Antimicrobial susceptibility of *Listeria monocytogenes*, *Listeria ivanovii,* and *Listeria* species isolated from swine processing facilities in Colombia

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Summary

Objective: To analyze distribution of *Listeria monocytogenes* serotypes and antimicrobial susceptibility of *Listeria* isolates from a domestic swine processing facility.

Materials and methods: Presumptive *Listeria* isolates (314) were molecularly identified to discriminate among *L monocytogenes*, *Listeria ivanovii,* and *Listeria* species. *Listeria monocytogenes* serotypes were identified by polymerase chain reaction (PCR) and PCR-restriction enzyme analysis (PCR-REA) and tested for antimicrobial susceptibility.

Results: Isolates were identified as *L monocytogenes* (259; 82.5%), *L ivanovii* (2; 0.6%), and *Listeria* species (53; 16.9%). Distribution of *L monocytogenes* serotypes: 4a/4c (0.4%), 4b (11.2%), 4d/4e (14%), 4b/4d/4e (9.3%), 1/2a (26.3%), 3a (7.7%), 1/2a/3a (6.2%), 1/2b/3b (1.2%), 1/2c (5%), 3c (1.2%), and 1/2c/3c (5.4%). Thirty-two *L monocytogenes* isolates (12.4%) were not typeable by PCR-REA, suggesting the possibility of serotypes 4ab/7. Susceptibility was 84.2% to 100% for most antimicrobials. Major resistance (R) and intermediate (I) susceptibility were found for clindamycin (R = 36.7%, I = 39.8% for *L monocytogenes*; R = 100% for *L ivanovii*; and R = 14%, I = 86% for *Listeria* species). Drugs of choice for treatment of human listeriosis (penicillin, ampicillin, and trimethoprim-sulfamethoxazole) remained effective; 1.2% of *L monocytogenes* were ß-lactam resistant. Multidrug resistance was found only in *L monocytogenes* (26.6%) and *Listeria* species (26.4%), with (clindamycin[I or R]-erythromycin[R]-azithromycin[R]) and (ciprofloxacin[I]-clindamycin[I]) the most frequent phenotypes.

Implications: Resistance to clindamycin and ciprofloxacin are shared between *L monocytogenes* and untyped *Listeria.* Although erythromycin is a drug of choice for prophylaxis in Colombian swine, resistance is low. No specific relationships between serotypes, sources, and antimicrobial susceptibility were found.

Resumen - Susceptibilidad antimicrobiana de las especies de *Listeria moncytogenes*, *Listeria ivanovii*, y *Listeria* aisladas de de plantas procesadoras de cerdos en Colombia

Objetivo: Analizar la distribución de serotipos de *Listeria monocytogenes* y la susceptibilidad antimicrobiana de los aislados de *Listeria* de plantas procesadoras de cerdos doméstica.

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Materiales y métodos: Los supuestos (314) aislados de *Listeria* fueron identificados molecularmente para distinguir entre las especies de *L monocytogenes*, *Listeria ivanovii*, y *Listeria*. Se identificaron los serotipos de *Listeria monocytogenes* a través de la reacción en cadena de la polimerasa (PCR por sus siglas en inglés) y del análisis de restricción de enzima (PCR-REA) y en busca de susceptibilidad antimicrobiana.

Resultados: Los aislados fueron identificados como especies de *L monocytogenes* (259; 82.5%), *L ivanovii* (2; 0.6%), y *Listeria* (53; 16.9%). La distribución de serotipos de *L monocytogenes*: 4a/4c (0.4%), 4b (11.2%), 4d/4e (14%), 4b/4d/4e (9.3%), 1/2a (26.3%), 3a (7.7%), 1/2a/3a (6.2%), 1/2b/3b (1.2%), 1/2c (5%), 3c (1.2%), y 1/2c/3c (5.4%). Treinta y dos aislados de *L monocytogenes* (12.4%) no fueron tipificables a través de PCR-REA, sugiriendo la posibilidad de los serotipos 4ab/7. La susceptibilidad fue de 84.2% a 100% para la mayoría de los antimicrobianos. Se encontró mayor resistencia (R por sus siglas en inglés)

y susceptibilidad intermedia (I por sus siglas en inglés) a la clindamicina (R = 36.7%, I = 39.8% para la especie *L monocytogenes*; R = 100% para la *L ivanovii*; y R = 14%, I = 86% para la *Listeria*). Las drogas de elección para el tratamiento de listeriosis humana (penicilina, ampicilina, y trimethoprim-sulfamethoxazole) permanecieron efectivas; 1.2% de *L monocytogenes* fueron resistentes a la β-lactam. La resistencia a fármacos múltiples se encontró sólo en las especies de *L monocytogenes* (26.6%) y *Listeria* (26.4%), con (clindamycina[I ó R]-erytromicina[R]-azitromicina[R]) y (ciprofloxacina[I]-clindamycina[I]) siendo los fenotipos más frecuentes.

Résumé - Sensibilité antimicrobienne de *Listeria monocytogenes*, *Listeria ivanovii*, et *Listeria* sp isolés d'une usine de transformation de porcs en Colombie

Objectif: Analyser la distribution des sérotypes de *Listeria* monocytogenes et la sensibilité antimicrobienne d'isolats de *Listeria* provenant d'une usine de transformation de porcs.

Matériels et méthodes: Des isolats présumés de *Listeria* (314) ont été identifiés de façon moléculaire afin de distinguer *L monocytogenes*, *Listeria ivanovii* et *Listeria* sp. Les sérotypes de *L monocytogenes* ont été identifiés par réaction d'amplification en chaîne par la polymérase (PCR), PCR-analyse par enzyme de restriction (PCR-REA) et testés pour leur sensibilité aux antibiotiques.

Résultats: Les isolats furent identifiés comme étant *L monocytogenes* (259; 82,5%), *L ivanovii* (2; 0,06%), et *Listeria* sp (53; 16,9%). La distribution des sérotypes de *L monocytogenes* était la suivante : 4a/4c (0,4%), 4b (11,2%), 4d/4e (14%), 4b/4d/4e (9,3%), 1/2a (26,3%), 3a (7,7%), 1/2a/3a (6,2%), 1/2b/3b (1,2%), 1/2c (5%), 3c (1,2%), et 1/2c/3c (5,4%). Trente-deux isolats de *L monocytogenes* (12,4%) étaient non-typables par PCR-REA, suggérant la possibilité de sérotypes 4a/7. La sensibilité envers la plupart des antimicrobiens variait de 84,2% à 100%. Les principales résistances (R) et sensibilités intermédiaires (I) ont été notées pour la clindamycine (R = 36,7%, I = 39,8% pour *L monocytogenes*; R = 100% pour *L ivanovii;* et R = 14%, I = 86% pour *Listeria* sp). Les antibiotiques de choix pour le traitement de la listériose humaine (pénicilline, ampicilline, et trimethoprime-sulfaméthoxazole) demeurent efficaces; 1,2% des isolats de *L monocytogenes* étaient résistants aux β-lactames. De la résistance multiple fut détectée chez *L monocytogenes* (26,6%) et *Listeria* sp (26,4%), les phénotypes les plus fréquents étant (clindamycine[I ou R]-érythromycine[R]-azithromycine[R]) et (ciprofloxacine[I]-clindamycine[I]).

