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Antimicrobial resistance of *Listeria monocytogenes* isolated from seafood and humans in Iran

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ABSTRACT

Fourteen *Listeria monocytogenes* isolates previously collected from seafood (n = 7) and human patients (n = 7) were studied for their antimicrobial susceptibility against eight common antimicrobials (ampicillin, penicillin, gentamicin, streptomycin, tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol, and cefotaxime). A high resistance level to ampicillin, cefotaxime (100%), and pencillin (57% in seafood isolates and 71.4% in clinical isolates) was observed in this study. However, all of the isolates were susceptible to trimethoprim-sulfamethoxazole, chloramphenicol, and tetracycline. Simultaneous resistance was identified in 4 clinical isolates (57.1%). Genotypic characterization of fish isolates (isolated from three fish species) was performed by pulsed-field gel electrophoresis (PFGE). A high diversity among fish isolates was observed. PFGE analyses distinguished the 4 isolates into 4 reproducible pulsotypes. There was no correlation between the antibiograms with pulsotypes. In conclusion, the resistance of seafood isolates to the antibiotics commonly used to treat listeriosis could be a potential health hazard for consumers.

1. Introduction

Listeria monocytogenes is a foodborne pathogen that can contaminate food products during or after processing. The organism is well adapted to very different environmental conditions encountered in foods; it can grow at a wide range of pH values (4.1–9.6), low water activity (less than 0.9), and high salt concentrations (10%) [1]. This pathogen is responsible for human listeriosis, a severe infection that may result in meningitis, encephalitis, septicemia, or abortion, with a considerable mortality rate (10–40%) [2,3]. The outcome of listeriosis depends on the early administration of antibiotics having rapid and bactericidal activity against *L. monocytogenes*. Although almost all *Listeria* strains are susceptible to most of the common antibiotics, the cure rate is only ~70% [2,3].

During 1998–2008, twenty-four confirmed listeriosis outbreaks were reported in the United States [4]. The number of listeriosis cases in European Union increased 19.1% in 2009 (n = 1645) compared to 2008 and remained almost at the same level in 2010 (n = 1601) [5]. *L. monocytogenes* strains associated with outbreaks of listeriosis have been isolated in various kinds of food products including vegetable, raw meat, dairy and seafood products [6].

Currently, a combination of ampicillin or amoxicillin with gentamicin is the primary therapy for human listeriosis, whereas vancomycin, trimethoprim-sulfamethoxazole and erythromycin are regarded as second-choice drugs to treat pregnant women [3,6]. *L. monocytogenes* is usually susceptible to a wide range of antibiotics. For example, a comprehensive study on 4816 clinical *L. monocytogenes* isolates in France demonstrated that only 1.27% of human isolates were resistant [2]. However, a continuing increase of antibiotic resistance of *L. monocytogenes* has been reported [7] and it is not clear yet why antibiotic therapy fails in about >30% of cases.

Currently, pulsed field gel electrophoresis (PFGE) was used as the "gold standard" method for molecular subtyping of bacteria. PFGE shows a high level of sensitivity for discrimination of *L. monocytogenes* strains, and is often considered the current gold standard for discriminatory ability.

The levels of antibiotic resistance are influenced by antimicrobial usage and geographical differences [6,8]. Therefore, regular monitoring of the antibiotic susceptibility of *L. monocytogenes* in different geographical regions is important to ensure the effectiveness of antimicrobials for listeriosis treatment. To date, very limited data is available on the resistance patterns of *L. monocytogenes* in seafood products in Iran. Therefore, the purpose of this study was to determine the an-

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tibiotic resistance patterns of *L.* monocytogenes isolated from seafood products and to compare the resistance patterns with clinical isolates.

2. Material and methods

2.1. Bacterial isolates

A total of 14 *L. monocytogenes* isolates from seafood samples and clinical samples were characterized by antimicrobial susceptibility tests. *Staphylococcus aureus* and seven clinical *L. monocytogenes* isolates (isolated from blood and vaginal swabs by another research group) were obtained from the Microbiology Department of Iran University of Medical Sciences, Iran. The seafood isolates were isolated between October 2014 and August 2015 in Karaj and Tehran, Iran (n = 237). The standard strain of *L. monocytogenes* was obtained from Persian Type Culture Collection.

