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The Determination of the Antimicrobial Susceptibility and Antimicrobial Resistance Gene Patterns in *L. monocytogenes*

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ABSTRACT

Background: Listeriosis, a fatal disease for vulnerable groups, has become common in the last decade due to extensive consumption of dairy and meat products. *Listeria monocytogenes* is an important foodborne opportunistic human pathogen and causal agent of listeriosis. There is a little information about the antibiotic susceptibility and resistance genes pattern of *L. monocytogenes*.
Methods: The present study aimed to evaluate the antibiotic susceptibility and resistance genes pattern of *L. monocytogenes* isolates from different clinical and environmental sources.
Results: The results showed that 88% of the isolates are resistant to streptomycin and 83% to TMP-SMX. Polymerase chain reaction (PCR) amplification of resistance genes showed that the prevalence of *ermA*, *ermB*, *strA*, *tetS*, *tetA*, and *ermC* genes in *L. monocytogenes* isolates were 0% (0/55), 10.9% (6/55), 78.81% (43/55), 0% (0/55), 27.27% (15/55), and 0% (0/55), respectively.
Conclusion: The resistance of the isolates to the antibiotics represents a potential public health risk and indicates the necessity of the bacteriological controls to reduce the contamination of the food samples.

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Introduction

The genus *Listeria* harbors Gram-positive and facultatively anaerobic species that are widely distributed in the environment and can be found in animal feces, soils, sewage, decaying vegetation, silage, and water. Moreover, they are frequently carried in the intestinal tract of humans and animals (1-5). Eight species are described in the *Listeria* genus including *L. innocua*, *L. rocourtiae*, *L. seeligeri*, *L. marthii*, *L. welshimeri*, *L. grayi*, *L. ivanovii*, and *L.monocytogenes* (6-8). However, only *L. ivanovii* and *L. monocytogenes* are considered to be pathogenic to humans and animals (9, 10). Human listeriosis is a disease caused by *L. monocytogenes* which is fatal in vulnerable groups such as pregnant women, immune-compromised individuals, elderly persons, and neonates. In spite of its low prevalence, the *L. monocytogenes* infections account for 28% of all deaths from foodborne illnesses and 4% of all hospitalizations. The relatively high case fatality rate of listeriosis (30%) makes it a serious foodborne and onward disease (9, 11). Over the last decades, several outbreaks of listeriosis have been reported all over the world including Canada, England, the USA, France, and other (12). The main transfer route of *L. monocytogenes* to human is through the environmental impurity of ready-to-eat (RTE) food products (13).

The administration of antimicrobial agents is the main treatment of listeriosis. Currently, the combination of ampicillin or penicillin G combined (or not) with an aminoglycoside is recommended treatment for listeriosis. In general, all antibiotics except cephalosporins and fosfomycin are effective on the most Listeria species. However, several studies reported the isolation of antimicrobial resistant strains from the environment, food, and human listeriosis (14-16). The concern for *L. monocytogenes* has increased because of the high prevalence of the resistant isolates to the clinically important antimicrobial agents (17-21). Researchers proposed that the use of antimicrobials in animals is the main reason for the antimicrobial resistance development in zoonotic bacteria. The antimicrobials are being used in food-producing animals not only for disease prophylaxis and therapy but also to increase animal growth and feed efficiency. Generally, the food-producing animals are carriers of many foodborne opportunistic bacteria and pathogens which these microorganisms can enter milk and meat products at milking, during slaughter, and even contaminate raw vegetables (22).

The genetic mechanisms behind these resistances are an interesting topic for researchers and clinicians. Some studies suggested that *L. monocytogenes* can acquire resistance genes from streptococci, staphylococci, and enterococci (23, 24). The objectives of the present study were to determine the antimicrobial susceptibility and antimicrobial resistance gene patterns in *L. monocytogenes* isolated from varied resources sources.

Materials and Methods

Bacterial isolates

In the present study, 55 *L. monocytogenes* isolates (food isolates, n=15; animal isolates, n=10; and clinical isolates, n=30) were obtained from Microbiology Department of Iran University of Medical Sciences, Tehran, Iran.

Enrichment, culturing, morphological and biochemical identification

The isolates were transferred to *Listeria* selective agar (Himedia, India) and PALKAM Agar (Merck, Germany) and then, plates were incubated at 37 °C for 24-48 h. The microbiological and biochemical tests, including gram staining, oxidase test, Christie Atkins

Munch Petersen (CAMP) test, catalase reaction, hemolysis on Sheep Blood Agar, Voges-Proskauer (MR-VP), fermentation of sugars (xylose, rhamnose, mannitol, and methyl α-D-mannopyranoside) and Methyl Red tests were used to verify the grown colonies.

