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Antibiotic Susceptibility Profiles of *Listeria monocytogenes* Strains Isolated from Food Products and Clinical Samples

Profiluri de rezistență ale unor tulpini de *Listeria monocytogenes* izolate din probe clinice și produse alimentare

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Abstract

Listeria monocytogenes has a ubiquitous distribution in nature and could contaminate food of animal origin, causing severe infections in humans. Till present, little is known about the antibiotic resistance profiles of these strains in Romania. The aim of this study was to determine the antibiotic susceptibility patterns of 37 *L. monocytogenes* strains isolated from animal derived foods and from clinical samples. Food samples were collected from meat and dairy products, between 2009 and 2013. Clinical samples were collected from patients with septicemia, meningitis/meningo-encephalitis, abortion cases and newborns, hospitalized during April 2010 - April 2013 in three medical institutions from Bucharest: Babes Hospital, Elias Hospital, National Institute of Infectious Diseases (INBI) Matei Bals. All tested isolates exhibited resistance to cephalosporins and nalidixic acid; one strain isolated from boiled shell snails was resistant to trimethoprim/sulfamethoxazole. The resistance to the first choice antibiotic ampicillin in *L. monocytogenes* strains isolated from severe infections is underlining the need of in vitro antibiotic susceptibility testing of each clinical isolate to establish the efficacy of different antibiotics, as well as of extended epidemiological studies to highlight the resistance profiles of *L. monocytogenes* strains circulating in our country.

Key words: *Listeria monocytogenes*, food, invasive infections, antibiotics, resistance

Rezumat

Listeria monocytogenes are o distribuție ubiquitară în natură și poate contamina produsele alimentare de origine animală, provocând infecții severe la om. Până în prezent există foarte puține date cu privire la profilurile de rezistență ale tulpinilor circulante în România. Scopul acestui studiu a fost determinarea pattern-urilor de rezistență la antibiotice ale unor tulpini de *L. monocytogenes* (n=37) izolate din produse alimentare de origine animală și din probe clinice. Probele din alimente (carne și produse lactate), au fost colectate în perioada 2009-

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2013. Probele clinice au fost recoltate de la pacienți cu septicemie, meningită/meningo-encefalită, cazuri de avort spontan și nou-născuți, spitalizați în perioada Aprilie 2010 - Aprilie 2013 în trei Instituții Medicale din București: Spitalul Elias, Spitalul Victor Babes și Institutul Național de Boli Infecțioase (INBI) Matei Bals. Toate tulpinile testate au prezentat rezistență la cefalosporine și acidul nalidixic; o tulpină izolată din melci fierți a fost rezistentă la Trimetoprim/sulfametoxazol. Rezistența unora dintre tulpinile analizate la ampicilina, antibiotic de elecție pentru terapia infecțiilor cauzate de *L. monocytogenes*, subliniază necesitatea testării *in vitro* a sensibilității la antibiotice a fiecărui izolat clinic pentru a stabili eficiența diferitelor antibiotice, precum și a unor studii epidemiologice extinse în scopul stabilirii profilurilor de rezistență ale tulpinilor de *L. monocytogenes* circulante în țara noastră.

Cuvinte cheie: *Listeria monocytogenes*, alimente, infecții invazive, antibiotice, rezistență

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Introduction

Listeria monocytogenes is the main pathogenic species of the respective genus, found both in humans and animals (1, 2). *L. monocytogenes* is a Gram-positive rod, capable of growing in conditions such as low temperature, acidic pH, high salt concentration, resistant to the procedures applied in the food industry to inactivate pathogenic microorganisms (3, 4).

L. monocytogenes is often implicated in human listeriosis, a severe disease which can determine septicemia, meningitis/meningo-encephalitis, abortion and baby illness at birth (5). Susceptible population groups include the elderly people, immunocompromised individuals, pregnant women and their fetuses or neonates (1). Consumption of food products contaminated with this microorganism is the main cause of human listeriosis.

