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RESEARCH NOTE

Comparison of phenotypic methods for penicillinase detection in *Staphylococcus aureus*

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ABSTRACT

Penicillinase testing is required for *Staphylococcus aureus* isolates with a penicillin MIC of ≤0.12 mg/L. This study compared five phenotypic assays with a PCR for *blaZ* when testing 197 *S. aureus* isolates. The starch–iodine plate method and nitrocefin tests had low sensitivities of 42.9% and 35.7%, respectively. The cloverleaf assay and the penicillin zone-edge determination method had sensitivities of 67.8% and 71.4%, respectively, and these methods might be appropriate in many circumstances, but were not as sensitive as *blaZ* PCR.

Keywords *blaZ*, PCR, penicillinase, phenotypic assays, sensitivity testing, *Staphylococcus aureus*

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Resistance to penicillin in *Staphylococcus aureus* is mediated by production of a penicillinase, encoded by *blaZ*. Four types of penicillinase (termed A–D) have been described in *S. aureus*, all of which are Ambler class A β-lactamases [1]. Reliable detection of penicillinase production is important because penicillin is considered to be superior to oxacillin against isolates that do not produce a penicillinase. An erroneus report of penicillin susceptibility could result in potentially inadequate therapy of *S. aureus* infections. A penicillin MIC of ≤0.12 mg/L is formally in the sensitive range, but the CLSI recommends that additional testing should be performed [2]. The aim of the present study was to compare five phenotypic methods for penicillinase detection, i.e., penicillin disk-diffusion, penicillin zone-edge determination, a cloverleaf assay, nitrocefin tests and a starch–iodine plate method, against a PCR assay for *blaZ* when testing the sub-population of isolates with a penicillin MIC in the formally sensitive range according to the Vitek 2 system (bioMérieux, Durham, NC, USA).

Consecutive non-duplicate isolates of *S. aureus* (n = 197) were collected if their penicillin MIC according to Vitek 2 was ≤0.12 mg/L. Disk-diffusion tests for penicillin were performed according to CLSI recommendations [2] (10-U disk; Oxoid, Basingstoke, UK). The penicillin zone-edge was checked for a sharp edge with discrete full-sized colonies right at the edge, or for a tapered edge with a gradual decrease in growth [3]. A sharp edge was considered to be indicative of penicillinase production. Zone inhibition diameters were measured, with a diameter of ≤28 mm for penicillin being considered to be penicillinase-positive [2]. The cloverleaf assay was performed as described previously [4], except that *S. aureus* ATCC 25923 was used as the indicator strain on Mueller–Hinton agar (Merck, Darmstadt, Germany). Testing with nitrocefin sticks (Oxoid) was performed according to the manufacturer's instructions, with colonies located at the penicillin zone-edge, and was read after 5, 15 and 60 min. The starch–iodine plate method was performed as described previously [4], except that oxacillin (Sigma, Taufkirchen, Germany) 0.2 mg/L was used for penicillinase induction instead of methicillin. Decolourisation under and around the colonies was considered to be a positive result.

Primers for amplification of *blaZ*, designed according to *blaZ* sequences published previously, were stau-blaZ-fwd (5′-CAAAGATGA TATAGTTGCTTATTCTCC) and stau-blaZ-rev

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(5′-TGCTTGACCACTTTTATCAGC). PCRs were performed in 50-μL volumes containing 200 μM each dNTP, 1 μM each primer, 10 μL template DNA, 1.25 U *Taq* polymerase (GE Healthcare, Munchen, Germany) and the reaction buffer supplied by the manufacturer. Cycling conditions comprised 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 7 min. The PCR was evaluated with known *S. aureus* strains producing penicillinases A–D [1], kindly provided by D. Kernodle (Vanderbilt University, Nashville, TN, USA). The *S. aureus*-specific PCR for SA442 [5] was also performed for all isolates as a control.

Of 197 *S. aureus* isolates with a penicillin MIC of ≤0.12 mg/L according to Vitek 2, 28 (14.2%) were positive for *blaZ* according to the PCR assay. All isolates with a penicillin MIC of 0.03 mg/L according to Vitek 2 were *blaZ*-negative. Five (6.2%) of 81 isolates with a penicillin MIC of 0.06 mg/L, and 23 (23.2%) of 99 isolates with a penicillin MIC of 0.12 mg/L were *blaZ*-positive. Of all 28 *blaZ*-positive isolates, 19 (67.8%) were detected in the cloverleaf assay, 20 (71.4%) were detected by zone-edge determination, 12 (42.9%) were detected by the starch–iodine plate method, and seven (25%), ten (35.7%) and 11 (39.3%) were detected by the nitrocefin assay after incubation for 5, 15 and 60 min, respectively. The use of a breakpoint of ≤28 mm in penicillin disk-diffusion tests, recommended by the CLSI for categorising an isolate as penicillin-resistant [2], resulted in a sensitivity of 57.1%. Overlapping zone inhibition diameters for *blaZ*-positive and -negative isolates were found for penicillin (Fig. 1). None of the isolates that had a positive phenotypic test for penicillinase production was negative according to the *blaZ* PCR assay.

