

Antimicrobial Susceptibility of *Listeria monocytogenes* Food Isolates from Different Cities in Colombia

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Abstract

One hundred eight *Listeria monocytogenes* food isolates from four cities in Colombia and previously confirmed by multiplex polymerase chain reaction were characterized for antimicrobial susceptibility. Isolates were evaluated against 17 antimicrobials contained in the MICroSTREP plus[®]3 panel (MicroScan system). Susceptibility found for ampicillin, amoxicillin/clavulanic acid, and chloramphenicol was 100%, whereas it was 98% for other antimicrobials such as trimethoprim/sulfamethoxazole, 97% for azithromycin, 92% for vancomycin, 90% for erythromycin, 86% for tetracycline, 84% for penicillin, 70% for ciprofloxacin, 57% for rifampin, 56% for meropenem, and 32% for clindamycin. Natural resistance to cephalosporins was confirmed in all cases, and 16% of isolates were nonsusceptible to penicillin. Using *Staphylococcus* spp. or *Enterococcus* spp. breakpoints, 48% of isolates displayed multidrug resistances, and the major resistance phenotypes were against rifampin, clindamycin, ciprofloxacin, azithromycin, and erythromycin. Colombian food isolates displayed high resistance to clindamycin, meropenem, rifampin, and ciprofloxacin (30%–65%), and the primary drugs of choice against listeriosis remain effective for most of isolates (84%).

Introduction

LISTERIA MONOCYTOGENES is an emergent food-borne pathogen responsible for major outbreaks and sporadic food-related cases of listeriosis (Posfay-Barbe and Wald, 2009) worldwide (Torres *et al.*, 2004), rendering this food-transmitted disease a public health concern.

L. monocytogenes can affect the central nervous system, causing neurological sequels or leading to death. Newborns, pregnant women, old people, and immunologically suppressed people are more susceptible to the illness. The clinical forms of the disease vary according to the infected group and the most common manifestations are meningitis, meningoencephalitis, septicemia, abortion, and prenatal infection. Mortality by this disease oscillates between 20% and 30% in risk groups (Korkeala and Siitonen, 2003). The noninvasive form of listeriosis causes gastrointestinal syndrome (Torres *et al.*, 2005).

A large variety of foods have been implicated as vehicles in sporadic outbreaks and epidemics; among them are milk, cheeses, vegetables, poultry, and beef products, particularly ready-to-eat products (Gallegos *et al.*, 2008; Vanegas *et al.*, 2009).

L. monocytogenes is naturally resistant to cephalosporins, aztreonam, fosfomicin, piperimic acid, dalfopristin/quinupristin, and sulfamethoxazole (STX) (Charpentier and Courvalin, 1999; Troxler *et al.*, 2000). The primary drugs of choice against *L. monocytogenes* causing human listeriosis are penicillin and ampicillin alone or combined with an aminoglycoside such as streptomycin or gentamicin; patients allergic to penicillin may be treated with trimethoprim/STX (CLSI, 2008b; Lyon *et al.*, 2008; Chen *et al.*, 2010). Vancomycin, erythromycin, tetracycline, and chloramphenicol have also been used in listeriosis treatment (Charpentier and Courvalin, 1999); however, it is possible to find case reports involving the use of other antibiotics against *L. monocytogenes*, such as linezolid and rifampin (Salamano *et al.*, 2005; Morosi *et al.*, 2006).

Several studies on antimicrobial resistance of *Listeria* spp. clearly show that genetic material involved in short-term evolutionary adaptation may be successfully transferred from *Bacillus subtilis*, *E. coli*, and *Streptococcus* spp. to *Listeria* spp. and also from *Listeria* spp. to other Gram-positive bacteria phylogenetically related, such as *Enterococcus* spp. and *Staphylococcus* spp. (Lyon *et al.*, 2008). Further, the genetic

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heterogeneity of *Listeria* spp. indicates that both vertical and horizontal gene transfers are relatively common in these bacteria (Charpentier and Courvalin, 1999).

Although government recommendations do not support this practice, in Colombia, as in many other countries, it is a common practice to provide large doses of antibiotics to livestock and poultry, assuming a "disease prevention and growth promoters effect" and thus increasing the risk of stimulating a dissemination cycle of antibiotic-resistant microorganisms (Lozano and Arias, 2008).

As there are few works on antimicrobial susceptibility of Colombian *Listeria* spp. isolated from food (Gallegos *et al.*, 2008), the aim of the present retrospective study was to describe the antimicrobial susceptibility profile of *L. monocytogenes* food isolates of four cities of Colombia. It is important to remark that because of a low and variable number of isolates from certain food products or cities, it is not possible to establish relation between the resistance pattern and the food origin.

