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Antimicrobial resistance of *Listeria monocytogenes* serogroups IIa and IVb from food and food-production environments in Poland

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Introduction: *Listeria monocytogenes* is an important foodborne pathogen responsible for human listeriosis, which is a disease with high hospitalisation and mortality rates. The bacteria are usually susceptible to most antibacterial substances, but resistance to some of them has been recently observed. The present study introduces the evidence on the emergence of antibiotic resistance among *L. monocytogenes* strains isolated from food and food-production environments in Poland. **Material and Methods:** A total of 283 *L. monocytogenes* isolates classified into serogroups IIa and IVb which had been recovered from food and food production environments were tested with 17 antimicrobials. These included those that are recommended for treatment of severe listeriosis cases in humans. A multiplex PCR was used to identify serogroups, and a microbroth dilution method was applied for the determination of antibiotic resistance among the isolates tested. **Results:** Only 34 (12.0%) strains were susceptible to all the antimicrobials used in the study. The remaining 249 (88.0%) strains displayed different instances of resistance to the antimicrobials tested, from insusceptibility to one (112 strains; 39.6%) to resistance to four antibacterial substances (6 strains; 2.1%). Among them, there were 38 strains (13.4%) with multiresistance patterns. **Conclusion:** Polish food and its processing environments may be a potential source of antimicrobial-resistant *L. monocytogenes*, which may pose a potential health risk to consumers in the country.

Keywords: *Listeria monocytogenes*, antimicrobial resistance, meat, ready-to-eat food, food-production environments.

Introduction

Listeria monocytogenes is a facultative anaerobic bacterium and an opportunistic pathogen that causes listeriosis in humans, one of the most severe foodborne zoonotic diseases (9). According to the recent European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) report, 2,183 human cases of listeriosis were noted in the European Union (EU) in 2021, including 120 in Poland (12). The disease has the highest hospitalisation and mortality rates among all food-borne zoonoses. Most human listeriosis cases are caused by the consumption of contaminated food, which is frequently ready-to-eat products (RTE) (12). There are also other routes of transmission of *L. monocytogenes*, such as direct contact with infected but asymptomatic animals or with food-production environments where the bacteria are present (26).

Based on molecular biology techniques, *L. monocytogenes* has been classified into four phylogenetic lineages and four molecular serogroups with specific serotypes (10, 25, 32). The majority of listeriosis epidemics worldwide are caused by *L. monocytogenes* lineage I (of serogroup IVb and serotypes 4b and 1/2b) or, to a lesser extent, lineage II (of serogroup IIa and serotypes 1/2a and 3a), which suggests a higher pathogenic potential of lineage I strains than of other evolutionary lines (25). On the other hand, the higher prevalence of isolates of lineage II in food may be due to their higher resistance to environmental conditions in food-production areas (25).

This bacterium is susceptible to most antibacterial substances used against Gram-positive bacteria (24). It possesses a natural resistance to the first generation of quinolones, fosfomycin and the third generation of cephalosporins (2, 16, 24). However, the invasive form of human listeriosis requires effective antimicrobial therapy, which usually includes ampicillin, penicillin with gentamicin, meropenem, or a combination of sulfamethoxazole and trimethoprim (3).

Antimicrobial resistance is more frequently observed among *L. monocytogenes* from animals and food than among clinical isolates (14, 31). Although the percentage of such resistant strains is usually low, *L. monocytogenes* resistant to some antimicrobial agents used in human medicine, such as penicillin, ampicillin and gentamicin has been observed (13, 28). Furthermore, there are also reports of the identification of multiresistant strains from various sources, especially from food and food-production environments (7). Such *L. monocytogenes* may spread through food to humans and became a serious public health problem (17, 33, 37).

The presence of antimicrobial resistant, especially multiresistant *L. monocytogenes* isolates in food and its production environments may pose a risk for public health (7). There are several studies showing increasing resistance among foodborne bacteria around the world, including *L. monocytogenes* (14, 23, 31). Therefore, monitoring of resistance and resistance trends among such microorganisms allows effective strategies to be established to prevent a further spread of such resistant isolates (27).

