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ANTIMICROBIAL SUSCEPTIBILITY

OF LISTERIA ISOLATED FROM FOOD PRODUCTS

G. S. Skitovich[1], K. V. Serova[2], N. B. Shadrova[3], O. V. Pruntova[4]

[1] Senior Researcher, Candidate of Science (Biology), FGBI "ARRIAH", Vladimir, *e-mail: skitovich@arriah.ru*

[2] Leading Veterinarian, FGBI "ARRIAH", Vladimir, *e-mail: serova@arriah.ru*

[3] Head of Laboratory, Candidate of Science (Biology), FGBI "ARRIAH", Vladimir, *e-mail: shadrova@arriah.ru*

[4] IAC Chief Expert, Doctor of Science (Biology), FGBI "ARRIAH", Vladimir, *e-mail: pruntova@arriah.ru*

SUMMARY

The paper demonstrates results of testing food products and raw materials for *Listeria monocytogenes* in 2011–2016. In 2011, the share of detected positive samples amounted to 0.3% (n=1100), in 2016 the detection level amounted to 7% (n=698). Basic types of products containing *Listeria monocytogenes* include meat and semi-finished meat products (87.2%; n=34), poultry meat and semi-finished poultry meat products (10.3%; n=4), and pasteurized cream (2.6%; n=1). The test results showed that all *Listeria monocytogenes isolates* demonstrated resistance to cephalosporins, aminoglycosides and fluroquinolones.

Key words: *Listeria monocytogenes*, antibiotics, antibiotic resistance.

INTRODUCTION

All *Listeria* species are gram-positive rod-shaped 0.4–2 μm bacteria, single or arranged in short chains or rarely in long strands. *Listeria* are not resistant to acids, they do not form spores and capsules, facultative anaerobes, chemoorganotrophs. These bacteria are widely spread in nature and can survive in extreme environmental conditions including wide range of temperatures and presence of bactericides [6].

Listeria monocytogenes is a facultative intracellular pathogen of food that induces rare but lethal disease in humans with compromised immunity, i.e. listeriosis [5]. Listeriosis is not a wide spread infection. The number of confirmed identified cases is significantly lower as compared to salmonellosis and campylobacteriosis but the severity of clinical disease and mortality are higher. Thus out of 2518 listeriosis cases identified in the USA in 1997 20% were lethal and 92% of cases required hospitalization [5, 7, 8].

In the Russian Federation the listeriosis morbidity has been officially reported since 1997. The majority of large scale listeriosis epidemics with high mortality resulted from consumption of food, first of all, cheese and other dairy products, salads, and to a lesser extent – meat, poultry and fish products [6].

The importance of alimentary transmission of listeriousis is well illustrated by the data received by the US Centers for Disease Control and Prevention (CDC) demonstrating that 11% of all products stored in household refrigerators are contaminated with listeria.

Analysis of listerium properties demonstrated that ubiquity, thermotolerancy and psycophillic properties of the agent are extremely favorable for the bacterium replication and persistence in food products. Epidemic monitoring of listeriosis while maintaining traditional approach to continuous monitoring of epidemics in farm animals and rodents involves continuous monitoring of listerium absence in food during its manufacture and storage.

One more important aspect for control of pathogenic microorganisms is decrease of antimicrobial preparations' activity over time due to formation of drug resistance in microbes. Resistant strains of microorganisms emerge due to modification of the bacterial cell genome resulted from spontaneous mutations. The acquired resistance becomes fixed and it is inherited by subsequent bacterial generations thus increasing the risk of spread of resistant strains in humans [1].

L. monocytogenes are sensitive to the wide range of antibiotics excluding novel cephalosporines and fos-

Table 1
Detection of *L. monocytogenes* in food in 2011–2016

Year	Total number of tests	Number of positives (%)
2011	1100	3 (0.3)
2012	839	2 (0.2)
2013	1807	42 (2.3)
2014	1007	13 (1.3)
2015	1532	27 (1,8)
2016	1004	66 (6.6)
Total	7289	153

