

<https://doi.org/10.15388/SMVK.2026>

Vilniaus universitetas
Medicinos fakultetas



STUDENTŲ MOKSLINĖS VEIKLOS TINKLO LXXVIII KONFERENCIJA



Vilnius, 2026 m. gegužės 8 d.

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Leidinį sudarė VU MF
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2026

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ISSN 2783-7831 (skaitmeninis PDF)

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RELIABILITY OF B-LACTAMASE DETECTION METHODS AND THEIR ROLE IN ASSESSING PENICILLIN SUSCEPTIBILITY IN METHICILLIN-SUSCEPTIBLE *STAPHYLOCOCCUS AUREUS* ISOLATES

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Background. Recent studies report an increasing prevalence of penicillin-susceptible *Staphylococcus aureus*, ranging from 9% to 30% globally, depending on the region. Penicillin was originally the drug of choice for *S. aureus* infections due to its high activity and low toxicity. β -lactamase production is a major mechanism of penicillin resistance; therefore, its detection is important for accurate assessment of penicillin susceptibility in MSSA isolates.

Aim. To evaluate the reliability of β -lactamase detection methods and their role in assessing penicillin susceptibility in MSSA isolates.

Materials and methods. *S. aureus* strains from adult patients (≥ 18 years) were collected between January and December 2025 at Vilnius City Clinical Hospital. Penicillin susceptibility was determined by disc diffusion using 1 U (EUCAST) and 10 U (CLSI) benzylpenicillin discs. β -lactamase production was assessed using cefinase (*Liofilchem*[®], Italy) and cloverleaf tests. In the cefinase assay, β -lactamase activity was indicated by color change, while in the cloverleaf test, growth indentation at the inhibition zone edge indicated β -lactamase production.

Results. A total of 122 MSSA strains were examined. Most MSSA isolates were isolated from skin and soft tissue samples (70.5%), followed by blood (16.4%), urine (5.7%) and other specimens (7.4%). Of the 122 MSSA isolates, 70 (57.4%) were penicillin-resistant. β -lactamase production was detected in 68 (55.7%) isolates by the cefinase test and in 71 (58.2%) by the cloverleaf test. Results of the two methods were concordant in 119/122 isolates (97.5%), with three discrepancies observed. Comparison with disc diffusion showed complete agreement for the cloverleaf test (58.2% resistant, 41.8% susceptible; $p < 0.0001$), while the cefinase test showed three discordant results (55.7% resistant).

Conclusions. A considerable proportion of MSSA isolates were susceptible to penicillin. β -lactamase detection showed high agreement with penicillin susceptibility testing, highlighting the importance of detecting β -lactamase production for accurate assessment of penicillin susceptibility in MSSA. Three discordant results were observed between β -lactamase tests, and comparison with disc diffusion indicated that the cloverleaf test more accurately reflected penicillin susceptibility than the cefinase test.

Keywords. MSSA; β -lactamase; penicillin susceptibility.