Materials and Methods

Isolates

One hundred eight food isolates of *L. monocytogenes* previously confirmed by PCR (Poutou *et al.*, 2005) were used. Isolates were distributed as follows: Bogotá ($n=44$; 41%), Madrid-Colombia ($n=8$; 7%), Funza ($n=42$; 39%), Pamplona-Colombia ($n=12$; 11%), and 2 (2%) of unknown provenience. Isolates were from poultry ($n=28$; 26%), cheese ($n=20$; 19%), lettuce ($n=8$; 7%), spinach ($n=34$; 31%), raw cow milk ($n=17$; 16%), and 1 (1%) of unknown food-type origin.

Antimicrobial susceptibility test

Currently, there are no interpretative criteria for antimicrobial susceptibility of *Listeria* spp., except for ampicillin, penicillin, and trimethoprim/STX. Therefore, breakpoints recommended by CLSI for *Enterococcus* spp. and *Staphylococcus* spp. were applied in this study for the other antimicrobials tested (Conter *et al.*, 2009; CLSI, 2010; De Nes *et al.*, 2010).

The antimicrobial susceptibility of the 108 isolates of *L. monocytogenes* was evaluated with the broth microdilution technique (MicroScan system). This system was approved by the "Instituto Nacional de Vigilancia de Medicamentos y Alimentos" (INVIMA), and the panel MICroSTREP plus[®]3 (INVIMA 2006RD-0000092) allowed the fulfillment of the requirement stated in the M45A document by CLSI (2008), related with the use of lysed horse blood for detection of antimicrobial susceptibility of exigent microorganisms (CLSI, 2008a).

A cell suspension equivalent to 0.5 McFarland scale (Müller-Hinton plus lysed horse blood) was inoculated into the MICroSTREP plus3 (Siemens) and incubated at 37°C as recommended by the manufacturer. The panel included the following antimicrobials: penicillin, ampicillin, cefotaxime, cephadrine, cefepime, chloramphenicol, trimethoprim/STX, cefuroxime, rifampin, meropenem, amoxicillin/clavulanic acid, clindamycin, tetracycline, azithromycin, erythromycin, vancomycin, and ciprofloxacin. We used *S. pneumoniae* (ATCC 49619) as a control for the antimicrobial susceptibility tests (CLSI, 2008a). Descriptive statistical analysis was carried out with the software Whonet 5.6 (2010) (Miranda *et al.*, 2006b).

Results and Discussion

Colombia, as a tropical country, lacks four seasons and shows slight climatic variations within a region; on the contrary, noticeable climatic differences occur between regions located at different altitudes or "thermal floors." In a previous study, we detected a certain influence of "thermal floors" and their associated environmental conditions (temperature, relative humidity, oxygen pressure, and ecological factors) on the food recovery of *L. monocytogenes* (Gallegos *et al.*, 2008). The present retrospective study included *L. monocytogenes* food isolates from cold-climate cities.

The resistance of *L. monocytogenes* is emerging and has been previously documented; in this sense, it is known that the standard antibiotic therapy choices for effective treatment of human listeriosis are penicillin, ampicillin, and trimethoprim/STX (CLSI, 2008a); for these antimicrobials, our food isolates of *L. monocytogenes* displayed 84%–100% susceptibility (Table 1).

The rare occurrence of penicillin resistance among food isolates of *L. monocytogenes* allows using a category different to susceptible, as it was recommended (CLSI, 2008a). A noteworthy 16% (17/108) of the isolates were nonsusceptible to penicillin, with minimum inhibitory concentrations (MICs) of 4 or 8 $\mu\text{g mL}^{-1}$ (Table 1); all of these isolates have to be submitted to a reference laboratory for susceptibility confirmation. On the contrary, recent studies have reported penicillin resistance levels of 66.7% (Santos Mantilla *et al.*, 2008), susceptibility levels of 5% (Pesavento *et al.*, 2010), and intermediate levels of about 83% (Chen *et al.*, 2010), which are higher than our results.

The ampicillin MIC₉₀ coincided with the susceptibility breakpoint, whereas penicillin MIC₉₀ was 4 $\mu\text{g mL}^{-1}$, exceeding the susceptibility breakpoint (Table 1). This result suggests an increase in antimicrobial resistance of some *L. monocytogenes* isolates when compared with clinical isolates

TABLE 1. ANTIMICROBIAL SUSCEPTIBILITY TEST FOR PENICILLIN, AMPICILLIN, AND TRIMETHOPRIM/SULFAMETHOXAZOLE OF *LISTERIA MONOCYTOGENES* FOOD ISOLATES

Antimicrobial	Breakpoints (mg mL^{-1})						No. of isolates (%)			
	R	I	S	MIC range	MIC ₅₀	MIC ₉₀	R	I	S	NS
PEN	—	—	≤ 2	0.25–8	1	4	0 (0)	0 (0)	91 (84)	17 (16)
AM	—	—	≤ 2	0.25–2	0.5	2	0 (0)	0 (0)	108 (100)	0 (0)
SXT	$\geq 4/76$	1/19–2/38	$\leq 0.5/9.5$	0.125–1	0.25	0.25	0 (0)	2 (2)	106 (98)	0 (0)

Breakpoints for *Listeria monocytogenes* were used for interpretation (CLSI, 2008a, 2010).

