Evaluation of a 10 μg cefoxitin disc for the detection of methicillin resistance in Staphylococcus aureus by BSAC methodology


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Sir,

The detection of methicillin resistance in Staphylococcus aureus has always presented problems for diagnostic laboratories because test conditions have a marked effect on the expression of resistance. Detection of the mecA gene or PBP2a are considered the reference methods,1 but are impractical for routine use and phenotypic methods have therefore been used.2 A recent publication has shown the value of using cefoxitin as an indicator of resistance.3 The aim of this study was to evaluate a 10 μg cefoxitin disc by the BSAC standardized disc methodology.

Two hundred consecutive clinical isolates (repeat isolates from the same patient were excluded) of S. aureus, were disc tested in each of the five centres. Briefly, the method used was Iso-Sensitest agar (ISA; Oxoid, Basingstoke, UK) poured to a depth of 4 mm (±0.5 mm), an inoculum equivalent to semi-confluent growth, a 10 μg cefoxitin disc and incubation at 35°C (±1°C) for 18–20 h. Obvious zones of inhibition were measured using a ruler or callipers, but zones were examined carefully in good light to detect colonies within the zone of inhibition, in which case the organism was considered to be resistant. Multiplex PCR for the amplification of mecA and nuc was carried out using primers and conditions as previously described.4 Template DNA was prepared by boiling bacterial colonies in water.

Figure 1 shows the zone diameter distribution for the organisms tested, all of which were nuc-positive. Of the isolates studied, 328 were mecA-positive and 224 (68%) of these gave no zone of inhibition. The zones of inhibition for the remaining mecA-positive isolates (104, 32%) ranged from 7–19 mm. Zones of inhibition for the methicillin-susceptible population ranged from 21–34 mm. A susceptible zone diameter breakpoint of ≥22 mm was chosen to avoid interpreting mecA-positive isolates at the tail end of the resistant zone distribution as falsely susceptible. Using this criterion, all mecA-positive isolates were correctly categorized as resistant, but two (0.2%) mecA-negative organisms were incorrectly categorized as resistant. Resistant control strain NCTC 12493 and susceptible control NCTC 6571 gave zones of 6 mm (no zone), and a range of 24–30 mm, respectively.

The BSAC currently recommends using Columbia agar supplemented with 2% salt and incubation at 30°C for testing methicillin against both S. aureus and coagulase-negative staphylococci (CoNS).2 Although a reliable method of testing, this has the disadvantages that the medium and incubation temperatures differ from those used for testing other anti-staphylococcal agents, that strains sensitive to salt appear falsely susceptible and that some hyper-producers of β-lactamase appear falsely resistant and require latex or PCR tests for confirmation of resistance.2 The method described above using cefoxitin as the indicator antibiotic circumvents many of these disadvantages in particular that hyper-producers of β-lactamase appear susceptible. One important feature of the method is that the incubation temperature must not exceed 36°C. Experiments have shown that there is unacceptable merging of the

![Figure 1](https://example.com/f1.png)

**Figure 1.** Zone diameter distribution for 1000 isolates of S. aureus tested on ISA medium with an inoculum equivalent to semi-confluent growth, a 10 μg cefoxitin disc and incubation at 35°C for 18–20 h. A zone diameter breakpoint of ≥22 mm was chosen to interpret susceptibility. Black bars, mecA negative; grey bars, mecA positive.
resistant and susceptible populations when tests are incubated at 37°C (data not shown). Although presently not recommended for CoNS an evaluation of the method for testing these organisms is currently in progress.

References


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Sir,

The increasing prevalence of AmpC β-lactamase-mediated resistance among Escherichia coli and Klebsiella pneumoniae is of clinical concern.1 Both of these organisms can acquire AmpC β-lactamases on plasmids and additionally, E. coli can hyper-produce its chromosomal AmpC β-lactamase, which is normally only produced at very low levels.2 The recognition of AmpC β-lactamase producers can be difficult, although resistance to cefotaxin can help in identifying them. Unfortunately, cefotaxin resistance is not only due to AmpC β-lactamase production, but may also be due to decreased permeability. Several methods, based on the ability of cell-free extracts of organisms to hydrolyse cefotaxin, have been proposed as confirmatory tests for the production of AmpC β-lactamases.3,4 Most are too time-consuming for use in routine diagnostic laboratories and may not detect all AmpC β-lactamases.4 There is a need, therefore, for alternative methods that can be integrated into diagnostic laboratories and ideally do not rely on cefotaxin as the indicator antibiotic. We report our preliminary findings with a combination disc susceptibility method that uses cefpodoxime as an indicator and the AmpC β-lactamase inhibitor benzo[b]thiophene-2-boronic acid (BZBTH2B).1,5

