CONCISE COMMUNICATION

Mecillinam susceptibility as an indicator of β-lactamase production in *Staphylococcus aureus*

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The use of direct susceptibility testing from specimens has led to the fortuitous observation that penicillin-susceptible strains have larger inhibition zones for mecillinam than do β-lactamase producers. The current study was, therefore, undertaken to test 179 *Staphylococcus aureus* isolates for mecillinam susceptibility by Rosco Neo-Sensitabs and to compare the results with commonly used tests for β-lactamase production, i.e. size and character of penicillin inhibition zones, chromogenic cephalosporin (nitrocefin) tests and clover leaf assays. Agreement between methods was reached for 175 of 179 strains when disregarding the results of the nitrocefin tests, 88 isolates being found susceptible and 87 being found to be β-lactamase producers. All 88 susceptible isolates had mecillinam zones of >22 mm, with the great majority being >25 mm; double zones did, however, occur. The 87 β-lactamase producers had zones <14 mm or no zones. Four isolates presenting problems in had mecillinam zones of ≤20 mm and were without tapering growth at the penicillin inhibition zone edge. In conclusion, the size of the mecillinam inhibition zone is found to be a useful supplementary test in the clinically important distinction between β-lactamase-producing and non-producing isolates of *S. aureus*.

Keywords Mecillinam susceptibility, *S. aureus*, β-lactamase

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The present study was undertaken following personal observations on the part of both investigators, of β-lactamase-producing *S. aureus* strains sometimes being misread as penicillin-susceptible. As reporting of penicillin susceptibility leads to treatment of *S. aureus* infections with penicillin, due to this agent's greater activity against penicillin-sensitive staphylococci compared to β-lactamase-stable penicillins, faulty reporting of penicillin susceptibility can have dire consequences. It is, therefore, of great importance to be able to make correct distinctions between β-lactamase-producing and non-producing strains in the clinical microbiology laboratory.

The routine use of direct susceptibility testing of specimens in Danish laboratories has led to the fortuitous observation that penicillin-susceptible *S. aureus* isolates have larger inhibition zones for mecillinam than do β-lactamase-producing isolates. The aim of the present study was, to test a collection of *S. aureus* strains for mecillinam susceptibility and to compare the results with those of other commonly used tests for detection of β-lactamase production.

One hundred and forty-four clinical *S. aureus* isolates consecutively collected from 144 patients were studied. Presumptively, penicillin-susceptible and -resistant isolates were collected independently of each other in order to obtain a sufficient number of penicillin-susceptible strains. Also included was a recent clinical isolate that presented problems in interpretation. Results were contradictory in various tests for β-lactamase production (no. 37) that had prompted the study, 19 strains from the Czechoslovak Collection of Microorganisms (including *S. aureus* ATCC 12600, ATCC 3538P, ATCC 13566, ATCC 19095, NCTC 4163, NCTC 4136), *S. aureus* ATCC 29213 (the

quality control strain for the Cefinase™ and the Cefinase Plus™ tests), and 14 clinical strains from the Staphylococcal Reference Laboratory, Statens Serum Institut, Copenhagen, Denmark. Strains were stored at −80 °C until ready for use. All 179 strains were examined in a blinded fashion without knowledge of results from previously performed tests.

Susceptibility testing was performed according to the agar diffusion method using NeoSensitabs (Rosco, Taastrup, Denmark) with Penicillin Low (V-penicillin, 5 μg) and mecillinam (33 μg) on Danish blood agar ([1], Statens Serum Institut) with semiconfluent growth [2]. This method has been found to give fair to good agreement with MIC values for mecillinam (agar dilution method on Mueller–Hinton), for Gram-positive and Gram-negative bacteria [3] and to minimum inhibitory concentration (MIC) values for mecillinam (E test, AB Biodisk, Solna, Sweden) for *Escherichia coli* [4]. These tablets have a diameter of 9 mm. Incubation was at 35 °C overnight. Inhibition zones were measured in millimeters. A note was made as to whether bacterial growth tapered out ('ghost zones' [5]) or was clear-cut at the inhibition zone edge. A zone of 28 mm is the breakpoint between penicillin-susceptible and β-lactamase-producing strains [2]. No recommendations exist for mecillinam in this regard.

Paper disks containing the chromogenic cephalosporins nitrocefin (Cefinase™, Becton Dickinson, Glostrup, Denmark) and cefisone (Cefinase Plus™, Becton Dickinson) [6] were used according to the manufacturer's instructions, to test for β-lactamase production. Disks were examined for the appearance of a pink–red colored dot for up to 60 min with *S. aureus* grown on a plate.