In contrast to previous reports, an unacceptably low sensitivity of 39.3% was found for nitrocefin tests, even after induction by penicillin and incubation for 60 min. Sensitivities reported previously for nitrocefin tests were 82% [6], 86.2% [7], 95.6% [8], 70.8–97.9%, depending on the induction method and agar used [4], and 62.1–100%, depending on the manufacturer of the nitrocefin assay [9]. In addition, a sensitivity of 100% has been reported previously for the starch–iodine plate method following penicillinase induction [4]. In the present study, the most sensitive phenotypic tests were penicillin zone-edge determination (71.4%) and the cloverleaf assay (67.8%). Sensitivities of 93.8% [8], 97.9% [4] and 96.6% [9] for the cloverleaf assay have been reported previously. Overlapping inhibition zone diameters for *blaZ*-positive and -negative isolates were found when performing disk-diffusion with penicillin disks, also as reported previously. Isolates with a zone diameter of ≤28 mm, as recommended by CLSI [2], were all *blaZ*-positive in the present study, but a larger diameter did not exclude the presence of *blaZ*.

The reduced sensitivities of phenotypic tests in the present study might have been caused by the

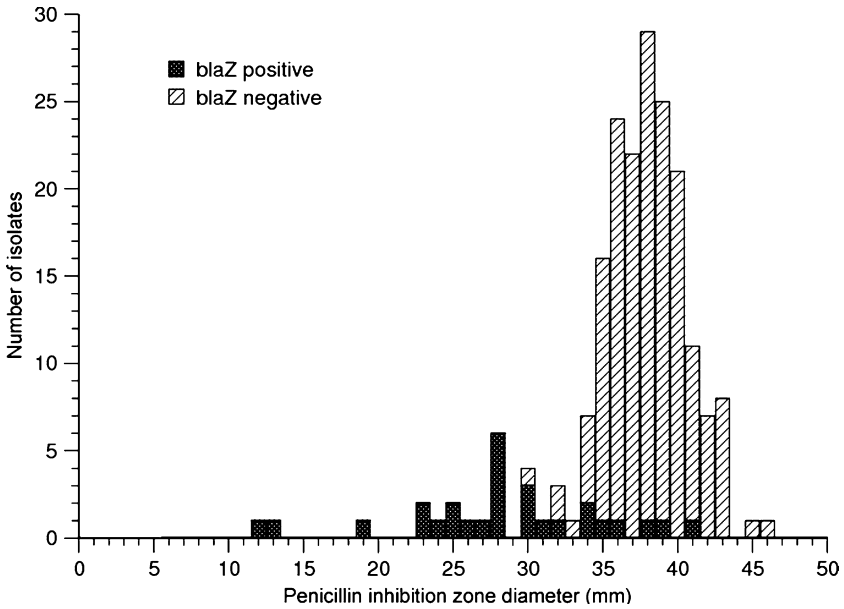

Fig. 1. Inhibition zone diameters obtained with a 10-U penicillin disk in disk-diffusion tests performed according to CLSI recommendations [2].

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use of a highly selected collection of isolates, all of which had a low penicillin MIC according to Vitek 2. In contrast to previous studies, a molecular method was used as the reference standard. Pitkälä *et al*. [9] also used PCR detection of *blaZ* as the reference standard, but only with bovine *S. aureus* isolates.

In conclusion, the results of this study discourage the use of nitrocefin tests or the starch–iodine plate method as additional assays for isolates with penicillin MICs of 0.06 or 0.12 mg/L according to the Vitek 2 system. Higher sensitivities were found for penicillin zone-edge determination and the cloverleaf assay, but it might be insufficient to report an isolate from a serious infection as penicillin-sensitive without performing a PCR to detect *blaZ*.

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RESEARCH NOTE

Detection and molecular characterisation of plasmidic AmpC β-lactamases in *Klebsiella pneumoniae* isolates from a tertiary-care hospital in Dublin, Ireland

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ABSTRACT

This study determined the types of AmpC enzymes produced by *Klebsiella pneumoniae* isolates resistant to third-generation cephalosporins and the clonality of these isolates. The presence of AmpC enzymes was identified by cephalosporin–cloxacillin synergy tests. Genes encoding AmpC enzymes were characterised by PCR and sequencing. Pulsed-field gel electrophoresis (PFGE) was used to type the isolates. Fifteen *K. pneumoniae* isolates were positive for bla_{AmpC}, 13 were positive for $bla_{\text{ACC-1}}$ and two were positive for $bla_{\text{DHA-1}}$. Production of the DHA-1 enzyme was inducible. The *ampR* gene was identified upstream of the $bla_{\text{DHA-1}}$ gene. PFGE demonstrated the polyclonal origin of the isolates carrying $bla_{\text{ACC-1}}$.

Keywords ACC-1, AmpC β-lactamase, DHA-1, *Klebsiella pneumoniae*, resistance, typing

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