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Prevalence, Characterization, and Antimicrobial Resistance of *Listeria monocytogenes* Isolates from Bovine Hides and Carcasses

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Listeria monocytogenes **isolates from bovine hides and carcasses (*n* = 812) were mainly of serogroup 1/2a. All strains were positive for internalin genes. Several isolates were resistant to oxacillin (72.2%) or clindamycin (37.0%). These findings indicate that *L. monocytogenes* of beef origin can be considered a public health concern.**

Listeria monocytogenes may contaminate food of animal origin, including beef meat (7, 17). Although infection of humans due to *L. monocytogenes* has a low incidence, it is associated with a high mortality rate (6, 19). Serological and PCR techniques identified at least 13 *L. monocytogenes* serotypes; however, mainly isolates of 1/2a, 1/2b, 1/2c, and 4b serogroups are recovered from food and patients (4). Several putative virulence factors of *L. monocytogenes* have been described, and among them, *inlA*, *inlC*, and *inlJ* (encoding internalin-like proteins), *lmo2672* (responsible for transcriptional regulator), and *llsX* (for listeriolysin S expression) seem to be the most important in the pathogenesis of human listeriosis (1, 3, 5, 9).

There is little information concerning *L. monocytogenes* antimicrobial resistance and especially little information about the isolates recovered from beef that has been determined by the microdilution method. It is known that *Listeria* strains are usually susceptible to most antibiotics; however, several resistant isolates have also been identified (3, 15, 16).

The aim of the present study was to determine the prevalence of *L. monocytogenes* in hides and carcasses of cattle slaughtered in Poland and to identify the virulence markers and antimicrobial resistance of the isolates.

A total of 406 cattle were used in the study. The samples from bovine hides and the corresponding carcasses (*n* = 812) were collected using a swab method, and *L. monocytogenes* was identified by the ISO 11290-1:1999 standard. Serotype determination was performed by multiplex PCR as described previously (4). The presence of virulence genes *inlA*, *inlC*, *inlJ*, *lmo2672*, and *llsX* was tested by PCR (Table 1) (10, 11). Antimicrobial susceptibility of the *L. monocytogenes* isolates was determined by using a Sensititre GPN3F plate according to the manufacturer's instructions (Trek Diagnostic Systems). The CLSI guidelines were used for the interpretation of the obtained MICs (2, 12).

It was found that 44 out of 406 hide samples (10.8%) were contaminated with *L. monocytogenes*, whereas 10 (2.5%) corresponding bovine carcasses were positive for this pathogen as shown by the presence of the 370-bp *prs* gene amplicon. Further PCR serotyping revealed that the majority of the isolates (47; 87.0%) were of the 1/2a serotype, and only 4 *L. monocytogenes* isolates were classified as 1/2c (Fig. 1). The remaining 3 strains

TABLE 1 PCR primers used for determination of *L. monocytogenes* virulence marker genes

PCR test	Primer name	Sequence (5′→3′)	Primer final concn (μM)	Target gene	Size of PCR amplicon (bp)	Reference
mPCR1	inlAF	ACGAGTAACGGGACAAATGC	0.25	*inlA*	800	11
	inlAR	CCCGACAGTGGTGCTAGATT	0.25			
	inlCF	AATTCCCACAGGACACAACC	0.15	*inlC*	517	
	inlCR	CGGGAATGCAATTTTTCACTA	0.15			
	inlJF	TGTAACCCCGCTTACACACAGTT	0.05	*inlJ*	238	
	inlJR	AGCGGCTTGGCAGTCTAATA	0.05			
PCR2	lmo2672F	CGGCACACTTGGATTCTCAT	0.3	*lmo2672*	481	10
	lmo2672R	AGGGCTAGTGACGGATGCTA	0.3			
PCR3	llsXF	TTATTGCATCAATTGTTCTAGGG	0.2	*llsX*	200	1
	llsXR	CCCCTATAAACATCATGCTAGTG	0.2			

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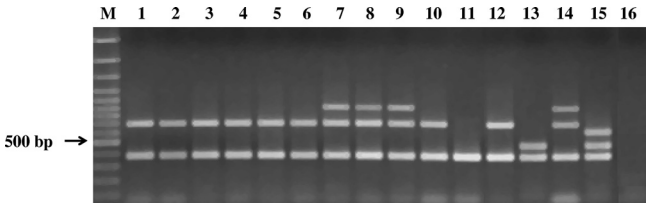

FIG 1 Agarose gel electrophoresis of examples of DNA amplicons generated by multiplex PCR for identification of *L. monocytogenes* serotypes. Lanes 1 to 6, *L. monocytogenes* 1/2a (hide samples); lanes 7 to 9, *L. monocytogenes* 1/2c (hide and carcass samples); lane 10, *L. monocytogenes* 1/2a (carcass sample); lane 11, *L. ivanovii* ATCC 19119; lane 12, *L. monocytogenes* 05CEB424LM (1/2a); lane 13, *L. monocytogenes* 06CEB406LM (1/2b); lane 14, *L. monocytogenes* 06CEB405LM (1/2c); lane 15, *L. monocytogenes* 06CEB422LM (4b); lane 16, negative control.

belonged to serotype 1/2b (1 isolate) or 4b (2 isolates). All strains were positive for the *inlA*, *inlC*, *inlJ*, and *lmo2672* virulence marker genes. Furthermore, 4 *L. monocytogenes* isolates from hides and belonging to serotype 4b (3 isolates) or 1/2a (1 strain) had another virulence marker gene, *llsX*. The antimicrobial resistance results for the 54 *L. monocytogenes* strains showed that all isolates were susceptible to ampicillin, gatifloxacin, levofloxacin, penicillin, rifampin, streptomycin, trimethoprim-sulfamethoxazole, and vancomycin (Table 2). On the other hand, many isolates were resistant to oxacillin (72.2% strains) and several were resistant to clindamycin (37.0%) or ceftriaxone (13.0%).