Table 1 shows the origin of the *L. monocytogenes* isolates used in this study. In order to confirm all *Listeria* isolates, *prs* and *inlA* primers were employed to detect *Listeria* spp. and *L. monocytogenes*, respectively (Table 2). PCR assays were conducted in a 25 μl reaction mixture using the previously described PCR conditions [9].

2.2. Antimicrobial susceptibility test

Antibiotic susceptibility of all *L. monocytogenes* isolates were assessed using the disc-diffusion method according to the Clinical and Laboratory Standards Institute on Mueller-Hinton agar (Liofilchem, Italy) + 5% (v/v) blood plates (CLSI, 2006). The plates were incubated at 37 °C for 18–20 h. The following discs were used: penicillins (ampicillin, 10 μg; penicillin, 10 μg), aminoglycosides (gentamicin, 10 μg; streptomycin, 10 μg); tetracyclines (tetracycline, 30 μg), sulfonamides (trimethoprim-sulfamethoxazole (SXT), 25 μg), phenicols (chloramphenicol, 30 μg); cephalosporins (cefotaxime 30 μg). The diameters of growth inhibitory zones were measured and interpreted according to the breakpoints recommended by the CLSI. The inhibition zones were classified as sensitive, intermediate susceptibility and resistant. *Staphylococcus aureus* ATCC 25923 was used as quality control strain.

2.3. Pulsed-field gel electrophoresis (PFGE)

PFGE of 4 fish isolates was performed using the standard CDC PulseNet Protocol [11]. Briefly, isolates were cultured on Brain Heart Infusion (Liofilchem, Italy) agar plates and incubated at 37 °C for 18 h. Bacterial cultures were embedded in 1% agarose plugs, lysed, washed, and digested with the restriction enzymes *AscI* and *ApaI* for at least 5 h at 37 °C and 30 °C, respectively. Size separation of DNA fragments was done in 1% agarose gels by using a CHEF-DR II apparatus (Bio-Rad Laboratories, Richmond, CA). The banding patterns were then interpreted and compared using the GelCompar II software (Applied Maths, Saint-Matins-Latem, Belgium) [11].

3. Results and discussion

In the current study, the food and clinical isolates were tested for the presence of *prs* and *inl*A genes. All food and clinical isolates were positive for *prs* and *inl*A genes.

Antibiotic resistance frequencies of *L. monocytogenes* isolates are shown in Table 3. Resistance to ampicillin and cefotaxime was the most common resistance phenotype and was detected in 100% of *L. monocytogenes* isolates.

The primary therapy for invasive listeriosis consists of supportive therapy along with penicillin or ampicillin in combination with gentamicin [3]. Vancomycin or trimethoprim/sulfamethoxazole can be used for patients who are allergic to pencillin. In this study, four

Table 1
Isolates of *L. monocytogenes* used in the antibiotic resistance study.

Source	Sample type	No. *L. monocytogenes*	*Ref.*
Seafood	Rainbow trout	1	This study
	Kilka fish	4	
	Tilapia	2	
Standard strain	Persian Type Culture Collection	1	PTCC collection, Iran
Clinical samples	Human samples	7	Previous study [10]

Table 2
List of primers used for confirmation of *L. monocytogenes*.

References	Target	Size of amplicon	Primer sequence (5′-3′)	*Name*
[35]	All *Listeria* spp.	370 bp	F:GCTGAAGAGATTGCGAAAGAAG R:CAAAGAAACCTTGGATTTGCGG	*Prs*
[36]	Internalin A	800 bp	F: ACGAGTAACGGGACAAATGC R: CCCGACAGTGGTGCTAGATT	*inlA*

Table 3
Percentages of resistance, intermediate susceptibility and sensitivity to the antimicrobials tested on the seafood and clinical isolates.