MIC, minimum inhibitory concentration; R, resistant; I, intermediate; S, susceptible; NS, nonsusceptible; PEN, penicillin; AM, ampicillin; SXT, trimethoprim/sulfamethoxazole.

reported (Martínez-Martínez *et al.*, 2001), which showed MIC₉₀ of about 1 µg mL⁻¹.

Resistance to trimethoprim/STX of *L. monocytogenes* was previously documented in 1997 (Morvan *et al.*, 2010), with MICs of 1,024 µg mL⁻¹. In our present work, 2% of the isolates were intermediate (MIC: 1 µg mL⁻¹) against SXT (Table 1). Considering that an intermediate strain will become a resistant one, our results agree with recent studies reporting 1.6%

(Conter *et al.*, 2009) and 0.6% (Lyon *et al.*, 2008) of resistance; however, some authors have reported higher resistance levels of up to 66% (Yücel *et al.*, 2005). The MIC₉₀ for STX was lower than the susceptibility breakpoint, proving the effectiveness of the antibiotic (Table 1).

Figure 1 (bottom graph) shows that only 1% (1/108) of the isolates were nonsusceptible to penicillin (MIC: 4 µg mL⁻¹) and also intermediate for trimethoprim/STX (MIC: 1 µg mL⁻¹);

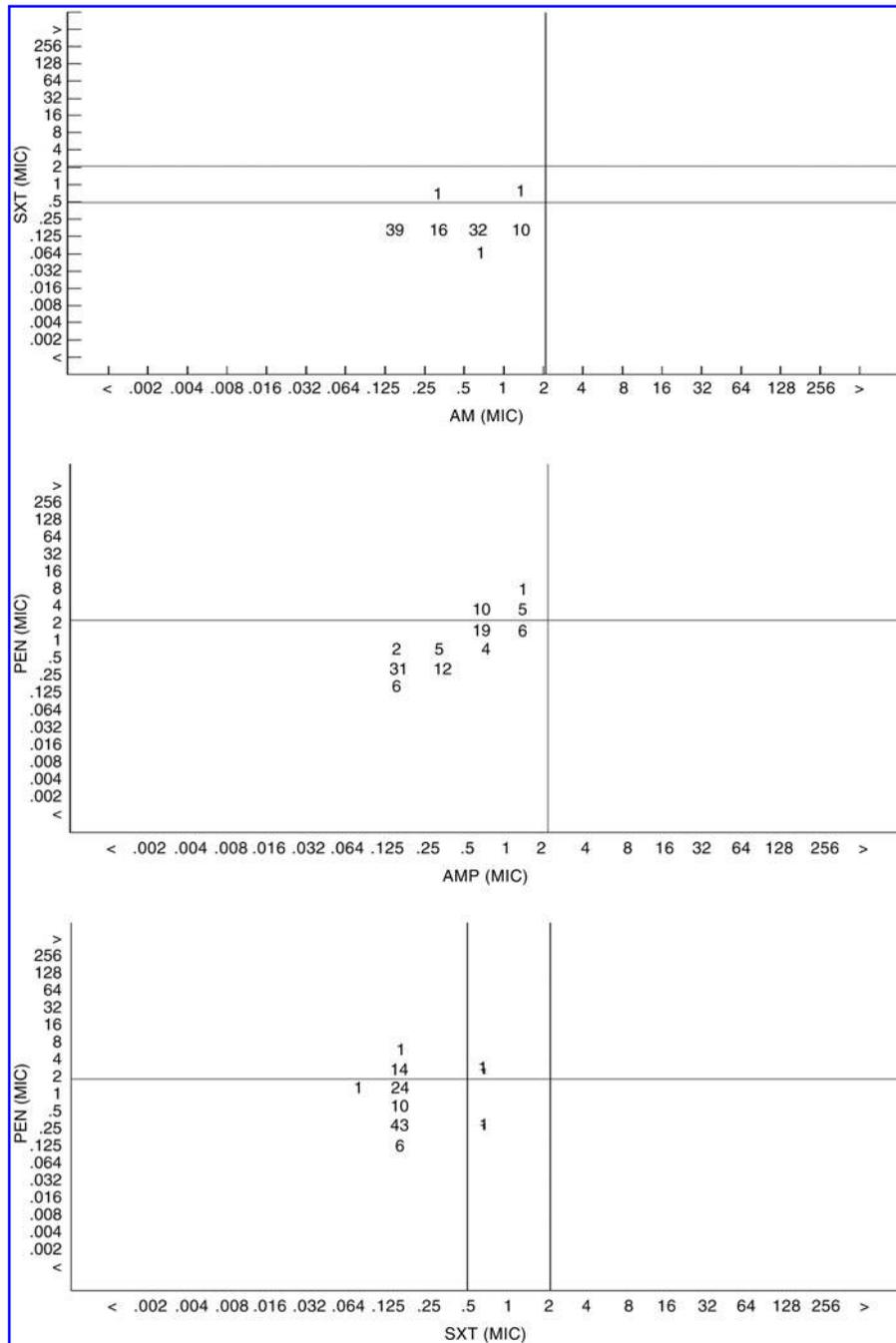


FIG. 1. Whonet 5.6 (2010) graphs. Isolates distribution expressed in percentage as a function of *Listeria monocytogenes* MICs. Top: trimethoprim/SXT versus AM. Middle: PEN versus AM. Bottom: PEN versus trimethoprim/SXT. Internal lines remark susceptibility and resistance breakpoints for *L. monocytogenes*, and the space between two vertical or horizontal lines shows the intermediates MIC for SXT. In the case of AM and PEN, only one line appears, remarking the susceptibility breakpoint; in both cases, resistance breakpoint has not been defined (1). MIC, minimum inhibitory concentration; SXT, sulfamethoxazole; AM, ampicillin; PEN, penicillin.

TABLE 2. ANTIMICROBIAL SUSCEPTIBILITY TEST OF *LISTERIA MONOCYTOGENES* FOOD ISOLATES AGAINST THE OTHER ANTIMICROBIALS ASSAYED, EXCEPT VANCOMYCIN

Antimicrobial	Breakpoints (mg mL ⁻¹)			MIC range	MIC ₅₀	MIC ₉₀	No. of isolates (%)		
	R	I	S				R	I	S
Amox/Clav	≥32	4	≤8	0.125–1	0.5	0.5	0 (0)	0 (0)	108 (100)
Meropenem	≥16	8	≤4	0.12–8	4	8	0 (0)	48 (44)	60 (56)
Rifampin	≥4	2	≤1	0.5–4	0.5	4	44 (41)	2 (2)	62 (57)
Ciprofloxacin	≥4	2	≤1	0.12–2	0.5	2	0 (0)	32 (30)	76 (70)
Clindamycin	≥4	1–2	≤0.5	0.25–8	4	4	70 (65)	3 (3)	35 (32)
Azithromycin	≥8	4	≤2	0.125–8	0.25	2	2 (2)	1 (1)	105 (97)
Erythromycin	≥8	1–4	≤0.5	0.125–8	0.25	1	1 (1)	10 (9)	97 (90)