The aim of the present study was to determine the antimicrobial resistance of serogroup IIa and IVb *L. monocytogenes* isolated from food and food-production environments in Poland.

Material and Methods

***Listeria monocytogenes* isolates.** A total of 283 *L. monocytogenes* isolates classified into the IIa (n = 146) and IVb (n = 137) serogroups were used in the study. They were obtained from raw meat (31 IIa strains and 21 IVb strains), RTE food (63 and 108 strains, respectively), and food production environments (52 and 8 strains). Several kinds of RTE food were tested, *i.e.* smoked offal, brawn, smoked sausage, smoked ham, scalded smoked meat, thick sausage, country ham, kabanos sausage, Krakow sausage and smoked tenderloin. Samples from the food production environments included swabs from the production tables, knives, saws, cutting boards and meat containers. In addition, swabs from the surfaces that had direct contact with food such as sewage grates, shoes, conveyor belts, the buckets of the conveyor and other equipment were also used for the study. The raw meat category included poultry meat and mechanically deboned poultry meat, minced beef and beefburgers, wild boar meat, venison and pork.

Strains of *L. monocytogenes* were isolated from food in official veterinary inspectorate laboratories located all over Poland and sent to the National Veterinary Research Institute in Puławy. The isolates were stored at −80°C in a Viabank cryoprotection system (BioMaxima, Lublin, Poland) until analysis. Extraction of DNA was carried out using the Genomic Mini kit and the manufacturer's protocol (A&A Biotechnology, Gdańsk, Poland), modified by adding 20 μL of lysozyme (10 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) and incubating at 37°C for 30 min. The serogroups of *L. monocytogenes* were determined with PCR as previously described (10, 35). Amplification of DNA was carried out in a thermal cycler (Biometra, Jena, Germany) under the following conditions: initial DNA denaturation at 95°C for 5 min; 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min; and completion with a final cycle at 55°C for 2 min and 72°C for 5 min. The primer sequences used in the PCR are shown in Table 1. The following primers were specific for *L. monocytogenes* serogroup determination: *prs* and *lmo0737* for IIa; *prs* and *ORF2819* for IIb; *prs*, *lmo1118* and *lmo0737* for IIc; and *prs*, *ORF2110* and *ORF2819* for IVb.

Table 1. PCR primers used for determination of *L. monocytogenes* serogroups

Target gene	Primer name	Primer sequence (5′ → 3′)
lmo0737	lmo0737F	AGGGCTTCAAGGACTTACCC
	lmo0737R	ACGATTTCTGCTTGCCATTC
lmo1118	lmo1118F	AGGGGTCTTAAATCCTGGAA
	lmo1118R	CGGCTTGTTCGGCATACTTA
ORF2819	ORF2819F	AGCAAAATGCCAAAACTCGT
	ORF2819R	CATCACTAAAGCCTCCCATTG
ORF2110	ORF2110F	AGTGGACAATTGATTGGTGAA
	ORF2110R	CATCCATCCCTTACTTTGGAC
Prs	prsR	GCTGAAGAGATTGCGAAAGAAG
	prsF	CAAAGAAACCTTGGATTTGCGG