Antimicrobial drugs are used routinely to treat listeriosis in humans and animals. For the therapy of listeriosis, the first choice antibiotic treatment is represented by ampicillin or a combination of ampicillin with an aminoglycoside such as gentamycin or streptomycin (6). In the last years, some studies have reported *L. monocytogenes* strains that showed multiple drug resistance to different antimicrobial substances, with significance for the public health, therefore requiring a rigorous monitoring of the antimicrobial susceptibility of *L. monocytogenes* strains (7-9).

The present study evaluates the susceptibility profiles of 37 *L. monocytogenes* strains, isolated from animal food products and clinical human samples in Romania, to 15 antibiotics used for the treatment of human and animal bacterial infections.

Materials and methods

Bacterial strains

Out of the total number of 37 *L. monocytogenes* tested strains, 19 were collected between 2009 and 2013 from various meat and dairy products, including raw minced meat (n=4), pork muscular tissue (n=1), beef muscular tissue (n=1), sausages (n=5), smoked bacon (n=2), bacon in processing (n=1), poultry carcass (n=1), chicken frankfurters (n=1), boiled shell snails (n=1) and cheese products (n=2). The rest of 18 *monocytogenes* strains were isolated from clinical samples, represented by blood culture (n=7) and cerebrospinal fluid (n=11), from patients with septicemia, meningitis/meningo-encephalitis, abortion and newborns, hospitalized from April 2010 to April 2013 in three medical institutions in Bucharest: Babes Hospital, Elias Hospital, National Institute of Infectious Diseases (INBI) Matei Bals (containing two isolate-pairs, i.e. blood-culture and cerebrospinal fluid, from two patients with sepsis and meningitis/meningo-encephalitis), respecting the ethical issues concerning studies involving human samples.

These samples were processed according to SR EN ISO 11290 standards part 1 and part 2, (10-12), and colonies grown on PALCAM agar were identified as *L. monocytogenes*. The identification of *L. monocytogenes* was performed in INBI "Matei Bals" and the confirmation was performed by the National Reference Center for Zoonotic Infections in National Institute for Research and Development in Microbiology and Immunology (NIRDMI)-Cantacuzino. Additionally, *L. monocytogenes* ATCC 19111 type 1 was used as reference strain. The *L. monocytogenes* isolates were stored at -80°C in Brain Heart Infusion (BHI) broth (Oxoid) containing 20% (v/v) glycerol. Before use, the pathogenic strains of *L. monocytogenes* were activated in BHI at 37°C for 24 hrs.

Antimicrobial susceptibility testing

The disk diffusion method was used in order to assess the resistance profiles of both the clinical and the food-borne *L. monocytogenes* strains. The susceptibility testing was performed using a number of 15 antibiotics (Oxoid), with the following concentrations: 10U Penicillin, 10µg Ampicillin, 30µg Amoxicillin/ clavulanic acid, 10µg Imipenem, 30µg Cefuroxime, 30µg Amikacin, 10µg Gentamicin, 30µg Neomycin, 30µg Nalidixic acid, 5µg Ciprofloxacin, 30µg Tetracycline, 15µg Erythromycin, 30µg Chloramphenicol, 30µg Rifampicin, 1.25µg / 23.75µg Trimethoprim/Sulfamethoxazole. The results were interpreted according to CLSI (Clinical and Laboratory Standards Institute), 2006 criteria. *L. monocytogenes* ATCC 19111 type 1 and *Staphylococcus aureus* ATCC 29213 were used as control strains. E-test strips (AB bioMérieux) were used in order to determine the MICs of different antibiotics in the case of a clinical *L. monocytogenes* strain that showed resistance to beta-lactam antibiotics (i.e. ampicillin, amoxicillin+clavulanic acid, imipenem). The strips containing different antibiotic exponential gradients

(expressed in µg), i.e. 0.002-32 Ciprofloxacin, 0.016-256 Clindamycin, 0.016-256 Erythromycin, 0.064-1024 Fosfomycin, 0.016-256 Gentamicin, 0.002-32 Imipenem, 0.016-256 Oxacillin, 0.016-256 Phenoxymethylpenicillin, 0.002-32 Rifampicin, 0.016-256 Teicoplanin, 0.016-256 Tetracycline, 0.016-256 Vancomycin.