Sixty non-replicate AmpC β-lactamase-producing clinical isolates of E. coli (n = 57) and K. pneumoniae (n = 3), collected from City Hospital, Birmingham (August 2001–March 2004) were tested. All of the isolates hydrolysed cefoxitin, as determined by the method of Nasim et al.5 and their cefoxitin MICs, as determined by the BSAC standardized agar dilution method,6 were reduced ≥4-fold in the presence of a fixed 100 mg/L concentration of clavulanic acid.7 Fourteen of the isolates were positive by PCR for plasmid-borne bla_{ampC} using primers and conditions described by Pérez-Pérez and Hanson.8 Seventy cefotaxin-resistant, AmpC β-lactamase-negative isolates of E. coli (n = 50) and K. pneumoniae (n = 20) were used as negative controls. All isolates were identified by API 20E (bioMérieux, Basingstoke, UK). Six laboratory strains of E. coli producing known AmpC β-lactamas (BIL-1, ACC-1, ACT-1, MIR-1, FOX-4, CMY-2) were also tested.

Combination discs were produced in-house using commercially available susceptibility discs (Oxoid, Basingstoke, UK and Mast, Bootle, UK) to which the inhibitor BZBTH2B was added. Stock solutions of BZBTH2B (VWR International Ltd, Lutterworth, UK) were made in DMSO and further diluted in 0.1 M NaOH and water. BZBTH2B was added to discs containing cefpodoxime 10 µg or cefpodoxime 10 µg + clavulanic acid 1 µg to give 64 µg BZBTH2B per disc. Blank discs containing the inhibitor alone were used to check for possible intrinsic antimicrobial activity. IsoSensitest agar plates were inoculated with the test organisms to give semi-confluent growth using the BSAC standardized disc susceptibility method.6 Cefpodoxime and cefpodoxime + clavulanic acid discs with and without BZBTH2B inhibitor, and discs with the inhibitor alone, were spaced over the agar surface. After overnight incubation in air at 35–37°C, the zones of inhibition were measured. For comparison, the method was repeated using cefotaxin 30 µg discs with and without BZBTH2B (64 µg) or clavulanic acid (100 µg).

A ≥5 mm increase in the zone diameter around the combined disc compared with that for cefotaxin, cefpodoxime or cefpodoxime + clavulanic acid alone, was considered significant. Using this cut-off, none of the combined discs gave a positive result with the cefotaxin-resistant, non-AmpC β-lactamase-producing isolates. BZBTH2B alone gave no zones of inhibition with any of the organisms tested. The combined disc methods cefotaxin + clavulanic acid, cefotaxin + BZBTH2B, cefpodoxime + BZBTH2B and cefpodoxime + clavulanic acid + BZBTH2B correctly identified 57 (86.4%), 59 (89.4%), 64 (97%) and 66 (100%) of the AmpC β-lactamase producers, respectively. Significantly, the cefotaxin-based disc methods failed to detect the ACC-1 β-lactamase-producing strain, which is cefotaxin susceptible. ACC-1 β-lactamase-producing clinical isolates of both E. coli and K. pneumoniae have been described.4 Cefpodoxime + BZBTH2B did not detect two isolates, which concomitantly produced an AmpC β-lactamase and an extended spectrum β-lactamase (ESBL). The activity of the ESBL masked the effects of BZBTH2B. Cefpodoxime + clavulanic acid + BZBTH2B was the only method to detect all the AmpC β-lactamase producers, as the addition of clavulanic acid inhibited the activity of the ESBLs. This test is simple enough to be easily integrated into routine diagnostic laboratories and has the potential to greatly simplify the detection of AmpC β-lactamases in E. coli and K. pneumoniae.

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Disc methods for detecting AmpC β-lactamase-producing clinical isolates of Escherichia coli and Klebsiella pneumoniae

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