F – forward; R – reverse

Determination of antimicrobial susceptibility. All 283 *L. monocytogenes* isolates were tested for their antimicrobial resistance using a microbroth dilution method as previously described (36). Sensititre GPN3F plates (Thermo Fisher Scientific, Waltham, MA, USA) containing 17 antimicrobials were used. The following antibiotics and concentrations were used: ampicillin (AMP; dilution 0.12–16 mg/L), ceftriaxone (AXO; 8–64 mg/L), ciprofloxacin (CIP; 0.5–2 mg/L), clindamycin (CLI; 0.12–2 mg/L), erythromycin (ERY; 0.25–4 mg/L), gatifloxacin (GAT; 1–8 mg/L), gentamicin (GEN; 2–16 mg/L), levofloxacin (LEVO; 0.25–8 mg/L), linezolid (LZD; 0.5–8 mg/L), oxacillin (OXA; 0.25–8 mg/L), penicillin (PEN; 0.06–8 mg/L), quinupristin/dalfopristin (SYN; 0.12–4 mg/L), rifampicin (RIF; 0.5–4 mg/L), streptomycin (STR; 512–1,024 mg/L), tetracycline (TET; 2–16 mg/L), trimethoprim/sulfamethoxazole (SXT; 0.5–4 mg/L), and vancomycin (VAN; 1–128 mg/L). The minimal inhibitory concentration (MIC) values were read using the Vision system (TREK Diagnostic System; Thermo Fisher Scientific). The antimicrobial resistance of the *L. monocytogenes* isolates was determined according to the Clinical and Laboratory Standards Institute guidelines and literature data (5, 6, 11, 22). Isolates showing intermediate resistance were classified

together with strains susceptible to the respective antimicrobial agent.

Reference strains. The following *L. monocytogenes* reference strains were used in the study: 05CEB424LM and 13CEB102LM of serogroup IIa, 06CEB406LM and 06CEB435LM of group IIb, 06CEB405LM and 13CEB1022LM of group IIc, and 06CEB422LM and 16CEL724LM of group IVb. The *L. innocua* reference strain was ATCC 33090, and the *L. ivanovii* strain was ATCC 19119. The reference strain of *Staphylococcus aureus* selected was ATCC 29213. The strains either originated from the European Union Reference Laboratory for *Listeria* (ANSES, Maisons-Alfort, France) or were commercially available.

Statistical analysis. The statistical analyses based on Fisher's exact test (Statistica version 10.0, StatSoft, now TIBCO, Palo Alto, CA, USA) were performed as previously described (34). A P-value < 0.05 was considered significant.

Results

Of the 283 (12.0%) strains tested, 34 were susceptible to all 17 antimicrobials used in the study. All *L. monocytogenes* isolates, regardless of the serogroup, were susceptible to ampicillin, gatifloxacin, gentamicin, penicillin, quinupristin/dalfopristin, streptomycin, trimethoprim/sulfamethoxazole, and vancomycin (Table 2). The isolates were mainly resistant to ceftriaxone (221; 78.1%), oxacillin (133; 47.0%), and clindamycin (60; 21.2%). Isolates resistant to ceftriaxone were mainly of serogroup IVb and were recovered from all three sample categories tested (Table 2). A statistically significant difference ($P < 0.01$) was observed between the number of AXO-resistant isolates classified to serogroup IVb and the number of these classified to serogroup IIa. Furthermore, strains resistant to oxacillin were predominantly identified in *L. monocytogenes* classified to serogroup IVb that were mainly isolated from RTE food and raw meat. The number of OXA-resistant isolates of serogroup IVb was significantly higher ($P < 0.01$) than the number of such strains belonging to serogroup IIa (Table 2). On the other hand, serogroup IIa strains resistant to clindamycin outnumbered serogroup IVb strains (33; 22.6% and 27; 19.7%, respectively), although this difference was not statistically important ($P > 0.05$) (Table 2). In the case of *L. monocytogenes* resistant to the remaining antimicrobials, no statistically significant difference in the number of isolates was observed ($P > 0.05$) between those affiliating to serogroup IIa and those in serogroup IVb.

Altogether 249 (88.0%) strains displayed resistance to the antimicrobials tested, most resisting one (112; 45.0% strains) and the fewest resisting four antibacterial substances (6; 2.4% strains) (Table 3). A large group of *L. monocytogenes* isolates (99; 39.8%) were resistant to two antimicrobials, mainly to the β-lactams ceftriaxone and oxacillin (72; 28.9%) and, to a lesser extent, to ceftriaxone and clindamycin (14; 5.6%, only isolates of serogroup IIa). Furthermore, 27 (10.8%) strains displayed resistance to three antimicrobials, namely ceftriaxone, oxacillin and clindamycin. Additionally, six isolates of serogroup IVb were resistant to four antimicrobials: ceftriaxone, oxacillin, clindamycin, and either linezolid or levofloxacin (Table 3).

