

# Characterization of *Listeria monocytogenes* isolates recovered from human clinical cases occurring in Portugal between 2008 and 2012



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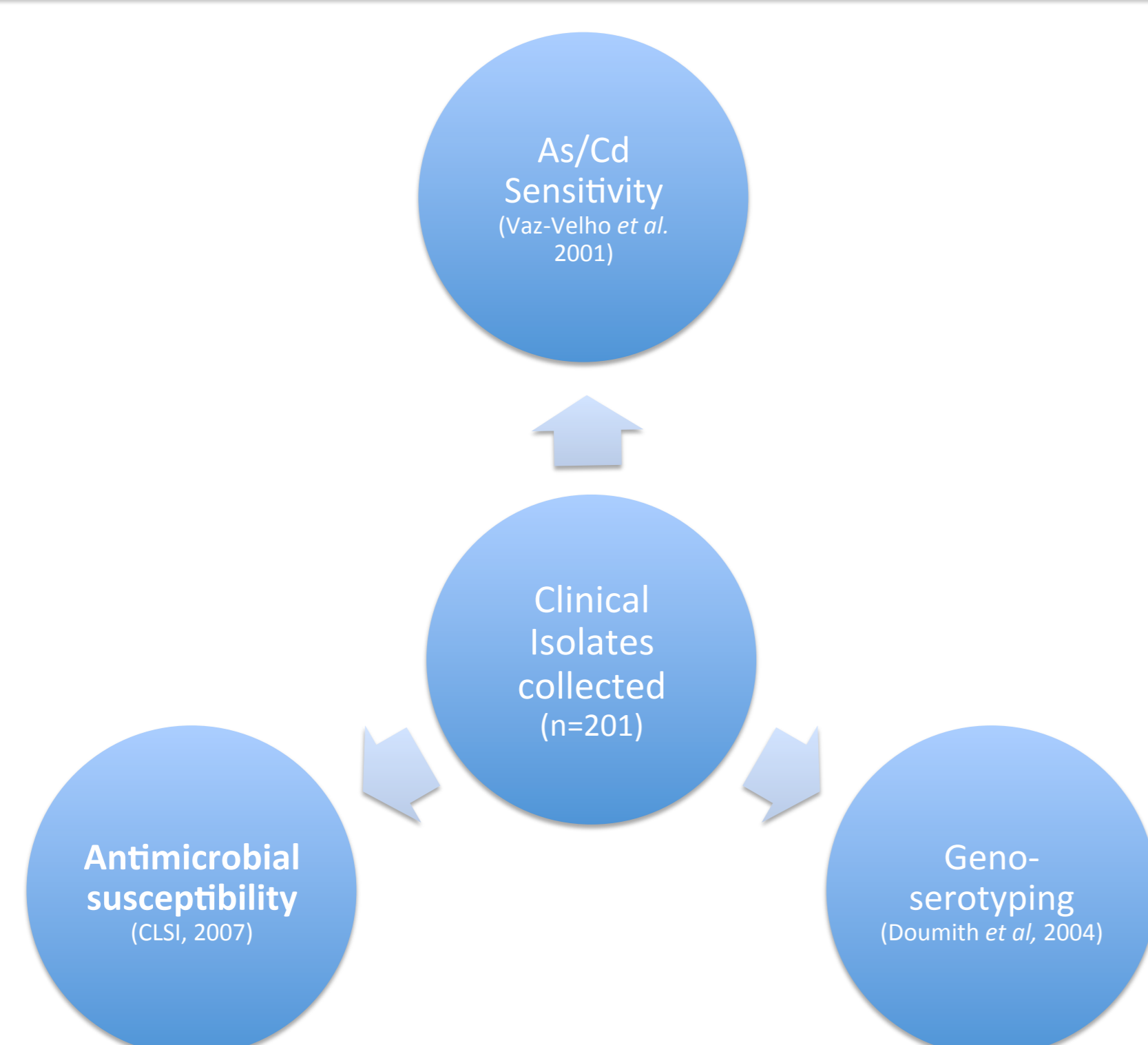
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## Introduction

*Listeria monocytogenes* is an environmental saprotroph, commonly isolated from soil and decaying vegetation, and simultaneously a threatening pathogen able to cause a severe bacterial disease, listeriosis. Contaminated food is the predominant route of transmission of listeriosis to humans; milk and dairy products, fish and meat products, vegetables and ready-to-eat (RTE) foods have been implicated in several outbreaks during the past decades (1). Once it infects the host, *L. monocytogenes* crosses the intestinal epithelium barrier, via transcytosis, and rapidly spread through the lymph or blood to the mesenteric lymph nodes, the spleen, and the liver (2). The most severe clinical presentations of listeriosis include septicaemia, meningitis, meningoencephalitis, and other central nervous system (CNS) infections (3). Individuals with impaired T-cell-mediated immunity (e.g. HIV/AIDS, cancer/receiving immunosuppressive therapy, organ transplant), chronic diseases (e.g. diabetes, alcoholism, liver and renal disease), or aged over 60, are at increased risk for invasive listeriosis (4).

