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Antibiotic Resistance of *Listeria monocytogenes* Isolated from Meat-Processing Environments, Beef Products, and Clinical Cases in Brazil

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The present study aimed to assess the antimicrobial resistance and the presence of virulence markers in 137 *Listeria monocytogenes* isolates obtained from meat-processing environments, beef products, and clinical cases. All isolates were subject to molecular serogrouping and their antibiotic resistance profiles were assessed against 12 antimicrobials. In addition, isolates were subjected to detection of virulence marker genes (*inlA*, *inlC*, *inlJ*). The isolates were classified into serogroups 4b, 4d, 4a, or 4c (46%), 1/2c or 3c (27%), 1/2a or 3a (13.9%), and 1/2b or 3b (13.1%). All tested isolates presented sensitivity to the majority of the tested antimicrobials, but most of them presented resistance or intermediate resistance to clindamycin (88.3%) and oxacillin (73.7%). Virulence markers were detected in all isolates, demanding further analysis to better characterize their pathogenic potential.

Introduction

Listeria monocytogenes is a gram-positive intracellular foodborne pathogen that is widely distributed in the environment. This pathogen can survive on a diversity of surfaces, resulting in persistence in food-processing environments and cross contamination with end products.[15] Listeriosis is the disease caused by *L. monocytogenes* and it is considered an emergent illness since the 1980s, when a number of cases and outbreaks were reported and associated with the consumption of foods contaminated with the pathogen.[8,20,23] This disease is characterized by severe infections in high-risk groups requiring rapid treatment with antibiotics.[31,32]

Serogrouping of *L. monocytogenes* isolates obtained from clinical and food samples is important to predict the possible risks to consumers. Strains from serotypes 1/2a, 1/2b, and 4b are considered the most virulent and are frequently associated with human listeriosis cases and outbreaks.[5,31]

A diversity of proteins are associated with the virulence activity of *L. monocytogenes*, such as internalins, listeriolysins, and phospholipases.[13] The internalin-related genes (*inlA*, *inlC*, and *inlJ*) are involved with the passage through the intestinal barrier, cell adhesion, and invasion, and their presence in *L. monocytogenes* isolates suggests a potential pathogenicity for consumers.

Most *L. monocytogenes* isolates are susceptible to the antibiotics that are usually employed to fight gram-positive bacteria, and the first report of a multiresistant strain was described in 1990 by Poyart-Salmeron *et al.*[27] Nowadays, several studies have shown resistance among isolates obtained from food-processing environments and foods.[17,18,21,25,34] The emergence of antimicrobial resistance has serious consequences for public health, such as failures in the treatment of diseases and limitations in therapeutic choice, and may require the use of more modern drugs.[2]

The present study aimed to assess the antimicrobial resistance and the presence of virulence markers among *L. monocytogenes* isolates obtained from different sources (environment, food, clinical cases) and different Brazilian regions.

Materials and Methods

Microorganisms

A total of 137 *L. monocytogenes* isolates from 11 different states of Brazil were analyzed in this study. They were obtained from different food-processing environments, foods, and clinical cases, between the years 1978 and 2013. All isolates were identified by biochemical tests according Pagotto *et al.*[26] and stored at −20°C in trypticase soya broth (TSB; Oxoid Ltd., Basingstoke, England) supplemented

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with glycerol at 20% (v/v). At the time of use, isolates were transferred to a trypticase soya agar (Oxoid) and incubated at 35°C.

Molecular serogrouping

Isolated colonies from each isolate were transferred to TSB and incubated at 35°C for 24 hr, and the obtained cultures were subjected to DNA extraction and purification using the Wizard Genomic DNA Purification kit (Promega Corp., Madison, WI). PCR serogrouping was conducted based on the assay described by Borucki and Call.[5] The amplification mix composed 12.5 μl of the GoTaq Green Master Mix (Promega), 2 μl DNA, 40 μM of each of the primers D1 (F: CGATATTTTATCTACTTTGTCA; R: TTGCTCCAAAGCAGGGCAT) and D2 (F: GCGGAGAAAGCTATCGCA; R: TTGTTCAAACATAGGGCTA), and ultrapure PCR water (Promega) to 25 μl. Two additional reactions were then conducted as described above, using the primers FlaA (F: TTACTAGATCAAACTGCTCC; R: AAGAAAAGCCCCTCGTCC) and GLT (F: AAAGTGAGTTCTTACGAGATTT; R: AATTAGGAAATCGACCTTCT). Amplification conditions were the same as that described by Borucki and Call.[5] Five-microliter aliquots of the PCR products were electrophoresed on 2.0% (w/v) agarose gels in a 0.5× Tris/Borate/EDTA buffer (TBE), stained with GelRed (Biotium, Inc., Hayward, CA), and visualized in a transilluminator. For each target DNA region, the following PCR product sizes were observed: 214 bp for D1, 140 bp for D2, 538 bp for FlaA, and 483 bp for GLT. In all molecular assays, *L. monocytogenes* strains Scott A, ATCC 7644, and ATCC 15313 were tested in parallel as positive controls.