Chloramphenicol	≥32	16	≤8	4–8	4	4	0 (0)	0 (0)	108 (100)
Tetracycline	16	8	≤4	0.1–8	1	8	0 (0)	15 (14)	93 (86)

Common breakpoints for *Staphylococcus* sp. and *Enterococcus* sp. were used for interpretation (CLSI, 2008a, 2010). Un, unknown; Amox/Clav, amoxicillin/clavulanic acid (Augmentin).

the figure also shows that 15% of penicillin-nonsusceptible isolates were sensitive to trimethoprim/STX. In Figure 1, the middle graph shows that 16% of isolates nonsusceptible to penicillin are susceptible to ampicillin and the top graph shows that 2% of intermediates for trimethoprim/STX isolates were sensitive to ampicillin, which is an encouraging result in terms of possible treatment.

The results of the antimicrobial susceptibility tests for the other antimicrobials are shown in Tables 2 and 3. The other 14 antimicrobial agents were tested, but *L. monocytogenes* showed natural resistance as expected to all the cephalosporins included in the MICroSTREP plus3 panel (Troxler *et al.*, 2000), namely cefotaxime, cefepime, cephadrine, and cefuroxime axetil; therefore, they were excluded from Tables 2 and 3.

More than 90% of isolates were susceptible to other antimicrobials, such as amoxicillin/clavulanic acid (Augmentin), azithromycin, erythromycin, chloramphenicol, and vancomycin (Tables 2 and 3). On the contrary, there is a remarkable number of isolates with intermediate MICs for rifampin, ciprofloxacin, meropenem, and tetracycline and with a high percentage of resistance to clindamycin and rifampin; all of them showed a MIC₉₀ of about 2 or 4 µg mL⁻¹. This MIC₉₀ ranging between an intermediate and a resistant strain suggests a potentially inefficient antimicrobial action in the future (Table 2).

Rifampin is a wide-spectrum antimicrobial because of its action on the RNA polymerase β subunit and is active against mycobacteria and Gram-positive bacteria. Its resistance has

been previously documented (Morse *et al.*, 1999) and different levels have been found: 50% (Santos Mantilla *et al.*, 2008), 3% (Conter *et al.*, 2007), and 1.6% (Conter *et al.*, 2009). The levels found in our work, that is, 41% of intermediates plus 2% of resistants (Table 2), should be considered as alarming values because rifampin is the major choice in therapeutic treatment against *Mycobacterium tuberculosis* when no multidrug resistance pattern is detected (Miranda *et al.*, 2006a).

