Part 1. Veterinary Medicine

UDC 619:579.869.1:615.281

DETERMINATION OF ANTIBIOTIC SUSCEPTIBILITY OF *LISTERIA* SPP.

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Summary. The aim of the research is studying of *Listeria* spp. sensitivity to antibiotics. The article presents the results of the sensitivity studying of species *L. monocytogenes, L. ivanovii, L. seeligeri* to penicillins, cephalosporins, carbopenems, aminoglycosides, macrolides, linzosides, tetracyclines, quinolones, nitrofurans, chloramphenicol, vancomycin, polymyxine, and rifampicin. The study and interpretation of the results were conducted using the disc-diffusion method according to European Committee for the Evaluation of Antimicrobial Susceptibility (EUCAST) methodology and guidelines 'Determination of the Sensitivity of Microorganisms to Antibacterial Drugs' in accordance with the Decree No. 167 of the Ministry of Healthcare of Ukraine from 05.04.2007. As a result of the conducted researches, the sensitivity features of the studied bacteria species *L. monocytogenes, L. ivanovii, L seeligeri* to antibiotics were established.

Keywords: *Listeria* spp., strains, antibiotics, sensitivity, resistance

Introduction. Listeriosis is an infectious zooanthroponotic disease, common in all countries, regardless of climate and social well-being (BIOHAZ, 2012).

The sources of the infection are rodents, livestock, cattle, pigs, dogs, wolves, cats, birds, fish and seafood, monkeys, people infected with listeriosis (Andrews, 1992).

The pathways of infection with human listeriosis are alimentary, aerogenic, contact, transplacental infections of the fetus and postpartum infections of the newborn baby; in animals — fecal-oral, alimentary, contact, air-drip, sexual, transmissive, transplacental (Bauwens, Vercammen and Hertsens, 2003).

The genus *Listeria* belongs to the Listeriaceae family, that includes 16 species at the moment of November 2015, among which the most relevant in the etiology of *Listeria* is *L. monocytogenes*; also known cases of human infection with species *L. ivanovii* and *L. seeligeri* (OIE, 2014; Guillet et al., 2010; Zhang et al., 2007).

The pathogenicity of these species is due to the presence of specific pathogenicity factors, which include listeriolysin O — hemolysin, the 'main factor' of the listeria pathogenicity. It has a pronounced toxic effect, and phosphatidylcholine — lecithinase, which plays an important role in the survival and reproduction of listeria in the infectious process and lysis of secondary vacuoles (Alberti-Segui, Goeden and Higgins, 2007; Churchill, Lee and Hall, 2006; Ermolaeva et al., 2003).

Antibiotics are used as etiotropic therapy in listeriosis treatment. *Listeria* spp. isolates are sensitive to penicillin derivatives (especially doominopenicillin), most macrolides (except azithromycin and spiramycin), aminoglycosides, tetracycline, glycopeptides (vancomycin) and lipopeptides (daptomycinum), oxazolidinones (Aureli et al., 2003).

There is the evidence that most drugs in the quinolone group have moderate activity against *Listeria* spp. At the same time, some researchers report that new fluoroquinolones are active against other strains, not only *L. monocytogenes* (EFSA and ECDC, 2012; Doganay, 2003).

The question of the pathogenic microorganisms resistance to antibiotics is being studied intensively throughout the world, because infectious pathogens undergo adaptation changes under the influence of anthropogenic factors (uncontrolled use of antibiotics, preservatives, disinfectants, etc.) that are expressed in the change of biological properties. These changes are manifested in the polymorphism of the pathogen populations, the appearance of avirulent and weakly virulent mutants and the resistance to antibacterial drugs of certain groups or field resistance (Clayton et al., 2014; Johnson et al., 2004; Volokhov et al., 2007).

The study of the specific sensitivity characteristics to antibiotics of certain bacteria species and genus is also relevant.

The aim of the study was to characterize the antibiotic susceptibility of *Listeria* spp. (*L. monocytogenes, L. ivanovii, L. seeligeri*).

