

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

Susana Serrano^{1,2†}, Maria V. Ferreira^{3†}, Cinthia Alves Barroco^{1,2}, Maria Teresa Barreto Crespo^{4,5} and Teresa Semedo-Lemsaddek^{1,2}

¹CIISA – Center for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.

²Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS).

³Faculty of Sciences (FCUL), University of Lisbon, Portugal.

⁴IBET, Institute of Experimental Biology and Technology, PO box 12, 2781-901 Oeiras, Portugal.

⁵ITQB, Institute of Chemical and Biological Technology António Xavier, Nova University of Lisbon, Republic Avenue, 2780-157 Oeiras, Portugal.

† These authors contributed equally to this work.

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 the disease. Hence, evaluating antimicrobial resistance of food-related bacteria is crucial to assess associated risks. In the present study, PDO-cheese samples from Portugal were collected over six years (2016-2022) and submitted to microbial isolation procedures; genomically distinct enterococci were further characterized regarding antibiotic susceptibility.

Overall, a total of 73 enterococci (selected based on RAPD-patterns from a larger microbial collection) were screened by susceptibility testing, using various antibiotics representing distinct drug classes and bacterial targets.

Resistance levels among the cheese enterococci were as follows: quinupristin/dalfopristin (92%), streptomycin (74%), and tetracycline (71%), while lower levels of resistance (<10%) were observed for ampicillin, erythromycin, chloramphenicol, ciprofloxacin, norfloxacin, and vancomycin. All the isolates were sensitive to gentamicin and linezolid, but approximately 16% showed a multidrug-resistant (MDR) phenotype. No statistically significant differences were observed in antibiotic resistance patterns among enterococci recovered from cheeses produced in different years ($p < 0.05$).

In conclusion, our results revealed that the resistance level of the isolates recovered from PDO-cheeses was maintained over six years. However, the presence of MDR-enterococci constitutes a matter of concern and further emphasizes the need for active surveillance procedures.

Keywords: Traditional cheese, *Enterococcus*; antibiotic resistance; multidrug-resistant (MDR).

Acknowledgments

This project is supported by National Funds through FCT – Foundation for Science and Technology under the projects UIDP/00006/2020, LA/P/0059/2020 - AL4AnimalS and PTDC/OCE-ETA/1785/2020. Teresa Semedo-Lemsaddek is financially supported by national funds through FCT under the Transitional Standard — DL57/2016/CP1438/CT0004. Susana Serrano holds a fellowship supported by national funds through FCT - UI/BD/153073/2022.