Detection of virulence markers

Multiplex PCRs were conducted to identify the presence of virulence marker genes involved in the processes of host cell invasion and cell–cell spread: *inlA* (F: ACGAGTTAACGGGACAAATGC; R: CCCGACAGTGGTGCTAGATT), *inlC* (F: AATTCCCACAGGACACAACC; R: CGGGAATGCAATTTTTCACTA), and *inlJ* (F: TGTAACCCCGCTATCACAGTT; R: AGCGGCTTGGCAGTCTAATA).[19] PCRs were composed of 12.5 μl GoTaq Green Master Mix, 2.0 μl DNA, 10.0 μM of each primer, and PCR ultrapure water to a final volume of 25 μl. Amplification conditions were the same as described by Liu *et al.*[19] Five-microliter aliquots of the PCR products were electrophoresed on 1.5% (w/v) agarose gels in 0.5× TBE, stained with GelRed, and visualized in a transilluminator. For each target DNA region, the following PCR product sizes were observed: 800 bp for *inlA*, 517 bp for *inlC*, and 238 bp for *inlJ*. *L. monocytogenes* ATCC 7644 were used as a positive control for the assessed genes.

Antimicrobial resistance

L. monocytogenes strains were subjected to phenotypical analysis to characterize their resistances against 12 antimicrobials (10 μg ampicillin, 10 units penicillin G, 1 μg oxacillin, 2 μg clindamycin, 15 μg erythromycin, 10 μg gentamicin, 10 μg imipenem, 5 μg rifampin, 30 μg chloramphenicol, 30 μg tetracycline, 25 μg trimethoprim/sulfamethoxazole, and 30 μg vancomycin) using the disk diffusion method (Oxoid). Cultures were transferred to brain and heart infusion (Oxoid), incubated at 35°C overnight, and diluted in 0.85% NaCl (w/v) until the turbidity was similar to 0.5 MacFarland. Diluted cultures were swabbed onto the surface of the Mueller–Hinton agar (Oxoid), and the antimicrobial disks were added (three disks per plate). After incubation at 35°C for 18 and 24 hr, the results for each antimicrobial agent were recorded and their resistance profiles were classified as sensitive, intermediate, and resistant, as described by Cockerill[10] to *Staphylococcus* spp. Reference strain *Staphylococcus aureus* ATCC 25923 was used as the control.

Results

Table 1 shows the different origins of *L. monocytogenes* isolates and also the serogrouping results. Among 69 isolates from meat-processing environments, a predominance of isolates from serogroups 4b, 4d, 4a, or 4c, and 1/2c or 3c was observed, and among 43 isolates from beef products, most were identified as belonging to serogroups 1/2b or 3b, followed by 4b, 4d, 4a, or 4c, and 1/2c or 3c. Finally, among 25 clinical strains, the most common serogroups were 4b, 4d, 4a, or 4c.

All *L. monocytogenes* isolates presented positive PCR results for the internalin genes (*inlA*, *inlC*, and *inlJ*) and were susceptible to ampicillin (10 μg), penicillin G (10 units), erythromycin (15 μg), gentamicin (10 μg), imipenem (10 μg), rifampin (5 μg), chloramphenicol (30 μg), tetracycline (30 μg), trimethoprim/sulfamethoxazole (25 μg), and vancomycin (30 μg). However, resistance to clindamycin (2 μg) and oxacillin (1 μg) was found in most of the *L. monocytogenes* isolates (Table 2). The detailed results for each isolate and related to each antibiotic are presented in the Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/mdr).

Discussion

The conventional agglutination method is the reference protocol for serotyping *L. monocytogenes* isolates obtained from clinical and food samples. However, molecular methodologies have been proposed for identification of the main *L. monocytogenes* serogroups associated with listeriosis. These methods provide rapid and low-cost results, but the exact identification of the serotype is not possible since these protocols propose a serogroup categorization that includes different serotypes, that is, usually the most prevalent serotype and other nonfrequent serotypes.[5,14]

In this study, we showed that most isolates recovered from different Brazilian states from food-processing environments and foods belonged to serogroups 4b, 4d, 4a, or 4c (Table 1), which is in agreement with previous studies in Brazil.[3,6,22] Moreover, serotypes 1/2a or 3a, 1/2b or 3b, and 1/2c or 3c also have been identified in food-processing environments and foods in different states, similar to previous studies.[6,7,24] All isolates from clinical cases belonged to serogroups 4b, 4d, 4a, or 4c, 1/2a or 3a, and 1/2b or 3b (Table 1), which are usually associated with the majority of outbreaks and sporadic cases of listeriosis.[31]

The internalin-related genes play important roles in the virulence mechanisms of *L. monocytogenes* and their presence suggests potential pathogenicity. The *inlJ* gene is directly related to the *L. monocytogenes* passage through the

