

[SBE 8]

Prevalence of *Staphylococcus aureus* and staphylococcal enterotoxins in raw milk from Northern Portugal

<u>Ricardo Oliveira</u>^{1,2}, Eva Pinho¹, Gonçalo Almeida^{1,3}, Nuno Filipe Azevedo², Carina Almeida^{1,2,4,*}

¹National Institute for Agrarian and Veterinarian Research (INIAV, I.P.), Vairão, Vila do Conde, Portugal

²LEPABE, Laboratory for Process Engineering, Environment, Biotechnology and Energy, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto,

Portugal ³Center for Study in Animal Science (CECA), ICETA, University of Oporto, 55142 Oporto,

Portugal

⁴Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

*e-mail: carina.almeida@iniav.pt

Staphylococcus aureus and its enterotoxins (SEs) are a serious and costly concern for milk industry and public health [1]. This work aimed to characterize the prevalence of S. aureus and enterotoxins in raw milk collected in the main dairy basin region of Portugal mainland. The presence of S. aureus was confirmed in 53% of refrigerated raw milk samples collected from the bulk tank of 100 dairy farms according to the ISO standards. Nonetheless, S. aureus was always below 10⁶ CFU/mL, the minimal concentration expected for enterotoxin production [2]. The presence of enterotoxins- (sea, seb, sec, sed, see, seq, seh, sei, sej, sep, ser) and methicillin resistance-encoding genes (mecA and mecC) was evaluated by PCR. Five isolates were found to be methicillin-resistant Staphylococcus aureus (MRSA) and 29 isolates contained enterotoxinencoding genes. One isolate was positive for sea, 3 isolates were positive for seh, 4 isolates were positive for sec, 25 isolates were positive for sei and 26 isolates were positive for seg. Consistent with other reports, seg and sei coexisted in most isolates, seg was only detected independently of the other gene in one isolate [3, 4]. Sec was only found in conjunction with seg and sei. Seh was detected alone or together with sea. Overall, the occurrence of non-typical enterotoxin genes (seg, seh and sei) was higher than the "top five" genes (sea-see). The detection of SEs (SEA-SEE), according to the EU-RL standard, revealed one positive sample. Interestingly, S. aureus was not detected on the positive sample, demonstrating that SEs can be present without requiring the presence of the bacteria [5]. These results suggest that raw milk can be an important source of MRSA, enterotoxigenic S. aureus and enterotoxins. Surveillance and postharvest handling practices might be crucial to prevent the spread along the food chain.

Acknowledgments: This work was financially supported by: Base Funding - UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE - funded by national funds through the FCT/MCTES (PIDDAC); Project POCI-01-0145-FEDER-028659, funded by FEDER funds through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES. The authors also thank FCT for the PhD Fellowship SFRH/BD/138883/2018.

References:

[1] J. Kadariya, T.C. Smith, and D. Thapaliya: "Staphylococcus aureus and Staphylococcal Food-Borne Disease: An Ongoing Challenge in Public Health," /pmc/articles/PMC3988705/, (2014).

[2] H. Fujikawa and S. Morozumi: "Modeling Staphylococcus aureus growth and enterotoxin production in milk." *Food Microbiol.* vol. 23, no. 3, pp. 260–267, 2006.

[3] G. Blaiotta, V. Fusco, C. Von Eiff, F. Villani, and K. Becker: "Biotyping of enterotoxigenic Staphylococcus aureus by enterotoxin gene cluster (egc) polymorphism and spa typing analyses." *Appl. Environ. Microbiol.* vol. 72, no. 9, pp. 6117–6123, 2006.

[4] S. Johler, G. Macori, A. Bellio, P.L. Acutis, S. Gallina, and L. Decastelli: "Short communication: Characterization of Staphylococcus aureus isolated along the raw milk cheese production process in artisan dairies in Italy." *J. Dairy Sci.* vol. 101, no. 4, pp. 2915–2920, 2018.

[5] M.Á. Argudín, M.C. Mendoza, and M.R. Rodicio: "Food Poisoning and Staphylococcus aureus Enterotoxins," http://www.mdpi.com/2072-6651/2/7/1751, (2010).