# Enterococcal community in traditional PDO cheeses: a roadmap on antibiotic resistance over the years

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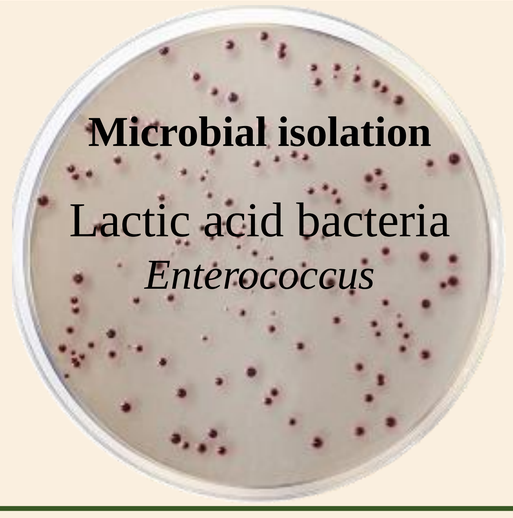
## Introduction

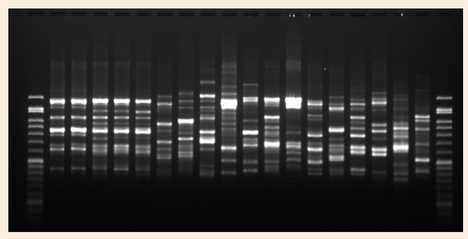
Protected Designation of Origin (PDO) labels are attributed to foods produced at a given geographic area. Traditional PDO-cheeses harbor autochthonous microbiota involved in natural fermentation/maturation processes, contributing to specific organoleptic features. Among microorganisms with technological potential, the genus *Enterococcus* has been extensively researched. On the other hand, enterococci are known opportunistic pathogens that exhibit resistance to several clinically relevant antibiotics, contributing to the persistence of disease. Hence, evaluating antimicrobial resistance of food-related bacteria is crucial to assess associated risks. In the present study, PDO-cheese samples from Azeitão and Nisa were collected over six years (2016-2022) and submitted to microbial isolation and genetic comparison procedures; genomically distinct enterococci were further characterized regarding antibiotic susceptibility.