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Table 1. *Listeria monocytogenes* Isolates Obtained from Meat-Processing Environments, Beef Products, and Clinical Samples Used in the Study

			Serogroup			
Source	*Sample*	n	*1/2a or 3a*	*1/2b or 3b*	*1/2c or 3c*	*4b, 4d, 4a, or 4c*
Processing environments	Bovine hides	9	3	1	2	3
	Bovine carcass	13	1	1	2	9
	Swine carcass	1	1	—	—	—
	Conductive mat chicken	9	—	—	—	9
	Plastic box	5	—	—	2	3
	Table	9	—	—	7	2
	Floor of refrigeration room	2	—	—	1	1
	Meat handlers	9	1	—	5	3
	Meat tenderizer	5	—	—	1	4
	Grinder	5	1	—	3	1
	Knife	2	—	—	1	1
Beef products	Refrigerated beef	25	2	6	11	6
	Frozen beef	13	4	5	2	2
	Minced beef	1	—	—	—	1
	Seasoned beef	2	—	2	—	—
	Cooked beef	1	—	—	—	1
	Frozen cooked beef	1	—	—	—	1
Clinical	Blood	7	1	1	—	5
	Cerebrospinal fluid	16	4	2	—	10
	Placenta	1	1	—	—	—
	Sheep brain	1	—	—	—	1
Total	—	137	19	18	37	63

intestinal barrier; the *InlC* gene contributes to the post-intestinal steps of infection; and the *inlA* gene plays an important role to entry into host cells.[19,32] In this study, all *L. monocytogenes* strains were positive for virulence markers *inlA*, *inlC*, and *inlJ*, as observed in a number of other studies with strains from serotypes 1/2a, 1/2c, 1/2b, and 4b.[19,30,34] However, additional characterization, such as sequencing the entire *inlA* gene, is important to properly assess the virulence potential of *L. monocytogenes* strains since some mutations are associated with the expression of truncated proteins, which can result in low virulence potential.[28,30]

Treatment of listeriosis is done using β-lactam antibiotics (ampicillin or amoxicillin) in association, or not, with an aminoglycoside (gentamicin). However, other drugs can also be used, such as erythromycin, tetracycline, chloramphenicol, rifampicin, trimethoprim/sulfamethoxazole, and linezolid.[31,33] In our study, all isolates were susceptible to antibiotics used in listeriosis treatment, but a high level of resistance and intermediate resistance was observed against clindamycin and oxacillin.

Resistance to lincosamides and penicillins has been described previously.[12,16–18,29,34] Antimicrobial resistance in microorganisms can occur due to endogenous and exogenous factors, and the environment plays an important role in allowing interactions with other bacteria and consequent gene or plasmid transfer.[9,11,18,21] Oxacillin and clindamycin resistance has been attributed to efflux pumps or 23S ribosomal RNA modifications,[1,18] as well as excessive use of both drugs in veterinary medicine.[1] However, Bertsch *et al.*[4] suggested that *Listeria fleischmannii*, which is resistant to clindamycin,

Table 2. Antimicrobial Resistance Profile of *L. Monocytogenes* Isolates Tested in This Study

		Number of isolates (%)		
Antibiotic class	*Antimicrobial*	*Resistant*	*Intermediate*	*Susceptible*
Aminoglycoside	Gentamicin	—	—	137 (100.0)
Anfenicol	Chloramphenicol	—	—	137 (100.0)
Ansamycin	Rifampin	—	—	137 (100.0)
Carbapenem	Imipenem	—	—	137 (100.0)
Glycopeptide	Vancomycin	—	—	137 (100.0)
Lincosamide	Clindamycin	72 (52.5)	49 (35.8)	16 (11.7)
Macrolide	Erythromycin	—	—	137 (100.0)
Penicillin	Ampicillin	—	—	137 (100.0)
	Penicillin G	—	—	137 (100.0)
	Oxacillin	78 (56.9)	23 (16.8)	36 (26.3)
Potentiated sulfonamide	Trimethoprim–sulfamethoxazole	—	—	137 (100.0)
Tetracycline	Tetracycline	—	—	137 (100.0)

may possess a transferable transposon that remains to be identified. While these drugs are not used to treat listeriosis, the emergence of resistance demands attention due to the possibility of horizontal transfer to other bacteria.[25]

This study showed the prevalence of pathogenic serogroups among isolates from food-processing environments, foods, and clinical cases in Brazil. Despite having susceptibility to most of the antibiotics used to treat listeriosis, the presence of high antimicrobial resistance to oxacillin and clindamycin is a serious concern for public health, and more in-depth research is needed to better understand the mechanisms of antimicrobial resistance. In addition, all isolates harbored virulence marker genes, demanding further analysis to properly characterize their pathogenic potential.

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Disclosure Statement

No competing financial interests exist.

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