Coagulase negative staphylococci and enterococci in fermented meat products: presence of virulence and antibiotic resistance determinants

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Abstract: This study was undertaken to achieve information on the presence of antibiotic resistance determinants in coagulase negative staphylococci (CNS) and enterococci (Ec) that are a significant component of the fermentation process of cured meat products. Vancomycin resistant Ec and CNS were isolated thorough the production process from raw pork meats to final products and, furthermore, many Ec strains were found to be positive in PCR experiments for fsrA and ace, virulence expression factors.

Keywords: vancomycin, bacterial pathogens, PCR, virulence factors

Introduction: Enterococci (Ec) and coagulase negative staphylococci (CNS) are often part of the natural microflora involved in fermentation and flavour development of cured meats. The Enterococcus genus is of particular medical relevance because of its increased incidence in hospital acquired infections due to their enhanced multiple antibiotic resistance. Coagulase negative staphylococci are also involved in human infections and enterotoxin production has been reported in strains of this bacterial group. Aim of this work was to assess whether fermented sausages and cured pork meats could be a possible source of vancomycin resistant strains of Ec and CNS.

Materials and Methods: Samples were collected from European industrial and traditional fermented pork meat products, sausages and cured meats, throughout the production process, from raw meat to the retailer. Isolation of staphylococci was performed onto Baird Parker medium and enterococci onto Slanetz and Bartley agar plates. For the isolation of vancomycin resistant strains, vancomycin (8 or 32 mgL⁻¹) was added to the selective media. Strain identification was achieved using a RAPD fingerprinting protocol whereas taxonomic identification of isolated strains was performed by 5' region of the 16S rDNA sequencing (Ba-
Virulence determinants and vancomycin resistance genes were detected using PCR experiments (Clark et al., 1993; Nallapareddy et al., 2000; Qin et al., 2000), whereas hemolysin activity was tested on sheep blood agar.

**Results:** In the course of this work, vancomycin resistant staphylococci and enterococci were identified in all the 12 industrial fermented meat products. An example of the ratio between the vancomycin insensitive and total Ec and CNS from four different meat products is reported in fig. 1. Staphylococci and enterococci resistant to vancomycin (8 mg L⁻¹) were not found in traditional southern Italian sausages. When isolation was performed throughout the production process (fig. 2), vancomycin resistant enterococci were found in raw meat and decreased during the ripening process. Differently, vancomycin insensitive CNS increased in number during fermentation and viable cells were present at the end of the production process. The 16S rDNA sequencing made it possible to identify staphylococci strains as *S. epidermidis*, *S. equorum*, *S. saprophyticus*, *S. succinus*, *S. warneri* and *S. xylosus*.

![Graph 1](image1.png)

**Fig. 1** Ratio between vancomycin intensive and total Ec and CNS of four meat products

![Graph 2](image2.png)

**Fig. 2** Kinetics of vancomycin resistant Ec and CNS in one meat product

Three strains of *E. casseliflavus* and one strain of *E. pseudoavium* were found in traditional southern Italian sausages whereas *E. faecalis* strains were the most frequently ones isolated from the other pork’s products.

Particularly harmful was the presence of *E. faecalis* strains in pork’s meat products. PCR techniques showed that all tested vancomycin resistant enterococci from industrial products harboured *VanA* gene and that *ace* and *fsr* virulence genes were present in 100 and 80% of *E. faecalis* strains, respectively. Hemolysin production was found in all *E. faecalis* strains. The 33% of the insensitive staphylococci isolated were resistant to 32 mg L⁻¹ of vancomycin, even if no *Van* gene was found using primers related to vancomycin resistance genes of *Enterococcus*. 
resistant strains. The search of Ec virulence factors in the *E. casseliflavus* and *E. pseudoavium* strains didn’t show any positive result.

**Discussion:** The microbiological analyses revealed a widespread high presence of Ec and CNS in the fermented pork products analysed. Enterococci and staphylococci endowed with technological properties have been found in many processed meat products but clinical infections and food-borne outbreaks are increasing. The survival of Ec and CNS carrying pathogenic factors, in pork’s meat products, underlines that an improvement of hygienic conditions needs to be performed starting from meats used. The presence of virulence expression factors in Ec will lead to assay the CNS strains for *agr* and *sar* virulence expression factors, recently found in some CNS (Frebourg et al., 2000). The new isolates will be screened to assess which is their role in industrial and traditional processing in order to define starter cultures able to reduce pathogenic enterococci and staphylococci colonization of pork meat.

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**References**