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DETERMINATION *LISTERIA* SPP. (*L. WELSHIMERI, L. GRAYI, L. MURRAYI, L. INNOCUA*) SENSITIVITY TO ANTIBIOTICS

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Summary. This research was conducted to determine the sensitivity of *Listeria* spp. (*L. welshimeri*, *L. grayi*, *L. murrayi*, *L. innocua*) to following antibiotic groups: penicillins, cephalosporins, carbapenems, aminoglycosides, macrolides, lincosamides, tetracyclines, quinolones, nitrofurans, chloramphenicol, and vancomycin. Studying cultures were identified with microbiological analysis of soil samples, raw materials of plant origin, and rodent feces. The study and interpretation of the results were carried out using diffusive method in a comparative aspect with the reference strains of *Listeria* spp. appropriate species. As a result, the sensitivity studied groups *Listeria* spp. to antibiotics was determined.

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

Introduction. For today, international requirements regulate the study of animal food products to detect *Listeria monocytogenes* — the listeriosis causative agent. However, cases of people infected by the *Listeria* spp., which are considered as non-pathogenic, have been recently reported. In this regard, it is relevant to study the sensitivity of *Listeria* spp. to antibiotics, in particular those that were considered as non-pathogenic until recently.

Bacteria of the *Listeria* genus are widespread in nature. They have different adaptive capabilities and, apart from humans and animals, they can live in the environment. *Listeria*, as facultative psychrophile, grows in a wide temperature range of 1–45 °C, capable of accumulation and virulence persistence in environmental objects at low temperatures (soil, water, plants), and even become more virulent under favorable conditions. This leads to increasing of *Listeria* concentration in environmental objects in spring and autumn, but it significantly decreases in the soil in summer (Euzéby and Parte, 2018; EFSA and ECDC, 2010). Winter freezing of the soil does not have negative impact on their viability. The viability and reproductive activity are significantly affected by water balance and optimal, close to neutral, pH values. They are characterized by high metabolic ductility, the ability to transform from saprophytic to parasitic lifestyle and back (Euzéby and Parte, 2018; EFSA and ECDC, 2010).

The *Listeria* spp. genus includes 16 species (Euzéby and Parte, 2018), where *L. monocytogenes, L. ivanovii,* and *L. seeligeri* are pathogenic (Zhang et al., 2007; Guillet et al., 2010; Rocourt et al., 1986).

However, there are reports of human infection with species which are considered as non-pathogenic. A fatal case of a 62-year-old patient after septicemia caused by *L. innocua* against cholangitis is reported (Perrin, Bemer and Delamare, 2003).

The cases *L. welshimeri* (Andre and Genicot, 1987) and *L. grayi* (Rapose, Lick and Ismail, 2008; Salimnia et al., 2010) isolated in adult patients are described in literature. *Listeria* spp. are sensitive to penicillin, macrolides (except azithromycin and spiramycin), fluoroquinolones, aminoglycosides, tetracycline, and vancomycin (EFSA and ECDC, 2010; Doganay, 2003; Volokhov et al., 2007; Johnson et al., 2004; Clayton et al., 2014).

However, to sensitivity of *L. monocytogenes* species to antibiotics is most extensively studied (EFSA and ECDC, 2010; Zhang et al., 2007; Volokhov et al., 2007; Johnson et al., 2004). Therefore, the issue of species sensitivity within the genus is constantly studied and remains relevant for today.

The aim of the study was to determine the sensitivity of *Listeria* spp. (*L. welshimeri*, *L. grayi*, *L. murrayi*, *L. innocua*) to following antibiotic groups: penicillins, cephalosporins, carbapenems, aminoglycosides, macrolides, lincosamides, tetracyclines, quinolones, nitrofurans, chloramphenicol, and vancomycin.

Materials and methods. Sensitivity of 14 isolates of *Listeria* spp. (4 isolates of *L. welshimeri*, 5 isolates of *L. grayi*, 3 isolates of *L. murrayi*, and 2 isolates of *L. innocua* isolated from rodent feces, soil samples, and plant material) and reference cultures of *L. welshimeri* 1, *L. grayi* LMG 16490, *L. murrayi* LMG 16491 (Belgian Coordinated Collections of Microorganisms), *L. innocua* ATCC 33090 to antibiotics was determined.