Table 2. Antimicrobial resistance by sample origin of *Listeria monocytogenes* isolates from food and food-production environments in Poland

Antimicrobial agents	Number (%) of isolates *							
	Serogroup IIa (n = 146)				Serogroup IVb (n = 137)			
	Raw meat (n = 31)	Ready-to-eat food (n = 63)	Food-production environment (n = 52)	Total (%)	Raw meat (n = 21)	Ready-to-eat food (n = 108)	Food-production environment (n = 8)	Total (%)
AMP	0	0	0	0	0	0	0	0
AXO	17	38	40	95 (65.1)**	21	98	7	126 (92.0)**
CIP	0	1	2	3 (2.1)	0	1	0	1 (0.7)
CLI	8	17	8	33 (22.6)	2	25	0	27 (19.7)
ERY	0	0	1	1 (0.7)	0	0	0	0
GAT	0	0	0	0	0	0	0	0
GEN	0	0	0	0	0	0	0	0
LEVO	0	0	0	0	0	1	0	1 (0.7)
LZD	1	1	0	2 (1.4)	0	5	0	5 (3.6)
OXA	9	25	6	40 (27.4)**	11	79	3	93 (67.9)**
PEN	0	0	0	0	0	0	0	0
SYN	0	0	0	0	0	0	0	0
RIF	0	1	0	1 (0.7)	0	0	0	0
STR	0	0	0	0	0	0	0	0
TET	0	1	0	1 (0.7)	0	1	0	1 (0.7)
SXT	0	0	0	0	0	0	0	0
VAN	0	0	0	0	0	0	0	0

AMP – ampicillin; AXO – ceftriaxone; CIP – ciprofloxacin; CLI – clindamycin; ERY – erythromycin; GAT – gatifloxacin; GEN – gentamicin; LEVO – levofloxacin; LZD – linezolid; OXA – oxacillin; PEN – penicillin; SYN – quinupristin/dalfopristin; RIF – rifampicin; STR – streptomycin; TET – tetracycline; SXT – trimethoprim/sulfamethoxazole; VAN – vancomycin

* – Column totals exceed n values because some isolates were simultaneously resistant to more than one antimicrobial; ** – $P < 0.01$

Table 3. Antimicrobial resistance profiles of *Listeria monocytogenes* isolates from food and food-production environments in Poland

Antimicrobial resistance profile	Number (%) of resistant isolates		
	Serogroup IIa (n = 119)	Serogroup IVb (n = 130)	Total (n = 249)
AXO	59 (49.6)*	35 (26.9)*	94 (37.8)
OXA	14 (11.8)*	3 (2.3)*	17 (6.8)
CLI	1 (0.8)	0	1 (0.4)
AXO-OXA	9 (7.6)*	63 (48.5)*	72 (28.9)
AXO-CLI	14 (11.8)*	0*	14 (5.6)
CLI-OXA	8 (6.7)**	1 (0.8)*	9 (3.6)
AXO-CIP	1 (0.8)	1 (0.8)	2 (0.8)
AXO-TET	0	1 (0.8)	1 (0.4)
CLI-LZD	1 (0.8)	0	1 (0.4)
AXO-CLI-OXA	7 (5.9)*	20 (15.4)*	27 (10.8)
AXO-CIP-TET	1 (0.8)	0	1 (0.4)
AXO-OXA-RIF	1 (0.8)	0	1 (0.4)
AXO-CIP-OXA	1 (0.8)	0	1 (0.4)
AXO-CLI-ERY	1 (0.8)	0	1 (0.4)
AXO-CLI-LZD	1 (0.8)	0	1 (0.4)
AXO-CLI-LZD-OXA	0**	5 (3.8)**	5 (2.0)
AXO-CLI-LEVO-OXA	0	1 (0.8)	1 (0.4)