Antimicrobial Susceptibility Test

The disk diffusion method (Kirby Bauer) was used to perform the antibiotic susceptibility test (13). After incubation, the turbidity of broth was adjusted with sterile saline to achieve turbidity comparable to 0.5 McFarland standards. Clinical and Laboratory Standards Institute (guidelines M45-A2) was used to interpret the obtained results. Four antibiotic disks, including tetracycline (25.0 µg), chloramphenicol (10.0 µg), streptomycin (10.0 µg), penicillin G (10U), ampicillin (10.0 µg), TMP-SMX (2.0 µg for TMP and 38.0 µg for SMX) and erythromycin (15.0 µg) (Himedia, India) were used for the disk diffusion method. *L. monocytogenes* ATCC 7644 was used as the reference strain. The quantity and the quality of the extracted DNA were determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Detection of the genes by PCR technique

The standard PCR assay was used to identify *ermA*, *ermB*, *strA*, *tetS*, *tetA* and *ermC* genes in *L. monocytogenes* isolates. The DNA extraction kit (Roche Co, New York, USA) was used to extract the genomic DNA according to the manufacturer's protocol for Gram-positive bacteria. The primers sequences and predicted sizes for PCR amplification of the genes are listed in Table 1.

The reaction mixture (25 µl total volume) consisted of 1µ of the prepared DNA (10 µg/ml), 13.3 µl sterile distilled water and 0.7 µl of 10 pmol/µL of each primer, 10 µL of 1X Master Mix (Ampliqon Co., Denmark).

A DNA thermal cycler (BioRad Laboratories, Pittsburgh, PA) was used to perform the PCR amplification according to the following protocol: initial denaturation for 4 min at 95 °C, 35 cycles of denaturation for 1 min at 94 °C, annealing at 55-60 °C for 45 s and extension at 72 °C for 20 s. PCR was ended with an extra extension cycle for 30 s at 72 °C to produce the complete products. No template control (NTC) was used as the negative control.

The amplified products were electrophoresed in 1.5 % agarose gel with a power of 100V for 80 min and the bands were visualized with a PCR products Gel Documentation system. The PCR product size was estimated based on a 100 bp plus DNA ladder (Fermentas, Waltham, Massachusetts, USA) as the DNA size reference marker.

Results

Antibiotic resistance profiles

The antibacterial susceptibility of *L. monocytogenes* isolates to the various antibiotics are listed in Table 2. The results showed that the isolates are highly susceptible to Ampicillin and Erythromycin with the susceptible percent of 85 and 62%, respectively. On the other hand, the isolates are resistant to Streptomycin and TMP-SMX with the resistivity percent of 7 and 11%, respectively.

PCR amplification of the genes

The results showed that the prevalence of *ermA*, *ermB*, *strA*, *tetS*, *tetA*, and *ermC* genes in *L. monocytogenes* isolates were 0% (0/55), 10.9% (6/55), 78.81% (43/55), 0% (0/55), 27.27% (15/55), and 0% (0/55), respectively (Table 3). The PCR-amplified DNA products of these genes are shown in Table 3.

Table 1. The primers used for detection of genes encoding resistance to different antimicrobials in *L. monocytogenes* isolates.

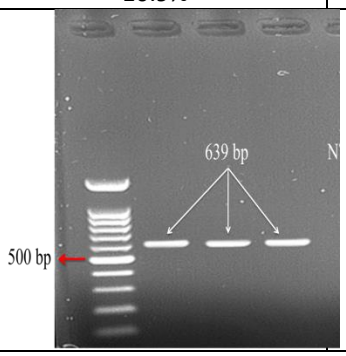
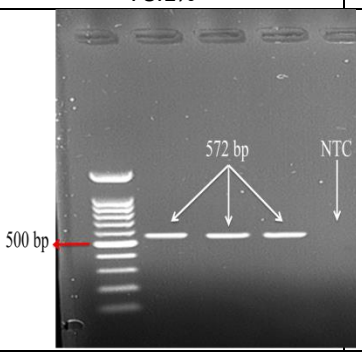
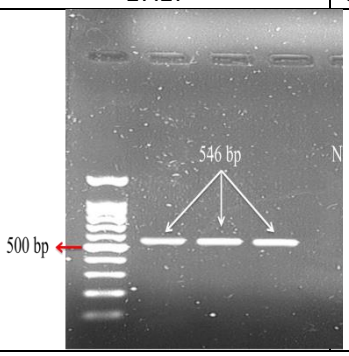
Specificity	Primer sequence (5′→3′)	Product size (bp)
ermA	F: TATCTTATCGTTGAGAAGGGATT R: CTACACTTGGCTTAGGATGAAA	139
ermB	F: GAAAAGGTACTCAACCAAATA R: AGTAACGGTACTTAAATTGTTTAC	639
strA	F: CTTGGTGATAACGGCAATTC R: CCAATCGCAGATAGAAGGC	572
tetS	F: TCCTTTGGGTAGTGGCATTC R: AAGCATTCGGAAATCTGCTG	420
tetA	F: GGCCTCAATTTCCTGACG R: AAGCAGGATGTAGCCTGTGC	546
ermC	F: CAAAACATAATATAGAT R: CTAATATTGTTTAAATCGTCAAT	641

Table 2. Susceptibility of *L. monocytogenes* Strains to 7 Antimicrobial Agents.*

Antibiotic	R %	I %	S %
Chloramphenicol	48	12	40
Penicillin G	40	12	28
Streptomycin	88	5	7
Tetracycline	33	11	56
TMP-SMX	83	6	11
Ampicillin	6	10	85
Erythromycin	33	5	62

* Abbreviations: I, intermediate resistance; R, resistant; and S, susceptible.

