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ANTIBIOTIC SUSCEPTIBILITY AND ABILITY TO FORM BIOFILM OF *LISTERIA MONOCYTOGENES* STRAINS ISOLATED FROM FROZEN VEGETABLES

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L. monocytogenes poses a serious threat to public health, since most cases of listeriosis are connected with eating contaminated food. *L. monocytogenes* is often detected both in fresh and frozen vegetables.

The aim of this study was to evaluate the antibiotic susceptibility and ability to form biofilm of *L. monocytogenes* strains isolated from frozen vegetable mixtures in Poland.

Ninetynine genetically different strains were found among 100 isolates of *L. monocytogenes*. Among the 99 strains, 80 (80.8%) were susceptible to all tested antibiotics. Nineteen (19.2%) strains were resistant to one or more antibiotics. From this group of *L. monocytogenes* strains, most strains were resistant to erythromycin (16; 16,1%), penicillin (15; 15.1%), meropenem (12; 12.1%), cotrimoxazole (12; 12.1%), and ampicillin (3; 3.1%). According to the obtained results, differences in intensity of biofilm, both between those isolated in successive years and in the particular year, were observed. Performed analysis showed statistically insignificant faint negative correlation (r= –0.088) between the number of antibiotics to which strains were resistant and the intensity of biofilm formation by them.

Food contamination with *L. monocytogenes* poses a threat to consumers, therefore it is necessary to monitor their antibiotic susceptibility, ability to form biofilm, and genetic similarity, in order to evaluate the strains persistence time in plant.

Keywords: *Listeria monocytogenes,* antibiotics resistance, biofilm formation, vegetables

L. monocytogenes causes listeriosis and is one of the most virulent zoonotic pathogenic agents transmitted by food (Schlech, 2000). In 2015, European Food Safety Authority (EFSA) reported 2206 confirmed human cases of listeriosis (0.46 cases per 100 000 population, which was similar to 2014) (EFSA, ECDC, 2016).

Transmission of *L. monocytogenes* occurs most often through contaminated food. The last outbreak of listeriosis, connected with vegetables, was recorded in the United States and Canada associated with consumption of packaged salad (Self et al., 2016). The main cause of pollution is the use of contaminated farmyard manure to fertilize soil and contaminated water to wash fruit and vegetables (Szymczak et al., 2014). Ability of these bacteria to survive in a wide range of environmental conditions and to form biofilm makes their eradication difficult. *L. monocytogenes* form various structures of biofilm, including a monolayer of adherent cells, flat unstructured multilayers, and a knitted-chain network (Chae & Schraft, 2000). Bacteria forming biofilm show better ability to survive and resist various environmental

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factors both chemical (heavy metals, acids, antibacterial agents, and disinfectants) and physical (drying, UV radiation) (Bridier et al., 2015).

L. monocytogenes is naturally susceptible to a wide range of antibiotics. The level of resistance varies by antimicrobial use in humans and animals, as well as geography. It is very important to monitor antibiotic susceptibility of *L. monocytogenes* isolated from different environments (Jamali et al., 2013).

The aim of this study was to evaluate the antibiotic susceptibility and ability to form biofilm of *L. monocytogenes* strains isolated from frozen vegetable mixtures in Poland.

1. Materials and methods

1.1. Research material

L. monocytogenes strains were isolated from samples of frozen vegetable mix (broccoli, carrot, green beans, green pea, corn, red beans, onion, red pepper, and potato) produced in vegetable freezing plant in Poland in 2003–2007 (Table 1). Each year the same frozen vegetables mix was tested. The prepared homogenate was tested for presence of *L. monocytogenes* according to PN EN ISO 11290-1 standard. All 504 isolates were identified using PCR method, and their genetic similarities were evaluated with RAPD technique (described below). Two hundred eighty eight strains were genetically different. Twenty strains from each year were used in the study. These strains were randomly chosen for research.

Table 1. L. monocytogenes occurrence in frozen vegetables

Year	Number of samples	Number of positive samples	Number of *L. monocytogenes* strains (genetically different)	Number of tested *L. monocytogenes* strains
2003	1250	93	44	20
2004	2300	141	62	20
2005	1600	120	96	20
2006	2850	88	67	20
2007	1100	62	19	19
TOTAL		504	288	99

1.2. Strains identification

Identification of obtained strains on the species level was carried out by PCR technique. DNA from the tested *L. monocytogenes* strains was isolated using the Genomic Mini kit (A&A Biotechnology). To determine the strains belonging to the *Listeria* genus, two specific primers were designed based on the 16S rRNA sequence (Border et al., 1990). To identify *L. monocytogenes* species, two additional primers were designed based on the sequence of the gene coding listeriolysine O (*hly*A) (Bansal, 1996). The standard strain *L. monocytogenes* ATCC 19111 was used as the control strain.