Materials and methods. There was studied the sensitivity of 15 isolates of *Listeria* spp. to the antibiotics: 5 isolates of *L. monocitogenes* (isolated from minced meat of chicken — 2 cultures, beef meat — 2 cultures, dried milk — 1 culture); 5 isolates of *L. ivanovii* (isolated from

aborted fetuses); 5 isolates of *L. seeligeri* (isolated from pathological material from animals) and reference cultures *L. monocitogenes* ATCC 19112, *L. ivanovii* ATCC 19119, *L. seeligeri* ATCC 35967. The studies were conducted using nutrient media and disks with minimum concentrations of the active ingredient produced by 'HiMedia'. Determination of antibiotic sensitivity was provided by diffusion method and the evaluation of the obtained results was carried out according to EUCAST methodology and guidelines 'Determination of the Sensitivity of Microorganisms to Antibacterial Drugs' in accordance with the Decree No. 167 of the Ministry of Healthcare of Ukraine from 05.04.2007 (Johnson et al., 2004; Volokhov et al., 2007; MHU, 2007).

Results. From the penicillin group, we studied the sensitivity of *Listeria* spp. to benzylpenicillin, piperacillin and ampicillin. It is known fact, that *Listeria* spp. (in particular, *L. monocytogenes*) is highly susceptible to natural and semisynthetic penicillins (Table 1). According to the results of our research, the strains of the studied species showed sensitivity to the indicated antibiotics. The level of sensitivity in *L. monocytogenes* isolate was higher than in *L. ivanovii* and *L. seeligeri* isolates. The group of cephalosporins, which is characterized by a lack of significant activity against *L. monocytogenes*, determined the sensitivity of *Listeria* spp. cephalosin (III), cephalexin (III), ceftazidime (III), cefipix (IV), ceftazidine (III), cephalexin (I), cefuroxime (II), cefaclor (II), cefemandole (II), cefotaxime (III), ceftriaxone (III) (Table 1). According to our results, the studied cultures were generally insensitive, with some exceptions: the strain *L. monocytogenes* ATCC 19112 and *L. seeligeri* ATCC 35967 are susceptible to cefamandol (II), strains *L. ivanovii* ATCC 19119 and *L. seeligeri* ATCC 35967 are sensitive to cefepime (IV).

Carbapenems act on many groups of gram-positive, gram-negative, and anaerobic microorganisms. These results coincide with this statement: the strain *L. monocytogenes* ATCC 19112 was sensitive to carbopenems (imipenem, meropenem), isolates — insensitive; strain *L. ivanovii* ATCC 19119 was highly susceptible to imipenem and insensitive to meropenem; epizootic isolates *L. ivanovii* were, in general, insensitive to imipenem and insensitive to meropenem (Table 1). The strain *L.seeligeri ATCC 35967* was sensitive to imipenem and meropenem; isolates — insensitive.

Table 1 — Sensitivity of *Listeria* spp. to the antibiotics (penicillins, cephalosporins, carbapenems)

Name of antibiotic / Content of antimicrobial substance, μg (ED)	Diameters of inhibition of cultural growth, mm																	
	Listeria monocytogenes						*Listeria ivanovii*						*Listeria seeligeri*					
	ATCC 19112	2/0811	3/0811	6/0811	7/0811	13/0811	ATCC 19119	1/09	3/09	4/09	1/0811	5/0811	ATCC 35967	22	4/0811	8/0811	23	24
Penicillins																		
Benzylpenicillin, 10	28	22+*	27+*	26+*	19+*	16+	19	17	18+*	20+*	16	19+*	20	11+*	12+*	15+*	14+*	15+*
Piperacillin, 100	24	19	25	20	24+*	22	19	14+*	13+*	17+*	13	19+*	18	18+*	19+*	17+*	18+*	17+*
Ampicillin, 10	22	24+*	19+*	18+*	21	24	0	0	0	0	0	0	19	17+*	17+*	18+*	17+*	17+*
Cephalosporins																		
Cefazolin, 30	24	22+*	24+*	20+*	21+*	19	16	16+*	14+*	16+*	12	16	26+*	21+*	20+*	19+*	21+*	19+*
Cefalexin, 30	22+*	20	17+	15+*	18+*	20+*	9	8	7	9	8	6	16	8	9	16+*	7	17+*
Cefuroxime, 30	12	19	20	27+*	25	17	8	9	7	8	9	8	19	8+*	9+*	9	8+*	10
Cefaclor, 30	22	12	20+*	21+*	17+*	20+*	14	14	12	10+*	11+*	9	18	11	12	16+*	11	15+*
Cefotaxime, 30	12	21+*	19+*	22+*	27*	22+*	7	0	7	9	0	0	17	14+*	14+*	12	13+*	11
Ceftriaxone, 30	14	23+*	21+*	21	19+*	17	11	10	9	8	10+*	0	14	13+*	12+*	10	12+*	10
Cefoperazone, 75	17	22*	19+*	23+*	23	26+*	9	9	7	0	9	6	19	14	15	14	14	15
Ceftazidime, 30	10	7	11+*	10+*	12	9	8	8	6	7	0	0	22+*	19+*	20+*	16	21+*	15
Cefepime, 30	0	16	11	0	13+*	10	31	17+*	21+*	19+*	24+*	22+*	28	18	19	24	18	23
Cefamandole, 30	28	26+*	29+*	14	22+*	19+*	9	0	8	0	7	7	29	16	17	16+*	16	15+*
Carbapenems																		
Imipenem, 10	24	19+*	22+*	21	19+*	23+*	49+*	22+*	25+*	24+*	20+*	24+*	33	25+*	25+*	27+*	28+*	25+*