The clover leaf assay used was an in-house modification of the test described by Østravik and Ødegaard [7]: *S. aureus* ATCC 25923 was used as the indicator strain. Care was taken to produce a dense but not confluent growth on 5% horse blood agar plates ([1], Statens Serum Institut) with the indicator strain. The test strain was then streaked as a cross on the blood agar plate, starting each of the four arms of the cross at the periphery. A Penicillin Low tablet was placed in the center of the cross and the plates were incubated overnight at 35 °C. The test was read as positive for β-lactamase production if *S. aureus* ATCC 25923 grew along the streaks of the test strain toward the penicillin tablet, resulting in a clover leaf configuration. The test was read as negative if the inhibition zone was circular.

No single method was regarded as the definitive reference method for detection of β-lactamase in this study. Each strain was tested twice in agar diffusion tests for penicillin and mecillinam, as reading of inhibition zones for these agents is known to be subject to some uncertainty. Discrepancies between the results of different methods resulted in retesting of the involved strains at least once, in order to ensure against mistakes such as interchange of strains, errors of transcription, etc. Determinations of MICs for G-penicillin with the E test were carried out according to the manufacturer's recommendations [8] in an attempt to resolve the discrepancies.

Lack of agreement was found between the results of the nitrocefin and cefisone β-lactamase tests for 12 strains, which were all nitrocefin negative and cefisone positive. As the results of the agar diffusion test and the clover leaf assay clearly indicated β-lactamase production for these 12 strains, only the results of the cefisone test were subsequently taken into account. The nitrocefin test's lack of sensitivity for β-lactamases of *S. aureus* is in agreement with the finding of others [6,9].

Considering the following parameters: inhibition zones for penicillin with clear-cut or tapering edges of growth ('ghost zones'), results of cefisone tests and clover leaf assays, complete agreement among methods was reached for 175 of 179 isolates regarding β-lactamase production. Thus, four isolates were found to be problematic, and are discussed below. Using these 175 isolates, comprising 88 penicillin-susceptible and 87 β-lactamase-producing isolates, as the basis, the use of mecillinam inhibition zones as a supporting test for penicillin susceptibility could be evaluated. A total of 88 penicillin-susceptible isolates, including 10 with penicillin zones of 28–30 mm (on or very close to the breakpoint of 28 mm), were found to have mecillinam zones of >22 mm, with the great majority showing zones of >25 mm. Three penicillin-susceptible isolates, however, produced double zones for mecillinam and retesting confirmed these results for two of the isolates. All 87 of the β-lactamase-producing isolates lacked zones for mecillinam, except for three isolates with small zones of 10–13 mm.

The results for the four isolates that presented problems in interpretation which were all clinical, are shown in Table 1. Three of the four isolates

Table 1 Results of tests used for detection of β-lactamase in four problem isolates

Isolate no.	Penicillin V zone (mm)	Tapering growth	Cefisone	Clover leaf	Mecillinam zone (mm)	Penicillin G MIC (mg/L)
37	26–28	–	–	+	19–20	0.125
105	25–27	–	–	(+)	17–19	0.125
136	24–26	–	–	–	17–20	0.064
146	21–23	–	–	+	0–13	0.125

– Negative results, + positive results, (+) weakly positive results.

(nos. 37, 105 and 146) were judged to be β-lactamase producing on the basis of an overall evaluation, with mecillinam zones of ≤20 mm, lack of tapering growth and MICs for penicillin of >0.1 mg/L [2]; cefisone tests were, however, negative upon repeated testings. The fourth isolate (no. 136) was not assessable due to contradictory results: size of penicillin zone and lack of 'ghost zone' indicating β-lactamase production, but the remaining tests being indicative of penicillin sensitivity.

As 10 of 88 penicillin-susceptible isolates had penicillin zones close to the breakpoint of 28 mm as stated above, a mecillinam zone of >22 mm for the β-lactamase non-producers actually discriminates better than the size of penicillin zones. Thus, the presence of mecillinam zones of >22 mm is found to be a useful supplementary test for the detection of penicillin-susceptible *S. aureus* when reading directly performed susceptibility tests using NeoSensitabs on Danish blood agar. Mecillinam zone diameter interpretive standards for this use can undoubtedly also be worked out for other susceptibility testing systems. In a few cases, equivocal readings will, however, necessitate secondary susceptibility tests as described above.

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