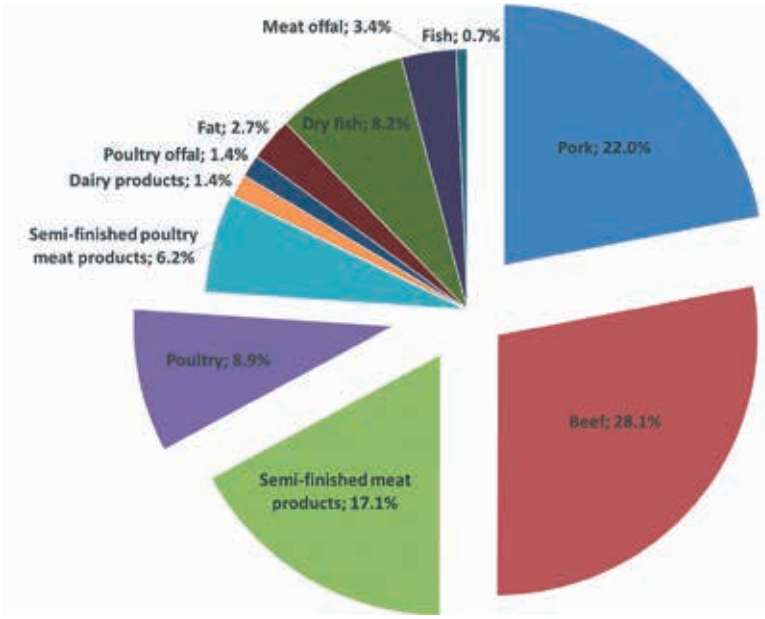
fomycin. Some isolates recovered from food products demonstrate higher resistance to such antibiotics as streptomycin and kanamycin. Antimicrobial resistance is most often predetermined by relative plasmids excluding tetracycline as the gene of resistance to it is included in the chromosome [9].

Listeria are sensitive to penicillin derivatives and resistant to cephalosporins. The majority of macrolides are effective against *L. monocytogenes* excluding azithromycin and spiramycin in particular. Aminoglycosides are highly active against listeria.

The majority of antibiotics demonstrate *in vitro* bacteriostatic but not bacteriocidal effect against listeria. This is typical for β-lactam antibiotics, macrolides, tetracyclines, chloramphenicol, rifampicin [9]. Quinolones demonstrate weak bacteriocide effect against listeria. Antibacterial effect is demonstrated by aminoglycosides, teicoplanin, cotrimoxazole in association with trimethoprim [8].

In view of high frequency of contamination of retailed meat and poultry products with *L. monocytogenes* demonstrating expressed pathogenic properties and increasing impact on pathology in humans, monitoring of antimicrobial resistance of such microorganisms isolated from food products is of topical importance.

Fig. Incidence of Listeria monocytogenes in food products (n=146)



In this respect the study was aimed at recovery of *L. monocytogenes* isolates from food samples and determination of antimicrobial resistance of isolated microorganisms.

MATERIALS AND METHODS

Reagents and nutrient media. For study purposes the following reagents and media were used: tryptic soy agar (TSA) (Merck, Germany); soybean casein digest broth (HiMedia, India); Fraser broth for primary enrichment (Merck, Germany); Fraser broth for secondary enrichment (Merck, Germany); Oxford agar (Merck, Germany); Ottaviani Agosti agar (ALOA) (Merck, Germany).

Control strains: Listeria monocytogenes strain 19115 (ATCC, USA); *Escherichia coli* strain 25922 (ATCC, USA); *Staphylococcus aureus* 6538 (ATCC, USA); *Rhodococcus equi* 6939 (ATCC, USA).

Isolates. 57 *L. monocytogenes* isolates were used that were recovered from different food products during microbiological tests performed in 2011-2016.

L. monocytogenes isolation. Isolation and identification were performed according to GOST 32031-2012 [2]. Weighted product sample (25±0.1) g prepared for testing was transferred into sterile plastic bag containing 225 cm^3 of Fraser broth for primary enrichment. The sample was homogenized for 1 min and incubated at (30±1) °C during (24±2) hours.

Following primary enrichment, irrespective of changes in the medium, 0.1 cm^3 of the sample was plated on 10 cm^3 of Fraser broth for secondary enrichment. The sample was incubated at (37±1) °C for 48 hours.

Following incubation streak inoculation using inoculating loop was performed on two media: Ottaviani Agosti agar and Oxford agar. The inoculated media were incubated at (37±1) °C for 48 hours.