Notes: * — stimulation of culture growth around the zone of inhibition; + — the normal growth of resistant colonies in the zone of inhibition of culture growth.

From the group of aminoglycosides, to which *Listeria* spp. are considered to be naturally sensitive, we studied sensitivity to streptomycin (I), kanamycin (I), neomycin (I), gentamicin (II), netilmicin (II), tobramycin (II), amikacin (III) (Table 2). The reference strains *L. monocytogenes* ATCC 19112 and *L. seeligeri* ATCC 35967 showed sensitivity to aminoglycosides; isolates *L. monocytogenes* and *L. seeligeri* — less susceptible mostly. Reference strain and *L. ivanovii* isolates showed resistance to the drugs of this group.

In the macrolide group (erythromycin, oleandomycin, azithromycin) to which *Listeria* spp. are considered to be sensitive, the reference strains *L. monocytogenes* ATCC 19112 and *L. seeligeri* ATCC 35967 showed sensitivity to erythromycin and were less susceptible and insensitive to azithromycin and oleandomycin. Isolates of these species were insensitive to certain drugs (Table 2). Reference strain and isolates *L. ivanovii* showed resistance to macrolides. All studied cultures were low sensitive or resistant to lincosamides (lincomycin, clindamycin). Only reference strain *L. monocytogenes* ATCC 19112 was susceptible to tetracyclines (tetracycline, doxycycline), which are considered inhibitors of *Listeria* spp. The epizootic isolates were low susceptible. *L. ivanovii, L. seeligeri* (referential and epizootic) were insensitive and insensitive (Table 2).

Table 2 — Sensitivity of *Listeria* spp. to the antibiotics (aminoglycosides, macrolides, lynkozamides, tetracycline)

Name of antibiotic / Content of antimicrobial substance, μg (ED)	Diameters of inhibition of cultural growth, mm																	
	Listeria monocytogenes						*Listeria ivanovii*						*Listeria seeligeri*					
	ATCC 19112	2/0811	3/0811	6/0811	7/0811	13/0811	ATCC 19119	1/09	3/09	4/09	1/0811	5/0811	ATCC 35967	22	4/0811	8/0811	23	24
Aminoglycosides																		
Streptomycin, 30	19	17	19+*	12	23+*	24+*	10	9	7	0	0	8	28	18+*	19+*	22+*	21+*	18+*
Kanamycin, 30	30	22*	19	24+*	20+*	19+*	8	7	0	7	8	0	29	21+*	20+*	18+*	19+*	20+*
Neomicin, 30	18	17	19	21+*	17	19*	9	6	0	7	9	7	30+*	19+*	19+*	20+*	21+*	18+*
Gentamicin, 10	24	20+*	22+*	19	21+*	19	13	10+*	8	7	0	9	22	16+*	17+*	18+*	17+*	16+*
Netilmicin, 30	15	13	19+*	21+*	19+*	17*	7	0	8	0	6	7	19	12+*	13+*	11	12	14+*
Amicacin, 30	20	18+*	17	22+*	19+*	21*	11	10+*	8	9	0	8	18	11	12	13+*	11+*	11
Tobramicin, 10	26	15	20+*	24+*	19	22*	7	0	9	8	0	7	25	16+*	17+*	16+*	15+*	15+*
Macrolides																		
Erythromycin, 15	26	18+*	22+*	19+*	21+*	20+*	18	11+*	13+*	11	16+*	14+*	27	19+*	20+*	18+*	17+*	21+*
Azithromycin, 15	16	17+*	19+*	22+*	19+*	20+*	12	10	14+*	9	15	9	13	7	9	8	6	8
Oleandomycin, 15	8	9	12	19+*	20+*	14	21	15+*	20+*	17+*	16	22+*	24	16+*	17+*	19+*	18+*	16+*
Lynkozamides																		
Lincomycin, 15	13	16+*	12	14	10	11	11	7	9	10	9	8	14	11+*	10+*	11+*	10+*	11+*
Clindamycin, 2	15	15	14	20+*	16	19+*	9	0	7	0	9	0	17	6	8	8	9	8
Tetracycline																		
Tetracycline, 30	26	18	16	21	19+*	25+*	19	9	12	15	11	10	22	14+*	15+*	14+*	13+*	14+*
Doxycycline, 30	28	26+*	17	22+*	20+*	27+*	10	10	12	10	11	9	14	10	9	12	13	9