1.3. Evaluation of strains genetic similarity

Evaluation of genetic similarity of the studied strains was performed with RAPD technique using OPA-11 primer with the sequence: 5'-CAATCGCCGT-3' (Ozbey et al. 2006). Each 25 µl reaction volume contained about 50 ng of template DNA.

To determine the degree of genetic similarity between isolates, a phylogenetic dendrogram was drawn in the program Phoretix 1 DPro (TotalLab). Cluster analysis was performed using hierarchical clustering with the UPGMA technique with Dice's coefficient.

1.4. Antibiotic susceptibility testing

Susceptibility of strains to penicillin, ampicillin, meropenem, erythromycin, and cotrimoxazole was assessed with the disk diffusion method on the Mueller Hinton Agar with addition of 5% horse blood and 20 mg l^{-1} NAD (bioMérieux). Incubation of antibiograms and interpretation of their results were carried out in accordance with the recommendations of EUCAST v. 6.0 (European Committee on Antimicrobial Susceptibility Testing, 2015).

1.5. Evaluation of biofilm formation

Biofilm formation by *L. monocytogenes* was assessed on the surface of polystyrene using crystal violet as previously described by Kwiecińska-Piróg and co-workers (2013). The experiment was carried out in triplicate for each strain.

Absorbance (A) was measured in the BIO-TEK spectrophotometer (The Synergy HT Multidetection) at the wavelength of 570 nm and read using the software KC4 v3.4 and KC4 Signature. The threshold value of absorbance (T) was proof of biofilm formation and was defined as the sum of the arithmetic mean of negative control and a triple value of its standard deviation ($T=xnc+3\delta$). Biofilm-forming strains were divided into 4 categories according to biofilm formation: poor (A: T–1000), moderately intensive (A: 1001–2000), strong (A: 2001–3000), and very strong (A≥3001).

1.6. Statistical analysis

Obtained results were subjected to statistical analysis in software STATISTICA 12 PL (StatSoft). The significance of differences between the frequency of antibiotic resistance profiles in total and particular years was verified with the post-hoc Bonferroni test ($\alpha=0.05$).

Also, the significance of differences between the value of absorbance for strains from successive years was checked using the post-hoc Bonferroni test ($\alpha=0.05$).

The existence of correlation between the level of drug resistance of the studied strains and their ability to form biofilm in plate wells was verified and interpreted according to the Stanisz scale (Stanisz, 1998).

2. Results and discussion

L. monocytogenes pose a serious threat to public health, since most cases of listeriosis are connected with eating contaminated food.

In our study, *L. monocytogenes* was found in 3.1–7.5% of the frozen vegetable samples, depending on the year (Table 1). García-Gimeno and co-workers (1996) and Kordowska-Wiater and co-workers (2007) showed the presence of *L. monocytogenes* in 30.0% and 10.3% samples of vegetables, respectively. Szymczak and co-workers (2014) isolated *L. monocytogenes* from 10.0% of the samples of strawberries, 15.0% of potatoes, and 5.0% of parsley. However, in the studies by Vitas and co-workers (2004) and Aguado and co-workers (2004), the percentage of occurrence of *L. monocytogenes* in frozen vegetables was much lower and amounted to 1.8% and 1.2%, respectively.

In the present study all considered isolates belonged to the *L. monocytogenes* species, which was confirmed by the results of identification by PCR technique. The occurrence of 99 strains was observed among 100 isolates classified into the species *L. monocytogenes*, one of which comprised two genetically identical isolates, based on our test, marked as 75 and 138, both from 2005 (Fig. 1). Almost all studied isolates were classified into two main monophyletic groups. The first group included 68 (68.0%) strains and the second one included 30 (30.0%) strains (Fig. 1). The exception was strain no. 3 from 2007, which could not be classified into any of the groups (Fig. 1).

