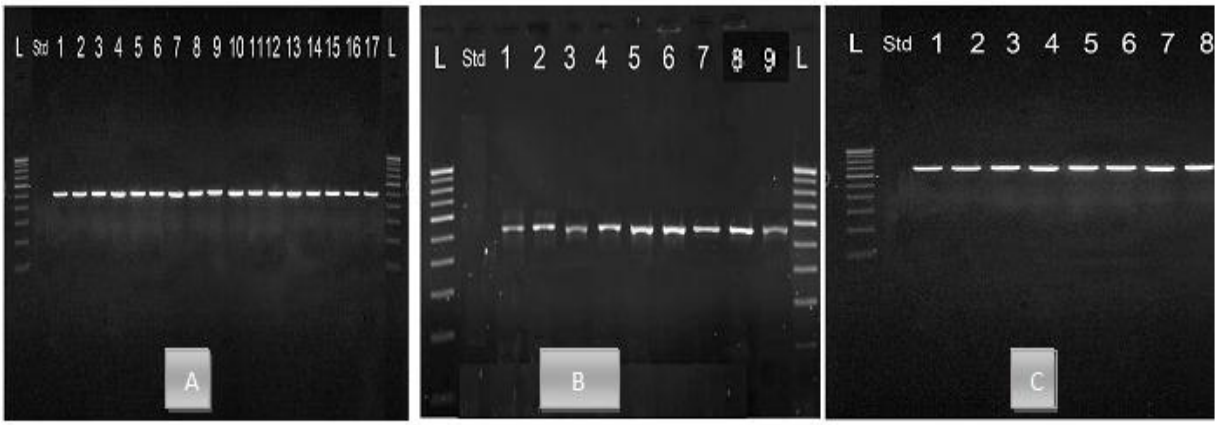

Photo 1: Amplification of Antibiotic resistance genes from different food isolates of *L.monocytogenes.*

*A.pen A (*500 bp); B. *amp C* (550 bp) and C. *cml A*(698 bp)

L stands for ladder DNA; STD – MTCC Standard L. monocytogenes; 1- 17 in A, 1-9 in B and 1-8 in C are isolates which shows amplification of antibiotic resistance gene.

DISCUSSION

In the present study, all isolates from food samples were subjected to wide range of antibiotics effective against gram-positive bacteria. The isolates which showed resistance to penicillin, ampicillin and chloramphenicol were subjected to DNA isolation and amplification with pen A, amp C and cml A gene. As India adopting an international practice of consuming processed foods, some of which are uncooked or undercooked. Such a tendency combined with the incidence of *L.monocytogenes* contamination reported food, thereby increasing the risk of listeriosis for consumers. Results of this study demonstrated that *L. monocytogenes* isolated from different food samples were resistant to a wide range of antimicrobials. Furthermore, most of the *L. monocytogenes* isolates in this study carried one or more antimicrobial resistance genes that may function as multidrug-resistant strains.

Resistance emerges from the use of antimicrobials in animals and subsequent transfer of antimicrobial resistant bacteria among animals and animal products.[10] Genetic methods may confirm the presence of specific genes conferring antimicrobial resistance; however, the presence of antimicrobial resistance genes alone does not necessarily imply resistance, as it is possible that resistance genes may not be expressed.[11] In the present study, all *L. monocytogenes* that carried antimicrobial resistance genes were also resistant to these antibiotic(s).

REFERENCES

1. Jones D, Seeliger HPR. The genus Listeria. In: Balows A, Truper HG, Dworkin M, Harder W, Schleifer KH, eds. (1992) The prokarotes. A handbook on the biology of bacteria: Ecophysiology, isolation, identi¢cation, applications. New York: Springer-Verlag, 1595 616.
2. Rocourt J, Wehmeyer U, Cossart P, Stackebrandt E. (1987) Proposal to retain Listeria murrayi and Listeria grayi in the genus Listeria. Int J. Syst Bacteriol, 37: 298-300.
3. Sallen B, Rajoharison A, Desvarenne S, Quinn F, Mabilat C. (1996) Comparative analysis of 16S and 23S rRNA sequences of Listeria species. Int J Syst Bacteriol, 46: 669-74.
4. Gouin E, Mengaud J, Cossart P. (1994) The virulence gene cluster of Listeria monocytogenes is also present in Listeria ivanovii, an animal pathogen, and Listeria seeligeri, a nonpathogenic species. Infect Immun, 62: 3550^3.
5. Leimeister-Wachter M, ChakrabortyT. (1989) Detection of listeriolysin, the thiol-dependent hemolysin in Listeria monocytogenes, Listeria ivanovii and Listeria seeligeri. Infect Immun, 57: 2350-7.
6. Soussy CJ. 2005. Edition de Janvier. Comite de L'antibiogramme de La Société Française de Microbiologie. Communiqué.
7. Wiggins GL, Albritton WL, Feeley JC. (1978) Antibiotic susceptibility of clinical isolates of Listeria monocytogenes. Antimicrob Agents Chemother, 13: 854–60.
8. Winslow DL, Pankey GA. (1982) In vitro activities of trimethoprim and sulfamethoxazole against Listeria monocytogenes. Antimicrob Agents Chemother, 22: 51–4.
9. Srinivasan. V, Nam H.M, Nguyen L.T., Tamilselvam B., Murinda S.E. and Oliver S.P. (2005) Prevalence of Antimicrobial Resistance Genes in *Listeria monocytogenes* Isolated from Dairy Farms, foodborne pathogens and disease, 2(3).
10. McEwen, S.A., and Fedorka-Cray P.J. (2002) Antimicrobial use and resistance in animals. Clin. Infect. Dis., 34: S93–106.
11. Michalova, E., P. Novotna, and J. Schlegelova. (2004) Tetracyclines in veterinary medicine and bacterial resistance to them. Vet. Med. Czech., 49: 79–100.