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Poster 10

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

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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.

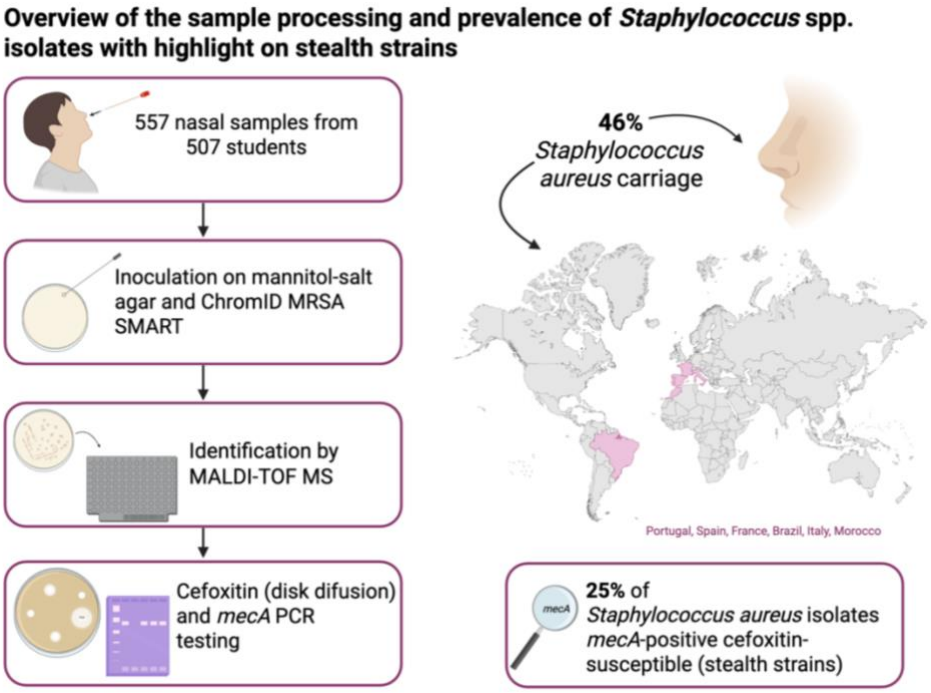

Figure 1. Overview of the sample processing and prevalence of *Staphylococcus* spp. isolates with a highlight on stealth strains.

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

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