In the case of ciprofloxacin, MIC₉₀ values (2 µg mL⁻¹) similar to that found in our present work (Table 2) have been reported for clinical isolates of *L. monocytogenes* (Martínez-Martínez *et al.*, 2001). The frequency of 30% intermediates in our study is higher when compared with the 2.4% (Li *et al.*, 2006) and 1.6% (Conter *et al.*, 2009) resistance levels previously reported, but all these results are supported by a reduction in susceptibility to ciprofloxacin as was recently reported (De Nes *et al.*, 2010).

Meropenem apparently has not been used in antimicrobial susceptibility testing of *L. monocytogenes* food isolates or in clinical treatment against listeriosis. In this sense, it is not useful to make an interpretation of MIC₉₀ beyond the fact that it coincides with the intermediate MIC (Table 2).

Apparently, the *L. monocytogenes* tetracycline resistance varies widely, being frequently found in food, environmental, human, and animal isolates (Conter *et al.*, 2007). Our results agree with those of Li *et al.* (2006), who found 18.6% of resistance (Table 2); however, higher levels (66.7%) were reported (Santos Mantilla *et al.*, 2008). Despite some other works reporting 6.3% (Walsh *et al.*, 2001), 6.4% (Rodas-Suárez *et al.*,

TABLE 3. ANTIMICROBIAL SUSCEPTIBILITY TEST OF *LISTERIA MONOCYTOGENES* FOOD ISOLATES AGAINST VANCOMYCIN

Antimicrobial	Breakpoints (mg mL ⁻¹)			MIC range	MIC ₅₀	MIC ₉₀	No. of isolates (%)		
	R	I	S				R	I	S
Vancomycin ^a	≥16	4–8	≤2	0.25–4	1	2	0 (0)	9 (8) ^c	99 (92)
Vancomycin ^b	≥32	8–16	≤4	0.25–4	1	2	0 (0)	0 (0)	108 (100)

^aBreakpoint for *Staphylococcus* sp.

^bBreakpoint for *Enterococcus* sp. used for interpretation (CLSI, 2008a, 2010).

^cThese results should not be considered alarming data, if we consider that the breakpoints for *Staphylococcus* spp., and *Enterococcus* spp. are very different. On the other hand, it is not convenient to generate alarm over the presence of phenotypes *L. monocytogenes* VA¹ without prior exhaustive confirmation.

2006), and 3% (Lyon *et al.*, 2008) of resistance, tetracycline is not considered a primary drug choice for listeriosis treatment and its use is not recommended in children and pregnant women.

Sixty-five percent of isolates displayed a strong resistance against clindamycin. This antimicrobial interferes with the protein synthesis in a similar way as erythromycin and chloramphenicol. All of them bind with the bacterial 50S ribosome subunit. Because of this reason, a cross-resistance among clindamycin, erythromycin, and chloramphenicol can sometimes be detected (Depardieu *et al.*, 2007). In our present work, only 5 of 52 multidrug-resistant isolates (10%) displayed resistance to both clindamycin and erythromycin, suggesting a possible modification of the 23S rRNA (Davis and Jackson, 2009). No resistance to chloramphenicol was detected. In contrast, the high resistance found against clindamycin (Table 2) may suggest that a mechanism of resistance such as a clindamycin-inactivating enzyme that modifies the antimicrobial structure could occur, as has been previously reported (Brisson-Noel *et al.*, 1988; Davis and Jackson, 2009; Chen *et al.*, 2010).

On the basis of the CLSI breakpoints for *Staphylococcus* spp. and *Enterococcus* spp., 48% of the isolates were classified as multidrug resistant (Table 4). In this study, intermediate MICs were considered as resistant at the moment of performing the

multidrug resistance patterns. These distributions show that resistances to rifampin, clindamycin, erythromycin, meropenem, tetracycline, and ciprofloxacin were the most frequent multidrug resistance phenotypes and were present in all the food types (Table 4).

The geographical distribution of multidrug resistance patterns was as follows: 50% (4/8) in Madrid (Colombia), 42% (5/12) in Pamplona (Colombia), 59% (26/44) in Bogotá, 36% (15/42) in Funza, and 100% (2/2) of unknown origin. In this sense, it is important to remark that, in the present study, it was not possible to test the hypothesis that the resistance pattern depends on the origin because of a low and variable number of isolates from certain food products or cities.

These results are supported by the fact that both poultry and cattle are treated with antibiotics as prophylactic agents and growth promoters and vegetables are irrigated with poultry manure (called "gallinaza" in Colombia).

