Microorganisms isolated from fresh meats and meat products sold for consumption – detection of virulence and antimicrobial resistance genes

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I. INTRODUCTION

Meat and meat products are a significant part of the Portuguese diet. These foodstuffs have microorganisms responsible for the deterioration processes that reduce their shelf-life, representing economic losses and a considerable environmental impact. Also, they are a source of pathogens, with an important impact on public health, being a potential cause of foodborne diseases [1]. This study aimed to conduct a microbiological analysis of fresh meat and meat products from small local shops and hypermarkets. *S. aureus* and *L. monocytogenes* were isolated from the samples, to evaluate their phenotypic and genotypic antimicrobial resistance, the presence of virulence factors, and the ability to form biofilms.

II. MATERIALS AND METHODS

A collection of 75 samples of fresh meat preparations and meat-based products was undertaken, in northern Portugal, from local shops and hypermarkets. All samples were evaluated for mesophiles, Enterobacteriaceae, LAB (lactic acid bacteria), *Pseudomonas* spp., *L. monocytogenes*, *S. aureus,* and *E. coli*, according to the ISO norms. After isolating *L. monocytogenes* e *S. aureus* from samples, antimicrobial resistance was determined using the Kirby-Bauer disk diffusion method, against 14 e 12 antimicrobial agents, respectively. Resistance and virulence genes were evaluated with PCR. Statistical analysis was performed with SPSS ® Statistics 29.0 for Windows, to determine if there were statistically significant differences in microorganisms' prevalence and counts.

III. RESULTS AND DISCUSSION

Total mesophilic counts presented the higher levels, followed by Enterobacteriaceae, LAB, *Pseudomonas* spp., *E. coli*, *S. aureus* and *L. monocytogenes* (Table 1). In general, the acceptability of samples was higher than 85%. Table 1 presents the microbiological counts (means and standard deviation) for type of product and microorganism evaluated.

Table 1- Microbiological counts (means and standard deviation) for type of product and microorganism evaluated.

Type of product	**Total mesophilic counts (TMC)**	**Enterobacteriaceae**	**LAB**	***Pseudomonas* spp.**	***E. coli***	***S. aureus***	***L. monocytogenes***
Meat-based products	6.06 ± 1.44 [a]	1.84 ± 2.55 [b]	3.08 ± 2.37	0.22 ± 0.70 [c]	0.50 ± 1.07	nd	0.18 ± 0.41
Meatballs and hamburgers	6.19 ± 1.06 [a]	2.57 ± 1.63 [ab]	3.33 ± 1.95	2.82 ± 0.66 [a]	1.24 ± 0.91	0.35 ± 0.71	0.04 ± 0.13
Meat skewers	6.32 ± 1.13 [a]	2.67 ± 1.86 [ab]	3.14 ± 1.89	2.30 ± 1.75 [ab]	0.90 ± 1.08	0.28 ± 0.56	0.13 ± 0.39
Breaded meat	4.57 ± 1.30 [b]	1.86 ± 0.71 [ab]	2.64 ± 1.57	0.85 ± 0.98 [bc]	0.23 ± 0.41	nd	0.20 ± 0.22
Minced meat	6.01 ± 0.73 [a]	3.79 ± 1.27 [a]	4.14 ± 0.96	1.35 ± 1.31 [b]	0.52 ± 0.81	0.21 ± 0.66	0.07 ± 0.29
Fresh sausage	5.08 ± 0.85 [ab]	2.03 ± 0.37 [ab]	3.36 ± 0.77	0.60 ± 0.73 [bc]	0.13 ± 0.21	nd	nd
Sig.	*	**	ns	***	ns	ns	ns

Sig. – level of significance; ns – not significant (p≥ 0.05); *significant (p< 0.05); **very significant (p< 0.01); *** highly significant (p< 0.001)
For each type of product, means that do not have the same letter, differ significantly (p < 0.05). nd – not detected

The prevalence of *S. aureus* and *L. monocytogenes* was 10.67% and 17.33%, respectively. *S. aureus* isolates were resistant to penicillin (52.6%), tetracycline (44.4%), chloramphenicol (36.8%) and tobramycin (26.3%). Virulence genes found were: *tet*K (31.58%), cat_{pc223} (21.05%) e *bla*Z e *ant*(a')-Ia (15.79%). *L. monocytogenes* isolates were resistant to trimethoprim-sulfamethoxazole (85.71%), ciprofloxacin (38.10%), meropenem (33.33%), tetracycline and erythromycin (28.57%), rifampicin (23.81%) and kanamycin (14.29%). Virulence genes found were: *tet*K (23.81%), *aad*A, *tet*L, and *bla*OXA-48 (14.29%), *erm*C and *msr*A/B (4.76%). Four *S. aureus* isolates and six *L. monocytogenes* isolates exhibited a multi-resistance profile. All isolates were able to form biofilms at 24h and 48h, and there were some highly productive biofilm strains.

IV. CONCLUSION

This study is of great relevance as it provides us with a better understanding of the microbiological quality of meat and, consequently, of good hygiene practices from animal slaughter to retail establishments. It is important to understand the characteristics of the bacterial strains, especially because these foodstuffs are a reservoir of pathogenic and spoilage bacteria that can cause food poisoning.

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