

# Characterization of enterococci strains isolated from "Alheira", a traditional fermented sausage produced in the north of Portugal

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## Abstract

Enterococci were isolated from four commercial brands of "alheira", a traditional fermented sausage produced in the north of Portugal. Gram+ and catalase- organisms isolated in Bile Esculine Azide Agar were tested for growth in 6.5% (w/v) NaCl, at 45°C and 10°C and at pH 9.6. Of the 75 presumptive *Enterococcus*, 45 were further identified as *E. faecalis*, 21 as *E. faecium* and 9 as *E. gallinarum/casseliflavus*. Antimicrobial activity against *Listeria innocua*, *Staphylococcus aureus* and *E. faecalis* was observed for 30.7%, 13.3% and 30.7% of the isolates, respectively. 2 of the *E. faecalis* isolates were  $\beta$ -haemolytic. All the isolates were sensitive to penicillin. 1.2%, 81.4% and 17.4% were classified as sensitive, intermediate and resistant to vancomycin, respectively. 2.9%, 27.1% and 72.8% were classified as sensitive, intermediate and resistant to tetracyclin, respectively.

## Introduction

Enterococci are present in the gut flora of man and production animals. Consequently they are frequently isolated from fermented foods. The use of *Enterococcus* in the food industry as starter cultures is increasing, mostly due to their potential to improve the sensorial quality of fermented products during maturation, production of bacteriocins and probiotic benefits. Their presence, however, is a matter of controversy as they may lead to severe diseases, specially in immunocompromised individuals. Increased frequency of resistance to antibiotics, such as vancomycin, is also cause of concern. Characterization of enterococci in terms of pathogenic traits is important when considering their use as starters or probiotics.

## Methods

From each sample of "alheira", 20% of the colonies grown on Bile Esculine Azide Agar were tested for Gram and catalase reactions. Gram + and catalase- isolates were further tested for growth in Nutrient Broth with 6.5% (w/v) NaCl, at pH 9.6, at 45 °C and 10°C.

Identification was performed by the Vitek system.

Antimicrobial activity against *Listeria innocua*, *Salmonella typhimurium*, *Staphylococcus aureus* and *E. faecalis* was evaluated by the disk diffusion assay in Triptone Soy Agar supplemented with 6% yeast extract.

Haemolytic activity was determined on Columbia Blood Agar containing 5% defibrinated horse blood. Susceptibility to vancomycin, tetracycline and penicillin G was determined according to the NCCLS antimicrobial susceptibility testing standards.

## Results

75 presumptive enterococci strains were isolated from four commercial brands of "alheira". These were further identified as *E. faecalis* (45), *E. faecium* (21) and *E. gallinarum/casseliflavus* (9) (Vitek System couldn't distinguished between this two species).

The percentage of the isolates with antimicrobial activity against target organisms is presented in table 1.

2 of the *E. faecalis* isolates were  $\beta$ -haemolytic positive.

All the isolates were sensitive to penicillin. 1.2%, 81.4% and 17.4% were classified as sensitive, intermediate and resistant to vancomycin, respectively. 2.9%, 27.1% and 72.8% were classified as sensitive, intermediate and resistant to tetracyclin, respectively.

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Table 1. Antimicrobial activity of *Enterococcus* against selected organisms

	<i>L. innocua</i>	<i>S. typhimurium</i>	<i>S. aureus</i>	<i>E. faecalis</i>
% of the isolates	30,7	0	13,3	30,7

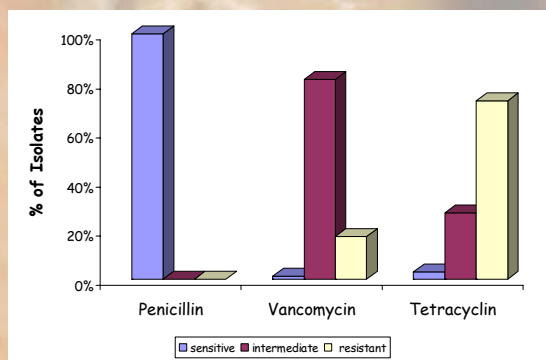


Fig. 1. Susceptibility of enterococci to antibiotics

## Conclusions

*E. faecalis* and *E. faecium* were the most frequently isolated species. Various strains were found to inhibit *L. innocua*, *E. faecalis*, and *S. aureus* but no one inhibited *S. typhimurium*. This antimicrobial activity will be further characterized.

Although all the strains were sensitive to penicillin, most of them presented an intermediate susceptibility to vancomycin and some were even resistant to this antibiotic. A high level of resistance to tetracycline was also observed. 2 strains showed haemolytic activity, a characteristic that is not often observed in food isolates. This work confirms that the presence of enterococci in foods needs to be carefully analyzed as it was demonstrated the presence of virulence traits in the *Enterococcus* isolated from "alheira".