Results

This paper reports the antibiotic susceptibility testing of 37 *L. monocytogenes* strains isolated from foodborne and clinical samples, identified using exclusively phenotypic tests, since molecular taxonomy is not yet implemented in the laboratories performing this study. Apart from penicillin, ampicillin and trimethoprim-sulfamethoxazole, for which clinical breakpoints for *Listeria* susceptibility testing are defined according to the Clinical and Laboratory Standard Institute (CLSI), in the present study CLSI criteria for staphylococci were used for the interpretation of antibiotic susceptibility results (13).

All *L. monocytogenes* food isolates were susceptible to the first choice antibiotic drugs, represented by penicillins (Table I). Concerning the antibiotic susceptibility spectrum, the food strains were fully susceptible to penicillin, ampicillin, amoxicillin+clavulanic acid, imipenem, amikacin, gentamicin, chloramphenicol, rifampicin, tetracycline. One of the 19 tested strains was resistant to trimethoprim/sulfamethoxazole. All tested strains proved to be resistant to nalidixic acid and cefuroxime. Six strains were intermediate resistant to ciprofloxacin, one to erythromycin and three to neomycin.

The clinical strains (Table II) proved to be susceptible to aminoglycosides, tetracyclines, chloramphenicol, rifampin and trimethoprim/sulfamethoxazole. Nine strains proved to be intermediate resistant to ciprofloxacin and one to erythromycin. One isolate-pair (from the blood-culture and cerebrospinal fluid of an im-

Table I. Antibiotic resistance/susceptibility spectrum of the food strains

Antibiotic /dose (µg/disc)			<i>L.monocytogenes</i> strains		
			S	I	R
Betactamins	Penicillins	Penicillin G 10U	19		
		Ampicillin 10µg	19		
		Amoxicillin + Clavulanic Ac 30µg	19		
	Cephalosporins	Cefuroxime 30µg			19
	Penems	Imipenem 10µg	19		
Aminoglycosides		Amikacin 30µg	19		
		Gentamycin 10µg	19		
		Neomycin 30µg	16	3	
Quinolones		Nalidixic Ac. 30µg			19
		Ciprofloxacin 5µg	13	6	
Tetracyclines		Tetracycline 30µg	19		
Macrolides		Erythromycin 15µg	18	1	
Phenicol		Chloramphenicol 30µg	19		
Other antibiotics		Rifampicin 30µg	19		
		Sulphamethoxazole/Trimethoprim 1,25µg/23,75µg	18		1

S = susceptible; I = intermediary; R = resistant

munocompromised patient with sepsis and meningitis/meningo-encephalitis) proved to be resistant to beta-lactam antibiotics (oxacillin, ampicillin, amoxicillin+clavulanic acid and imipen-

em) both in disk diffusion method and in E-test (Table III). Moreover, four strains were resistant to ciprofloxacin, from which one isolate-pair (blood-culture and cerebrospinal fluid) from a

Table II. Antibiotic resistance/susceptibility spectrum of the clinical strains

Antibiotic /dose (µg/disc)			<i>L.monocytogenes</i> strains		
			S	I	R
Betactamins	Penicillins	Penicillin G 10U	18		
		Ampicillin 10µg	16		2
		Amoxicillin + Clavulanic Acid 30µg	16		2
	Cephalosporins	Cefuroxime 30µg			18
	Penems	Imipenem 10µg	16		2
Aminoglycosides		Amikacin 30µg	18		
		Gentamycin 10µg	18		
		Neomycin 30µg	18		
Quinolones		Nalidixic Ac. 30µg			18
		Ciprofloxacin 5µg	5	9	4
Tetracyclines		Tetracycline 30µg	18		
Macrolides		Erythromycin 15µg	17	1	
Phenicol		Chloramphenicol 30µg	18		
Other antibiotics		Rifampicin 30µg	18		
		Trimethoprim/Sulfamethoxazole 1.25µg/23.75µg	18		