Notes: * — stimulation of culture growth around the zone of inhibition; + — the normal growth of resistant colonies in the zone of inhibition of culture growth.

From the group of quinolones, which are considered to be moderately active against *Listeria* spp. and fluoroquinolones (it has been experimentally established that levofloxacin (III) and moxifloxacin (IV), etc. were active against more than 99% of *L. monocytogenes* strains). We studied susceptible features of *Listeria* spp. to nalidixic acid (I), ciprofloxacin (II), norfloxacin (II), pefloxacin (II), floxacin, lomefloxacin (II), levofloxacin (III) (Table 3).

The cultures of *L. monocytogenes* were resistant to nalidixic acid. The strain *L. monocytogenes* ATCC 19112 was sensitive to ciprofloxacin, levofloxacin and low susceptible or resistant to other fluoroquinolones (lomefloxacin).

Epizootic strains of *L. monocytogenes* were insensitive. Reference strain *L. ivanovii* ATCC 19119 was sensitive to nalidixic acid and floxacin, epizootic isolates were insensitive to these drugs. *L. ivanovii* strains showed resistance to other preparations of this group. All *L. seeligeri* strains are resistant to nalidixic acid; the reference culture is susceptible to floxacin and lomefloxacin; to other drugs reference and epizootic cultures were low susceptible.

From the nitrofuran group (fusiidine, furadonine, furagin, furazolidone), the reference strain and some isolates of *L. monocytogenes* showed susceptibility to fusidine, culture *L. ivanovii* and *L. seeligeri* — resistant. All *Listeria* spp. exhibited resistance to furazolidone (Table 3).

L. monocytogenes ATCC 19112 and *L. seeligeri* ATCC 35967 are sensitive to chloramphenicol, epizootic isolates and reference strain are low susceptible.

L. ivanovii isolates are insensitive to vancomycin (glycopeptids, violations of the cell wall synthesis, active against *Listeria* spp.), *L. monocytogenes* are sensitive, *L. ivanovii*, *L. seeligeri* cultures are low susceptible.

All studied cultures were 100% resistant to polymyxin B. All strains of *L. monocytogenes* were susceptible to rifampin; *L. seeligeri* had low sensitivity; *L. ivanovii* were resistance.

Table 3 — Sensitivity of Listeria spp. to the antibiotics (fluoroquinolones, nitrofurans, and others)