The grown colonies were identified as *L. monocytogenes* by means of Gram staining, catalase activity testing, culture mobility determination, CAMP test, ELISA with mini VIDAS analyzer and biochemical analyses. Following identification the *L. monocytogenes* isolates were stored in 15% glycerol-containing soybean casein digest broth at -20 °C.

Preparation of test microorganism suspension. Pure day-old microorganism culture grown in solid nutrient ALOA was used for suspension preparation. Using inoculating loop several colonies were transferred to normal saline solution and optic density of the suspension was adjusted to 0.5 McFarland units. The suspension was used within 15 min after preparation.

Antibiotics. Standard paper disks impregnated with the following antibiotics were used: amoxicillin (20 μg/disc), sulfamethoxazole/trimethoprim (23.75/1.25 μg/disc), ampicillin (10 μg/disc), ampicillin/ sulbactam (10/10 μg/disc), gentamycin (10 μg/disc), doxycycline (30 μg/disc), enronit (300 μg/disc), ceftriaxone (30 μg/disc), cefepime (30 μg/disc), cefotaxime (30 μg/disc), laevomycetin (30 μg/disc), imipenem (10 μg/disc), meropenem (10 μg/disc), ticarcillin/ clavulanate (75/10 μg/disc), cefoperazone (75 μg/disc), ceftazidime (30 μg/disc), ciprofloxacin (5 μg/disc), ofloxacin (5 μg/disc), amikacin (30 μg/disc), azitronit (15 μg/disc) (FBIN RI Pasteur Institute of Epidemiology and Microbiology, RF).

Antimicrobial resistance determination. Susceptibility of *L. monocytogenes* isolates to antibiotics was examined according to standard methodical instructions [3, 4]. 20 ml of melted nutrient TSA was added onto 100 mm Petri

Table 2
Antibiotic resistance of *L. monocytogenes* isolates

No.	Antibiotic		Number of resistant isolates (%)		
	Group	Name	2016 (n=59)	2015 (n = 19)	Published data (n = 43)
1	Cephalosporins	Ceftazidime	57 (100)	n/t	6 (14)
2		Cefoperazone	57 (100)	n/t	n/t
3		Cefepime	57 (100)	19 (100)	n/t
4		Ceftriaxone	57 (100)	19 (100)	n/t
5		Cefotaxime	57 (100)	19 (100)	0
6	Aminoglycosides	Gentamycine	57 (100)	19 (100)	0
7		Amikacin	57 (100)	n/t	0
8	Fluoroquinolones	Ciprofloxacin	57 (100)	n/t	0
9		Ofloxacin	57 (100)	n/t	n/t
10	Penicillins	Ampicillin	0	1 (5)	9 (20,9)
11		Ampicillin/sulbactam	0	n/t	n/t
12		Amoxicillin	33 (58)	2 (11)	n/t
13		Ticarcillin/clavulanate	0	n/t	n/t
14	Tetracyclines	Doxycycline	35 (61)	19 (100)	0
15	Laevomycetins	Laevomycetin	57	n/t	n/t
16	Carbapenems	Meropenem	11 (19)	n/t	n/t
17		Imipenem	8 (14)	n/t	n/t
18	Macrolides	Azithronit	27 (47)	n/t	n/t
19	Polypeptides	Enronit	57	n/t	n/t
20	Sulfanilamides	Trimethoprim/sulfamethoxazole	14 (25)	19 (100)	5 (11,6)
21	Resistant to 1 antibiotic		0	0	15 (34.9)
22	Resistant to 2 antibiotics		0	0	20 (46.5)
23	Resistant to over 2 antibiotics		57 (100)	19 (100)	6 (14)

dishes. Before inoculation the medium surface was dried a little. 0.1 ml of bacterial suspension was evenly spread over the agar surface using spreader. Hereafter, different antibiotic impregnated discs were applied using forceps. Four antibiotics were tested in each dish. After disc application the Petri dishes were incubated bottom up at 37 °C for 18–24 hours. The results were evaluated by inhibition zones around the disks. Absence of test organism growth at the distance of over 10 mm from the disc was indicative of the strain susceptibility. If the test organism propagated close to the disc this microorganism was considered as resistant to the antibiotic. Diameter of inhibition zone including the disc diameter was measured within the accuracy of 1 mm [4]. *Escherichia coli* No. 25922 was used for quality control.

