EVALUATION OF ANTIBIOTIC SUSCEPTIBILITY OF *LACTOBACILLUS PLANTARUM* ISOLATED FROM TRADITIONAL PORTUGUESE SAUSAGE PRODUCTS

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Abstract – Nowadays the use of fermentative microbiota in food products is researched in order to confer protection to the products and furnish health benefits to consumers The aim of this work was to evaluate antibiotics susceptibility of *Lactobacillus plantarum* isolated from fermented traditional meat products in order to select them to be used as starters or protective bacteria on fermented meat products. The susceptibility of different *L. plantarum* isolates (n=44) from fermented/dry/smoked meat products of three different Portuguese industries were tested by Agar disc diffusion method for Vancomycin, Quinupristin/Dalfopristin, Rifampicin, Penicillin G, Erythromycine, Tetracycline, Gentamicin, Lincomycin and Chloramphenicol. Most of the studied *L plantarum* isolates were susceptible for Tetracycline (75%) and Erythromycin (71%) and could be used safely as starter cultures. Those that presented resistance need to be genetically evaluated since the mechanism of resistance is probably related to mobile genetic elements carried by *L plantarum*.

Key Words –Antibiotic resistance, LAB, *L. plantarum*, Traditional meat products

- INTRODUCTION

Fermented meat products are in Portuguese food for a long time. In fact, the fermentative process contributes to preserve meat of slaughtered animals, presently due to the taste and use versatility these products are much appreciated [1]. The method of preservation of traditional meat products, done with lactic fermentation by lactic acid bacteria's (LAB) gives them these characteristics. The LAB are responsible for the texture, flavour and protection ability against pathogens through production of antimicrobial substances [2].

Nowadays, the use of fermentative microbiota in food products is researched in order to confer protection to the products and, due to their probiotic characteristics, furnish health benefits to consumers [3, 4, 5].

In LAB group are included the *Lactobacillus plantarum*, which showed probiotic effects, by surviving and colonizing the gastrointestinal human tract and by conferring the therapeutic and prophylactic proprieties in individuals with particular diseases [6, 7, 8].

On the other hand it is important to ensure that isolates to be used as starters are completely safe, in order to minimize risks consumer's health. One of the greatest hazards in the use of these microorganisms it is the ability of resistance to antibiotics. This may be due to the existence of different mechanisms of resistance, however those more problematic will be the mobile resistance genes carried by plasmids in the genetic material of the bacteria, and in the case of verifying that existence, the more likely is that resistance will be transmitted to pathogenic bacteria [4, 9, 10]. Also, Lactobacilli intentionally added to the food chain should

not carry transferable antibiotic resistance genes according to EFSA (2007).

For the reasons described it's very important to select LAB strains without profiles of resistance against Tetracycline and Vancomycin whose resistance mechanisms it's known to be associated with the presence of mobile genetic elements [11] .
The aim of this work was to evaluate the antibiotics susceptibility of *Lactobacillus plantarum* isolated from fermented traditional meat products in order to select them to be used as starters or protective bacteria on fermented meat products.

- MATERIALS AND METHODS

2.1. Collection of Isolates: The isolates (n=44) were selected from a collection belonging to the Laboratory of Food Technology and Safety, Faculty of Veterinary Medicine of Lisbon. This collection was obtained from various fermented meat products of 3 different industries on south region of Portugal.

2.2. Antibiotic susceptibility screening: All isolates were tested by Agar disc diffusion method for following antibiotics: Vancomycin (30µg), Quinupristin/Dalfopristin (15ug), Rifampicin (5µg), Penicillin G (10 IU), Erythromycine (15ug), Tetracycline (30µg), Gentamicin (10mg), Lincomycin (15ug) and Chloramphenicol (30µg). Strains were grown on MRS Agar for 24 hours, at 30 ◦C under anaerobic conditions. Strains were then prepared as described in CLSI 2007 [13] and Eucast 2013 [14] for the mentioned antibiotics, and culture was incubated during 48 hours, at 30 ◦C in anaerobic conditions. After this period the diameters of inhibition zones were measured. Strains susceptibility categorization was carried out according to CLSI 2007 [13] and Eucast 2013 [14] break points. Quality control strains used were *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212.

