COMPARATIVE GENETIC CHARACTERIZATION OF *Listeria monocytogenes* ISOLATES FROM HUMAN CLINICAL CASES AND READY-TO-EAT MEAT-BASED FOODS IN PORTUGAL

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Abstract – The aim of this study was to genetically characterize *Listeria monocytogenes* isolates from ready-to-eat (RTE) meat-based foods and comparing their genetic profile with *L. monocytogenes* human clinical isolates, in order to assess a possible source of infection.

A total of 85 *L. monocytogenes* isolates were evaluated regarding their serogroup by a multiplex PCR assay in order to cluster isolates into five serogroups: IIa, IIb, IIc, IVa and IVb; secondly, a Pulsed-Field Gel Electrophoresis (PFGE) was performed with restriction enzymes *Apa*I and *Asc*I.

Results showed that the main serogroup in food items was IVb (39%) and IVa (28%), while in human isolates the most common were IVb (65%) and IIb (18%). Two main pulsogroups were obtained and there were some cases of more than 85% of similarity between RTE and human *L. monocytogenes* isolates. Taken together, our results show a close genetic relatedness between RTE and human *L. monocytogenes* isolates, stressing the importance of preventive measures implementation at food production and retail level. Also, an understanding of the genetic variability of strains found in humans and foods will contribute to a better awareness for this important food-borne pathogen.

Key Words – *L. monocytogenes*, pathogenic potential, pulsotypes, ready-to-eat foods.

I. INTRODUCTION

In the last decade, European member states have reported thousands of human listeriosis confirmed cases per year, with high fatality rates among these cases [1]. This trend has also been reported by the Centers for Disease Control and Prevention in United States of America [2].

RTE meat-based food products are one of the most consumed food products around the world [3]. Their long shelf-lives in refrigerated storage, as well as the fact that they do not need to undergo any heat-treatment prior to consumption, makes them an important source of food-borne disease, especially by *L. monocytogenes* [1].

There are several ways by which *L. monocytogenes* can remain in the finished RTE product, namely by its recontamination following a listericidal treatment [4] as a result of the contact with contaminated processing equipments or surfaces [5; 6; 7]. Therefore, tracing isolates and matching with those from human cases to know the source of infection is of primary importance [8] to delineate preventive strategies for disease control.

Because of the importance of *L. monocytogenes* epidemiology to human health [8] and the notable diversity in the pathogenicity among its strains [9], subtyping and virulence characterization are of upmost importance. The four main *L. monocytogenes* serotypes identified in food and human patients are 1/2a, 1/2b, 1/2c and 4b, being the last one related to more disease cases. The IIa molecular serogroup includes 1/2a and 3a serotypes isolates, the IIb includes 1/2b and 3b, the IIc serogroup includes 1/2c and 3c, the 4b isolates are yielded in the IVb serogroup and the IVa includes 4c isolates [10]. Each serogroup has a different virulence potential due to factors that play an important role in the bacteria survival, environmental persistence and pathogenesis [8, 9].

Pulsed-field gel electrophoresis (PFGE) is considered as the "gold standard" method for *L. monocytogenes* epidemiological investigations, due to its high reproducibility, robustness and high discriminating power [11].

The aim of this study was to genetically characterize *L. monocytogenes* isolates from RTE meat-based foods collected in different retail establishments and compare their genetic

profile with *L. monocytogenes* human clinical isolates, in order to assess a possible source of infection.

II. MATERIALS AND METHODS

Food sampling and L. monocytogenes isolates collection: 120 RTE products of meat origin (prepacked in the original industrial package or sliced and packed by order) were collected in industrial plants (codified as 1 to 10) and in retail establishments (codified as A to I) of Lisbon metropolitan region. The samples were transported in an isothermic box (below 5ºC) to the laboratory in less than 2 hours. Food samples were prepared according to ISO 6887-2:2003. Detection of *L. monocytogenes* was performed according to ISO11290-1:1995. *L. monocytogenes* identification was confirmed by PCR, according to Simon *et al*. (1996). An initial collection of 36 *L. monocytogenes* isolates was obtained. These isolates were characterized for serogroups and representatives were selected (n=19) for posterior pulsotyping.

Human clinical isolates collection: 49 isolates of human listeriosis cases were kindly provided by Department of Clinical Microbiology, Hospital de Santa Maria, Lisbon. These isolates were collected between 2007 and 2013.

Genetic methods: L. monocytogenes isolates (n=68) serogrouping was done by multiplex PCR assay, according to Kérouanton *et al*. (2010).

PFGE of the isolates was performed according to the CDC PulseNet standardized procedure for *L. monocytogenes* typing [11]. Electrophoresis of resulting DNA fragments was performed using a CHEF-Dr III System apparatus (Bio-Rad Laboratories, Hercules, USA). Gels stained with ethidium bromide (Sigma, St. Louis, MO, USA) were photographed under UV transillumination. A dendrogram of the selected *L. monocytogenes* isolates was constructed based on the PFGE patterns and serogroups average from experiments in BioNumerics software package (Sint-Martens-Latem, Belgium, version 6.6). Cluster analysis was performed using the unweighted pair group method with arithmetic averages (UPGMA) and band-based Dice correlation coefficient with an optimization and position tolerance of 1.5%. Serogrouping was performed by simple matching with a binary conversion limit of 50%.

III. RESULTS AND DISCUSSION

From the 120 food products analyzed, 15 samples were positive (12.5%) for *L. monocytogenes* presence. These findings are slightly above the ones described in similar studies [15, 16].

