SUBTYPING AND VIRULENCE-RELATED CHARACTERIZATION OF *Listeria monocytogenes* ISOLATED IN THE PORTUGUESE READY-TO-EAT MEAT-BASED PRODUCTS FOOD-CHAIN

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Abstract – *Listeria monocytogenes* isolates (n=15) recovered from ready-to-eat meat-based food products (RTEMP) collected in industrial processing plants and retail establishments were genetically characterized in order to assess possible sources and routes of food contamination in the RTEMP food-chain and delineate preventive measures . It was found that 12.5% of the RTEMP samples were contaminated with *L. monocytogenes.* All the isolates were assessed by multiplex PCR for serogroup determination and detection of virulence-associated genes *inl*A, *inl*B, *inl*C, *inl*J, *plc*A, *hly*A, *act*A and *iap*. Serogroups IIb and IVb dominated and all isolates were positive for the assessed virulence genes. Pulsed-field gel electrophoresis (PFGE) using restriction enzymes *Apa*I and *Asc*I, revealed genetic variability and differentiated the isolates in two major clusters. However, some PFGE profiles of particular RTEMP collected in industries and retail establishments seem to be highly related exhibiting more than 95% of similarity, suggesting a possible common source and strain persistence over time.

The close genetic relatedness of RTEMP strains stressed the importance of preventive measures implementation throughout the food-chain continuum.

Key Words – *L. monocytogenes*, delicatessen, PFGE.

I. INTRODUCTION

L. monocytogenes can lead to listeriosis, a severe illness with high mortality rates and long-term sequelae, which is mainly transmitted through the ingestion of contaminated foods [1]. Different *L. monocytogenes* serogroups diverge in their pathogenicity to humans, and more than 90% of human listeriosis is linked to serogroups IIa, IIb and IVb [2].

Ready-to-eat meat-based food products (RTEMP) do not require a heat treatment prior to consumption and are commonly associated to listeriosis [3]. *L. monocytogenes* can establish environment niches in food processing plants and retail establishments, where it is introduced through raw materials of different sources, or by personnel [4], contaminating final products by cross-contamination from the processing environment after the listericidal treatment step. The use of highly discriminatory typing methods is essential to reveal potential sources and routes of food contamination, further assisting the RTEMP food-chain in delineating preventive strategies for *L. monocytogenes* control.

In this study, *L. monocytogenes* isolates from food samples of the RTEMP food-chain were genetically characterized and subtyped to assess possible sources and routes of contamination and delineate preventive measures.

II. MATERIALS AND METHODS

One hundred and twenty RTEMP samples were collected in ten industrial producing units and nine retail establishments located in the central region of Portugal, either pre-packed or packed by order. Industrial RTEMP samples were coded with the letter F, and the collection order number and a letter (A or B), while retail samples were coded with the letter F and pbo (packed by order) or pp (pre-packed), and an additional number representing sample collection order.

L. monocytogenes detection was performed according to ISO11290-1. Fifteen isolates was

obtained that were confirmed by PCR, according to Simon *et al*. (1996). Serogrouping of all *L. monocytogenes* isolates was done by multiplex PCR [7], the protocol of Liu *et al. (*2007) was used for *inl*A, *inl*B, *inl*C and *inl*J virulence genes detection and for virulence-associated genes *plcA, hlyA, actA* and *iap* detection, Rawool *et al*. (2007) PCR protocol was followed.

Genetic characterization of the isolates was performed using the Centers for Disease Control and Prevention PulseNet standard procedure for *L. monocytogenes* typing using restriction enzymes *Apa*I and *Asc*I [10]. A dendrogram of all isolates (n=15) was constructed in BioNumerics software package version 6.10 (Applied Maths, Sint-Martens-Latem, Belgium). PFGE patterns were analyzed with an optimization setting of 1.5% and a band-position tolerance of 1.5%. Cluster analysis was performed using the unweighted pair group method with arithmetic averages (UPGMA) and band-based Dice correlation coefficient.

III. RESULTS AND DISCUSSION

L. monocytogenes was detected in 12.5% of all the analyzed RTEMP (15/120 samples), specifically in 5 of the 20 industrial samples and in 10 of the 100 retail samples.

L. monocytogenes frequency (25%) in the industrial segment of the RTEMP chain is higher than the ones reported by Modzelewska-Kapitula & Maj-Sobotka (2014) in which 1.8% of the analyzed RTEMP samples were positive and by Meloni *et al*. (2014) who found 8% of positive samples in fermented sausages processing plants. The high frequency of *L. monocytogenes* in industrial RTEMP seems to be due to specific non-conforming pre-requirements related to selection and control of raw materials, equipment preventive maintenance and hygiene program (Henriques *et al.*, 2014). *L. monocytogenes* frequency (10%) in the retail segment is in line with the ones reported in similar studies at retail level [14; 15] and might be related with the food handling practices and the hygiene at retail level.

