Calcium-supplemented daptomycin Etest strips for susceptibility testing on Iso-Sensitest agar

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Objective: Iso-Sensitest agar (ISA), which is recommended by the BSAC for routine susceptibility testing of staphylococci and enterococci, contains insufficient calcium for testing daptomycin. Isotonic agar supplemented with 50 mg/L calcium has been advocated, but is not routinely available in many laboratories. We evaluated a daptomycin Etest that incorporates a constant level of calcium throughout the daptomycin gradient, designed to give an appropriate concentration around the strip during testing, as an alternative for susceptibility testing on ISA.

Methods: Ninety-one isolates of Staphylococcus aureus (45 methicillin-susceptible, 46 methicillin-resistant) and 90 enterococci (47 Enterococcus faecalis, 43 Enterococcus faecium) were tested. Daptomycin Etest MICs were determined on ISA, whereas agar dilution MICs were determined in parallel on Isotonic agar supplemented with calcium to 50 mg/L as a control.

Results: The agar dilution and Etest MIC ranges of daptomycin for S. aureus were 0.25–1 mg/L (mode 0.5 mg/L), and 0.125–2 mg/L (mode 0.25 mg/L), respectively. The corresponding MIC values for enterococci were 0.25–4 mg/L (mode, 1 mg/L) and 0.125–4 mg/L (mode, 2 mg/L). For staphylococci, 86% of the Etest MIC results were within one dilution of the agar dilution values, and for enterococci, 90% of the Etest MIC results met these criteria. When results from the two methods were not identical, there was a tendency for the Etest MIC values to be lower than the agar dilution values.

Conclusions: This study shows that calcium-supplemented daptomycin Etests on ISA are an accurate and convenient alternative to calcium-supplemented Isotonic agar.

Keywords: lipopeptides, enterococci, Staphylococcus aureus, MRSA

Introduction

Daptomycin, a cyclic lipopeptide produced by Streptomyces roseosporus, has potent activity against most Gram-positive bacteria,¹ and has recently received approval by the US Food and Drug Administration for the treatment of complicated skin and skin structure infections.² Should daptomycin subsequently be licensed for clinical use in the UK, microbiology laboratories will need to include it among the antibiotics they test for activity against Gram-positive pathogens. However, testing is problematic, as daptomycin requires the use of medium containing calcium at 50 mg/L for optimal activity, and levels of calcium vary between different media and batches.³⁻⁵

A particular concern is that Iso-Sensitest agar (ISA), which is recommended for routine susceptibility testing by the British Society for Antimicrobial Chemotherapy (BSAC), is unsuitable for testing daptomycin, as the calcium content is insufficient.⁴ Although Isotonic agar supplemented with calcium to 50 mg/L has been evaluated and found suitable for use with daptomycin,³ the requirement to test daptomycin on a different medium from that used with other antibiotics is inconvenient at best. As an alternative, AB Biodisk (Solna, Sweden) has developed a daptomycin Etest strip that incorporates calcium at a constant level throughout the daptomycin gradient, designed to give an appropriate concentration around the strip during testing. The strip was developed by determining the amount of calcium that provided good correlation between the NCCLS reference method (broth microdilution with 50 mg/L calcium) and the strip result. We evaluated this Etest product for determining MICs for Staphylococcus aureus and enterococci on ISA. For comparison,
Evaluation of calcium-supplemented daptomycin Etests

Materials and methods

Bacteria
Ninety-one isolates of *S. aureus* (45 methicillin-susceptible, 46 methicillin-resistant) and 90 enterococci (47 *Enterococcus faecalis*, 43 *Enterococcus faecium*) from geographically diverse UK hospitals were tested.

Susceptibility testing
Daptomycin powder and calcium-supplemented daptomycin Etest strips were provided by Cubist Pharmaceuticals (Lexington, MA, USA). Agar dilution MICs of daptomycin were determined on Isotonic agar supplemented with Ca\(^{2+}\) to 50 mg/L (Mast Laboratories, Bootle, UK). Etest MICs were determined on Iso-Sensitest agar (Oxoid, Basingstoke, UK). Intermediate MIC values were rounded up to the nearest doubling dilution concentration.

Results
As shown in Figure 1, the agar dilution MICs of daptomycin for *S. aureus* were in the range 0.25–1 mg/L (mode 0.5 mg/L), whereas the Etest MICs were in the range 0.125–2 mg/L (mode, 0.25 mg/L). For enterococci, the corresponding values were 0.25–4 mg/L (mode, 1 mg/L) and 0.125–4 mg/L (mode, 2 mg/L).

The concordance between the MICs of daptomycin obtained by agar dilution and Etest for individual isolates is shown in Figure 2. With staphylococci, 86% of the Etest MIC results were either the same as (36%) or within one two-fold dilution (50%) of the agar dilution values, with the remainder of the isolates (14%) showing a four-fold difference in MIC between the two methods. For enterococci, 90% of the Etest MIC results were either identical to the agar dilution values (53.3%) or within one two-fold dilution (36.7%). Among seven enterococcal isolates (8%) showing a greater level of discrepancy, six showed a four-fold difference in MIC between the two methods, whereas one showed an eight-fold difference. For those results that were not concordant, there was a tendency, seen with both staphylococci and enterococci, for the Etest values to be lower than the agar dilution MIC values.

Discussion
Studies by several groups have shown that testing for susceptibility to daptomycin requires the use of medium containing calcium at 50 mg/L for optimal activity.\(^3\)–\(^5\) ISA, which is recommended for routine susceptibility testing by the BSAC, is unsuitable for testing daptomycin due to the low concentration of calcium.\(^4\) Although Isotonic agar in a commercially available formulation supplemented with calcium is suitable, it is inconvenient to have to routinely stock or source two different media for susceptibility testing, when daptomycin is to be tested alongside other agents.

This pilot study indicated that calcium-supplemented daptomycin Etest strips could be used on ISA to test staphylococci and enterococci for susceptibility to daptomycin. For 86% of the *S. aureus* isolates and 90% of the enterococci tested, the MIC values obtained by agar dilution on Isotonic agar supplemented with calcium, and calcium-supplemented Etests on ISA, were within one doubling
dilution of each other. With only one of 181 isolates tested was the difference in MIC greater than four-fold.

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References