AXO – ceftriaxone; CIP – ciprofloxacin; CLI – clindamycin; ERY – erythromycin; LEVO – levofloxacin; LZD – linezolid; OXA – oxacillin; RIF – rifampicin; TET – tetracycline
* – $P < 0.01$; ** – $P < 0.05$

Differences in the resistance patterns between serogroup IIa *L. monocytogenes* isolates and those of serogroup IVb were observed. Among the 146 strains of serogroup IIa, 119 (81.5%) were resistant to at least one antimicrobial tested, whereas of the 137 strains classified into serogroup IVb, 130 (94.9%) did not demonstrate any resistance ($P < 0.01$) (Table 3). Taking into account the particular resistance profiles presented in Table 3, statistically significant differences were observed between the number of IIa strains and that of IVb strains resistant to ceftriaxone, to oxacillin, to both ceftriaxone and oxacillin, to both ceftriaxone and clindamycin, to both clindamycin and oxacillin, and to all three antimicrobials ($P < 0.01$ in each case) (Table 3). Furthermore, a difference ($P < 0.05$) was observed between the number of serogroup IIa strains resistant to the combination of ceftriaxone, clindamycin, linezolid and oxacillin and the number of serogroup IVb strains with this profile (Table 3).

A total of 38 (13.4%) isolates, 12 classified into serogroup IIa (8.2%) and 26 into IVb (19.0%), displayed antimicrobial multidrug resistance (MDR) patterns, *i.e.* simultaneous resistance to drugs of at least three classes (Table 3). The vast majority of such strains (27; 84.4%) were resistant to the third generation cephalosporins (ceftriaxone), lincosamides (clindamycin) and β-lactams (oxacillin). They were mainly isolated from RTE food (23 isolates) and of the 23 from this source, most were affiliated to the IVb serogroup (18 isolates) (Fig. 1). Five of the 38 MDR *L. monocytogenes* isolates were of this serogroup and from the same source and were resistant to four antimicrobials, *i.e.* ceftriaxone, clindamycin, linezolid and oxacillin. The remaining six MDR strains were mainly of the IIa serogroup and were resistant to three (5 serogroup IIa isolates) or four antimicrobials (1 serogroup IVb isolate resistant to three of the previously mentioned four drugs in combination and levofloxacin) (Table 3).

The three most numerous groups of isolates showing resistance to at least one tested antimicrobial were further characterised in relation to their origin. Most strains were resistant to ceftriaxone alone (94 isolates), ceftriaxone in combination with oxacillin (72 isolates), or to oxacillin and clindamycin (27 strains) (Fig. 1). *Listeria monocytogenes* resistant to ceftriaxone, classified into either serogroup, were isolated from all three sources, *i.e.* raw meat, RTE food, and food-production environments. Statistically significant differences were observed between the number of strains of the IIa serogroup from RTE food and the number of these from food-production environments ($P < 0.01$) (Fig. 1). Among strains of the IVb serogroup, such a difference was found again between the number of isolates from RTE food and the number from food-production environments ($P < 0.01$). No other differences were noted between ceftriaxone-resistant isolates and serogroups, regardless of the source of strains ($P > 0.05$).

Among isolates resistant to ceftriaxone and oxacillin and ceftriaxone, oxacillin and clindamycin, no statistical differences between the sources and serogroups of strains were noted ($P > 0.05$).