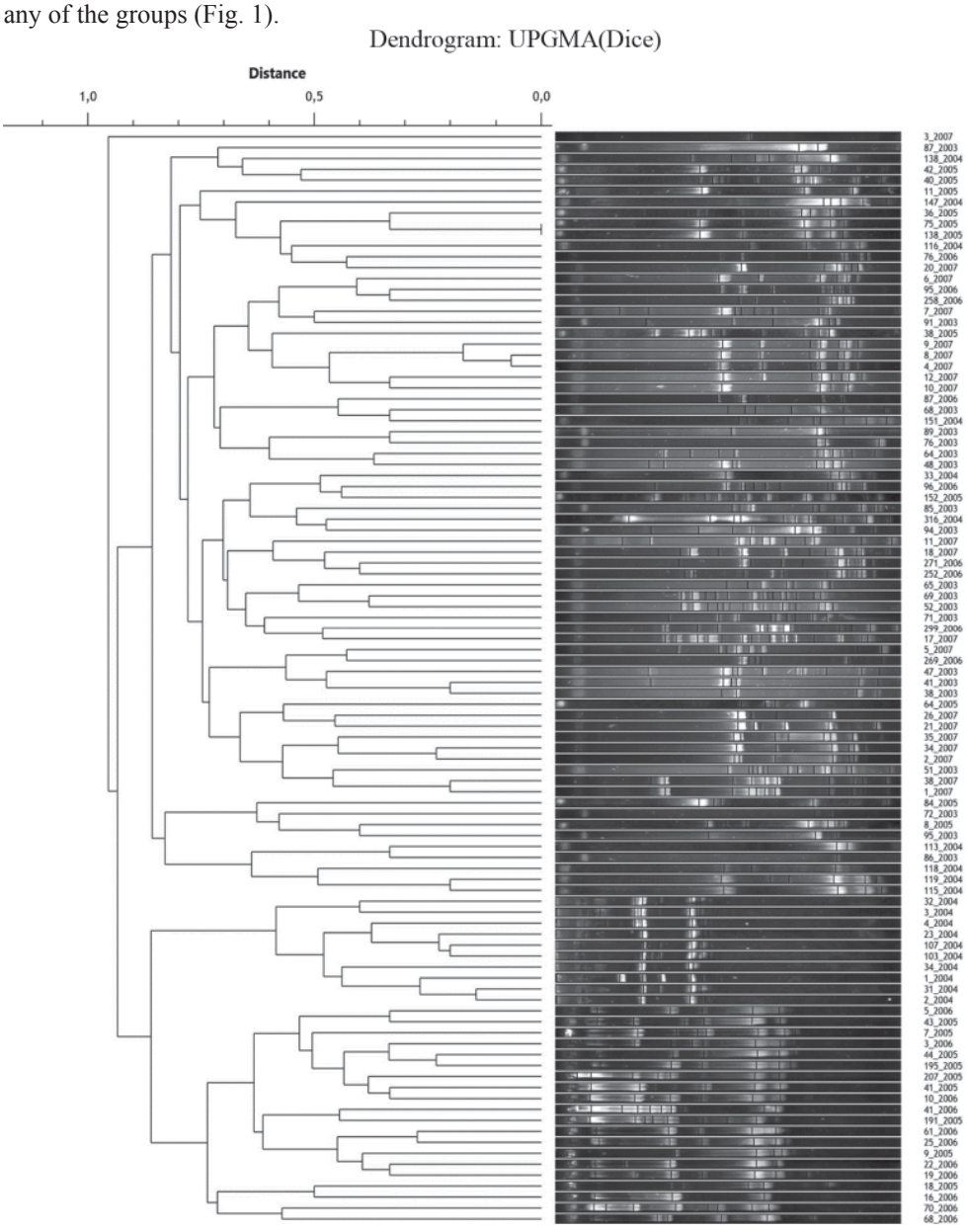

Fig. 1. Dendrogram for studied isolates of *L. monocytogenes* (n=100)

The performed antibiotic susceptibility assessment of *L. monocytogenes* strains allowed 9 profiles to be distinguished (Table 2). Of all 99 strains, 80 (80.8%) belonged to profile A and were susceptible to all tested antibiotics. Only 19.1% of all strains examined in this study displayed resistance to at least one of the five evaluated antibiotics. Of the resistant strains, resistance to erythromycin and penicillin was most frequently observed among the test group (n=16.2% and 15.2%, respectively). Similar results were obtained by Ruiz-Bolivar and co-workers (2011), according to whom the percentage of strains resistant to penicillin was 16.0%. In this study resistance to meropenem was reported for 12 (12.1%) strains, 12 (12.1%) strains to cotrimoxazole, and 3 (3.0%) strains to ampicillin (Table 2). Various antibiotic resistance was observed in different years, with the highest percentage of resistant strains in 2007. However, due to the fact, that only 20 strains were tested each year, it cannot be clearly stated that resistance to antibiotics increased among *L. monocytogenes* strains during the research period. Morvan and co-workers (2010) showed considerable differences in the values of minimal inhibitory concentrations (MIC) between strains isolated from humans in 1926 and strains isolated in 2007. Comparison of each of the periods covered by the study shows that the MIC_{50} value for ampicillin has more than doubled over 2005–2007 (MIC_{50}=0.5 μg ml^{-1}) as compared with the years 1926–1989 (MIC_{50}=0.19 μg ml^{-1}). Increased MIC_{50} values were also indicated for penicillin. Monitoring of resistance to ampicillin is an important element in eradication of these microorganisms.