The genus *Listeria* includes eight species,[1] but only *Listeria monocytogenes* is a human and animal pathogen. *Listeria ivanovii* has long been considered a ruminant pathogen with a few reported cases of human infections.[2-4] However, it has recently been proposed that *L ivanovii* is an opportunistic enteric human pathogen.[5] Lack of central nervous system (CNS) involvement is probably a general characteristic of *L ivanovii* infection regardless of host species.[6]

Listeria monocytogenes infection in humans can affect the CNS, causing death or neurological sequelae. The infection may cause meningitis, meningoencephalitis, septicemia, abortion, and prenatal infection (invasive form of listeriosis), while the non-invasive form causes a gastrointestinal syndrome.[7] In ruminants, listeriosis can cause four clinical syndromes: CNS infection causing meningoencephalitis in adults and meningitis in young animals; uterine infections characterized by abortion or neonatal septicemia; generalized septicemia with involvement of the liver and other organs; and mastitis in dairy cattle.[8]

In general, listeriosis affects at-risk groups such as infants, pregnant women,[9] the elderly, and immunocompromised people,[10] with mortality rate approximately 20% to 30% (within the risk groups).[11] Occurrence of disease is related to consumption of contaminated food, principally ready-to-eat products, but in sporadic outbreaks and epidemics, a wide variety of foods act as vehicles: milk, cheese, paté, beef, pork, poultry meat, vegetables, and seafood.[12,13]

All strains of *L monocytogenes* are considered potentially pathogenic, but their virulence is variable. Serotypes frequently involved in development of food-borne listeriosis are 4b, 1/2b, 1/2c, and 1/2a;[1] however, serotype 4b is considered the most virulent and is responsible for the majority of food-borne outbreaks and sporadic cases of illness.[14-16] This may be related to the fact that this serotype is more adapted to the mammalian host than are the strains corresponding to serotype 1/2.[7,17,18]

In recent years, pork production in Colombia has increased, which has expanded participation in the domestic market.[19-21] Clinical listeriosis rarely occurs in pigs. The primary manifestation of the disease in pigs is septicemia, with encephalitis and abortions occurring less commonly. However, infection with *L monocytogenes* is usually subclinical, ie, pigs are asymptomatic carriers,[8] with a prevalence of 10% to 50% in fecal samples.[22] It is difficult to find data on the economic impact of listeriosis on the swine industry. However, economics should not be the only motive for improving surveillance, detection methodologies, disinfection, and handling of this organism. It is also necessary to consider the role of animals as a reservoir and source of infection for humans. Human infection can occur by direct contact or through consumption of contaminated food from animal sources.[8]

Few published or accessible studies are available on antimicrobial susceptibility patterns of *L monocytogenes* in Colombia,[23] and few publications relate classical or molecular serotyping to food type and source.[24-26] A recent study[27] showed that the prevalence of *L monocytogenes* in pig carcasses, pig-meat cuts, and pig derivates in Colombia was approximately 13.8%. A study conducted by the Secretaría Distrital de Salud de Bogotá, Colombia, between 2001 and 2004 reported an overall incidence of 11.2% and incidences of 6.3%, 2.0%, and 0.2% in hams, chorizo (a type of highly seasoned pork sausage), and other sausages, respectively.[28]

In this study we included *Listeria* isolates obtained from two concurrent studies performed at processing in Colombia, 2010-2011. These studies were conducted to investigate prevalence, antimicrobial susceptibility, disinfectant tolerance, and serotypes of *L monocytogenes* in circulation, with the main objective to analyze *L monocytogenes* serotype distribution and antimicrobial susceptibility of *L monocytogenes, L ivanovii*, and *Listeria* species in Colombia's domestic pork industry.

Materials and methods

Sampling and processing
Sampling and processing were performed as described in Gamboa-Marín et al,[27] except for surfaces, which were sampled and processed as described by the United States Department of Agriculture Food Safety and Inspection Service.[29]

Sources of isolates
Swine industry activity in Colombia is grouped into six regions according to the geographical distribution of technologically advanced hog farms, where most industrial activity is concentrated in the Antioquia, Western, and Central regions (Figure 1).[21] A total of 314 presumptive *Listeria* species isolates from samples collected from swine processing facilities in the six pork-producing regions were used in this study.

Origins of the isolates were distributed as follows: meat deboning (n = 172; 54.7%), pig carcasses (n = 26; 8.3%), ham (n = 13; 4.1%), sausage (n = 12; 3.8%), chorizo (n = 14; 4.5%), "longaniza" (another type of highly seasoned pork sausage; n = 19; 6.1%), utensils (n = 9; 2.9%), equipment (n = 25; 8%), contact surfaces (in contact with meat during processing), (n = 8; 2.5%), non-contact surfaces (n = 13; 4.1%), hose water (n = 1; 0.3%), and worker stools (from workers who had diarrhea when other samples were collected; n = 2; 0.6%).

Genomic DNA purification and quantification
Presumptive isolates (314 isolates) were cultivated in brain heart infusion broth supplemented with 0.5% glucose (w/v) and incubated in a shaker for 12 hours at 37°C and 250 rpm.[25] A 1-mL sample of culture was collected for DNA purification using the Wizard Genomic DNA Purification Kit (Promega, Fitchburg, Wisconsin). Purity and concentration of DNA were determined with a spectrophotometer (Biospec 1601; Shimadzu, Nakag Kyoto, Japan) ($\lambda_{260}/\lambda_{280}$ nm) with background correction set at λ_{320} nm.[30]

Molecular identification
***Listeria monocytogenes* and *Listeria* species identification**. Two sets of primers, L1-Forward (CTC CAT AAA GGT GAC CCT) and U1-Reverse (CAG CMG CCG CGG TAA TWC), which yield a 938-bp product and identify the *Listeria* genus, and LF-Forward (CAA ACG TTA ACA ACG CAG TA) and LR-Reverse (TCC AGA

Figure 1: National distribution of pork-industry production in Colombia.[21]

GTG ATC GAT GTT AA), which yield a 750-bp product and amplify the *hlyA* gene typical of *L monocytogenes,* were employed in a multiplex-PCR to divide the isolates into two groups, *L monocytogenes* and *Listeria* species.[25] The final reaction volume was 35 µL, composed of 1× PCR buffer, 1.5 mM of $MgCl_2$, 0.2 mM of deoxyribonucleotides (dNTPs), 20 pmol of primers, and 2 U of Taq-deoxyribonucleic acid polymerase (TaqDNA Polymerase; Vivantis Technologies, Selangor Darul Ehsan, Malaysia). Five µL of DNA (approximately 100 ng) was used for amplification. Temperature settings were 95°C for 1 minute then 94°C for 30 seconds, followed by 40 cycles of 51°C for 20 seconds and 72°C for 30 seconds; then 72°C for 8 minutes.[25]

***Listeria ivanovii* identification.** Another set of primers, IvaI-Forward (CTA CTC AAG CGC AAG CGG CAC) and Lis1B-Reverse (TTA TAC GCG ACC GAA GCC AAC), were used in a single PCR to amplify a 1100-bp fragment of *L ivanovii*. The final reaction volume was 25 µL, composed of 1× PCR buffer, 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs, $1×10^{-5}$ mg primers, and 1.5 U of TaqDNA Polymerase (Vivantis). Five µL of DNA (approximately 100 ng) was used for amplification. Temperature settings

were 35 cycles of 95°C for 15 seconds, 62°C for 30 seconds, and 72°C for 50 seconds.[31] The PCR product expected is approximately 1100 bp.