Studies were conducted using nutrient media and disks with minimal concentrations of active ingredient produced by 'HiMedia'.

Determination of antibiotic sensitivity by diffusion method and evaluation of obtained results was carried out according to EUCAST prescriptions (EUCAST, 2018) and 'Determination of the Sensitivity of Microorganisms to Antibacterial Drugs' (MHU, 2007).

Results. Sensitivity of *L. welshimeri* 1, *L. grayi* LMG 16490, *L. murrayi* LMG 16491, and *L. innocua* ATCC 33090, as well as field cultures of *Listeria* spp. to

penicillins (benzylpenicillin, piperacillin, and ampicillin) has been determined. *L. welshimeri* 1 reference strain and *L. welshimeri* isolates #21–24 were highly sensitive to piperacillin and ampicillin (Table 1).

As for cephalosporins' group, the sensitivity of *Listeria* spp. was determined to cefazolin (I), cefalexin (I), cefuroxime (II), cefaclor (II), cefamandole (II), ceftazidime (III), cefotaxime (III), ceftriaxone (III), cefoperazone (III), and cefepime (IV). As a result of our research, it has been found that studied cultures were non-sensitive, but with some exceptions: strains *L. welshimeri* 1 and #21–24 are sensitive to cefazolin (I) and ceftriaxone (III), and *L. grayi* LMG 16490 strain is sensitive to cefuroxime (II) (Table 1).

L. welshimeri 1 and *L. murrayi* LMG 16491 reference strains, as well as *L. welshimeri* field cultures #21–24 were sensitive to carbapenems (imipenem, meropenem). *L. grayi* LMG 16490 reference strain showed sensitivity to imipenem and was not sensitive to meropenem. *L. murrayi* isolates 12/0811, 14/0811, 22 and *L. grayi* 2/09, 5/09, 6/09, 10/0811, 11/0811 are non-sensitive. *L. innocua* ATCC 33090 reference strain and *L. innocua* isolates #22 and #23 were insensitive to imipenem and resistant to meropenem (Table 1).

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																	
	L. welshimeri					*L. grayi*						*L. murrayi*				*L. innocua*		
	1	21	22	23	24	LMG 16490	2/09	5/09	6/09	10/0811	11/0811	LMG 16491	12/0811	14/0811	22	ATCC 33090	22	23
Penicillins																		
Benzylpenicillin, 10	19	22	23	20	24	20	17*	17*	18	18*	16*	28+	17*	22	21	25+*	26+*	24+*
Piperacillin, 100	40	39+*	36+*	39+*	32+*	28	18+*	15+*	19+*	18+*	20+*	29+	21+	18+	19+	24	23	25
Ampicillin, 10	34	37+*	35+*	29+*	37+*	32	25+	26+*	22+	27+*	23+*	30+	27+	22+	23+	34+	33+	35+
Cephalosporins																		
Cefazolin, 30	34	35	33	36	32	25	21+*	17+*	17+*	26+*	18	20+	16*	17	16	22	21	20
Cefalexin, 30	26	27+*	28+*	30+*	29+*	29	20+*	18+*	21+*	17+*	22	27+	20+*	21+*	22+*	20	19	21
Cefuroxime, 30	26	27+*	28+*	29+*	26+*	32	20	20	23	17	26	24	20	18	18	20	22	21
Cefaclor, 30	28	28+*	27+*	27+*	29+*	27	15	17	14	19	21	14+	9	6	8	15	16	16
Cefotaxime, 30	27	29+*	29+*	28+*	27+*	27	18+	19+	22+	16+	17+	19+*	13	14	15	17	15	17
Ceftriaxone, 30	32	33+*	30+*	31+*	34+*	11	0	8	16	0	12	11	8	0	0	12	11	11
Cefoperazone, 75	28	27+*	29+*	27+*	30+*	25	15+	12+	17+	18+	21+	26+	20	18	18	20	21	22
Ceftazidime, 30	12	11	14	12	13	12	8	7	0	9	0	17	12	13	13	10	11	11
Cefepime, 30	20	20+*	20+*	20+*	20+*	18	12	11	10	7	13	16	11	12	14	12	12	11
Cefamandole, 30	26	28+*	27+*	29+*	27+*	27	21	17	21	16	25	23+	17	20	19	23+	22+	24+
Carbapenems																		
Imipenem, 10	44	40	41	43	44	28	25	19	20	22	20+	28	20+	21	20	25+	26+	26+
Meropenem, 10	34	35+*	37+*	36+*	35+*	22	14	17	14	19	17	30	20	22	21	0	6	0

