Characterization of *L. monocytogenes* isolated from traditional Portuguese cheeses

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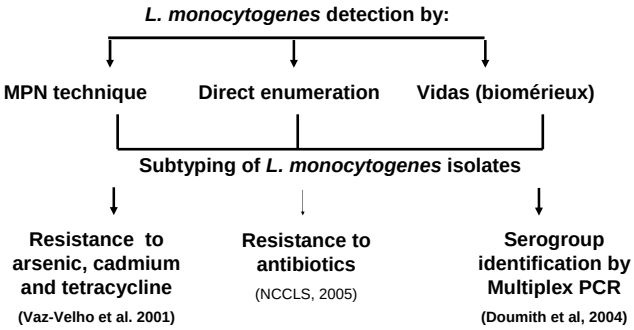
Introduction

L. monocytogenes is considered a serious public health hazard.
The incidence of human listeriosis is low (2-15 per million population) but the death rate among individuals at risk approaches 30%, far exceeding that of other common food-borne pathogens.
Consumption of contaminated foods is probably the primary source of human listeriosis and the prevalence of *L. monocytogenes* in foods is relatively high.
The aim of this study was the characterisation of 62 strains of *L. monocytogenes* isolated from 12 different Portuguese commercial cheeses according to the serotype, biotype, RAPD-PCR and resistance to antibiotics.

Materials and Methods



Results and Discussion

Table 1 shows the Minimal inhibitory concentration of various antibiotics(MIC_{50} and MIC_{90}). 90% of the isolates were found with a MIC of 128 μg/ml for nitrofurantoin. With the exception of penicillin and ampicillin, there are no breakpoints defined for antibiotics resistance *L. monocytogenes.* Therefore it was not possible to classify them according to their susceptibility to the antibiotics. However it was possible to conclude that there are differences between the strains and also between the origin of the strains and the susceptibility to antibiotics.
Table 2 shows the biotype and the serogroup determined for each *L. monocytogenes* strain. In general, the isolates from each cheese demonstrated a similar pattern.
For all the analysed cheeses and concerning the sensitivity to heavy metals three biotypes were present: As:s,Cd:s (90,5%); As:s,Cd:r (1,6%) and As:r,Cd:s (7,9%). Four serovars were found: 1/2a-3a (4,8 %), 1/2b-3b (71,4%), 1/2c-3c (1,59%) and 4b-4d-4e (21,9%).
The isolates were analysed and characterised according to the RAPD-PCR profile using primer UBC155. 5 different clusters were observed. Isolates from different cheeses were often included in the same cluster .

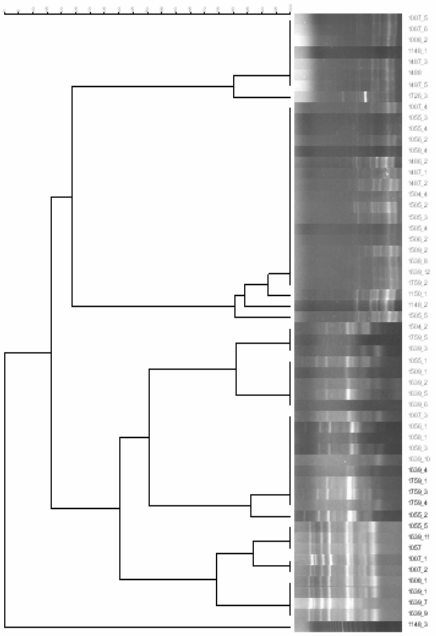
Table 1- MIC50 and MIC 90 for strains isolated from commercial cheeses

	MIC (μg/ml) for strain isolated from commercial cheeses (n=62)		
Drug	Range	50%	90%
Penicillin G	0,25 – 2	0,25	1
Ampicillin	0,0625 – 1	0,25	0,5
Gentamicin	0,03125 – 1	0,125	0,125
Ciprofloxacin	1 – 128	1	2
Erytromycin	0,5 – 16	1	1
Vancomycin	0,5 – 1	1	1
Choramphenicol	2 – 8	4	8
Tetracycline	1 – 128	4	4
Rifampicin	0,03125 – 0,25	0,125	0,25
Nitrofurantoin	32 – 128	128	128

Table 2- Characterization of cheese isolated strains.

isolate	cheese	Serogroup	Resistance to As, Cd and Tetra: As	Cd	Tetra
1	Q1	1/2a-3a	-	-	-
2		1/2b-3b	-	-	-
3		1/2b-3b	-	-	-
4		1/2b-3b	-	-	-
5		1/2b-3b	-	-	-
6		1/2b-3b	-	-	-
7	Q2	1/2b-3b	-	-	-
8		1/2b-3b	-	-	-
9		1/2b-3b	-	-	-
10		1/2b-3b	-	-	-
11		1/2b-3b	-	-	-
12		1/2b-3b	-	-	-
13		4b-4d-4e	-	-	-
14		4b-4d-4e	-	-	-
15		4b-4d-4e	-	-	-
16	Q3	4b-4d-4e	-	-	-
17		4b-4d-4e	-	-	-
18		4b-4d-4e	-	-	-
19		4b-4d-4e	-	-	-
20		4b-4d-4e	-	-	-
21		1/2b-3b	-	-	-
22	Q4	1/2b-3b	-	-	-
23		1/2b-3b	-	-	-
24	Q5	1/2b-3b	-	-	-
25		1/2b-3b	-	-	-
26		1/2b-3b	-	-	-
27	Q6	1/2b-3b	-	-	-
28		1/2b-3b	-	-	-
29		1/2b-3b	-	-	-
30		1/2c-3c	-	-	-
31		1/2b-3b	-	-	-
32		1/2b-3b	-	-	+
33		1/2b-3b	-	-	-
34		1/2b-3b	-	-	-
35		1/2b-3b	-	-	-
36	Q7	1/2b-3b	-	-	-
37		1/2b-3b	-	-	-
38		1/2b-3b	-	-	-
39		1/2b-3b	-	-	-
40		1/2b-3b	-	-	-
41		1/2b-3b	-	-	-
42	Q8	1/2a-3a	-	-	-
43		1/2a-3a	-	-	-
44		1/2b-3b	-	-	-
45	Q9	1/2b-3b	-	-	-
46		1/2b-3b	-	-	-
47		1/2b-3b	-	-	-
48		1/2b-3b	-	-	-
49		1/2b-3b	-	-	-
50		1/2b-3b	-	-	-
51		1/2b-3b	-	-	-
52	Q10	1/2b-3b	-	-	-
53		1/2b-3b	-	-	-
54		1/2b-3b	-	-	-
55		1/2b-3b	-	-	-
56		1/2b-3b	-	-	-
57		1/2b-3b	-	-	-
58	Q11	4b-4d-4e	-	-	-
59		4b-4d-4e	+	-	-
60		4b-4d-4e	+	-	-
61	Q12	4b-4d-4e	+	-	-
62		4b-4d-4e	+	-	-

Figure 1- Dendrogram obtained by RAPD-PCR.



Conclusions

This study demonstrated that other typing methods are necessary to be used in order to identify possible correlations between isolates.

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