Grouping *L monocytogenes* isolates by divisions

The *L monocytogenes* isolates were sorted into divisions by use of a multiplex PCR. Two pairs of primers were employed: D1-Forward (CGA TAT TTT ATC TAC TTT GTC A), D1-Reverse (TTG CTC CAA AGC AGG GCA T), which yields a 214-bp product and classifies isolates as division I (serotypes 1/2b, 3b, 4b, 4d, and 4e) or division III (serotypes 4a and 4c), and D2 Forward (GCG GAG AAA GCT ATC GCA), Reverse (TTG TTC AAA CAT AGG GCT A), which yields a 140-bp product and classifies the isolates as division II (serotypes 1/2a, 1/2c, 3a, and 3c). Temperature settings were 95°C for 3 minutes followed by 25 cycles of 95°C for 30 seconds, 59°C for 30 seconds, and 72°C for 1 minute, then 72°C for 10 minutes.[25]

PCR serotyping

Isolates belonging to division II were subtyped using the FlaA primer set Forward (TTA CTA GAT CAA ACT GCT CC) and Reverse (AAG AAA AGC CCC TCG TCC), to generate a 538-bp product characteristic of serotypes 1/2a and 3a. Absence of amplification identified serotype 1/2c or 3c. Temperature settings were 95°C for 3 minutes followed by 25 cycles of 95°C for 30 seconds, 54°C for 30 seconds, and 72°C for 1 minute, then 72°C for 10 minutes.[25]

Isolates belonging to divisions I and III were subtyped using the GLT primer set Forward (AAA GTG AGT TCT TAC GAG ATT T) and Reverse (AAT TAG GAA ATC GAC CTT CT) to obtain a 483-bp product characteristic of serotypes 1/2b and 3b. Temperature settings were 95°C for 3 minutes followed by 25 cycles of 95°C for 30 seconds, 45°C for 30 seconds, and 72°C for 1 minute, then 72°C for 10 minutes.[25]

The reaction mixture for D1/D2, FlaA, and GLT consisted of 25 μL reaction volume, 50 pmol per μL of each primer, 1U of Taq DNA Polymerase (Vivantis), 1× PCR buffer, 0.2 mM of dNTPs, 2.5 mM of MgCl₂, and 100 ng of DNA.[25]

Isolates that did not amplify a 483-bp band with the GLT primer set were assumed to be serotype 4 and thus were further subtyped with primers MAMA-C (LM4/LMB) Forward (CAG TTG CAA GCG CTT GGA GT) and Reverse (GTA AGT CTC CGA GGT TGC AA), yielding a 268-bp amplification product that identifies serotypes 4a and 4c.[25] Strains that did not amplify were considered to be serotype 4 (b, d, or e). The reaction volume was 50 μL containing 0.5 μmol of each primer, 2U Taq DNA Polymerase (Vivantis), 1× PCR buffer, 0.2 mM of dNTPs, 2.0 mM of MgCl₂, and 100 ng of DNA.[25] MAMA-C PCR temperature settings were 95°C for 10 minutes followed by 40 cycles of 95°C for 30 seconds, 55°C for 1 minute, and 72°C for 1 minute, then 72°C for 10 minutes.

PCR-restriction enzyme analysis for confirmation of serotypes 1/2a, 1/2c, and 4b

PCR for *iap* gene amplification. *Listeria monocytogenes* isolates that were classified as serotypes 1/2a and 3a (identified as FlaA-positive by amplification and generation of a 538-bp product) and those that did not amplify with FlaA (serotype 1/2c or 3c), and the isolates that did not amplify with MAMA-C were considered to be serotype 4b, 4d, or 4e and were subtyped with a single PCR, using the primer set CLM1-Forward (ACA GCT GGG ATT GCG GT) and CLM2-Reverse (CCC AGC CAG AGC CGT GGA), located within the *iap* gene of *L monocytogenes*, in order to amplify a 1395-bp fragment of the *iap* gene. The reaction volume was 50 μL containing 0.1 pmol of each primer, 1.25 U Taq DNA Polymerase (Vivantis), 1× PCR buffer, 0.125 mM of dNTPs, 1.5 mM of MgCl₂, and 100 ng of DNA. Temperature settings were 95°C for 5 minutes followed by 35 cycles of 95°C for 90 seconds, 54°C for 60 seconds, and 72°C for 3 minutes, then 72°C for 7 minutes.[32]

Restriction enzyme analysis. Five μL of each CLM1/CLM2 PCR product were digested with *Hin*dIII restriction enzyme (Vivantis) according to the instructions of the manufacturer.[32] All PCR procedure temperatures were controlled in a C1000 Thermal Cycler (BioRad, Hercules, California). All PCR products and PCR-REA products were visualized in 1.5% agarose electrophoresis gel (w/v) prepared in TAE IX (40 mM Tris acetate, 1 mM EDTA, pH 8 ± 0.2) and stained with ethidium bromide (0.3 μg per mL) and run at 100 V in power supply model PowerPac Basic (BioRad) for 1 hour, then visualized and photographed under UV light. A 100-bp ladder (Promega

or Axygen Biosciences, Union City, California) was used as a molecular size marker and *L monocytogenes* ATCC 19115[33] was used as a PCR control.