Table 2. Profiles of antibiotic resistance of studied *L. monocytogenes* strains (n=99)

Profile	Antibiotic resistance	Number of strains	Number of strains isolated in individual years				
			2003	2004	2005	2006	2007
A	S: P, AM, MEM, E, SXT R: ---	80**	18[a]	19[a]	16[a]	19[a]	8[b]
B	S: AM R: P, MEM, E, SXT	9*	1[a]		1[a]		7[b]
C	S: P, AM, MEM, SXT R: E	3*			2		1
D	S: MEM R: P, AM, E, SXT	1*		1			
E	S: AM, MEM, SXT R: P, E	2*					2
F	S: AM, SXT R: P, MEM, E	1*					1
G	S: P, MEM, E, SXT R: AM	1*	1				
H	S: E, SXT R: P, AM, MEM	1*				1	
I	S: AM, E, SXT R: P, MEM	1*					1
Total		**99**	20	19	20	20	20

*: number of strains representing the given profile marked with a different number of asterisks differs statistically significantly (P≤0.05)

a;b: number of strains representing the given profile in particular years marked with different letters differs statistically significantly (P≤0.05)

S: susceptible; R: resistant; AM: ampicillin; P: penicillin; MEM: meropenem; E: erythromycin; SXT: cotrimoxazole

All studied strains of *L. monocytogenes* formed biofilm, which for 42 (42.4%) strains was very strong, for 21 (21.2%) strong, for 28 (28.3%) moderately intensive, and for 8 (8.1%) poor (Fig. 2).

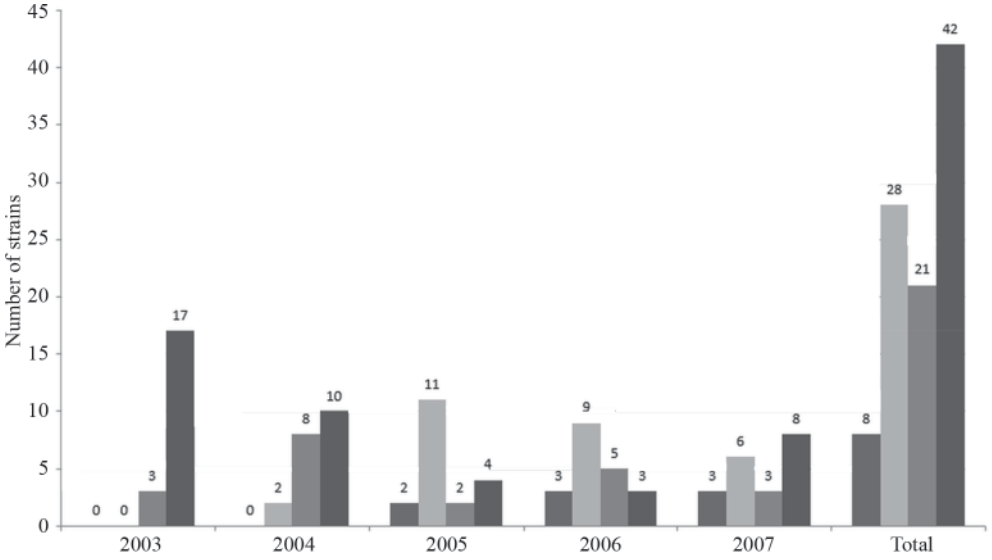

Fig. 2. Intensity of biofilm formation by *L. monocytogenes* strains in individual years
: weak; : moderate; : strong; : very strong

The most intensive biofilms (average value of absorbance – 3.479, Fig. 3) were formed by *L. monocytogenes* strains isolated in 2003. This value was statistically significantly higher ($P \leq 0.05$) than that determined for strains from the years 2005–2007 (Fig. 3). The lowest average value of absorbance (1.846) was indicated for *L. monocytogenes* strains from 2006. This value was statistically significantly lower ($P \leq 0.05$) than that determined for strains from the years 2003–2004 (Fig. 3). In the studies by Doijad and co-workers (2015) and Harvey and co-workers (2007) most strains formed poor or moderate intensive biofilm, only 26.5% and 5.0% of the strains isolated from food formed strong biofilm, respectively. Similar results were obtained by Meloni and co-workers (2012) and Barbosa and co-workers (2013), who classified most strains of *L. monocytogenes* isolated from food and surfaces having contact with food as strains forming poor or moderately intensive biofilm.