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

As for aminoglycosides, sensitivity to streptomycin (I), kanamycin (I), neomycin (I), gentamicin (II), netilmicin (II), tobramycin (II), and amikacin (III) was studied. *L. welshimeri* 1, *L. grayi* LMG 16490, *L. murrayi* LMG 16491, and *L. innocua* ATCC 33090 reference strains are mainly sensitive, and isolates mostly have low sensitivity (Table 2).

From the group of macrolides (erythromycin, azithromycin, and oleandomycin), *L. welshimeri* strains are sensitive to erythromycin, resistant to azithromycin and oleandomycin and *L. grayi* strains are resistant to these antibiotics. *L. murrayi* LMG 16491 strain is sensitive to erythromycin, but resistant to azithromycin and oleandomycin, *L. murrayi* isolates 2/09, 5/09, 6/09, 10/0811, and 11/08/11 are not sensitive to macrolides. *L. innocua* ATCC 33090 reference strain and field cultures are not sensitive to erythromycin, but resistant to azithromycin and oleandomycin (Table 2).

As for quinolones' group which is considered as moderately active against *Listeria* spp., the sensitivity of *Listeria* spp. to nalidixic acid (I), ciprofloxacin (II), norfloxacin (II), pefloxacin (II), ofloxacin (II), and

levofloxacin (III) was studied. All strains of *L. welshimeri*, *L. grayi*, and *L. murrayi* were resistant to nalidixic acid, and reference strains showed sensitivity to quinolones II–III generations in 60% of cases; isolates have low sensitivity. *L. innocua* strains are resistant to nalidixic acid and less sensitive to preparations II–III generations (Table 2).

Referring to drugs of the nitrofurans' group, the sensitivity of *Listeria* spp. to furazidine and furazolidone was determined. *L. welshimeri* cultures 1, 7/10, 22, 23, 24 and *L. murrayi* LMG 16491 were sensitive to furazidine and more resistant to furazolidone. Other cultures were not less sensitive to furazidine and furazolidone (Table 2).

Table 2 — Sensitivity of *Listeria* spp. to the antibiotics (aminoglycosides, macrolides, lincosamides, tetracyclines, fluoroquinolones, nitrofurans, chloramphenicol, vancomycin)