Antimicrobial susceptibility test. A broth microdilution technique (MicroScan System; Siemens Healthcare Diagnostics, Bogotá, DC, Colombia) was employed for antimicrobial susceptibility testing of *L monocytogenes* and *L ivanovii* isolates. A cell suspension equivalent to 0.5 on the McFarland scale, prepared in Müeller-Hinton medium supplemented with lysed horse blood, was inoculated into the MICroSTREP plus 3 panel (Siemens) (Table 1). Panels were incubated following the manufacturer's recommendations. The panel MICroSTREP plus 3 allowed fulfillment of the requirement stated in the M100-S22 document by Clinical and Laboratory Standards Institute (CLSI)[34] concerning use of lysed horse blood for detection of antimicrobial susceptibility of exigent microorganisms. *Streptococcus pneumoniae* (ATCC 49619) was used as a control for antimicrobial susceptibility testing. Whonet 5.6 software (World Health Organization, 2010; **http://www.whonet. org/dnn/Software/WHONET/tabid/97/ language/en-US/Default.aspx**) was used for descriptive statistical analysis.[23,25]

Results

Molecular identification and serotyping

All isolates were confirmed by PCR to be *Listeria* species, ie, amplification of a 938-bp fragment with primers L1/U1. Of these, 259 isolates were identified as *L monocytogenes* (82.5%), ie, amplification of a 750-bp fragment with primers LF/LR. Only two isolates were identified as *L ivanovii* (0.6%) (data not shown), ie, amplification of a 1100-bp fragment with primers IvaI/Lis1B. The other 53 isolates were classified as *Listeria* species (16.9%) because DNA of these isolates amplified only with primers L1/U1. The control strain (*L monocytogenes* ATCC 19115) showed the expected bands (Figure 2A, B, C, and D).

Molecular serotyping of *L monocytogenes* allowed detection of the following serotypes: 4a/4c (0.4%), 4b (11.2%), 4d/4e (14%), 4b/4d/4e (9.3%), 1/2a (26.3%), 3a (7.7%), 1/2a/3a (6.2%), 1/2b/3b (1.2%), 1/2c (5%), 3c (1.2%), and 1/2c/3c (5.4%). Thirty-two *L monocytogenes* isolates (12.4%) were not serotypes identified by the specific primers,

Table 1: Antibiotics, terms, and their abbreviations in a study of *Listeria* species in samples collected from Colombian swine processing facilities

Antimicrobial or term	Abbreviation
Penicillin	PEN
Ampicillin	AM
Cefotaxime	CFT
Cephradine	CFR
Cefepime	CPE
Chloramphenicol	C
Trimethoprim/sulfamethoxazole	TMP/SMX
Cefuroxime	CRM
Rifampin	RIF
Meropenem	MER
Amoxacillin/clavulanic acid	AOX/CLAV
Clindamycin	CD
Tetracycline	TET
Azithromycin	AZI
Erythromycin	E
Vancomycin	VA
Ciprofloxacin	CP
Minimum inhibitory concentration*	MIC
Susceptibility category resistant	R
Susceptibility category intermediate	I
Susceptibility category sensitive	S
Susceptibility category nonsusceptible†	NS

* MIC_{50} and MIC_{90} represent the MIC values of an antimicrobial at which the growth of 50% and 90% of the microbial population, respectively, are inhibited.

† Isolates with MICs above or zone diameters below the value indicated for the susceptible break point are reported as nonsusceptible (NS) due to the absence or rare occurrence of resistant strains.[34]

suggesting the possibility that they were serotypes 4ab or 7, but this remains to be demonstrated. The control strain (*L monocytogenes* ATCC 19115) was serotyped as 1/2b as expected.

Antimicrobial susceptibility test

The antimicrobial susceptibility, resistance, and intermediate patterns of the 314 isolates can be seen in Tables 2, 3, and 4. In this study, the susceptibility of isolates varied between 84.2% and 100% for most antimicrobials. Major resistance and intermediate values were found for clindamycin: R = 36.7%, I = 39.8% for *L monocytogenes* (Table 2); R = 100% for *L ivanovii* (Table 3); and R=14%, I=86% for *Listeria* species (Table 4). The primary drugs of choice against listeriosis (penicillin, ampicillin, and trimethoprim-sulfamethoxazole) remained effective for most isolates, and only 1.2% of *L monocytogenes* isolates were nonsusceptible to penicillin and ampicillin

On the basis of criteria established by the CLSI[34] for *Staphylococcus* species and *Enterococcus* species, 26.6% of *L monocytogenes* and 26.4% of *Listeria* species displayed multidrug resistance. A total of 30 multidrug-resistance patterns were identified in *L monocytogenes* isolates, whereas in *Listeria* species, only three patterns were identified. The most frequent phenotypes for *L monocytogenes* were clindamycin[I]-erythromycin[R]-azithromycin,[R] and clindamycin[R]-erythromycin[R]-azithromycin,[R] while for *Listeria* species, the most frequent

phenotype was ciprofloxacin[I]-clindamycin.[I] No multidrug-resistance was found in *L ivanovii*. It is important to note that 19 of 69 *L monocytogenes* multidrug-resistant isolates (27.5%) showed simultaneous resistance to clindamycin and erythromycin (Table 5); no chloramphenicol[R] phenotype was identified among these isolates. This combination was not found in any *L ivanovii* strains or in *Listeria* species (Table 6).

The results of antimicrobial susceptibility testing for cefotaxime, cefepime, cephradine, and cefuroxime were not reported in this paper. However it is important to note that resistance to cephalosporins was confirmed in more than 90% of isolates (data not shown).

Relationship between serotype, source, and antimicrobial resistance

The relationship between antimicrobial resistance (including intermediate isolates), *L monocytogenes* serotypes, and distribution by source revealed that, first, major resistance behavior was found against clindamycin. Second, major diversity of resistance patterns was found in serotypes 4b (17 patterns), 4b/4d/4e (15 patterns), 1/2c/3c (11 patterns), and 3a (nine patterns). Third, serotype 1/2b/3b was found uniquely in equipment, whereas only one isolate (serotype 4a/4c) was found on a contact surface. Fourth, the other serotypes (4b, 4d/4e, and 1/2a/3a) were found in all sources.

The most complex resistance phenotypes were found in isolates of serotypes 1/2a/3a and 1/2c/3c (with resistance to up to five antimicrobial agents plus intrinsic resistance to cephalosporin). It should be emphasized that presumptive isolates of serotype 4ab or 7 (to be confirmed) also showed an important antimicrobial resistance pattern.

Discussion

In a recent study, we reported an overall prevalence of *L monocytogenes* of approximately 13.8% from Colombian swine processing plants,[28] but prevalence alone should not be considered. Antimicrobial susceptibility in association with serotype distribution will provide important information for health and food agencies that must evaluate and control the microbiological risk in a population.