Certain differences in the ability to form biofilm were observed among *L. monocytogenes* strains isolated in particular years for which the genetic similarity was at least 80.0% (Fig. 1, Table 3). Similar values of absorbance were obtained for strains no. 23 and 107 and no. 1 and 33 isolated in 2004, as well as no. 8 and 9 from 2007 (Table 3). For genetically similar strains, no. 1 and 38 from 2007, a significant difference was indicated in the intensity of biofilm formation, the values of absorbance were 1.305 and 4.177, respectively (Table 3).

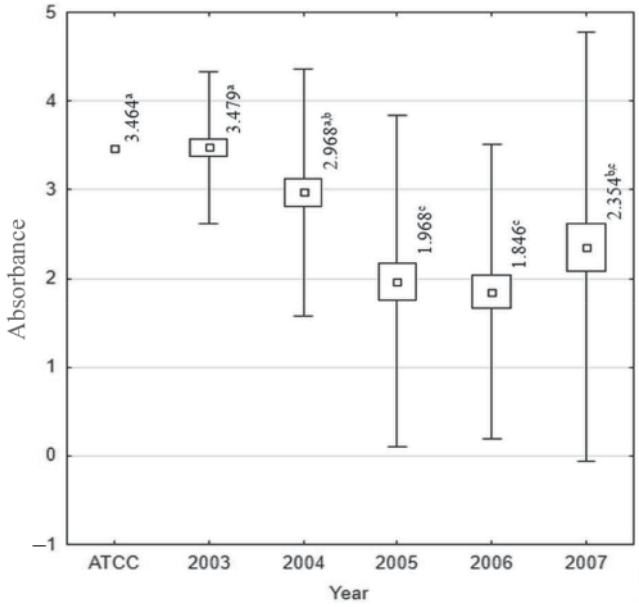

Fig. 3. Statistical significance of differences in ability to form biofilm by *L. monocytogenes* strains isolated in successive years
▫: Mean; □: Mean +/- Standard Error; ⊥: Mean +/- 2 Standard Deviation

Table 3. Differences in intensity of biofilm formation by studied *L. monocytogenes* strains (n=99)

Strain	2003			Strain	2004			Strain	2005		
	AM	STD	CV (%)		AM	STD	CV (%)		AM	STD	CV (%)
38/2003	3.487	0.285	8.17	1/2004	3.587	0.272	7.57	7/2005	1.908	0.164	8.61
41/2003	2.881	0.096	3.35	2/2004	3.508	0.192	5.46	8/2005	1.433	0.054	3.73
47/2003	3.283	0.217	6.59	3/2004	2.911	0.212	7.29	9/2005	3.756	0.098	2.61
48/2003	3.637	0.270	7.42	4/2004	2.873	0.659	22.95	11/2005	1.382	0.202	14.59
51/2003	3.323	0.488	14.69	23/2004	3.673	0.083	2.25	18/2005	2.670	0.068	2.55
52/2003	3.918	0.224	5.71	31/2004	3.193	0.276	8.65	36/2005	3.999	0.072	1.79
64/2003	3.673	0.440	11.98	32/2004	3.299	0.433	13.11	38/2005	1.141	0.186	16.27
65/2003	4.177	0.170	4.07	33/2004	3.583	0.203	5.67	40/2005	3.518	0.095	2.70
68/2003	3.149	0.336	10.66	34/2004	3.802	0.123	3.22	41/2005	0.992	0.068	6.81
69/2003	3.475	0.247	7.12	103/2004	2.528	0.332	13.12	42/2005	1.302	0.107	8.20
71/2003	3.499	0.207	5.91	107/2004	3.505	0.065	1.85	43/2005	1.687	0.145	8.60
72/2003	3.600	0.244	6.78	113/2004	1.278	0.074	5.83	44/2005	1.627	0.102	6.27
76/2003	4.258	0.158	3.72	115/2004	2.032	0.030	1.48	64/2005	1.908	0.076	3.97

Table 3. cont.