*L. monocytogenes* infections are also associated with bacteremia in pregnant women, frequently asymptomatic or presenting mild influenza-like symptoms, although it can develop to abortion, stillbirth, premature labor, or severe neonatal infection (e.g. sepsis, pneumonia or meningitis) (5). In Portugal listeriosis is not a notifiable disease, thus its incidence has been underestimated. Our research group has estimated an incidence rate of listeriosis of 2.3 cases per million inhabitants for the year 2007 (6). The aim of this study was to obtain epidemiological data on cases of listeriosis occurring in Portugal during 2008 – 2012. Isolates were collected from voluntary collaborating health care units and characterized by: (i) biotyping (cadmium and arsenic sensitivity); (ii) geno-serotyping by multiplex PCR; and, (iii) the minimal inhibitory concentration (MIC, µg/ml) of twelve antibiotics.

## Methods & Results



### Data collected from human episodes

A total of 201 *L. monocytogenes* isolates were collected from major Portuguese hospitals during the study period. Information regarding patient age, gender and clinical specimen tested was not accessible for all the cases. From the information available 17 cases (8.5%) corresponded to maternal/neonatal (MN) infections. For the 184 non-MN cases, strains were isolated from blood (62.5%), cerebrospinal fluid (20.1%), both blood and cerebrospinal fluid (3.3%), other specimens (5.4%); for 16 cases this information was unknown. The mean age of the 155 non-MN cases with documented age was 61 years, with 89 cases (57.4%) being equal or up to 60 years. The gender ratio (M/F) of confirmed non-MN cases was 1.7.

### Biotyping results by arsenic and cadmium sensitivity

Evaluation of sensitivity to heavy metals (arsenic and cadmium) differentiated the 201 isolates into four major groups, including: As<sup>S</sup>Cd<sup>S</sup> (66.7%); As<sup>R</sup>Cd<sup>S</sup> (14.9%); As<sup>S</sup>Cd<sup>R</sup> (10.9%); and, As<sup>R</sup>Cd<sup>R</sup> (7.5%) contains 46 isolates (48.4%).

### Geno-serotyping results

Three geno-serogroups were identified among the 201 *L. monocytogenes* isolates recovered from listeriosis cases. Molecular serotyping identified 155 (77.1%) isolates belonging to serogroup IVb (including serotypes 4b or 4d or 4e) serotype 4b), 33 (16.4%) isolates belonging to serogroup IIb (including serotypes 1/2b or 3b), and 13 (6.5%) isolates belonging to serogroup IIa (including serotypes 1/2a or 3a).

### Antimicrobial susceptibility

Table 1 shows the MIC<sub>90</sub> and MIC<sub>50</sub> values determined for *L. monocytogenes* isolates collected from 2008 to 2012. All the isolates were susceptible to ampicillin, the preferred agent to treat listeriosis. Resistances to ciprofloxacin (n=49), rifampicin (n=28), nitrofurantoin (n= 84), and to streptomycin (n=118) were observed. Twenty-nine isolates (14.4%) were resistant to two or more antimicrobials of different classes. No significant differences (p < 0.05) were found between antibiotic resistance and geno-serogroup, except for rifampicin that present higher MIC values for serogroup IVb.

Table 1: MIC<sub>90</sub> and MIC<sub>50</sub> values for *L. monocytogenes* clinical isolates between 2008-2012

Drug	MIC breakpoint*	Range	MIC (µg/ml) for strain isolated from human cases											
			2008 (n=26)		2009 (n=20)		2010 (n=55)		2011 (n=71)		2012 (n=29)		2008-2012 (n=201)	
			50%	90%	50%	90%	50%	90%	50%	90%	50%	90%	50%	90%
Penicillin G	2-4	0.125-2	0.25	0.5	0.25	0.5	0.25	0.5	1	2	0.25	1	0.25	1
Ampicillin	2-4	0.125-2	0.5	1	0.5	1	0.5	1	1	1	0.5	1	0.5	1
Gentamicin	4-16	0.03125-2	1	1	1	1	1	2	0.03125	0.0625	1	1	1	1
Ciprofloxacin	1-4	0.25-8	2	16	2	4	2	4	1	2	4	4	2	4
Erythromycin	0.5-8	0.0625-1	0.5	0.5	0.5	0.5	0.5	0.125	0.25	0.5	0.5	0.5	0.5	0.5
Vancomycin	4-32	0.03125-2	2	4	2	4	2	4	0.03125	0.03125	2	2	2	2
Chloramphenicol	8-32	4-2	16	16	16	16	16	16	8	16	16	16	16	16
Tetracycline	4-16	1-16	1	1	1	1	1	4	4	4	4	4	4	4
Rifampicin	1-4	0.125-32	2	4	2	4	2	4	0.25	1	0.25	2	1	4
Nitrofurantoin	32-128	64-128	64	64	64	64	64	128	128	128	64	64	64	128
Streptomycin	32	8-128	64	64	64	64	64	64	16	16	64	64	64	64

\* Specific breakpoints for *Listeria* susceptibility testing were those recommended by the CLSI for veterinary pathogens or CLSI criteria for staphylococci for the agar dilution method.