Table 3. The prevalence of *ermA*, *ermB*, *strA*, *tetS*, *tetA*, and *ermC* genes in *L. monocytogenes* isolates.

Loci	*ermA*	*ermB*	*strA*	*tetS*	*tetA*	*ermC*
	0	6	43	0	15	0
	0%	10.9%	78.1%	0%	27.27	0%
						

Discussion

L. monocytogenes is a food-borne pathogen widely distributed in the environment which causes life-threatening and severe infection mainly in high-risk groups of patients. *L. monocytogenes* is susceptible to a wide range of antibiotics except for fosfomycin and cephalosporins (3, 8). The gold standard treatment of listeriosis is the administration of penicillin G or ampicillin combined with an aminoglycoside, classically Gentamicin. In 1988, the first antibiotic resistant *L. monocytogenes*, resistant to >10µg/ml of tetracycline, was isolated in France (25). Since then, various studies reported the other resistant strains of *Listeria* spp. isolated from the environment, food or in sporadic cases of human listeriosis (26-29). Since *L. monocytogenes* has not been routinely isolated and reported in Iran and given the increasing number of resistant strains isolated worldwide, it is critical we obtain a wide understanding of the extent of antibiotic resistance and the resistance gene patterns of this pathogen. Accordingly, the aim of the present study was to characterize the antibiotic susceptibility profiles of *L. monocytogenes* isolated from food, animals and clinical samples, and the genetic mechanisms that confer resistance.

Based on our results the majority of the isolates belonged to serotypes 1/2c and 1/2a which can be attributed to better grow and survival of these serotypes, these results are in agreement with previous reports (30, 31). Based on the other studies, serotypes 4b, 1/2c, and 1/2a are responsible for more than 95% of human listeriosis cases (32). The isolation of these epidemiologically important serotypes from the different source indicates the wide distribution of these serotypes which are capable of causing disease.

The results of antibiotic resistance investigation showed that the presence of a high level of resistance to Streptomycin (88%) and TMP-SMX (83%) and a relatively high level of resistance to Chloramphenicol (48%) and Penicillin G (40%) in *L. monocytogenes* isolates. On the other hand, the isolates represented the acceptable

susceptibility to the currently used antibiotic such as Ampicillin (85%) and Erythromycin (62%) which indicated that there would not be any risk with using these traditional treatments. Charpentier et al. (23) reported multidrug resistance in *L. monocytogenes* isolates to gentamicin, chloramphenicol, kanamycin, erythromycin, rifampin, and streptomycin. In the present study, chloramphenicol, erythromycin, and streptomycin resistance were observed in 48, 33, and 88% of *L. monocytogenes*. Another study represented that only 1.02% of *L. monocytogenes* isolates were resistant to chloramphenicol and vancomycin (33).

These results revealed the emergence of *L. monocytogenes* from various sources that are resistant to one or more antibiotics. Moreover, the studied isolates were not resistant to Erythromycin, Ampicillin, and Tetracycline which are consistent with the results of the identification of the *tet*, *erm*, and *strA* resistance *L. monocytogenes*. Resistance to these antibiotics is relatively common in Europe and North America (1, 18, 33).

The PCR amplification was performed to recognize the resistance gene patterns *L. monocytogenes* isolates. The PCR results showed that *L. monocytogenes* isolates were positive for *ermB*, *strA*, and *tetA* virulence marker genes. *ermB* is responsible for a dimethylation of the adenine residue at position 2085 in 23S rRNA, which subsequently reduces the affinity between macrolide-lincosamide-streptogramin B anti-biotics and ribosomes. *strA* is an aminoglycoside O-phospho transferase which non-covalently but selectively interacts with ATP bonds a phosphate group to the antibiotic and performs its antibiotic resistance role. *tetA* is a metal-tetracycline/H+ antiporter which decreases the accumulation of the antibiotic in whole cells.

Conclusion

In the present study, the isolates represent a potential public health risk due to the presence of serogroup 1/2c and 1/2a as well as the virulence marker genes, which are involved in human listeriosis. The extent of the antibiotic resistance is due to the extensive use of the antibiotics in therapy and as a supplement in animal food. These data are applicable for public health and epidemiological studies of this pathogen.

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Conflict of interest

None declared.

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