Strain	2003			Strain	2004			Strain	2005		
	AM	STD	CV (%)		AM	STD	CV (%)		AM	STD	CV (%)
85/2003	2.786	0.339	12.16	116/2004	2.604	0.112	4.28	75(138) /2005	1.501	0.082	5.34
86/2003	3.497	0.404	11.55	118/2004	2.993	0.063	2.10	84/2005	0.879	0.063	7.14
87/2003	3.502	0.319	9.10	119/2004	3.577	0.120	3.36	152/2005	1.812	0.136	
89/2003	3.607	0.225	6.24	138/2004	2.514	0.059	2.33	191/2005	2.056	0.074	7.51
91/2003	3.271	0.165	5.04	147/2004	1.674	0.049	2.93	195/2005	1.686	0.063	3.59
94/2003	2.586	0.201	7.79	151/2004	3.303	0.098	2.97	207/2005	3.010	0.065	3.74
95/2003	3.968	0.157	3.95	316/2004	2.931	0.113	3.85	–	–	–	–
Negative control	0.084	0.011	13.26	Negative control	0.084	0.011	13.26	Negative control	0.084	0.011	13.26

Strain	2006			Strain	2007		
	AM	STD	CV (%)		AM	STD	CV (%)
3/2006	0.955	0.065	6.85	1/2007	1.305	0.146	11.15
5/2006	3.235	0.072	2.23	2/2007	3.304	0.182	5.52
10/2006	2.351	0.241	10.24	3/2007	1.549	0.101	6.50
16/2006	3.449	0.240	6.95	4/2007	1.305	0.125	9.60
19/2006	3.428	0.263	7.67	5/2007	2.159	0.066	3.04
22/2006	1.599	0.194	12.12	6/2007	1.425	0.139	9.77
25/2006	2.279	0.082	3.59	7/2007	0.619	0.194	31.36
41/2006	0.984	0.125	12.67	8/2007	0.620	0.053	8.49
61/2006	2.361	0.223	9.43	9/2007	0.690	0.091	13.21
68/2006	1.214	0.120	9.89	10/2007	1.171	0.122	10.41
70/2006	1.336	0.209	15.64	11/2007	1.842	0.046	2.50
76/2006	2.231	0.112	5.03	12/2007	2.717	0.065	2.38
87/2006	1.214	0.234	19.26	17/2007	3.487	0.285	8.17
95/2006	1.718	0.227	13.23	18/2007	2.881	0.096	3.35
96/2006	1.398	0.027	1.92	20/2007	3.283	0.217	6.59
252/2006	1.140	0.290	25.40	21/2007	3.637	0.270	7.42
258/2006	1.380	0.135	9.81	26/2007	3.323	0.488	14.69
269/2006	2.414	0.109	4.52	34/2007	3.918	0.224	5.71
271/2006	0.777	0.100	12.84	35/2007	3.673	0.440	11.98
299/2006	1.458	0.064	4.40	38/2007	4.177	0.170	4.07
Negative control	0.084	0.011	13.26	Negative control	0.084	0.011	13.26

AM: mean value of absorbance; STD: standard deviation; CV: coefficient of variation

The performed analysis allowed for determination of a statistically insignificant (P=0.380) poor negative correlation (r = –0.088) between the number of antibiotics to which the studied strains were resistant and the intensity of biofilm formation by them (absorbance) (Fig. 4). As a result, among studied strains there was a very weak tendency of a decrease in the intensity of biofilm formation along with an increase in their antibiotic resistance. Other authors also correlated biofilm production with antibiotic resistance and observed similar pattern as in our study (Atray and Atray, 2015; Neupane et al., 2016; Zaki et al., 2017).