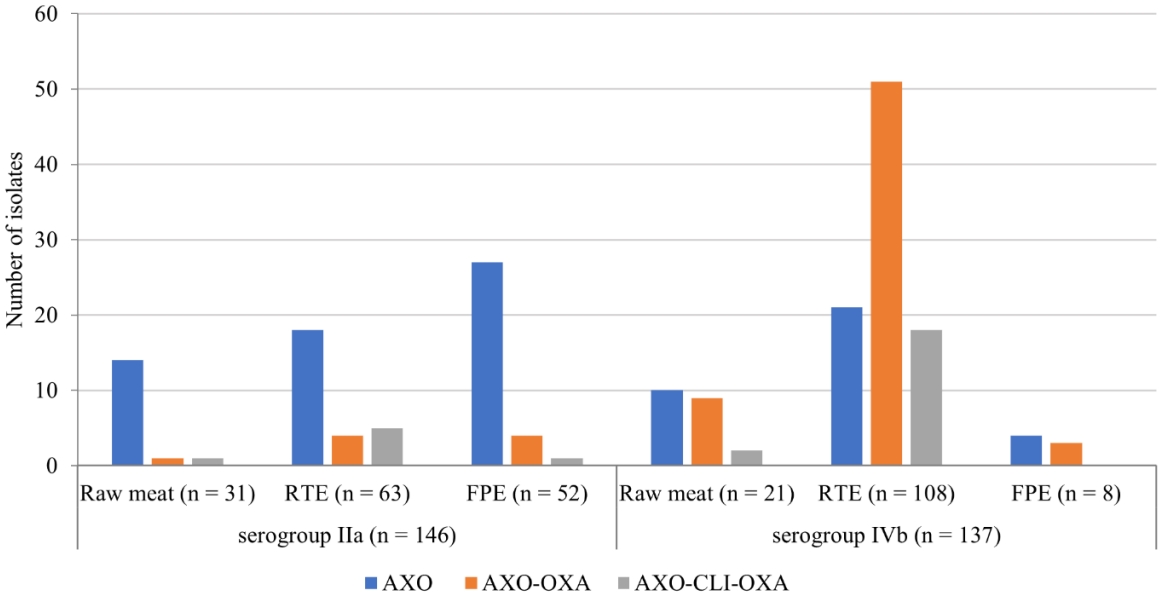

Fig. 1. Relationship between serogroup, source of isolate and antimicrobial resistance of *Listeria monocytogenes* identified among the most numerous antimicrobial resistance profiles
AXO – ceftriaxone; OXA – oxacillin; CLI – clindamycin; RTE – ready-to eat food; FPE – food production environment

Discussion

In the present study, *L. monocytogenes* isolated from food and food-production environments was tested for resistance to a panel of 17 antimicrobials, including those that are recommended for treatment of severe listeriosis cases in humans, *i.e.* β-lactams (penicillin and ampicillin) and aminoglycosides (gentamicin and streptomycin) (4). Additionally, resistance of the isolates against tetracyclines, erythromycin, chloramphenicol, vancomycin, and trimethoprim/sulfamethoxazole, *i.e.* the antimicrobials that may be used in cases of reduced sensitivity or resistance of *L. monocytogenes* to β-lactams, was also investigated. Resistance to all these antimicrobials was tested to assess the possible health risk to consumers from *L. monocytogenes* isolates recovered from raw meat and RTE food. It was shown that regardless of a strain's serogroup and origin, all of them were susceptible to ampicillin, penicillin, gentamicin, streptomycin, vancomycin, and trimethoprim/sulfamethoxazole; thus, they showed typical antimicrobial resistance characteristics for *L. monocytogenes*. Furthermore, only single isolates were resistant to other antimicrobials important in human treatment, *i.e.* tetracycline and erythromycin. On the other hand, several strains (37.7%) displayed resistance to ceftriaxone and they were identified in both serogroups and in samples from all sources tested. A high rate of resistance of *L. monocytogenes* isolated from food of marine origin to ceftriaxone (77.8% of isolates) was previously identified in Poland (8). A statistical correlation between resistance and serotype of *L. monocytogenes* was recently shown by Caruso *et al.* (1). Antimicrobial resistance to oxacillin was significantly more frequently identified in isolates of lineage I classified to serotype 4b/4e than in isolates of other serotypes. In addition, the percentages of strains of this serotype showing intermediate clindamycin or ciprofloxacin MIC values were also significantly higher. Furthermore, it was also described in another publication on isolates of food origin that *L. monocytogenes* serotype 1/2a was more frequently resistant to ciprofloxacin than serotype 4b (20).