### Incidence of listeriosis in Portugal

At least 201 cases were identified between 2008 and 2012. The highest annual number of cases per million inhabitants was recorded for 2010 (5.5) and 2011 (7.1) considering the resident population of Portugal as that given by the National Institute of Statistics. (Figure 1). This might be explained by the occurrence of an outbreak (data not shown) and enhanced surveillance during this period. The listeriosis incidence rate in 2008, 2009, and 2012 was 2.6, 2.2, and 2.9, respectively (Figure 1).

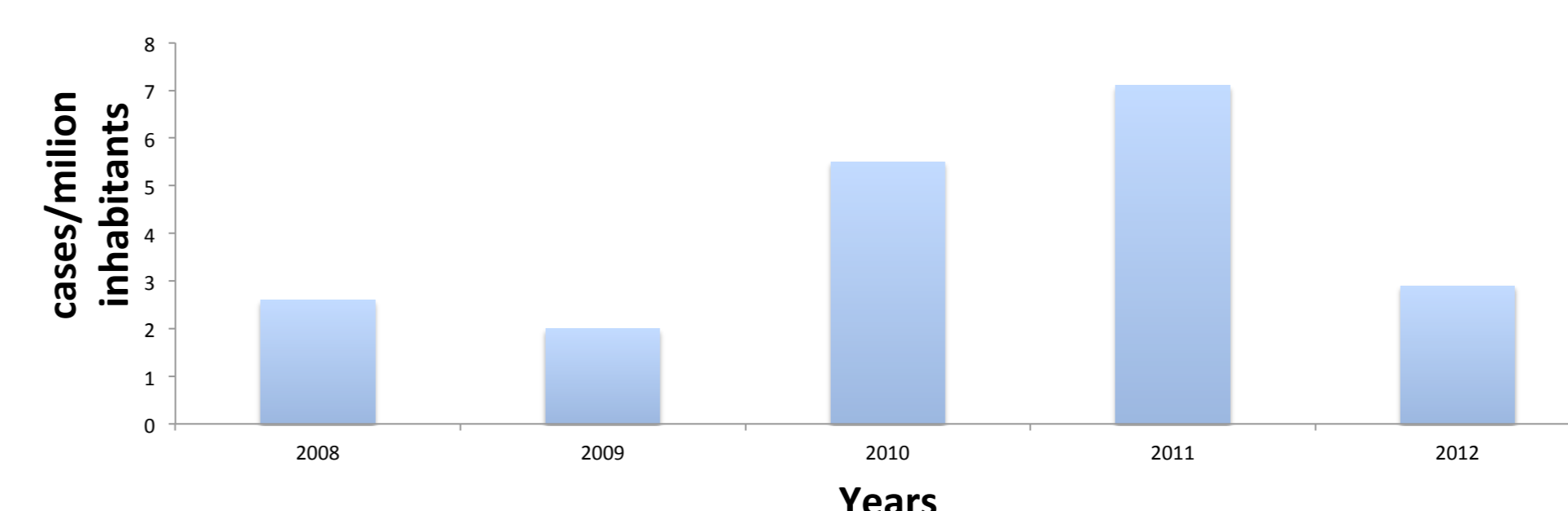


Figure 1: Incidence of listeriosis in Portugal between 2008-2012

## Conclusions

Although no official records exist for incidence of listeriosis in Portugal, this study demonstrates that the number of cases occurred during 2008 – 2012 are similar to those reported in other countries with implemented surveillance system (7). As observed for other European countries (Almeida *et al.*, 2010), it was observed an increased incidence of cases in adults aged more than 60 years old. Most of these cases were caused by geno-serogroup IVb strains.

The incidence of antibiotic-resistant isolates of *L. monocytogenes* was low but higher than that observed in Portugal during the period 2003 to 2007 (8). Given the increasing population at greater risk of listeriosis, namely, the elderly, the high mortality rate of the infection and the detection of resistant isolates, monitoring for antibiotic resistance in strains of *L. monocytogenes* on a large scale, and assessing the risk of infection by these strains, is highly recommended.

The implementation of national surveillance studies monitoring the incidence of listeriosis and antimicrobial resistance of strains of this clinically important pathogen would be most valuable, allowing identification of sporadic and outbreak cases, to detect general trends in antibiotic susceptibilities, and in parallel with food studies, to potentially identify food sources of clinical strains.

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