Name of antibiotic / Content of antimicrobial substance, μg (ED)	Diameters of inhibition of cultural growth, mm																	
	L. welshimeri					*L. grayi*						*L. murrayi*				*L. innocua*		
	1	21	22	23	24	LMG 16490	2/09	5/09	6/09	10/0811	11/0811	LMG 16491	12/0811	14/0811	22	ATCC 33090	22	23
Aminoglycosides																		
Streptomycin, 30	40	37+	38+	39+	37+	32	24	22+*	17	26+*	24+*	24+*	14	20+	21+	21	22	23
Kanamycin, 30	14	13+*	14+*	15+*	13+*	27	16	16	17+*	16	20	19	14	15	14	16	16	17
Neomycin, 30	20	21	20	21	22	24	17	19	14+*	20+*	20+*	22+*	18+*	17	16	24+*	23+*	24+*
Gentamicin, 10	26	26+*	27+*	28+*	27+*	26	19	20+*	19	18	17	23	20	18	18	20	22	21
Netilmicin, 30	22	22	23	22	24	28	25+	22+	25+	20+	21+	24+	17+*	18+	19+	25+*	24+*	23+*
Amikacin, 30	26	26+*	27+*	28+*	27+*	26	20	16	18	20	17	27	21	20	21	20	19	21
Tobramycin, 10	26	26+	28+	27+	26+	30	23+-	22	24	25+	26	27	22+*	20	19	30	33	29
Macrolides																		
Erythromycin, 15	30	30+	32+	31+	32+	24	17	19	20	20+	21	28	22+	20+	21+	25+	27+	26+
Azithromycin, 15	14	14	15	13	14	17	13	15	11	9	12	17	15	13	13	17	16	15
Oleandomycin, 15	16	16	15	17	16	14	9	10	14	9	10	13	9	10	9	14	13	15
Lincosamides																		
Lincomycin, 15	20	21+*	23+*	22+*	23+*	0	0	0	0	0	0	0	0	0	7	0	0	7
Clindamycin, 2	14	15	16	14	14	24	16	15	16+-	18+	12	22+	16+	14	15	24+	24+	25+
Tetracyclines																		
Tetracycline, 30	46	45+	46+	44+	43+	23	16	17+-	18	20+-	18	25+	18	21	20	22+	21+	22+
Doxycycline, 30	32	31+	30+	32+	31+	25	18	17	20	18	17	27	21	21	22	25	25	25
Quinolones																		
Nalidixic acid, 30	7	0	0	6	0	9	0	7	7	0	6	0	0	6	7	9	9	9
Ciprofloxacin, 5	30	31+*	30+*	29+*	32+*	17	12	13	10	10	9+-	27+	14+	16	15	17+	16+	17+
Norfloxacin, 10	15	14	15	15	13	28	17	20	21+-	25+-	19+-	29	17	19	18	18	18	19
Pefloxacin, 10	16	16	17	16	15	25	19	18	17	22+-	19+-	25	20+	21	20	19	20	19
Ofloxacin, 5	26	27+	26+	27+	28+	24	20	20	19	21	20+-	26	19	22+	20+	20	21	20
Levofloxacin, 5	30	28+*	28+*	26+*	27+*	15	14	10	12	11	10	20	16	14	15	15	16	14
Nitrofurans																		
Furazidine, 300	24	22	23	22	25	12	9	10+	0	9	7+	23	14	13+	12+	12	11	11
Furazolidone, 300	15	16	14	16	17	13	9	10	8	7	6	18	11	12	11	12+	12+	11+
Chloramphenicol, 30	26	27+*	28+*	27+*	26+*	17	15	10	14	16	14	24+	18+	17	16	17+*	16+*	15+*
Vancomycin, 30	36	36+*	35+*	34+*	33+*	27	22+	20+	21+	22+	20+*	23+*	20	22+*	20+*	20	19	21

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

Regarding to chloramphenicol, which is active against many types of Gram-positive and Gram-negative bacilli, *L. welshimeri* and *L. murrayi* reference strains are sensitive, as for field strains, they are low sensitive. *L. grayi,* and *L. innocua* reference and field cultures are low sensitive (Table 2).

Reference and field cultures of *L. welshimeri*, *L. grayi*, *L. murrayi*, and *L. innocua* are sensitive to vancomycin (Table 2).

Conclusions. 1. All studied *L. welshimeri*, *L. grayi*, *L. murrayi*, and *L. innocua* cultures showed sensitivity to natural, semi-synthetic penicillins, and vancomycin, but showed resistance to nalidixic acid.

2. Cultures showed selective sensitivity to carbapenems, macrolides, lincosamides, tetracyclines, aminoglycosides, cephalosporins, quinolones II and III generations, nitrofurans, and chloramphenicol.

3. Reference cultures of *Listeria* spp. differed in the increased level of sensitivity to antibiotics in comparison with isolates.

4. Differences in sensitivity of cultures to certain groups of antibiotics have been noted.

5. The question of specific sensitivity characteristics for certain cultures and serotypes to antibiotics needs further study using a wide range of active substances of existing pharmacological groups.

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