All these antimicrobial susceptibility results are also supported by the fact that in Colombia it is authorized to use more than 16 antimicrobial molecules with the intention of fattening or for therapeutic purposes (Lozano and Arias, 2008), which leads to resistance gene transfer and the presence of antibiotic residues in food products (Teuber, 1999; Phillips *et al.*, 2004; Lozano and Arias, 2008). Although since 2005 the

TABLE 4. DISTRIBUTION OF MULTIDRUG RESISTANCE PATTERNS BY FOOD TYPE

Phenotype	Food type origin No. of isolate phenotypes/no. of multidrug resistances (%)					
	Cheeses	Cow milk	Poultry	Lettuce	Spinach	Unknown
MER ^I RIF ^R CD ^R CP ^I	0 (0)	0 (0)	0 (0)	0 (0)	2/12 (16.6)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I	0 (0)	0 (0)	1/14 (7.2)	0 (0)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^I	0 (0)	1/8 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^I	0 (0)	1/8 (12.5)	1/14 (7.2)	1/3 (33.33)	2/12 (16.6)	1/2 (50)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	0 (0)	1/14 (7.2)	0 (0)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	1/8 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	0 (0)	0 (0)	1/3 (33.33)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	0 (0)	0 (0)	0 (0)	1/12 (8.3)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	0 (0)	0 (0)	0 (0)	1/12 (8.3)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	0 (0)	0 (0)	1/3 (33.33)	1/12 (8.3)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	8/14 (57.2)	2/8 (25)	3/14 (21.3)	0 (0)	2/12 (16.6)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	0 (0)	0 (0)	0 (0)	1/12 (8.3)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	0 (0)	0 (0)	0 (0)	1/12 (8.3)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	3/14 (21.2)	0 (0)	4/14 (28.6)	0 (0)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	0 (0)	1/14 (7.2)	0 (0)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	0 (0)	1/14 (7.2)	0 (0)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	1/14 (7.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	1/14 (7.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	1/8 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	0 (0)	1/14 (7.2)	0 (0)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	1/14 (7.2)	0 (0)	0 (0)	0 (0)	1/12 (8.3)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	1/8 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	0 (0)	1/14 (7.2)	0 (0)	0 (0)	0 (0)
MER ^I RIF ^R CD ^R CP ^I TE ^I AZI ^R E ^R	0 (0)	1/8 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)
No. of multidrug-resistant/no. of food-specific isolates (%)	14/20 (70)	8/17 (47)	14/28 (50)	3/8 (38)	12/34 (62)	1/2 (50)
No. of multidrug-resistant/total multidrug-resistant isolates (%)	14/52 (27)	8/52 (15)	14/52 (27)	3/52 (6)	12/52 (23)	1/52 (2)
No. of multidrug-resistant/total isolates (%)	14/108 (13)	8/108 (7)	14/108 (13)	3/108 (3)	12/108 (11)	1/108 (1)

Intermediates were considered inside the multidrug resistance pattern. Vancomycin intermediates were not included because breakpoints differ between *Staphylococcus* spp. and *Enterococcus* spp.

MER, meropenem; RIF, rifampin; CD, clindamycin; CP, ciprofloxacin; TE, Tetracycline; AZI, azithromycin; E, erythromycin.

Instituto Colombiano de Agricultura has taken action against the indiscriminate use of antibiotics and the national legislation has adopted several standards for animals, prohibiting the use of nitrofurans, chloramphenicol, gentian violet, and dimetridazole, and the application of therapeutic antimicrobials as growth promoters (Conpes, 2005, 2007a, 2007b), there remain shortcomings in monitoring and controlling the marketing of these products.

Conclusions

Despite having a nonhomogeneous isolate distribution, the results of this work show several important findings: first, the antibiotics of choice in the treatment of listeriosis (penicillin, ampicillin, and trimethoprim/STX) remain effective, despite the occurrence of nonsusceptible strains to penicillin; second, our results support the fact that the indiscriminate supply of antibiotics as prophylactics or metaphylactics has favored the acquisition of antimicrobial resistance genes, which has been documented by other authors (Phillips *et al.*, 2004; Lozano and Arias, 2008); third, it seems that rifampin resistance was high, which is alarming if we consider that rifampin is a very important choice in the treatment against *M. tuberculosis*; and fourth, MIC₉₀ value for vancomycin was low, which is a positive and significant fact taking into account the gene transfer between *L. monocytogenes* and *Staphylococcus aureus* (Lyon *et al.*, 2008), a vancomycin intermediate or resistant (VISA or VRSA) phenotype that has not yet been officially reported in Colombia (Contreras *et al.*, 2005).

Acknowledgments

This work was financed by Laboratorio de Microbiología de Alimentos, Grupo de Biotecnología Ambiental e Industrial (GBAI) de la Facultad de Ciencias de la Pontificia Universidad Javeriana (Project ID 00003436), Bogotá, Colombia, and the Instituto de Investigaciones Biológicas del Trópico (IIBT) at the Facultad de Medicina Veterinaria, Universidad de Córdoba, Montería, Colombia. Special thanks to Dr. Gilma J. Luna and Dr. María C. Vanegas for providing some isolates for this study.

Disclosure Statement

The first author is currently working for Rochem Biocare S.A. as a product specialist. This company is licensed by Siemens to distribute in Colombia the MICroSTREP plus3 panels used in this work for the antimicrobial susceptibility study. These findings are neither related to nor the result of pressures due to her job position. The selected panels allowed the fulfillment of the requirement stated in the M45A document by CLSI (2008), related with the use of lysed horse blood for detection of antimicrobial susceptibility of exigent microorganisms such as *L. monocytogenes*. Therefore, the authors declare that there are no conflicts of interests in this work.