Name of antibiotic / Content of antimicrobial substance, μg (ED)	Diameters of inhibition of cultural growth, mm																	
	Listeria monocytogenes						*Listeria monocytogenes*						*Listeria monocytogenes*					
	ATCC 19112	2/0811	3/0811	6/0811	7/0811	13/0811	ATCC 19119	1/09	3/09	4/09	1/0811	5/0811	ATCC 35967	22	4/0811	8/0811	23	24
Fluoroquinolones																		
Nalidixic ac. 30	0	0	0	0	0	0	28	19+*	18	20+*	18	19	9	5	6	0	0	6
Ciprofloxacin, 5	22	18	16	22	19	27+*	9	9	0	0	7	0	19+*	13+*	14+*	13+*	12+*	13+*
Norfloxacin, 10	14	24+*	20	19+*	20+*	17+*	8	8	0	0	7	0	18	12	13	14	15	12
Pefloksatsin. 10	12	11	23+*	12	24*	17	6	0	7	0	0	8	26	13	13	19	18	13
Floxacin, 5	16	19+*	24+*	17	20+*	22+*	35	21+*	19	22+*	19	21+*	36	21+*	20+*	20+*	21+*	21+*
Lomefloxacin, 30	0	19+*	24+*	9	25+*	20+*	8	0	0	6	9	0	29	19	20	21+*	20+*	19
Levofloxacin, 5	23	20+*	26+*	17	24+*	20+*	11	0	9+*	8	0	7	22	17+*	18+*	18+*	17+*	17+*
Nitrofurans																		
Fuzidin, 10	20	14+*	19+*	21+*	20+*	12	14	11+*	9	10	9	11+*	13	11	10	9	8	11
Furadonin, 300	14	13+*	20+*	15+*	22+*	12+*	8	9	7	9	8	9	16+*	10	11	10	11	10
Furamagum, 300	14	14+*	16+*	12	12	11	10	8	0	9	7	9	14	10	11	10	9	10
Furazolidone, 300	12	11	10	12	9	8	6	6	0	6	7	0	16	9	10	11	10	9
Others																		
Chloramphenicol, 30	31	17+*	20+*	22+*	19+*	19+*	18	18+*	17+*	16	17	18+*	27+*	18+*	19+*	22+*	21+*	18+*
Vancomycin, 30	22+*	15+*	20+*	19+*	22+*	20+*	19+*	17	16	18	17+*	17	17	17	16	14+*	13+*	17
Polymyxin, 300	0	0	0	0	0	0	9	0	6	0	7	0	9	6	7	7	6	6
Rifampicin, 5	20	18+*	20+*	19+*	22+*	24+*	9	0	8	0	0	0	20	17+*	18+*	14+*	13+*	19+*

Notes: * — stimulation of culture growth around the zone of inhibition; + — the normal growth of resistant colonies in the zone of inhibition of culture growth.

Thus, the reference and epizootic strains of the studied species (*L. monocytogenes*, *L. ivanovii, L. seeligeri*) were sensitive to penicillins in 100% of cases, to cephalosporins and carbopenems — mostly insensitive. The strain *L. ivanovii* showed resistance to the aminoglycosides. References cultures of *L. monocytogenes* ATCC 19112, *L. seeligeri* ATCC 35967 were sensitive, epizootic cultures were insensitive.

As for the group of macrolides, *L. monocytogenes, L. seeligeri* were sensitive to erythromycin, and were not susceptible to oleadomycin and azithromycin. Strains *L. ivanovii* were insensitive to macrolides. *Listeria* spp. was found insensitive and resistant to lincosamides. *Listeria* spp. was found preferably resistant to tetracyclines, with the exception of the reference strain *L. monocytogenes*.

The studied isolates of *Listeria* spp. were resistant and non-sensitive to quinoline, reference strains showed sensitivity to individual drugs. *Listeria* spp. were mostly resistant to nitrofuran and chloramphenicol with the exception of several *L. monocytogenes* cultures that exhibited sensitivity to fusidine, furadonine, chloramphenicol. *L. monocytogenes* culture was sensitive to vancomycin, *L. ivanovii*, *L. seeliger*i — low susceptible to vancomycin. The *L. monocytogenes* strains showed the susceptibility to rifampin. *L. seeligeri* showed low sensitivity and *L. ivanovii* — resistance to rifampin. The studied *Listeria* spp. was resistant to polymyxin B.

Conclusions. 1. The sensitivity study of *Listeria* spp. to antibiotics showed that both generic and species-specific features of sensitivity in the studied cultures have been established.

2. *Listeria* spp. isolates differed in the increased level of resistance to antibiotics compared with reference cultures.

3. Sensitivity level of *Listeria* spp. to drugs within a certain pharmacological group manifested individually.

4. It is appropriate to take into account the specific characteristics of cultures and to use a wide range of active substances during conducting the antibiotic resistance studies.

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