In Colombia, there are few published data or easily accessible documents concerning the presence and distribution of *L monocytogenes* serotypes.[25] It is well known[1]

Figure 2: Molecular identification and serotyping of *Listeria* isolates from samples collected in Colombian swine processing facilities. **Panel A**. 1, 9, and 16: 100-bp molecular size marker (Invitrogene); 2: reagent control for polymerase chain reaction (PCR); 3: DNA from *L monocytogenes* ATCC 19115 amplified with U1/L1-LF-LR; 4: DNA from *Listeria* species isolate amplified with U1-L1/LF-LR (938- and 750-bp bands identify *L monocytogenes*); 5 and 6: DNA from *Listeria* species amplified with U1/L1-LF-LR (938-bp band identifies *Listeria* genus); 7: DNA from *L monocytogenes* ATCC 19115 amplified with D1; 8: DNA from *L monocytogenes* isolates amplified with D1 (214-bp band identifies division I or III isolates); 10 and 11: DNA from *L monocytogenes* isolates amplified with D2 (140-bp band identifies division II isolates); 12 and 13: DNA from *L monocytogenes* isolates that do not amplify with D1/D2; 14 and 15: DNA from *L monocytogenes* isolates amplified with FlaA (538-bp band identifies serotypes 1/2a and 3a). **Panel B**. 1 and 9: 100-bp molecular size marker (Promega); 2 and 3: DNA from *L monocytogenes* isolates amplified with FlaA (538-bp band identifies serotypes 1/2a and 3a); 4, 5, and 7: DNA from *L monocytogenes* isolates amplified with GLT (483-bp band identifies serotypes 1/2b and 3b); 6: DNA from *L monocytogenes* isolates amplified with GLT; 8: Reagent control for PCR reaction. **Panel C**. 5: 100-bp molecular size marker (Axygen); 1, 3, 4, 7, and 8: DNA from *L monocytogenes* isolates amplified with CLM1-CLM2 (1395-bp band identifies the *iap* gene); 2, 6, and 9: empty wells. **Panel D**. 5: 100-bp molecular size marker (Axygen); 1, 2, 3, and 4: restriction enzyme analysis (REA) (*Hind*III) of DNA from *L monocytogenes* isolates after amplification with CLM1-CLM2 (693-, 425-, and 277-bp bands identifies serotypes 1/2a or 1/2c). **Panel E**. 1: 100-bp molecular size marker (Axygen); 2-4, 6, and 7: REA (*Hind*III) of DNA from *L monocytogenes* isolates after amplification with CLM1-CLM2 (1118- and 277-bp bands identifies serotype 4b); 5: negative REA (*Hind*III) of DNA from *L monocytogenes* isolates (no bands identifies serotypes 4d, 4e).
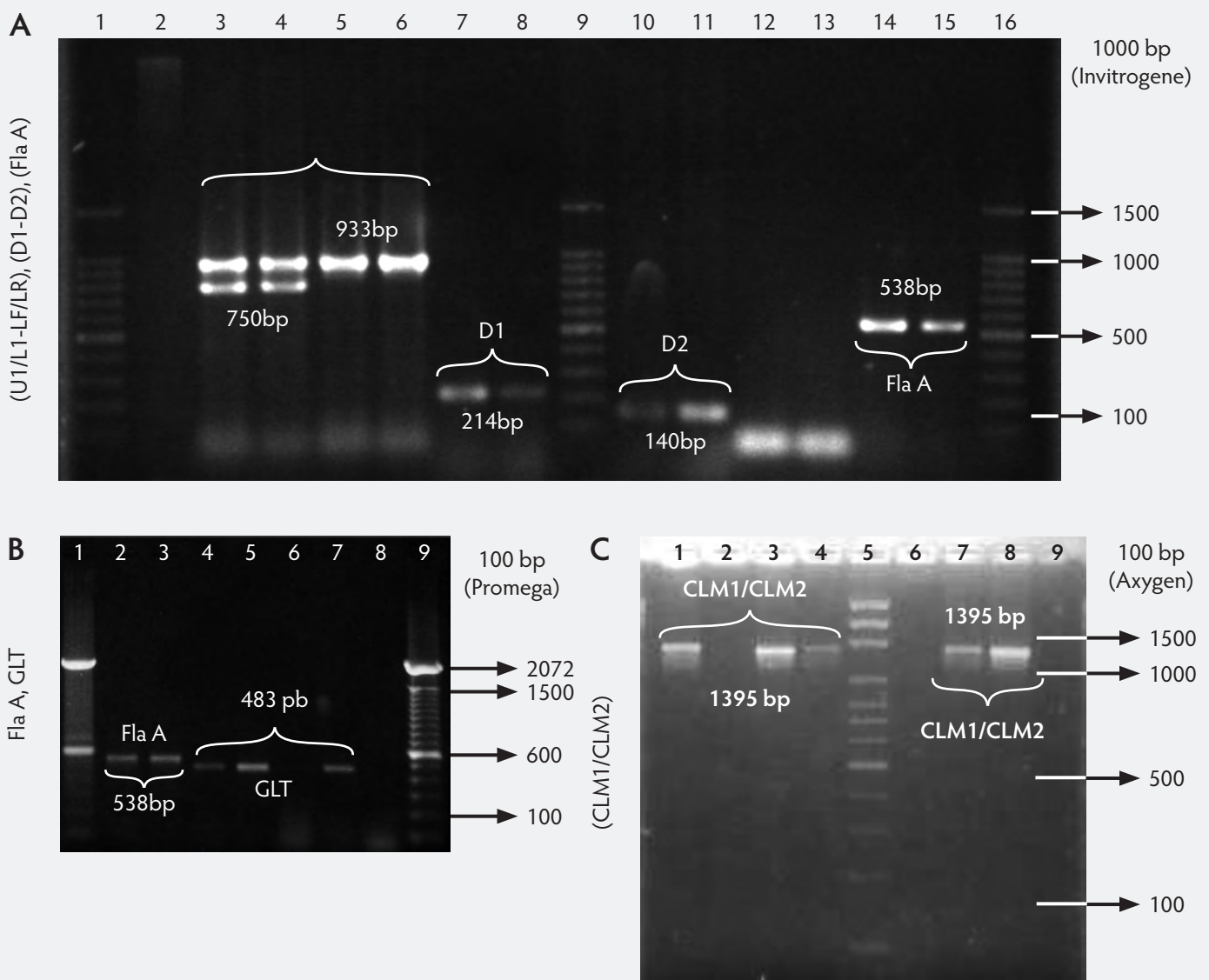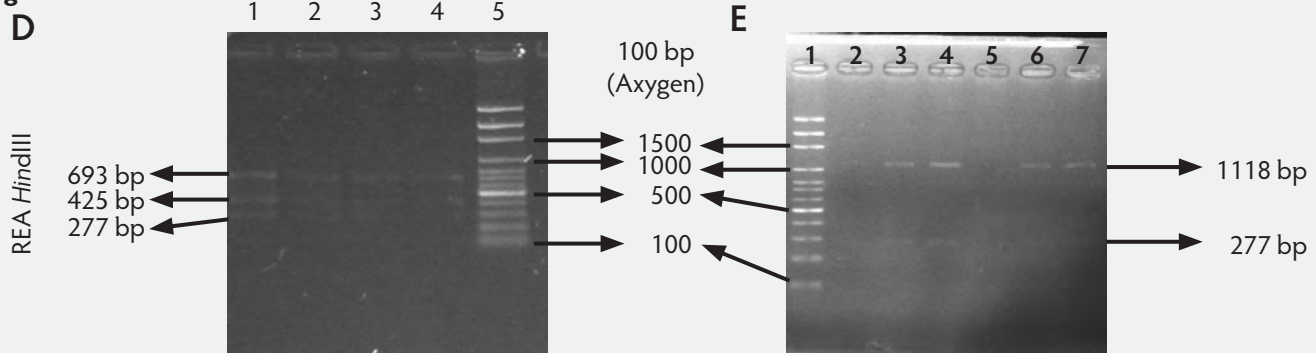
Figure 2