Our results indicate that *L. monocytogenes* may contaminate beef carcasses during the slaughter process. Molecular serotyping revealed that serotype 1/2a was predominant, irrespective of the sample origin. Very few isolates were of the 1/2c, 4b, or 1/2b serogroup.

Worldwide, several studies were conducted to determine the prevalence of *L. monocytogenes* in bovine samples. This pathogen was identified in cattle hides (13.3%) and carcasses (2.8%) at processing plants in the United States; the most prevalent serotype, as in the present study, was 1/2a, and only few strains were of serogroup 1/2b or 4b (8). Another study performed by Rivera-Betancourt et al. (18) indicated that the bacteria were found on 9.9% of 1,033 examined hide samples, whereas bacteria were found in the carcasses ($n = 522$) to a much smaller extent, i.e., 1.1%.

Many putative virulence markers in *L. monocytogenes* have been identified, and the surface-associated internalins are claimed to play a role in the pathogenesis of human listeriosis (14). In the present study, three main internalin genes (*inlA*, *inlC*, and *inlJ*) as well as the *lmo2672* marker were detected in all 54 *L. monocytogenes* isolates, which suggests that these strains may be potentially virulent for consumers. Similar results were obtained by Mammina et al. (13) in a study of 54 humans. The *L. monocytogenes* isolates belonged to three main serotypes (1/2a, 1/2b, and 4b).

A wide spectrum of antibiotics, of 11 different groups, were used in this study to assess the resistance of the *L. monocytogenes* isolates (Table 2). The results revealed that the strains were sensitive to most antibiotics tested, except oxacillin, to which 72.2% of the isolates were resistant; some strains were also resistant to clindamycin (37.0%). A similar set of antimicrobials was used by Lyon

TABLE 2 Antimicrobial resistance of *L. monocytogenes* isolates tested in the study

Antimicrobial group	Antimicrobial	Antimicrobial class according to WHO[a]	No. (%) of isolates		
			Susceptible	Intermediate	Resistant
Aminoglycosides	Gentamicin	CI	53 (98.1)	0	1 (1.9)
	Streptomycin	CI	54 (100)	0	0
Ansamycin	Rifampin	CI	54 (100)	0	0
Cephalosporins	Ceftriaxone	CI	32 (59.2)	15 (27.8)	7 (13.0)
Glycopeptides	Vancomycin	CI	54 (100)	0	0
Lincosamides	Clindamycin	I	5 (9.3)	29 (53.7)	20 (37.0)
Macrolides	Erythromycin	CI	53 (98.1)	1 (1.9)	0
Oxazolidinones	Linezolid	CI	51 (94.4)	3 (5.6)	0
Penicillins	Ampicillin	CI	54 (100)	0	0
	Penicillin	CI	54 (100)	0	0
	Oxacillin	HI	15 (27.8)	0	39 (72.2)
Fluoroquinolones	Ciprofloxacin	CI	43 (79.6)	11 (20.4)	0
	Gatifloxacin	CI	54 (100)	0	0
	Levofloxacin	CI	54 (100)	0	0
Streptogramins	Quinupristin-dalfopristin	CI	52 (96.2)	1 (1.9)	1 (1.9)
Potentiated sulfonamide	Trimethoprim-sulfamethoxazole	HI	54 (100)	0	0
Tetracyclines	Tetracycline	HI	53 (98.1)	0	1 (1.9)

[a] CI, critically important; I, important; HI, highly important (2).

et al. (12), who found that as many as 90% of *L. monocytogenes* isolates were resistant to oxacillin. Furthermore, the results for ceftriaxone, ciprofloxacin, and clindamycin susceptibility revealed that several strains displayed intermediate resistance, i.e., 38%, 34%, and 27% of isolates, respectively. Similar results for those antibiotics were also found in our studies (27.8%, 20.4%, and 53.7% of the strains tested, respectively).

The resistance of *L. monocytogenes* to antimicrobials currently used in human therapy was also investigated by other authors (3, 15, 16). Most of the strains isolated from raw meat, food, or food-processing environments were susceptible to antimicrobials except oxacillin; some isolates were also resistant to ampicillin, clindamycin, gentamicin, tetracyclines, and penicillin. These results are in agreement with the antimicrobial resistance pattern obtained in the present study. On the other hand, a recent study on *L. monocytogenes* isolates from food and the environment ($n = 202$) performed by Granier et al. (7) showed the resistance of 4 strains (2.0%) only; 2 of them were resistant to tetracycline (MIC > 8 μg/ml), and another 2 were resistant to erythromycin (MIC > 2 μg/ml). As shown in the present study, only 1 out of 54 isolates tested was resistant to tetracycline. All these results indicate the need for further investigation of the antimicrobial resistance profile of *L. monocytogenes*, especially the strains isolated from the food chain.

In summary, our results regarding the presence of virulence markers and antimicrobial resistance of *L. monocytogenes* indicate that contamination of beef may be a public health concern.

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