## Methods


Cheeses from 2016-2022



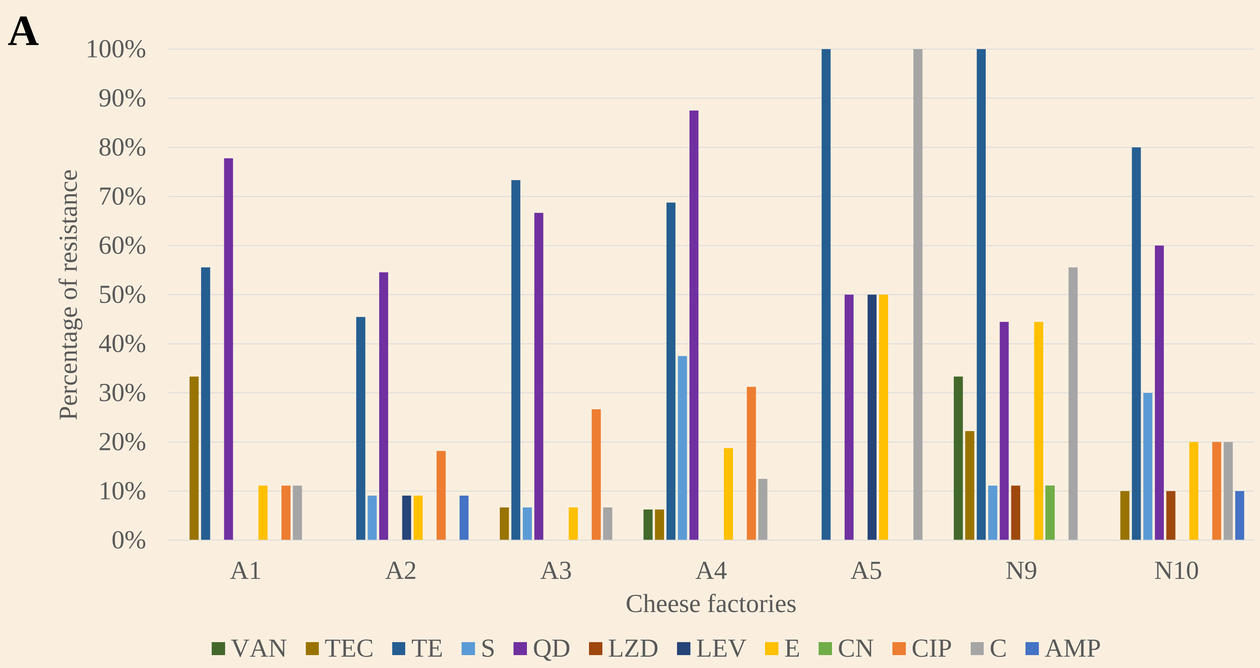

**RAPD-PCR**



**Antibiotic susceptibility**
Disk diffusion

## Results

A total of 73 enterococci were selected, using RAPD-PCR, as representative of each cheesemaking factory/production year. Results obtained are displayed in Figure 1A/B and show that within the same region isolates from distinct cheesemaking factories harbor similar resistance profiles, except for A5-enterococci which showed higher resistance levels for erythromycin, quinupristin-dalfopristin, ampicillin and chloramphenicol. Concerning resistance levels over time, teicoplanin was the antibiotic with higher frequency of resistance, except in 2022, in which quinupristin-dalfopristin showed levels above 90%.



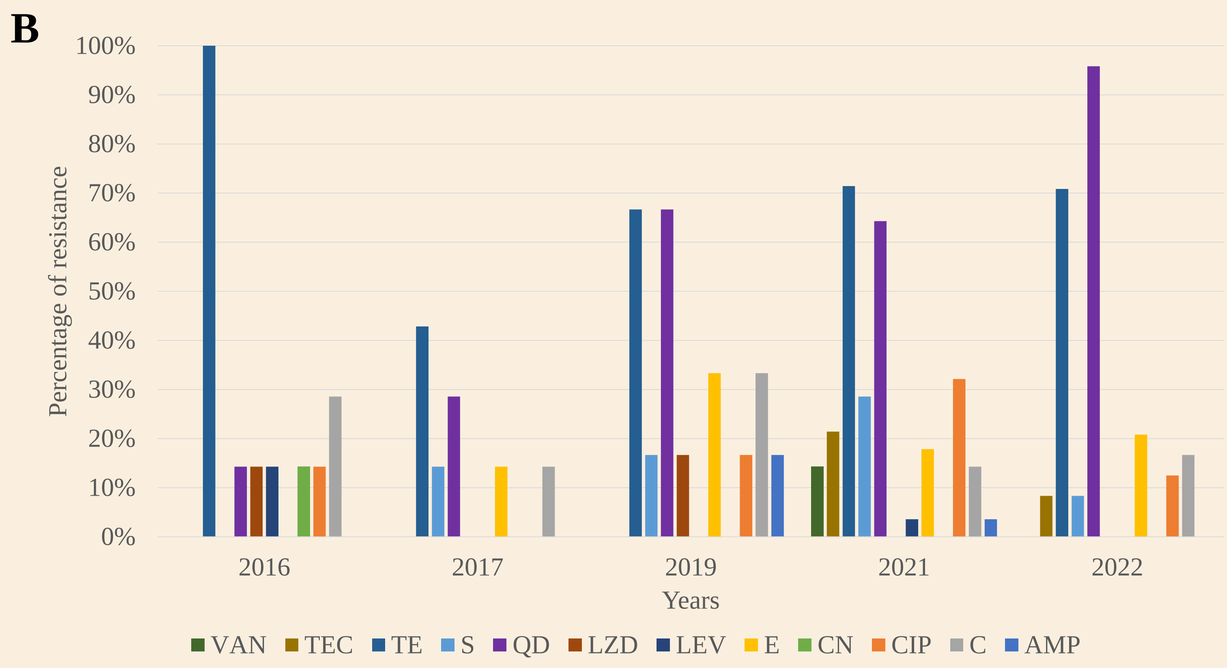




Figure 1 – In **A -** Resistance profiles per cheesemaking factory and **B** – resistance profiles per year. A1-A5 cheesemaking factories from Azeitão; N9-N10 cheesemaking factories from Nisa; VAN- Vancomycin (30 µg); TEC – Teicoplanin (30 µg); TE – Tetracycline (30 µg); S – Streptomycin (300 µg); QD - Quinupristin-dalfopristin (15 µg); LZD – Linezolid (30 µg); LEV - Levofloxacin (5 µg) E – Erythromycin (15 µg); CN – Gentamicin (120 µg); CIP – Ciprofloxacin (5 µg); C - Chloramphenicol (30 µg); AMP – Ampicillin (10 µg). * The resistance profiles were classified according to Clinical and Laboratory Standards Institute (CLSI).

## Conclusion

Overall, our results revealed that the resistance level of the isolates recovered from PDO-cheeses was maintained over six years. However, similar antibiotic surveillance must be continued to keep in check the changes in resistance profiles over-time.

## Acknowledgements

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