As can be seen in Table 1, serogrouping revealed that the most frequent serogroups in food samples were IVb (39%) and IVa (28%), followed by IIb (17%). In human clinical isolates the most frequent serogroups were also IVb (65%) and IIb (18%), being followed by IIa (10%) serogroup. These numbers are consistent with the ones reported by the European Food Safety Authority in the European summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013 [1].

Table 1 Serogroups of *L. monocytogenes* isolates determined by multiplex PCR assay

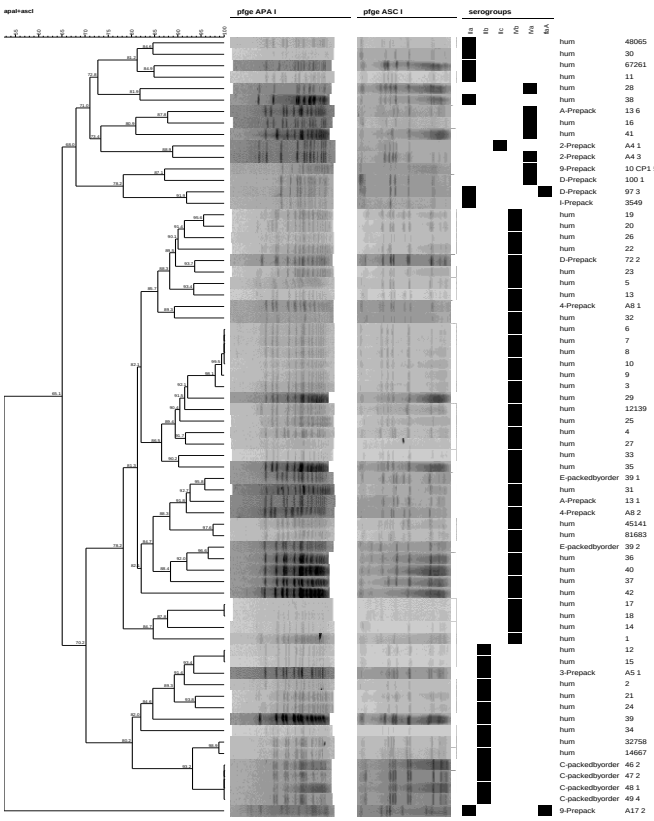
Multiplex PCR assay		*L. monocytogenes* isolates		
		Food (n=36)	Clinical (n=49)	Total (n=85)
Serogroups	IIa	4	5	9
	IIb	6	9	15
	IIc	2	0	2
	IVa	10	3	13
	IVb	14	32	46

Regarding PFGE analysis, the resulting dendrogram of all isolates for *Apa*I and *Asc*I restriction patterns can be seen in Figure 1.

Two major clusters can be seen in Figure 1. The first one is constituted by a mix of 15 food and human isolates. The second cluster (n=52) is constituted mainly by human isolates with 11 food isolates, and serogroups IIb and IVb show up as the most common ones. One food isolate ($A17_1$) appears not to match with any of the above-mentioned clusters; it was firstly classified as IIa serogroup, but as preconized by Kérouanton *et al*., 2010, it was submitted to an additional PCR assay based on the amplification of the *fla*A gene for atypical isolates, being confirmed its classification in IIa serogroup.

The same food sample revealed the presence of different strains of *L. monocytogenes*, as can be

Figure 1. Dendrogram based on PFGE patterns of 68 *L. monocytogenes* isolates



seen for RTE samples 39 (39_1 and 39_2 isolates, although belonging to the same serogroup – IVb – have different genetic profiles, presenting one more band in *Asc*I restriction pattern) and A8 ($A8_1$ and $A8_2$ isolates belong to serogroup IVb, but have a different restriction profile for *Asc*I). This fact suggests an adaptation of the original strain possibly to environment conditions [17].

Interestingly, food sample 13_6, a prepacked chicken salad from retailer A, was associated with human clinical sample 16 with more than 86% of restriction pattern similarity and sharing the same serogroup (IVa). Also, food sample 72_2, prepacked ham morcels from retailer D, was associated with human clinical isolate 23, having the same serogroup (IVb) and more than 94% of similarity of restriction patterns. Likewise, food sample $A8_1$, a beef salad from industry 4, was closely related to human isolate 32, sharing the same serogroup (again, IVb) and having 88% of matching. More, 96% of similarity was found for food isolate 39_2, a packed by order shredded cooked meat from retailer E, and human clinical isolate 36. Furthermore, food isolate $A5_1$ (prepacked cooked piglet from industry 3) had 93.4% of genetic relatedness with human isolates 12 and 15 that shared the same genetic profile between them. Lastly, food isolates 46_2, 47_2, 48_1 and 49_4, although being different RTE (pork head, "chourição", turkey breast and pork ham, respectively) were all sliced in the same slicing machine at the delicatessen section of retailer C and packed by order; they exhibit 100% of genetic similarity, which suggests a common source of contamination, most likely the slicing machine. These isolates also share 93.2% of genetic relatedness with human isolates 32758 and 14667.

Results show that there is a close genetic relatedness between RTE meat-based food products collected in industrial and retail establishments and human listeriosis isolates. This fact underlines the importance of preventive measures implementation in food establishments, especially those that prepare RTE foods, to prevent contamination.

IV. CONCLUSION

Genetic characterization of food and human isolates of *L. monocytogenes* is an important step not only at laboratorial level, but also for the establishment and improvement of public health policies, that should reflect the risk posed by *L. monocytogenes*. In Portugal, listeriosis is not a communicable disease, so more information is needed to understand the routes and food vehicles the pathogen chooses to thrive and infect human beings.

An understanding of the variability of strains found in humans and foods and the genetic profile presented by different strains will contribute to a better awareness for this important food-borne pathogen.

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