Antimicrobial class	Antimicrobial agent	Seafood isolates no. (%)			Clinical isolates no. (%)			Total no.(%)		
		S[a]	I	R	S	I	R	S	I	R
Pencillins	Pencillin	3(42.8)	–	4 (57)	2 (28.5)	–	5(71.4)	5(35.7)	0	9(64.2)
	Ampicillin	–	–	7 (100)	–	–	7(100)	0	0	14(100)
Aminoglycosides	Gentamicin	7(100)	–	–	5(71.4)	1(14.2)	1(14.2)	12 (85.7)	1 (7.1)	1(7.1)
	Streptomycin	7(100)	–	–	3(42.8)	–	4(57.1)	10 (71.4)	0	4(28.5)
Folate Pathway inhibitors	Trimethoprim-sulfamethoxazole	7(100)	–	–	7(100)	–	–	14 (100)	0	0
Cephems (parenteral)	Cefotaxime	–	–	7(100)	–	–	7(100)	0	0	14(100)
Phenicols	Chloramphenicol	7(100)	–	–	7(100)	–	–	14 (100)	0	0
Tetracyclines	Tetracycline	7(100)	–	–	7(100)	–	-	14 (100)	0	0

[a] S: sensitive, I: intermediate, R: resistant.

seafood strains (57%) and five clinical isolates (71.4%) were found to be resistant to pencillin. Issa et al. [12] found that most of the *L. monocytogenes* strains isolated from food were resistant to ampicillin and penicillin, whereas the other studies [13,14] reported a high susceptibility to these antibiotics. A high resistant to penicillin and ampicillin previously reported by Fallah et al. [15] in seafood products. The high levels of *L. monocytogenes* resistance to ampicillin and pencillin could be explained by the fact that they are the first antibiotic options, which are widely used in listeriosis treatment [8,16].

Resistance to trimethoprim/sulfamethoxazole, tetracycline and Chloramphenicol was not found in any *L. monocytogenes* isolates. These results concur with those found by Gomez, Azon [14] in meat isolates and Safdar and Armstrong [17] in human strains. In contrast, a high resistance level (57.1%) to streptomycin was observed among clinical isolates. The same result was reported by Srinivasan et al. [18] in dairy farm environments, however Sadeghi Kalani et al. [19] found no resistance.

A summarized list of *L. monocytogenes* antibiotic-resistant strains isolated from food products is given in Table 4. The levels of resistance vary and are influenced by antimicrobial use and geographical differences. Given the increasing number of antibiotic-resistant *L. monocytogenes* strains being isolated around the world, it seems that this pathogen rapidly acquire a wide variety of antibiotic resistance genes from the commensal bacteria found in foods and food processing environment [20].

Antibiotic resistance in *L. monocytogenes* is mostly caused by mobile genetic elements such as mobilizable plasmids, self-transferable plasmids, and conjugative transposons. In addition, existence efflux pumps have been reported in *Listeria* [20]. Therefore, factors which influence gene transfer may play an important role for acquisition of resistance genes in this pathogen [8]. For instance, the plasmid pIP501 which confers resistance to lincosamides phenicol, streptogramins, and macrolides, was firstly reported to be transferable by conjugation to *L. monocytogenes* as well as be transferable between species of *Listeria* [14]. Another plasmid, pAMβ1, conferring resistance to erythromycin, is transferable by conjugation from *Enterococcus faecalis* to *L. monocytogenes* [20].

For another example, Tn*916*, carrying the *tetM* tetracycline resistance gene, was firstly found in *E. faecalis*. *In vitro* and *in vivo* studies showed that Tn916 can transfer by conjugation of Tn*916* from *E. faeclis* to *Listeria* species and *L. monocytogenes* [21,22]. Bertrand et al. [23] demonstrated three *L. monocytogenes* strains and one *L. innocua* strain from 241 *Listeria* isolates were resistance to tetracycline due to the presence of the *tetM* gene.

Regarding simultaneous resistance, four clinical strains (57.1%) showed resistance to three antibiotics (penicillin, ampicillin, and streptomycin), two of which revealed resistance and intermediate susceptibility to gentamicin. Four seafood isolates (57.1%) demonstrated resistance to two antibiotics (penicillin and ampicillin). Comparing the results here with previous research, simultaneous resistance was also found in ready-to-eat food by Fallah et al. [24] who reported that 60.2% of *L. monocytogenes* strains isolated from poultry product samples were multi-resistant. Rodas-Suarez et al. [25] isolated 6% *L. monocytogenes* multi-resistant strains from oyster, fish, and seawater samples.

