

# Clinical isolates of *Listeria monocytogenes*: serotyping, biotyping and susceptibility to antibiotics.

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## Introduction

Listeriosis is a severe infection caused by *Listeria monocytogenes* particularly among the elderly, very young and immunocompromised individuals and has also been associated with late-term miscarriages in pregnant women. Within the context of the Risk Assessment process, it is necessary to gather data concerning the frequency, nature, severity and duration of illness, caused by the presence of the hazard in the food. The aim of this study was to characterise the strains isolated from human cases of listeriosis by serotyping and biotyping by cadmium, arsenic and tetracycline sensitivity, as well as to determine the minimal inhibitory concentration of some antibiotics (MIC).

## Materials and Methods

Serotypes were determined using antisera against O and H antigens (Denka Seiken Co., Ltd, Tokyo, Japan). Resistance to arsenium, cadmium and tetracycline were determined according to Vaz-Velho et al. (1) and minimal inhibitory concentration of some antibiotics (MIC) was performed following agar dilution assays (2).

## Results

Table 1 shows the results obtained for the 51 strains recovered from the 58 cases of listeriosis identified in Portugal between 1994 and 2005. Table 2 shows the MICs determined for 49 strains.

Table 1 – results of serotyping and biotyping

Case	Year of isolate	Serotype	As/Cd/tetra profile	Case	Year of isolate	Serotype	As/Cd/tetra profile	Case	Year of isolate	Serotype	As/Cd/tetra profile
1	1994	4b	R/S/S	18	2000	4b	S/S/S	35	2004	4b	R/S/S
2	1996	4b	S/S/S	19	2001	4b	S/S/S	36	2004	4b	S/S/S
3	1997	4b	S/S/S	20	2001	1/2b	S/S/S	37	2004	4b	R/R/S
4	1997	4b	S/S/S	21	2002	4b	S/S/R	38	2004	1/2b	S/S/S
5	1997	4b	S/S/S	22	2003	4b	S/S/S	39	2004	4b	R/S/S
6	1998	1/2b	S/R/S	23	2003	1/2b	S/S/S	40	2005	1/2b	S/S/S
7	1998	4b	R/S/S	24	2003	4b	S/S/S	41	2005	4b	R/R/S
8	1999	4b	S/S/S	25	2003	4b	S/S/S	42	2005	4b	S/S/S
9	1999	4b	S/R/S	26	2003	1/2b	S/S/R	43	2005	1/2b	S/R/S
10	1999	4b	S/R/S	27	2003	4b	R/S/S	44	2005	4b	S/S/R
11	1999	4b	R/R/S	28	2003	4b	R/R/S	45	2005	1/2b	S/R/S
12	2000	1/2b	S/S/S	29	2004	4b	R/R/S	46	2005	1/2b	S/R/S
13	2000	4b	S/R/S	30	2004	4b	S/S/S	47	2005	4b	R/S/S
14	2000	4b	S/R/S	31	2004	4b	S/R/S	48	2005	4b	S/R/S
15	2000	4b	S/S/S	32	2004	1/2b	S/S/S	49	2005	4b	S/S/S
16	2000	4b	S/S/S	33	2004	1/2b	S/S/S	50	2005	4b	S/R/S
17	2000	4b	S/R/S	34	2004	1/2b	S/S/R	51	2005	4b	S/R/S

Table 2 – MICs for *Listeria monocytogenes* isolated in Portugal during the period 1994 – 2005, and MIC for 90% of the strains for 2003, 2004 and 2005

Drug	MIC (µg/ml) for strain isolated					
	1996 – 2005 (n=49)			2003 (n=7) <sup>a</sup>	2004 (n=10) <sup>a</sup>	2005 (n=12) <sup>a</sup>
	Range	50%	90%			
Penicillin G	0,125 – 2	0,25	1	1	0,25	0,25
Ampicillin	0,125 - 0,25	0,25	0,25	0,25	0,25	0,25
Gentamicin	0,125 – 1	0,25	1	0,5	0,5	0,5
Ciprofloxacin	0,25 – 2	2	2	2	2	2
Erytromycin	0,125 – 0,5	0,25	0,50	0,25	0,25	0,25
Vancomycin	1 – 4	2	4	4	2	4
Choramphenicol	4 – 8	8	8	8	8	8
Tetracycline	1 – 2	1	2	2	2	2
Rifampicin	0,03125 – 0,5	0,125	0,25	0,5	0,25	0,25
Nitrofurantoin	32 – 128	64	128	128	128	128

<sup>a</sup> MICs for 90% of the strains

## Conclusions

51 strains were collected from 58 cases of listeriosis occurred between 1994 and 2005. Two different serotypes were found, 4b and 1/2b; 38 strains were serotype 4b (75%) and 13 strains were serotype 1/2b (25%). Concerning the sensitivity to the heavy metals and tetracycline, 5 groups were found with a predominant group with 23 strains (45%) with a profile sensitive to arsenite, cadmium and tetracycline. 43 strains were found with a MIC higher than 32 µg/ml for nitrofurantoin. This value corresponds to the MIC breakpoint applied for *S. aureus* (3). One of the strains isolated in 2004, from blood of an oncologic patient 65 years old who died, was resistant for penicillin, MIC breakpoint >1 (3).

## References

- (1)Vaz-Velho, M. Duarte, G., McLauchlin, J. and Gibbs, P. (2001) Characterization of *L. monocytogenes* isolated from production lines of fresh and cold-smoked fish. *J. Appl. Microbiol.* 91:556-562
- (2) National Committee for Clinical Laboratory Standards (2004) Performance standards for antimicrobial susceptibility testing: fourteenth informational supplement. NCCLS Document M100-S14. NCCLS.
- (3) The Swedish Reference Group for Antibiotics ([www.srga.org](http://www.srga.org)).

## Acknowledgments

This work was funded by Fundação para a Ciência e Tecnologia project POCTI/AGR/44889/2002.