References

Brisson-Noel A, Delrieu P, Samain D, and Courvalin P. Inactivation of lincosamide antibiotics in *Staphylococcus*: identification of lincosaminide o-nucleo-tidyltransferases and comparison of the corresponding resistance genes. *J Biol Chem* 1988;263:15880–15887.

[CLSI] Clinical and Laboratory Standards Institute. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infre-*

quently Isolated of Fastidious Bacteria; Approved Guideline. M45-A. Vol. 26, No. 19, 2008a, 76p.

[CLSI] Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement*. Vol. 27, No. 1, 2008b, pp. 1–188.

[CLSI] Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement*. M100-S20. Vol. 30, No. 1, 2010, 160p.

[Conpes] Consejo Nacional de Política Económica y Social. Política nacional de sanidad e inocuidad para la cadena porcícola, Conpes 3458 (Ministerio de Agricultura y Desarrollo Rural, Ministerio de Protección Social, Ministerio de Ambiente, Vivienda y Desarrollo Territorial, Ministerio de Comercio, Industria y Turismo, Bogotá, D.C. Colombia). 2007a, 42p.

[Conpes] Consejo Nacional de Política Económica y Social. Política nacional de sanidad e inocuidad para la cadena avícola, Conpes 3468 (Ministerio de Agricultura y Desarrollo Rural, Ministerio de Protección Social, Ministerio de Ambiente, Vivienda y Desarrollo Territorial, Ministerio de Comercio, Industria y Turismo, Bogotá, D.C. Colombia). 2007b, 37p.

[Conpes] Consejo Nacional de Política Económica y Social. Política nacional de sanidad e inocuidad para las cadenas de la carne bovina y de la leche, Conpes 3476 (Ministerio de Agricultura y Desarrollo Rural, Ministerio de Protección Social, Ministerio de Ambiente, Vivienda y Desarrollo Territorial, Ministerio de Comercio, Industria y Turismo, Bogotá, D.C. Colombia). 2005, 39p.

Conter M, Paludi D, D'Orio V, Vergara A, and Ianieri A. Antimicrobial susceptibility of *Listeria monocytogenes* isolated from food and food-processing environment. *Ann Fac Medic Vet di Parma* 2007;XXVII:157–164.

Conter M, Paludi D, Zanardi E, Ghidini S, Vergara A, and Ianieri A. Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. *Int J Food Microbiol* 2009;128:497–500.

Contreras GA, Gómez CA, Leal AL, Perez MJ, and Navarrete ML. *Staphylococcus aureus* resistentes a la vanomicina: una nueva amenaza. *Infectio* 2005;9:91–99.

Charpentier E and Courvalin P. Antibiotic Resistance in *Listeria* spp. *Antimicrob Agents Chemother* 1999;43:2103–2108.

Chen B-Y, Pyla R, Kim T-J, Silva JL, and Jung Y-S. Antibiotic resistance in *Listeria* species isolated from catfish fillets and processing environment. *Lett Appl Microbiol* 2010;50:626–632.

Davis JA and Jackson CR. Comparative antimicrobial susceptibility of *Listeria monocytogenes*, *L. innocua*, and *L. welshimeri*. *Microb Drug Resist* 2009;15:27–32.

De Nes F, Pelicoli Riboldi G, Guedes Frazzon AP, Alves d'Azevedo P, and Frazzon J. Antimicrobial resistance and investigation of the molecular epidemiology of *Listeria monocytogenes* in dairy products. *Rev Soc Bras Med Trop* 2010;43:382–385.

Depardieu F, Podglajen I, Leclercq R, Collatz E, and Courvalin P. Modes and Modulations of Antibiotic Resistance Gene Expression. *Clin Microbiol Rev* 2007;20:79–114.

Gallegos JM, Vanegas MC, Albarracín Y, Máttar S, Poutou RA, and Carrascal AK. Frequency of isolation of *Listeria* spp., in different retail foods in Colombia. *APRA* 2008;4:9–18.

Korkeala H and Siitonen A. *Listeria monocytogenes*. Isolates from invasive infections: variation of sero-and genotypes during an 11 year period in Finland. *J Clin Microbiol* 2003;41:1694–1700.

Li Q, Sherwood JS, and Logue CM. Antimicrobial resistance of *Listeria* spp. recovered from processed bison. *Lett Appl Microbiol* 2006;44:86–91.