In the present study several strains displayed resistance to more than one antimicrobial, especially to ceftriaxone and oxacillin (28.9% of isolates). Such *L. monocytogenes* isolates were mainly classified into serogroup IVb and identified in food and food-production environments. Interestingly, strains of food origin with the same antimicrobial resistance profile were also previously identified in Poland (30). Furthermore, other strains resistant to ceftriaxone and simultaneously to clindamycin were identified among isolates of serogroup IIa and were recovered from all sources tested in the study. This may suggest that ceftriaxone-resistant isolates may pose a potential health risk to consumers in Poland.

Some *L. monocytogenes* isolates were also resistant to clindamycin, either alone or in combination with other antimicrobials, mainly with ceftriaxone but also to a lesser extent with oxacillin, linezolid, clindamycin or levofloxacin. High resistance rates of *L. monocytogenes* strains to clindamycin were also described in Poland by Wiśniewski *et al.* (37). Such isolates were identified both in food and food-processing environments. Other studies also identified strains resistant to clindamycin in other countries, although the percentages of such isolates were rather low (7, 21). However, there are also reports of high

resistance rates of *L. monocytogenes* isolates to this antimicrobial (11, 15, 29).

In the present investigation a total of 38 (15.3%) strains tested displayed multiresistance patterns, *i.e.* they were resistant to at least three antimicrobial classes. Twenty-seven isolates showed resistance mainly to ceftriaxone (a third-generation cephalosporin), clindamycin (a lincosamide), and oxacillin (a β-lactam), and five also to linezolid (an oxazolidone). Resistance of *L. monocytogenes* strains to three or more antimicrobials was also described by Escolar *et al.* (11), although none of their isolates displayed multiresistance patterns identical to the current profiles.

In a study by Noll *et al.* (24) performed with a panel of *L. monocytogenes* strains identified in Germany over a period of 40 years from food, food-processing plant environments and human patients, 21% of isolates were resistant to three or more antibiotics. The strains were classified into serogroups IIa and IVb, as were those investigated in the present study. Unfortunately, the particular multiresistance profiles were not detailed in the report.

A relatively high prevalence of multiresistant *L. monocytogenes* strains in milk and milk products was recently identified in South Africa (17). Again, none of the strains was simultaneously resistant to the same antimicrobials as were resisted in combination in the present study. A further study by the same authors (18) revealed that multiresistance was relatively common in strains isolated from RTE food and such isolates showed a pattern of resistance to as many as 11 antimicrobials.

In the study by Kayode *et al.* (19) on *L. monocytogenes* of environmental water origin, several isolates showed multiresistance patterns, even against as many as 15 antimicrobials. The drugs included those tested in the present investigation, *i.e.* ampicillin, ceftriaxone, ciprofloxacin and erythromycin. A recent investigation by Kayode and Okoh (18) also demonstrated that several *L. monocytogenes* isolated from RTE food had multiresistance. These results may suggest that strains with multiple resistance patterns are more common than was previously thought and that such strains may be an underestimated group of *L. monocytogenes* pathogenic to humans.

Listeria monocytogenes of serogroups IIa and IVb isolated from food and food-production environments were susceptible to most of the tested antibacterial agents, including those used in the treatment of listeriosis in humans. However, some isolates were found to be resistant to ceftriaxone, oxacillin and clindamycin and a few of them showed multiresistance patterns. Therefore, foods of animal origin and their processing environments may be a potential source of antimicrobial-resistant *L. monocytogenes*, which may pose a potential health risk to Polish consumers. Therefore, continued study of the antimicrobial resistance of this microorganism is needed for an adequate background of information for selection of an effective treatment for severe listeriosis in humans.

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