Table 2: Number and the distribution of 259 *Listeria monocytogenes* isolates collected from Colombian swine processing facilities in terms of susceptibility to antibiotics commonly used in humans*

Antimicrobial	Break point (µg/mL)			MIC range	MIC$_{50}$	MIC$_{90}$	Antimicrobial susceptibility No. of isolates (%)			
	R	I	S				R	I	S	NS
PEN	NA	NA	≤ 2	0.06-16	0.25	0.5	0 (0)	0 (0)	256 (98.8)	3 (1.2)
AM	NA	NA	≤ 2	0.06-16	0.25	0.5	0 (0)	0 (0)	256 (98.8)	3 (1.2)
TMP/SMX	≥ 4/76	1/19-2/38	≤ 0.5/9.5	0.25-4	0.25	0.5	4 (1.5)	5 (1.9)	250 (96.5)	0 (0)
AOX/CLAV	≥ 32	16	≤ 8	0.25-2	0.25	0.5	0 (0)	0 (0)	259 (100)	0 (0)
MER	≥ 16	8	≤ 4	0.06-8	0.25	0.25	0 (0)	1 (0.4)	258 (99.6)	0 (0)
RIF	≥ 4	2	≤ 1	0.5-4	0.5	0.5	2 (0.8)	0 (0)	257 (99.2)	0 (0)
CP	≥ 4	2	≤ 1	0.06-4	1	1	3 (1.2)	16 (6.2)	240 (92.7)	0 (0)
CD	≥ 4	1-2	≤ 0.5	0.25-8	2	4	**95 (36.7)**	**103 (39.8)**	61 (23.6)	0 (0)
AZI	≥ 8	4	≤ 2	0.25-8	1	8	37 (14.3)	2 (0.8)	220 (84.9)	0 (0)
E	≥ 8	1-4	≤ 0.5	0.25-8	0.25	8	36 (13.9)	5 (1.9)	218 (84.2)	0 (0)
C	≥ 32	16	≤ 8	2-16	8	8	0 (0)	7 (2.7)	252 (97.3)	0 (0)
TET	≥ 16	8	≤ 4	1-8	1	2	0 (0)	17 (6.6)	242 (93.4)	0 (0)
VA†	≥ 16	8	≤ 4	1-8	1	2	0 (0)	17 (6.6)	242 (93.4)	0 (0)
VA‡	≥ 32	8-16	≤ 4	1-8	1	2	0 (0)	0 (0)	259 (100)	0 (0)

* Antimicrobial abbreviations and break-point categories are defined in Table 1. Bold print identifies the primary drugs of choice for treatment of human listeriosis and the greatest numbers of organisms in the R and I susceptibility categories.

† Break points for *Staphylococcus* species used.[23]

‡ Break points for *Enterococcus* species used.[23]

MIC = minimal inhibitory concentration; NA = not applicable (break points not yet established by the Clinical and Laboratory Standards Institute).

Table 3: Number and distribution of two *Listeria ivanovii* isolates collected from Colombian swine processing facilities in terms of susceptibility to antibiotics commonly used in humans*

| Antimicrobial | MIC Range | MIC$_{50}$ | MIC$_{90}$ | Antimicrobial susceptibility No. of isolates (%) | | |
				R	I	S
PEN	0.25-0.25	0.25	0.25	0(0)	0(0)	2(100)
AM	0.25-0.25	0.25	0.25	0(0)	0(0)	2(100)
TMP/SMX	0.25-0.25	0.25	0.25	0(0)	0(0)	2(100)
AOX/CLAV	0.25-0.25	0.25	0.25	0(0)	0(0)	2(100)
MER	0.25-0.25	0.25	0.25	0(0)	0(0)	2(100)
RIF	0.5-0.5	0.5	0.5	0(0)	0(0)	2(100)
CP	1-1	1	1	0(0)	0(0)	2(100)
CD	4-4	4	4	**2(100)**	0(0)	0(0)
AZI	1-1	1	1	0(0)	0(0)	2(100)
E	0.25-0.25	0.25	0.25	0(0)	0(0)	2(100)
C	8-8	8	8	0(0)	0(0)	2(100)
TET	1-1	1	1	0(0)	0(0)	2(100)
VA†	1- 8	1	2	0(0)	0(0)	2(100)
VA‡	1-8	1	2	0(0)	0(0)	2(100)

* Antimicrobial abbreviations and break-point categories are defined in Table 1. Bold print identifies the primary drugs of choice for treatment of human listeriosis and the greatest numbers of organisms in the R and I susceptibility categories.

† Break points for *Staphylococcus* species used.[23]

‡ Break points for *Enterococcus* species used.[23]

MIC = minimal inhibitory concentration.

that certain serotypes occur more often in food or in infected humans or animals, and it is also known that approximately 95% of strains isolated from cases of human listeriosis caused by *L monocytogenes* correspond mainly to four serotypes: 4b, 1/2b, 1/2c, and 1/2a. In two previous studies we used molecular methods to determine the serotypes of *L monocytogenes* isolates from different sources and types of food, and we then analyzed the antimicrobial susceptibility patterns of these isolates.[23,25] Herein we have started with a representative sample of the swine processing plants,[27] in order to analyze the antimicrobial susceptibility and serotype distribution along the pork-meat chain.

We combined the molecular serotyping methodology used in our earlier study[25] with a *Hin*dIII restriction enzyme analysis after *iap* gene amplification,[32] with the main objective of discriminating among *L monocytogenes* serotypes 1/2a, 1/2c, and 4b (Figure 2). There is an important discrimi-nation to be made between *L ivanovii* and *L monocytogenes,* as *L monocytogenes* is a human and animal pathogen, while *L ivano-vii* is a ruminant pathogen that has recently been recognized as an opportunistic human enteric pathogen.[5]

Our results show a high frequency of sero-types 1/2a (26.3%) followed by 4d/4e (14%), 4b (11.2%), and 4b/4d/4e (9.3%). These frequencies are lower than those obtained by other authors (64%,[35] 57%,[36] and 92.8%,[37] respectively); nonetheless, our results are in agreement that serotype 1/2a is the most frequent. The frequency of serotype 4b in this study is higher than reported by other authors,[38] who investigated the occurrence of *L monocytogenes* in sausages and found serovar 4b in 8.0% of isolates and the highest prevalence in serotype 1/2a (49.5%).