- RESULTS AND DISCUSSION

3.1. *Evaluation of antibiotics susceptibility*

In Figure 1 it is observed the percentage of susceptibility presented by *L. plantarum* isolates for each antibiotic.

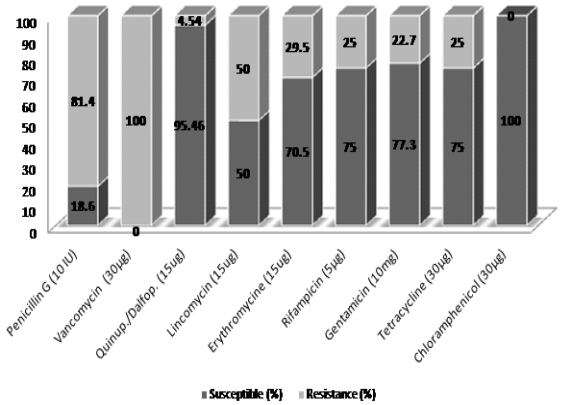

Figure 1. Antibiotic susceptibility presented by *L. plantarum* isolates (%).

L. plantarum isolates under study were resistant for Vancomycin (100%) and Penicillin G (81%). For Lincomycin more than 50% of the isolates were also resistant but in the same group of MLS_B (macrolide, lincosamide, streptogramin B compound) 30% of isolates were Eritromycin resistant and only 5% were Quinupristin/Dalfopristin resistant.

Regarding Gentamicin, Rifampicin and Tetracycline aproximattelly 25% of isolates were resistant. Chloramphenicol was able to inhibit the multiplication of *L. plantarum* isolates.

Heterofermentative *Lactobacilli* are intrinsically resistant to Vancomycin confirming the results presented (15). However, Penicillin resistance results is in contrast to the common opinion of lactobacilli being susceptible to penicillins in general, suggesting that further work must be done to confirm resistance by MIC (minimal inhibitory concentration) method and investigating points mutations of genes encoding Penicillin binding proteins (Pbp).

Most of the isolates seem to be susceptible for Tetracycline and Erythromycin. Those which presented resistance need to be genetically evaluated since the mechanism of resistance is probably related to mobile genetic elements carried by *L plantarum*. Genes *tet* (K, L, M, O, Q, S, W, 36) have been reported in various *Lactobacillus* species [16, 17, 18]. Overall, the most frequently found *tet* gene, *tet*(M), has previously been identified in strains of *L. plantarum* [19, 20]. Further work must be performed in order to screen the presence of mobile genes elements such as *tet* and *erm* genes on *L. plantarum* isolates under study, in order to identify strains with potentially nontransferable resistance genes.

- *Resistance profile associated to industries*:

Table 1 presents the resistance profile to different antimicrobials studied according to the origin of *L. plantarum* isolates.

Isolates from industry A and C presented higher level of resistance to the antibiotics studied than Industry B. For MLS_B group, isolates from Industry C presented higher level of resistance compared with the other industries. The same report was done for Tetracycline.

This higher level of resistance could be related with more promiscuous contact with *Enterococcus* or other microbial groups particularly in poor hygiene environments since this fact could increases the spread of resistant bacteria.

The strains *L. plantarum* S4B6 (Industry A), 2L2-8, 19P1-7, 1L2-6 (Industry B), P05-4, P05-15 and P05-67 (Industry C), were selected for potential use as starters since they were susceptible to antibiotics tested from MLS_B group and for Tetracycline.

Table 1. Resistance profile associated to each industry

Antibiotic	Industry A n=6 (%)	Industry B n=16 (%)	Industry C n=22 (%)
Penicillin G	83.3	68.8	95.5
Vancomycin	100	100	100
Quinup./Dalfop.	0	0	9.1
Lincomycin	83.3	18.8	63.6
Erythromycine	0	0	59
Rifampicin	66.7	0	31.8
Gentamicin	16.7	31.3	18.2
Tetracycline	16.7	18.8	31.8
Chloramphenicol	0	0	0

- CONCLUSION

Most of the studied *L plantarum* isolates were susceptible for Tetracycline 75% and Erythromycin 71% and could be used safely as starter cultures. Those which presented

resistance need to be genetically evaluated since the mechanism of resistance is probably related to mobile genetic elements carried by *L plantarum*.

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