PFGE analyses distinguished the 4 isolates into 4 reproducible pulsotypes (Fig. 1). The genotyping data demonstrated that the *L. monocytogenes* strains isolated form rainbow trout, tilapia, and kilka

Table 4
Antibiotic-resistant *L. monocytogenes* isolated from food products.

Antibiotic	Sample	R[a](%)	Location	Reference
Ampicillin	Delicatessen products, Cakes	9.5%	Poland	[26]
	Milk and dairy products	26.3%	Iran	[27]
	Ready-to-eat food	20%	Italy	[28]
	Turkish meat	66%	Turkey	[29]
	Dairy products	60%	Lebanese	[30]
	Food	100%	Malaysia	[12]
	Poultry products	44.9	Iran	[24]
	Seafood products and processing environments	38.5%	Iran	[15]
	Seafood, beef, chicken, pork	3%	China	[6]
	Fish and seawater samples	23.7%	Mexico	[25]
Penicillin	Dairy products	90%	Lebanese	[30]
	Dairy products	32%	Iran	[27]
	Poultry products	41.8	Iran	[24]
	Seafood products and processing environments	38.1%	Iran	[15]
	Seafood, beef, chicken, pork	4.6%	China	[6]
	Fish and seawater samples	22.5%	Mexico	[25]
Gentamicin	Dairy products	7%	Lebanese	[30]
	Dairy products	5%	Iran	[27]
	Vegetables	32%	Africa	[31]
	poultry products	10.2%	Iran	[24]
	Bovine hides and carcasses	1.9%	Poland	[32]
	Seafood products and processing environments	0.7%	Iran	[15]
	Seafood, beef, chicken, pork	6%	china	[6]
	Fish and seawater samples	2.3%	Mexico	[25]
SXT[b]	Italian meat products	3%	Italy	[33]
	Dairy products	17%	Lebanese	[30]
	Food	39%	Malaysia	[12]
	Seafood products and processing environments	2.5%	Iran	[15]
	Fish and seawater samples	12.7%	Mexico	[25]
Tetracycline	Italian meat products	3%	Italy	[33]
	Dairy, fish, meat	6%	Canada	[34]
	Poultry products	34.7%	Iran	[24]
	Seafood products and processing environments	18.7%	Iran	[6]
Chloramphenicol	Vegetables	58%	Africa	[31]
	Poultry products	24.5%	Iran	[24]
	Seafood products and processing environments	3.24%	Iran	[15]
Vancomycin	Seafood products and processing environments	20.9%	Iran	[15]
	Seafood, beef, chicken, pork	5.4	China	[6]

[a] Resistant.

[b] Trimethoprim-sulfamethoxazole.

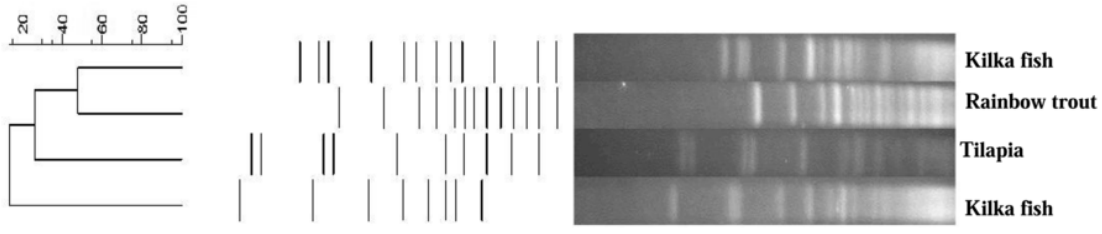

Fig. 1. PFGE patterns for *Listeria monocytogenes* isolated from three fish species.

fish were genetically diverse and heterogeneous. These genetically diverse patterns were showed previously in Miettinen and Wirtanen [37] study. There was no correlation between the antibiograms with pulsotypes. The lack of association could be due to the small sample size.

In conclusion, this report showed that raw seafood products in Iran were contaminated with *L. monocytogenes* and many food and clinical pathogens were resistant to common antibiotics. The resistance of the *L. monocytogenes* isolates to antibiotics commonly used to treat listeriosis is alarming and constitutes a serious hazard for public health. Hence, there is great need for a surveillance programs in Iran to monitor epidemiological information on the pathogen dispersion in different sources.

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