The presence and persistence of *L monocy-togenes* lineage II isolates (serotypes 1/2a, 1/2c, 3a, and 3c) in food and food-plant

environments may be associated with a greater capacity for growth and survival.[1] Strains of lineage II may be more competent than those belonging to lineage I (serotypes 1/2b, 3b, 4b, 4d, and 4e), which probably is associated with increased resistance to bacteriocins, although, after storage at 4°C, serotype 4b strains tend to be more resistant to heat treatment (60°C) than 1/2a strains.[1]

Previous studies[11,39,40] show that the strains of serotype 1/2 presented a higher food prevalence; nonetheless, serotype 4b is most often isolated from patients with listerio-sis, suggesting that this serotype is more pathogenic than the others.[1] Additionally, serotype 4b is more adapted to the human host.[17,18]

On the one hand, some investiga-tions[11,17,18,39,40] clearly show that serotype 1/2 has been isolated from various food products, suggesting that it has different eco-logical niches. On the other hand, our study found that serotypes 1/2a, 3a, and 4b/4d/4e were detected in all types of samples, which is important, considering that serotypes 1/2a and 4b have been the causes of several liste-riosis outbreaks,[41] including those associated with consumption of pork.

Considering that *L monocytogenes* is resis-tant to 3rd to 6th generation cephalosporins, that these antimicrobials are included in the MICroSTREP plus3 panel, and that cephalosporin resistance has been reported before,[41] patterns of cephalosporin resis-tance were not included in the multidrug resistance analysis and will not be further discussed here.

In a previous study,[23] we assayed the anti-microbial susceptibility of *L monocytogenes* isolates from several sources, finding major resistance (30% to 65% of isolates) to clindamycin, meropenem, rifampin, and ciprofloxacin, whereas penicillin, ampicillin, and trimethoprim-sulfamethoxazole, the primary drugs of choice against listeriosis,[23] remained effective for most isolates (84%). As in the previous study,[23] clindamycin resistance was the most common resistant phenotype (approximately 80.6% of phe-notypes) among all *Listeria* isolates, while resistance to meropenem, rifampin, and cip-rofloxacin comprised 0.4% to 24.5% of resis-tant phenotypes. Results of the present study do not agree with the recently reported decrease in susceptibility of *L monocytogenes* to ciprofloxacin.[42]

Table 4: Number and distribution of 53 *Listeria species* isolates collected from Colombian swine processing facilities in terms of susceptibility to antibiotics commonly used in humans*

Antimicrobial	MIC Range	MIC$_{50}$	MIC$_{90}$	No. of isolates (%)		
				R	I	S
PEN	0.25-0.5	0.25	0.5	0(0)	0(0)	53(100)
AM	0.25-1	0.5	0.5	0(0)	0(0)	53(100)
TMP/SMX	0.25-0.25	0.25	0.25	0(0)	0(0)	53(100)
AOX/CLAV	0.25-0.5	0.25	0.5	0(0)	0(0)	53(100)
MER	0.25-0.5	0.25	0.25	0(0)	0(0)	53(100)
RIF	0.5-0.5	0.5	0.5	0(0)	0(0)	53(100)
CP	0.5-4	0.5	2	1(1.9)	13(24.5)	39(73.6)
CD	2-4	2	4	6(11.3)	**47(88.7)**	0(0)
AZI	1-2	1	1	0(0)	0(0)	53(100)
E	0.25-1	0.25	0.25	0(0)	1(1.9)	52(98.1)
C	8-8	8	8	0(0)	0(0)	53(100)
TET	1-1	1	1	0(0)	0(0)	53(100)
VA†	1-2	1	2	0(0)	0(0)	53(100)
VA‡	1-2	1	2	0(0)	0(0)	53(100)

* Antimicrobial abbreviations and break-point categories are defined in Table 1. Bold print identifies the primary drugs of choice for treatment of human listeriosis and the greatest numbers of organisms in the R and I susceptibility categories.

† Breakpoints for *Staphylococcus* species used.[23]

‡ Break points for *Enterococcus* species used.[23]

MIC = minimal inhibitory concentration

All *Listeria* isolates studied displayed approximately 98% susceptibility to β-lactams, with only 1.2% of isolates classified as nonsusceptible. In our previous study,[23] 16% were classified as nonsusceptible.

More than 90% of isolates were susceptible to the antimicrobials assayed, with susceptibility to rifampin and vancomycin particularly notable because of their importance in treating *Mycobacterium tuberculosis* and *Staphylococcus aureus* infections, respectively.[23]

A phenomenon of inducible clindamycin resistance has been described in clinical isolates of *Staphylococcus* species, when an infection caused by an erythromycin[R]-clindamycin[I] or an erythromycin[R]-clindamycin[S] strain is being treated with clindamycin.[23,34] It is not possible yet to extrapolate this phenomenon to *Listeria* species, although it is important to note that in the present study, 19 of 69 *L monocytogenes* isolates (27.5%) displayed both clindamycin and erythromycin resistance, suggesting a possible modification of the 23S rRNA.[43] These values are higher than in our earlier reports

of 7%[23] and 10%[25] of *L monocytogenes* isolates resistant to clindamycin and erythromycin, respectively. In some countries (eg, Cuba[44] and Colombia[45]), erythromycin is commonly employed as a prophylactic antimicrobial in domestic pig herds.

In *Listeria* species, the multidrug-resistance patterns were limited to a combination of clindamycin-intermediate and ciprofloxacin-intermediate or resistant, but in *L monocytogenes*, 30 different patterns were detected. Most multidrug-resistant isolates (61.6%) were *L monocytogenes* isolated from pig carcasses, and the greatest number of multidrug-resistance patterns was found in deboned meat, with clindamycin, erythromycin, and azithromycin involved in most of the patterns. Resistance phenotypes reported in the present study were similar to those in the previous study,[23] in which 78% of resistance phenotypes involved clindamycin, erythromycin, azithromycin, tetracycline, ciprofloxacin, rifampin, or meropenem. However, in the present study, 22% of resistance phenotypes involved trimethoprim-sulfamethoxazole and chloramphenicol.

Relationships between the source, the organism, and resistance to specific antimicrobials were not found. However, all serotypes were distributed among all types of samples, as expected from the operational dynamics of the swine processing plants. Isolates belonging to *L monocytogenes* serotypes 4, 1/2a, 3a, and 1/2c/3c showed more varied resistance patterns.

The geographic sources of serotypes and resistant, intermediate, or multidrug-resistant isolates will remain confidential. However, serotypes and antimicrobial susceptibility patterns found in the swine processing facilities or in specific sources in those facilities will be privately revealed to the industry authorities so they can take action.