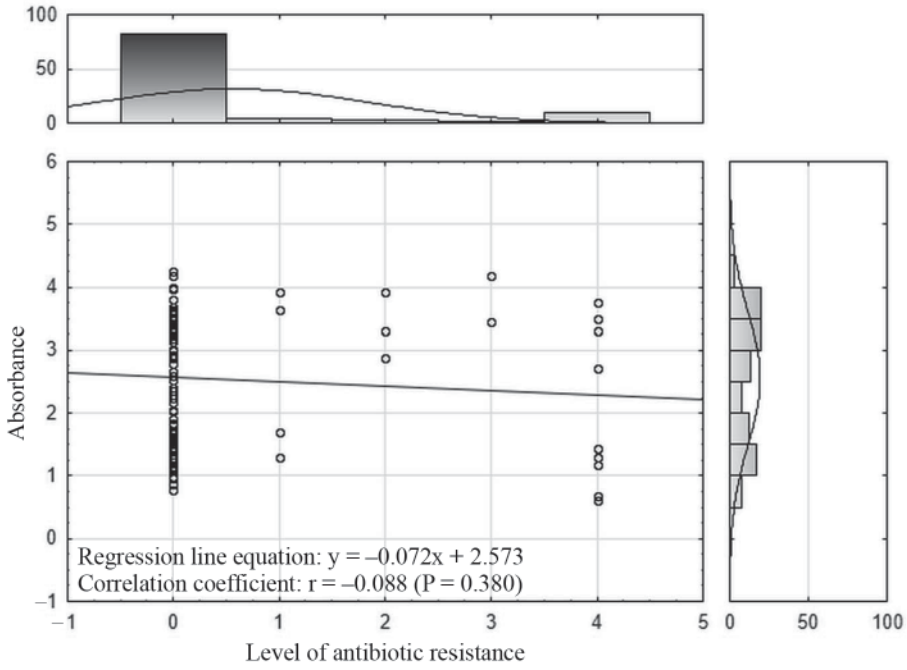

Fig. 4. Relationship between the intensity of biofilm formation and drug susceptibility of *L. monocytogenes* strains

3. Conclusions

In conclusion, this report showed that frozen vegetables in Poland may be contaminated with *L. monocytogenes*. Most studied strains were susceptible to the antibiotics, but some strains were resistant to penicillin, meropenem, erythromycin, and cotrimoxazole. Moreover, among the studied strains there was a very week tendency of decreasing intensity of biofilm formation with an increase in their antibiotic resistance.

The occurrence of *L. monocytogenes* in food production poses a threat to consumers. Hence, it is necessary to constantly monitor their antibiotic susceptibility, to assess the ability to form biofilm, and genetic similarity in order to assess the persistence of strains.

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References

Aguado, V., Vitas, A.I. & Garcia-Jalon, I. (2004): Characterization of *Listeria monocytogenes* and *Listeria innocua* from a vegetable processing plant by RAPD and REA. *Int. J. Food Microbiol., 90,* 341–347.

Atray, D. & Atray, M. (2015): Correlation between biofilm production and antibiotic resistance pattern in uropathogenic *Escherichia coli* in Tertiary Care Hospital in Southern Rajasthan, India. *Int. J. Curr. Microbiol. App. Sci., 4*(7), 640–646.

Bansal, N.S. (1996): Development of a polymerase chain reaction assay for the detection of *Listeria monocytogenes* in foods. *Lett. Appl. Microbiol., 22,* 353–356.

Barbosa, J., Borges, S., Camilo, R., Magalhães, R., Ferreira, V., Santos, I., Silva, J., Almeida, G. & Teixeira, P. (2013): Biofilm formation among clinical and food isolates of *Listeria monocytogenes*. *Int. J. Microbiol., 2013,* 1–6. http://dx.doi.org/10.1155/2013/524975

Border, P.M., Howard, J.J., Plastow, G.S. & Siggens, K.W. (1990): Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. *Lett Appl. Microbiol., 11,* 158–162.

Bridier, A., Sanchez-Vizuete, P., Guilbaud, M., Piard, J.C., Naïtali, M. & Briandet, R. (2015): Biofilm-associated persistence of food-borne pathogens. *Food Microbiol., 45,* 167–178.

Chae, M.S. & Schraft, H. (2000): Comparative evaluation of adhesion and biofilm formation of different *Listeria monocytogenes* strains. *Int. J. Food Microbiol., 62,* 103–111.

Doijad, S.P., Barbuddhe, S.B., Garg, S., Poharkar, K.V., Kalorey, D.R., Kurkure, N.V., Rawool, D.B. & Chakraborty, T. (2015): Biofilm-forming abilities of *Listeria monocytogenes* serotypes isolated from different sources. *PLoS One* 10:e0137046. https://doi.org/10.1371/journal.pone.0137046

EFSA, ECDC (2016): The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015 (2016) *EFSA J., 14*(12):4635.http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2016.4634/epdf (last accessed 08.06.2017).

European Committee on Antimicrobial Susceptibility Testing (2015): *Breakpoint tables for interpretation of MICs and zone diameters Version 6.0, valid from 2016-01-01* http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_6.0_Breakpoint_table.pdf (last accessed 25.07.2016).

García-Gimeno, R.M., Zurera-Cosano, G. & Amaro-López, M. (1996): Incidence, survival and growth of *Listeria monocytogenes* in ready-to-use mixed vegetable salads in Spain. *J. Food Safety*, *16,* 75–86.