- Lozano MC and Arias DC. Residuos de fármacos en alimentos de origen animal: panorama actual en Colombia. *Rev Colomb Cienc Pecu* 2008;21:121–135.
- Lyon SA, Berrang ME, Fedorka-Cray PJ, Fletcher DL, and Meinersmann RJ. Antimicrobial Resistance of *Listeria monocytogenes* Isolated from a Poultry Further Processing Plant. *Foodborne Pathog Dis* 2008;5:253–259.
- Martínez-Martínez L, Joyanes P, Suárez AI, and Perea EJ. Activities of gemifloxacin and five other antimicrobials agents against *Listeria monocytogenes* and Coryneform bacteria isolated from clinical samples. *Antimicrob Agents Chemother* 2001;45:2390–2392.
- Miranda J, Rios R, Clavijo A, Chacón C, and Mattar S. Estudio preliminar de la susceptibilidad antimicrobiana y variabilidad genética de *Mycobacterium tuberculosis* en un área del Caribe colombiano. *Colombia Méd* 2006a;37:275–286.
- Miranda MC, Pérez F, Zuluaga T, *et al.*. Resistencia a antimicrobianos de bacilos Gram negativos aislados en unidades de cuidado intensivo en hospitales de Colombia, WHONET 2003, 2004 y 2005. *Biomédica* 2006b;26:424–433.
- Morosi S, Francisci D, and Baldelli F. A case of rhombencephalitis caused by *Listeria monocytogenes* successfully treated with linezolid. *J Infect* 2006;52:e73–e75.
- Morse R, O'hanlon K, Virji M, and Collins MD. Isolation of rifampin-resistant mutants of *Listeria monocytogenes* and their characterization by *rpoB* gene sequencing, temperature sensitivity for growth, and interaction with an epithelial cell line. *J Clin Microbiol* 1999;37:2913–2919.
- Morvan A, Moubareck C, Lecrercq A, *et al.*. Antimicrobial resistance of *Listeria monocytogenes* strains isolated from human in France. *Antimicrob Agents Chemother* 2010;54:2728–2731.
- Pesavento G, Ducci B, Nieri D, Comodo N, and Lo Nostro A. Prevalence and antibiotic susceptibility of *Listeria* spp isolated from raw meat and retail foods. *Food Cont* 2010;21:708–713.
- Phillips I, Casewell M, Cox T, *et al.*. Does the antibiotics in food animals pose a risk to human health? A critical review of published data. *J Antimicrob Chem* 2004;53:28–52.
- Posfay-Barbe KM and Wald ER. Listeriosis. *Sem Fetal Neon Med* 2009;14:228–233.
- Poutou RA, Burbano ME, Sierra SC, Torres KJ, Carrascal AK, and Mercado M. Estandarización de la Extracción de ADN y Validación de la PCR Múltiple para Detectar *Listeria monocytogenes* en Queso, Leche, Carne de Res y Pollo. *Univ Sci* 2005;10:61–78.
- Rodas-Suárez OR, Flores-Pedroche JF, Betancourt-Rule JM, Quiñones-Ramírez EI, and Vázquez-Salinas C. Occurrence and antibiotic sensitivity of *Listeria monocytogenes* strains isolated from oysters, fish, and estuarine water. *Appl Environ Microbiol* 2006;72:7410–7412.
- Salamano R, Braselli A, Hoppe A, Monteghirfo R, and Silva T. Neurolisteriosis en adultos. A propósito de seis casos clínicos. *Arq Neuropsiquiatr* 2005;63:1063–1069.
- Santos Mantilla SP, Franco RM, Trindade de Oliveira LA, Barbosa Santos E, and Gouvea R. Resistência antimicrobiana de bactérias do gênero *Listeria* spp. isoladas de carne moída bovina. *Braz. J Vet Res Anim Sci* 2008;45:116–121.
- Teuber M. Spread of antibiotic resistance with food-borne pathogens. *Cell Mol Life Sci* 1999;56:755–763.
- Torres KJ, Sierra SC, Poutou RA, Carrascal AK, and Mercado M. Patogénesis de *Listeria monocytogenes*, Microorganismo Zoonótico Emergente. *Rev MVZ-Córdoba* 2005;10:511–543.
- Torres KJ, Sierra SC, Poutou RA, Vera H, Carrascal AK, and Mercado M. Incidencia y Diagnóstico De *Listeria monocytogenes*; Microorganismo Zoonótico Emergente En La Industria De Alimentos. *Rev UDCA Act Divulg Cient* 2004;7:25–57.
- Troxler R, Von Graevenitz A, Funke G, Wiedemann B, and Stock I. Natural antibiotic susceptibility of *Listeria* species: *L. grayi*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeligeri* and *L. welshimeri* strains. *Clin Microbiol Infect* 2000;6:525.
- Vanegas MC, Vasquez E, Martinez AJ, and Rueda AM. Detection of *Listeria monocytogenes* in raw whole milk for human consumption in Colombia by real-time PCR. *Food Cont* 2009;20:430–432.
- Walsh D, Duffy G, Sheridan JJ, Blair IS, and McDowell DA. Antibiotic resistance among *Listeria*, including *Listeria monocytogenes*, in retail foods. *J Appl Microbiol* 2001;90:517–522.
- Yücel N, Çıtak S, and Önder M. Prevalence and antibiotic resistance of *Listeria* species in meat products in Ankara, Turkey. *Food Microbiol* 2005;22:241–245.

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