OPEN ACCESS **Scientific Letters**

IV 1H-TOXRUN INTERNATIONAL CONGRESS 2025 | 08-09 MAY, 2025 Porto, Portugal

Poster 10

Diagnostic pitfalls in *Staphylococcus* spp. surveillance: lessons from a multinational university student cohort

Bárbara Pinheiro [1,2,*], Luís M. Gomes-Sampaio [1], Patrícia Pacheco [1], Carla Campos [3,4], Maria Pranto Braz [5], Cristina Coelho [5], Carla Miranda [1,6], Sandra Quinteira [1,7,8,9] and Ana R. Freitas [1,2,10]

[1] UCIBIO - Applied Molecular Biosciences Unit, Translational Toxicology Research Laboratory, University Institute of Health Sciences (1H-TOXRUN, IUCS-CESPU), 4585-116 Gandra, Portugal
[2] Associate Laboratory i4HB - Institute for Health and Bioeconomy, University Institute of Health Sciences - CESPU, 4585-116 Gandra, Portugal
[3] Instituto Português de Oncologia do Porto Francisco Gentil, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
[4] Escola Superior de Saúde, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
[5] UNIPRO, Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal
[6] LAQV-REQUIMTE—Associated Laboratory for Green Chemistry of the Network of Chemistry and Technology, University NOVA of Lisbon, Campus da Caparica, 1099-085 Caparica, Portugal
[7] CIBIO—Research Center in Biodiversity and Genetic Resources, InBIO, Research Network in Biodiversity and Evolutionary Biology, Associated Laboratory, University of Porto, Campus de Vairão, Rua Padre Armando Quintas 7,4485-661 Vairão, Portugal
[8] BIOPOLIS Program in Genomics, Biodiversity and Land Planning, Campus de Vairão, Rua Padre Armando Quintas 7, 4485-661 Vairão, Portugal
[9] Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
[10] UCIBIO, Unidade de Ciências Biomoleculares Aplicadas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

* Correspondence: barbara.spinheiro@gmail.com

Abstract

Background: *Staphylococcus aureus* is a leading cause of severe and hard-to-treat human infections, particularly when resistant to cefoxitin due to the presence of the *mec*A gene [1]. However, diagnostic challenges arise from the misidentification of *S. aureus* and related species when relying on classical identification methods (mannitol fermentation; coagulase production), as well as from the detection of strains carrying the *mec*A gene but phenotypically susceptible to cefoxitin - known as "stealth" strains [2]. **Objective:** Building upon a previous collection of *S. aureus* from healthy students' nares [3], we aimed to expand this collection with new samples, assess the occurrence of "stealth" isolates, and further investigate cases of incongruent identification. **Methods:** Nasal swab samples (*n*=557) from 507 students (median-23-years; 9 countries) attending a large university (Porto district) were collected between March 2022 and November 2024. They were inoculated onto mannitol-salt agar and, in parallel, enriched in brain-heart-broth with 6.5% NaCl further plated onto ChromID® MRSA-SMART. Isolates deriving from mannitol-salt (only fermenting colonies) and chromogenic (all typical colonies) agar media were stored for species identification (MALDI-TOF MS), cefoxitin-susceptibility (disk-diffusion), and *mecA* gene screening (PCR). **Results:** *Staphylococcus aureus* was identified in 46% (256/557; 6 countries) of cases. Other *Staphylococcus* species included *S. haemolyticus* (*n*=5), *S. capitis* (*n*=3), *S. warneri* (*n*=3), *S. saprophyticus* (*n*=1), *S. simulans* (*n*=1), and *S. ureilyticus* (*n*=1). These isolates expressed variable coagulase production (7 positive, 7 negative). On another hand, a non-fermenting *S. aureus* was detected (cromogenic medium). Ten (1.8%) students were colonized with methicillin-resistant staphylococci species carrying *mec*A including *S. aureus* (*n*=6), *S. haemolyticus* (*n*=3), *S. ureilyticus* (*n*=1). The *mec*A gene was also detected in 4/16 (25%; 3 Portuguese, 1 Italian) *S. aureus* susceptible to cefoxitin, the so-called "stealth" strains. Screening is ongoing in more isolates. **Conclusions:** Our study highlights the importance of integrating both phenotypic and genotypic methods for *Staphylococcus* accurate identification. Furthermore, the detection of stealth strains in healthy students underscores the need for robust community-based screening, as *S. aureus* carriage may be underestimated. Future studies will unveil whether these strains are capable of reversion to resistência.