S = susceptible; I = intermediary; R = resistant

Table III. MIC levels to different antibiotics of penicillin resistant strain

Antibiotic	Penicillin	Ampicillin	Oxacillin	Imipenem	Gentamycin	Ciprofloxacin	Erythromycin	Clindamycin	Teicoplanin	Vancomycin	Tetracycline	Fosfomycin	Rifampicin
MIC	0.25	>1	≥4	≥1	≤0.5	1	≤0.25	≤0.25	≤0.5	≤0.5	≤1	≥128	≤0.5
Interpretation	S	R	R	R	S	I	S	S	S	S	S	R	I

deceased woman with meningitis, and one from a patient with abortion in the 2¹/₂ month. All clinical strains were resistant to nalidixic acid and cefuroxime.

Discussion

Till present, little is known about the antibiotic resistance profiles of these strains in Romania. The present study, although performed on a small number of strains, is among the first reports on susceptibility profiles of food borne and clinical *L. monocytogenes* strains isolated in our country. The limited number of clinical strains can be explained by the fact that listeriosis is less common in Romania compared to other European countries, while food isolates of *L. monocytogenes* are usually unreported, thus making it difficult to gather a large collection of strains. Also, a recent study had shown that out of twenty five samples positive for *L. monocytogenes* by classical microbiological method, only 22 were confirmed by real-time PCR, suggesting

that using only the standard method can lead to false results (14).

The standard antibiotic therapy for listeriosis is represented by penicillin or ampicillin with gentamicin. For the patients with allergy to penicillin, the treatment may use a combination of vancomycin with an aminoglycoside, as well as cotrimoxazole with rifampin (6, 15, 17, 18).

All tested *L. monocytogenes* strains proved to exhibit natural resistance phenotypes to the second and third generation cephalosporins and nalidixic acid, as mentioned by other literature data (7, 16, 19). Our results are similar to those obtained by Ennaji et al, showing that almost all the analyzed strains (43 strains) of *L. monocytogenes* were susceptible to a wide range of antibiotics effective against Gram-positive bacteria, belonging to aminoglycosides group and glycopeptides, but were completely resistant to nalidixic acid, colistine, and second and third generations cephalosporins (19). For this reason, nalidixic acid is incorporated into selective media for the isolation of *L. monocytogenes*. The high

level of innate resistance of *L. monocytogenes* to cephalosporins may be especially significant since members of this family of β -lactams are frequently used to treat sepsis of unknown etiology (9, 20).

The high rate of ciprofloxacin resistance and intermediate resistance among foodborne and clinical strains is a matter of concern, this trend being recently reported in other studies, particularly in food chain isolates of *L. monocytogenes* 1/2a. It has been demonstrated that ciprofloxacin exposure could co-select resistance to other antimicrobial agents too (21).

The fact that 94.5% *L. monocytogenes* strains isolated from food products and clinical samples were susceptible to penicillins, the antibiotics commonly used for both veterinary and human listeriosis treatment, suggests that ampicillin or penicillin plus an aminoglycoside could remain the treatment of choice for listeriosis in Romanian patients. However, the fact that two *L. monocytogenes* strains, isolated from severe infections, showed resistance to different beta-lactam antibiotics, including ampicillin and amoxicillin+clavulanic acid is underlining the need of *in vitro* antibiotic susceptibility testing of each clinical isolate in order to establish the efficacy of different antibiotics, as well as of extended epidemiological studies to highlight the resistance profiles of *L. monocytogenes* strains circulating in our country. It has to be taken into account that, although the prevalence of acquired antibiotic resistance in *Listeria* isolates still remains low, the transferability of the resistance markers proven for *Enterococcus faecalis* recipient could facilitate further spread in the future. The high susceptibility rate to trimethoprim/sulfamethoxazole (97.2%) indicates that this antibiotic could be used in association with rifampicin as a second-choice therapy in penicillin-allergic patients. The preserved susceptibility of foodborne, clinical, and environmental *Listeria* isolates to trimethoprim/sulfamethoxaz-

ole was confirmed also in a recent, large scale studies, performed on a large number of strains (22, 23).

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Disclosure

There is no conflict of interests to be declared.

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