In conclusion, various *L monocytogenes* and *Listeria* species strains have circulated in the domestic pork industry, and the multidrug-resistance phenotypes identified in this study showed 77% similarity to those detected by Ruiz-Bolivar et al.[23] Considering the period of sampling and the isolate sources (food type and geographical region), this suggests "a stable spread of resistance patterns among *L monocytogenes* circulating in the country."[23] In contrast, it appears that these genes have not been disseminated to the *Listeria* species isolates, because in this study, only two resistance phenotypes (clindamycin and ciprofloxacin; 22%) were shared between *L monocytogenes* and *Listeria* species. It is not yet possible to associate a specific serotype with an antimicrobial resistance pattern. It seems that antimicrobial susceptibility related to the circulating strains of *L monocytogenes* has been stable over the past 5 years, at least in the studied areas. Further studies are required to determine relationships between isolates obtained from food, animals, and humans, and to extend the studies to other production chains.

Implications
- Under the conditions of this study, only two resistance phenotypes (clindamycin and ciprofloxacin) are shared between *L monocytogenes* and untyped *Listeria*.
- Under the conditions of this study, resistance is low to erythromycin, a drug of choice for prophylaxis in the domestic Colombian pork industry.
- Under the conditions of this study, specific relationships between serotypes, sources of isolates, and antimicrobial susceptibility are not detectable.

Table 5: Distribution of *Listeria monocytogenes* MDR isolates in samples collected from Colombian swine processing facilities*

MDR patterns†	Distribution of isolates-phenotypes by origin						
	No. isolate-specific phenotypes/no. specific MDR isolates (%)						
Phenotype	Meat deboning	Pig carcass	Sausage	Ham	Utensils	Equipment	Non-contact surface
CD^I, E^R, AZI^R	6/37 (16.2)	5/16 (31.3)	0/1 (0)	0/3 (0)	0/1 (0)	1/6 (16.7)	0/5 (0)
CD^I, E^R, AZI^R, TET^I	0/37(0)	1/16 (6.3)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^I, E^R, AZI^R, CP^I	0/37(0)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	1/6 (16.7)	0/5 (0)
CD^I, E^R, AZI^R, TMP/SMX^R	1/37(2.7)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^R, E^R, AZI^R	12/37 (32.4)	1/16 (6.3)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^R, E^R, AZI^R, CP^I	1/37(2.7)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^R, E^R, AZI^R, TET^I	0/37(0)	1/16 (6.3)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^R, E^R, AZI^R, TET^I, RIF^R	0/37(0)	1/16 (6.3)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^R, E^R, AZI^R, CP^I	0/37(0)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	1/6 (16.7)	0/5 (0)
CD^R, E^R, AZI^R, TET^I, CP^R	0/37(0)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	1/5 (20)
CD^R, E^R, AZI^R	0/37(0)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	1/5 (20)
CD^R, E^I, AZI^I, TET^I, RIF^R	0/37(0)	1/16 (6.3)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^I, E^I	1/37(2.7)	1/16 (6.3)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
E^R, AZI^R	1/37(2.7)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^I, AZI^R	0/37(0)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	1/6 (16.7)	0/5 (0)
CD^I, AZI^R	0/37(0)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	1/6 (16.7)	0/5 (0)
CD^I, TET^I	0/37(0)	4/16 (25)	0/1 (0)	0/3 (0)	1/1 (100)	0/6 (0)	0/5 (0)
CD^R, TET^I, CP^I	0/37(0)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	1/5 (20)
CD^R, TET^I	0/37(0)	1/16 (6.3)	0/1 (0)	2/3 (66.7)	0/1 (0)	0/6 (0)	0/5 (0)
TET^I, MER^I	0/37(0)	0/16 (0)	1/1 (100)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^R, CP^I	5/37 (13.5)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^R, CP^R	1/37(2.7)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^R, CP^I	0/37(0)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	1/6 (16.7)	1/5 (20)
CD^I, CP^I	1/37(2.7)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^I, CP^R	1/37(2.7)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^R, CP^I, C^I	0/37(0)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	1/5 (20)
CD^I, TMP/SMX^I	3/37 (8.1)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^I, TMP/SMX^R	1/37(2.7)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
CD^I, C^I	1/37(2.7)	0/16 (0)	0/1 (0)	1/3 (33.3)	0/1 (0)	0/6 (0)	0/5 (0)
CD^R, C^I	2/37 (5.4)	0/16 (0)	0/1 (0)	0/3 (0)	0/1 (0)	0/6 (0)	0/5 (0)
No. MDR isolates/ no. origin-specific isolates (%)	37/172 (21.5)	16/26 (61.5)	1/12 (8.3)	3/13 (23.1)	1/9 (11.1)	6/25 (24)	5/13 (38.5)
No. MDR isolates/ total MDR isolates (%)	37/69 (53.6)	16/69 (23.2)	1/69 (1.4)	3/69 (4.3)	1/69 (1.4)	6/69 (8.7)	5/69 (7.2)
No. MDR isolates/*L monocytogenes* total isolates (%)	37/259 (14.3)	16/259 (6.2)	1/259 (0.4)	3/259 (259)	1/259 (0.4)	6/259 (2.3)	5/259 (1.9)

* Antimicrobial abbreviations and break-point categories are defined in Table 1.

† MDR patterns occurred when MIC values for an isolate classified it in the I or R susceptibility category for two or more classes of antimicrobials, regardless of intrinsic resistance to cephalosporins.

MDR = multidrug-resistant; MIC = minimal inhibitory concentration.

Table 6: Distribution of *Listeria* species MDR isolates in samples collected from Colombian swine processing facilities*

MDR patterns	Distribution of MDR phenotypes of isolates by origin							
	No. isolate-specific phenotypes/no. specific MDR isolates (%)							
Phenotype	Sausage	Sausage "chorizo"†	Sausage "longaniza"†	Ham	Utensils	Equipment	Contact surface	Water
CD^I, CP^I	1/1 (100)	1/1 (100)	4/5 (80)	0/1 (0)	1/1 (100)	0/1 (0)	1/3 (33.3)	0/1 (0)
CD^R, CP^I	0/1 (0)	0/1 (0)	0/5 (0)	1/1 (100)	0/1 (0)	1/1 (100)	1/3 (33.3)	1/1 (100)
CD^I, CP^R	0/1 (0)	0/1 (0)	1/5 (20)	0/1 (0)	0/1 (0)	0/1 (0)	1/3 (33.3)	0/1 (0)
No. MDR isolates/ no. origin-specific isolates (%)	1/1 (100)	1/7 (14.3)	5/19 (26.3)	1/1 (100)	1/4 (25)	1/5 (20)	3/6 (50)	1/1 (100)
No. MDR isolates/ total MDR isolates (%)	1/14 (7.1)	1/14 (7.1)	5/14 (35.7)	1/14 (7.1)	1/14 (7.1)	1/14 (7.1)	3/14 (21.4)	1/14 (7.1)
No. MDR isolates/ *Listeria* species total isolates (%)	1/53 (1.9)	1/53 (1.9)	5/53 (9.4)	1/53 (1.9)	1/53 (1.9)	1/53 (1.9)	3/53 (5.7)	1/53 (1.9)

*   Antimicrobial abbreviations and break-point categories are defined in Table 1.

†   Highly seasoned specialty sausages.

MDR = multidrug-resistant.

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