RESULTS AND DISCUSSION

During the period of 2011–2016 the Microbiology Laboratory of the FGBI "ARRIAH" tested food samples for safety under the National Plan for Monitoring of Residues of Banned and Harmful Substances in Animals, Animal Products and Feed, government contracts and orders of manufacturers of raw food materials and food products.

Determination of *L. monocytogenes* in such food products as milk and meat is regulated by Technical Regulations (TR) of the Customs Union (CU) such as CU TR 033/2013 and CU TR 034/2013, respectively. 7289 tests for *L. monocytogenes* were performed during the above mentioned period. 153 positive samples were identified. Analysis of data on *L. monocytogenes* detection during food tests in 2011–2016 are shown in Table 1.

Table 1 demonstrates that the number of recovered *L. monocytogenes* isolates annually increases. Thus 66 positive samples (6.6%) were detected in 2016 that is 3.5-fold more as compared to the values obtained in the previous year.

Distribution of *L. monocytogenes* isolates by types of food products in 2011–2016 is shown in the figure.

The majority of *L. monocytogenes* isolates were recovered from raw meat and meat-containing products such as pork, beef, chicken carcasses, pork legs, stuffed bell pepper, ravioli, fried sausages, chicken cutlets, beef offal. Two isolates were recovered from ready-to-eat dairy products: pasteurized cream and hard cheese. High level of the microorganism detection was also reported in vacuum-packed dry fish (17.1%) and salted fat (2.7%). The highest amount of *L. monocytogenes* isolates were recovered during testing of beef (28.10%).

Over the recent years the key issue is spread of resistant microorganisms and decrease of efficiency of a number of antibiotics.

During the study antimicrobial resistance of 57 *L. monocytogenes* isolates recovered in 2016 during tests of food products was examined. Sensitive and resistant isolates were distinguished by the type of test culture growth. Study results are demonstrated in Table 2.

According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) *L. monocytogenes* possess expressed natural resistance to cephalosporins including ceftazidime. During the study a number of cephalosporins were tested. It was demonstrated that all tested isolates were resistant to such cephalosporin antibiotics as ceftazidime, cefoperazone, cefepime, ceftriaxone and cefotaxime. These data are consistent with the results obtained in 2015.

Aminoglycosides demonstrate bacteriocidal effect against all *L. monocytogenes* isolates. They bind to 16S rRNA of bacterial ribosome 30S subunit and thus inhibit protein synthesis [4].

However, mutations in 16S rRNA and methylation in particular result in high level resistance to such aminoglycosides as gentamycin, amikacin, etc [6]. Our own data demonstrate (Table 2) that all *L. monocytogenes* isolates recovered in 2015 and 2016 demonstrated total resistance to this group of antimicrobials.

Fluoroquinolones demonstrate bacteriocidal effect. However, mutations in genes of type II topoisomerases (gyrA и gyrB), which are preferable targets for gram-positive microorganisms lead to the development of high level resistance that is being supported by the study results.

It should be emphasized that in the RF fluoroquinolones were officially approved for therapeutic use in farm animals and poultry in 1994 [6]. Total resistance of *L. monocytogenes* to fluoroquinolones identified during this study is indicative of the global consequences of the preparations' use involving formation of resistance to them.

The recovered isolates turned out to be resistant to such antibiotics as laevomycetin and enronit.

Out of all studied antibiotics antimicrobial effect was demonstrated by penicillines: ampicillin, ampicillin/sulbactam and ticarcillin/ clavulanate excluding amoxicillin, resistance to which is typical for 58% of the isolates. Moreover, the resistance to amoxicillin increased as compared to 2015.

L. monocytogenes demonstrated partial resistance to carbapenems (19 and 14%), macrolides (47%) and sulfanilamides (25%). Large group of isolates are resistant to doxycycline (61%).

CONCLUSION

The study results demonstrate high resistance of *L. monocytogenes* circulating in the RF to the tested antibiotics.

Therefore, much attention should be paid to testing of food products for *L. monocytogenes*; biological properties of the recovered isolates should be examined and diagnostic tools should be improved.

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