Harvey, J., Keenan, K.P. & Gilmour, A. (2007): Assessing biofilm formation by *Listeria monocytogenes* strains. *Food Microbiol., 24,* 380–392.

Jamali, H., Radmehr, B. & Thong, K.L. (2013): Prevalence, characterization, and antimicrobial resistance of *Listeria* species and *Listeria monocytogenes* isolates from raw milk in farm bulk tanks. *Food Control, 34,* 121–125.

Kordowska-Wiater M., Janas P., Sosnowska B., Waśko A., Nowak A. & Kluza B. (2007): Występowanie bakterii patogennych oraz drobnoustrojów wskaźnikowych zanieczyszczenia fekalnego w mrożonych warzywach (Occurrence of pathogenic bacteria and faecal indicators in frozen vegetables). Żywność. *Nauka. Technologia. Jakość, 2*(51), 134–144.

Kwiecińska-Piróg, J., Skowron, K., Zniszczol, K. & Gospodarek, E. (2013): The assessment of *Proteus mirabilis* susceptibility to ceftazidime and ciprofloxacin and the impact of these antibiotics at subinhibitory concentrations on *Proteus mirabilis* biofilms, *Biomed. Res. Int., 2013,* 1–8. DOI: 10.1155/2013/930876

Meloni, D., Mazza, R., Piras, F., Lamon, S., Consolati, S.G., Mureddu, A. & Mazzette, R. (2012): The biofilm formation ability of *Listeria monocytogenes* isolated from meat, poultry, fish and processing plant environments is related to serotype and pathogenic profile of the strains. *Vet. Sci. Dev.,* 2:e12. https://doi.org/10.4081/vsd.2012.4012 (last accessed: 12 May 2018)

Morvan, A., Moubareck, C., Leclercq, A. & Hervé-Bazin, M. (2010): Antimicrobial resistance of *Listeria monocytogenes* strains isolated from humans in France. *Antimicrob. Agents Ch., 54,* 2728–2731.

Neupane, S., Pant, N.D., Khatiwada, S., Chaudhary, R. & Banjara, M.R. (2016): Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta lactamase production in uropathogenic *Escherichia coli* isolated from the patients suspected of urinary tract infections visiting Shree Birendra Hospital, Chhauni, Kathmandu, Nepal. *Antimicrob. Resist In., 5,* 5. doi: 10.1186/s13756-016-0104-9. eCollection 2016.

Ozbey, G., Ertas, H.B. & Kok, F. (2006): Prevalence of *Listeria* species in camel sausages from retail markets in Aydin province in Turkey and RAPD analysis of *Listeria monocytogenes* isolates. *Irish Vet. J., 59,* 342–344.

PN EN ISO (2017): *Microbiology of the food chain – Horizontal method for the detection and enumeration of* Listeria monocytogenes *and of* Listeria *spp. – Part 1: Detection method).* PN EN ISO 11290-1:2017

Ruiz-Bolivar, Z., Neuque-Rico, M.C., Poutou-Piñales, R.A., Carrascal-Camacho, A.K. & Mattar, S. (2011): Antimicrobial susceptibility of *Listeria monocytogenes* food isolates from different cities in Colombia. *Foodborne Pathog. Dis., 8,* 913–919.

Schlech, W.F. (2000): Foodborne listeriosis. *Clin. Infect. Dis., 31,* 770–775.

Self, J.L., Conrad, A., Stroika, S., Jackson, A., Burnworth, L. ... & Basler, C. (2016): Notes from the field: outbreak of listeriosis associated with consumption of packaged salad — United States and Canada, 2015–2016, *MMWR Morbid. Mortal. W., 65,* 879–881.

Stanisz, A. (1998): *Przystępny kurs statystyki* (Accessible course of statistics). T. 1. Statsoft Polska, Kraków, 362 pages.

Szymczak, B., Szymczak, M., Sawicki, W. & Dąbrowski, W. (2014): Anthropogenic impact on the presence of *L. monocytogenes* in soil, fruits and vegetables. *Folia Microbiol., 59*(1), 23–29.

Vitas, A.I., Aguado, V. & Garcia-Jalon, I. (2004): Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain). *Int. J. Food Microb., 90,* 349–356.

Zaki, N.H., Sachit, R.M., Salman, I., Al-Moosawi, L.H. & Sachit, S.M. (2017): Correlation between biofilm, protease production and antibiotic resistance in clinical bacterial isolates. *J. Genet. Environ. Resour. Conserv., 5*(1), 28–32.