Serogroup distribution of the isolates revealed that the most frequent serogroup was IIb (33%) followed by IVb (27%), IIa (20%), IVa (13%) and IIc (7%). Wang *et al*. (2015a) also found serogroup IIb to be the most frequent in RTEMP collected in China and previous works on *L. monocytogenes* serotype distribution in RTEMP refer serotypes 4b (included in serogroup IVb) and 1/2a (included in serogroup IIa) as the most frequently reported [16].

All the isolates presented the virulence markers *inl*A, *inl*B, *inl*C, *inl*J, *plc*A, *act*A, *hly*A and *iap* genes. However, isolates belonging to serogroups IVa and IVb were not positive for the *inl*B gene, which is due to the inability of the used primers to recognize the *inl*B gene of serogroups IVa and IVb [8]. PFGE characterization of the isolates resulted in the dendrogram presented in Figure 1. At about 50% of similarity, two major clusters and a single strain (F9A1) can be seen. This strain has a distinct PFGE profile and belongs to serogroup IIa, in which several atypical strains are included [7].

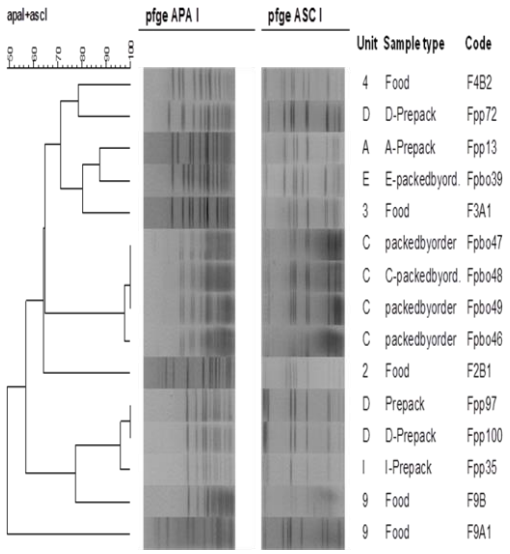
The first cluster is constituted by a mix of 10 retail and industrial isolates that belong in the vast majority to serogroups IIb and IVb. In this cluster, the PFGE profiles of *L. monocytogenes* isolates from RTEMP samples Fpbo46, Fpbo47, Fpbo48 and Fpbo49, are highly related (>97% of similarity) and shared the same serogroup (IIb) and although these are four different RTEMP samples, they were all prepared sequentially in the same slicing machine of the retail delicatessen and hence a common source of contamination could be identified for these samples. These results are consistent with the suggestion that there are stable clonal groups of *L. monocytogenes* that might be present not only in foods but also in food-related environments. Also in this first cluster, the PFGE profiles of RTEMP isolates Fpbo39 (a packed by order sliced chicken ham collected in retailer E) and Fpp13 (a pre-packed shredded chicken collected in retailer A) were associated displaying more than 85% of similarity and all belonging to serogroup IVb. In these cases, the possibility of a common source should not be discarded. In fact, the upstream food-chain continuum should be addressed in a root-cause analysis to understand the origin and persistence of a common strain, including the animal husbandry farm, slaughterhouse and food-producing industry, where *L. monocytogenes* might persist in refrigerated environment for long periods, even years.

In the second cluster, different sources seem to have highly related PFGE profiles, as featured by

samples Fpp97, Fpp100 and Fpp35; this fact might be explained by the ubiquitous nature of *L. monocytogenes*. Sample Fpp97 (a prepacked sliced ham) and sample Fpp100 (a prepacked shredded ham) were collected in the same retail establishment and, according to its labels, produced in the same industry, revealing 100% of similarity and also shared the same serogroup (IIa). This may point toward a common contamination source within the producing industry, because these samples were not manipulated in the retail establishment, as they were prepacked RTEMP. Strain Fpp35 presented a highly similar PFGE type (96%) with Fpp97 and Fpp100, but was collected in a different retail establishment and, according to its label, was produced in a different industry.

The persistence of strains over time and the possibility of common sources and routes of infection emphasize the need for preventive measures improvement along the RTEMP food-chain continuum. Most importantly, the strict selection and control of raw material suppliers, food handlers' health status control enhancement, workers training conducing to proper behaviors/ attitudes towards food preparation, consistent hygiene procedures with an adequate equipment sanitizing frequency and programmed maintenance operations to eliminate eventual environment niches of *L. monocytogenes* should be carefully considered and planned.

Figure 1. Dendrogram of *L. monocytogenes* isolates



IV. CONCLUSION

Overall, *L. monocytogenes* was detected in 12.5% of the samples and the majority of the isolates belonged to serogroups IIb and IVb and all presented the same virulence genes. PFGE typing revealed genetic diversity of the isolates that were gathered in two different clusters. Some isolates shared the same pulsotype or presented high similarity, which might point towards a persistent stable strain and a common source related to cross-contamination from the food producing environment, personnel involved in food processing operations or raw materials. Our work reinforces the need to address all the RTEMP food-chain stakeholders when designing and implementing preventive and control measures for *L. monocytogenes*, in order to reduce the potential risk that these foods might pose to the consumer in the transmission of foodborne listeriosis.

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