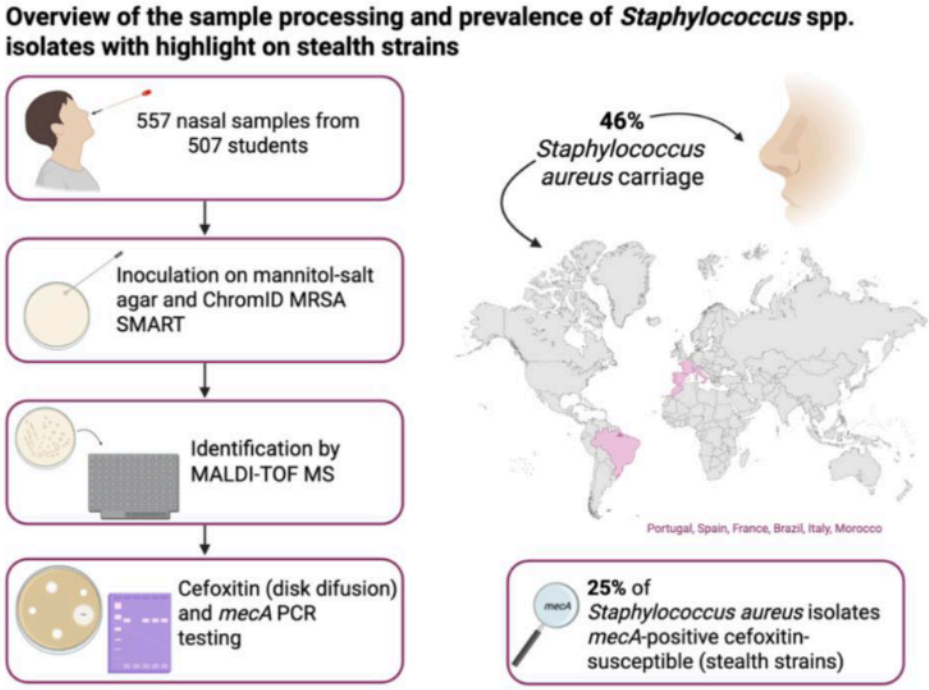

Figure 1. Overview of the sample processing and prevalence of *Staphylococcus* spp. isolates with a highlight on stealth strains.

Keywords: *Staphylococcus* spp.; *mec*A; university students

Acknowledgments/Funding

We greatly acknowledge all IUCS students volunteering to participate in this study approved by the Ethical Commission of CESPU (23/CE-IUCS/2022). This research was funded by CESPU, under the NARES_GI2-CESPU-2023 project and through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU).

References

1. GBD 2019 Antimicrobial Resistance Collaborators. Global Mortality Associated with 33 Bacterial Pathogens in 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. *Lancet* **2022**, *400(10369)*, 2221–2248, doi: 10.1016/S0140-6736(22)02185-7.
2. Liang, B. et al. Genomic Basis of Occurrence of Cryptic Resistance among Oxacillin- and Cefoxitin-Susceptible *mecA*-Positive *Staphylococcus aureus*. *Microbiol Spectr* **2022**, *10(3)*, e0029122, doi: 10.1128/spectrum.00291-22.
3. Sampaio, L.M. et al. Nasal colonization by *Staphylococcus aureus* in Health Sciences students and analysis of risk factors under a One Health perspective. *Scientific Letters* **2023**, *1(Sup 1)*, doi: 10.48797/sl.2023.79.

In *Scientific Letters*, works are published under a CC-BY license (Creative Commons Attribution 4.0 International License at https://creativecommons.org/licenses/by/4.0/), the most open license available. The users can share (copy and redistribute the material in any medium or format) and adapt (remix, transform, and build upon the material for any purpose, even commercially), as long as they give appropriate credit, provide a link to the license, and indicate if changes were made (read the full text of the license terms and conditions of use at https://creativecommons.org/licenses/by/4.0/legalcode).