

EUCAST disk diffusion and quality control criteria for *Staphylococcus aureus* with ceftaroline

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Introduction

Ceftaroline, the active metabolite of ceftaroline fosamil, is a novel cephalosporin with activity against many methicillin-resistant *Staphylococcus aureus* (MRSA) due to enhanced affinity for the penicillin-binding protein 2a (PBP2a). Recently, EUCAST established clinical MIC breakpoints for ceftaroline vs. *S. aureus*, S \leq 1 mg/L and R $>$ 1 mg/L.

Objectives

The objectives of this study were to evaluate ceftaroline 5-, 10- and 30- μ g disks and to establish disk diffusion breakpoints and quality control criteria for *S. aureus*.

Methods

Antimicrobial susceptibility testing was performed during 2012 on a challenge set of 100 *S. aureus*, including a large proportion of MRSA. MIC determination was performed at one site with broth microdilution according to the ISO standard 20776-1, 2006. A consensus MIC was calculated from a minimum of three separate tests for each isolate. Disk diffusion was performed on all isolates at two sites with ceftaroline 5-, 10- and 30- μ g disks according to EUCAST methodology. *mecA*-positive isolates (confirmed with PCR) with MICs close to the EUCAST breakpoint (n=17) were tested for reproducibility at both sites. Disk diffusion was also performed on additional isolates of *S. aureus* (n=180), categorised as MSSA (methicillin susceptible) or MRSA (methicillin resistant) according to EUCAST and CLSI MRSA screening. A tentative quality control (QC) range for *S. aureus* ATCC 29213 and ceftaroline 5 μ g was established by a two-site study. Disk diffusion was performed with ceftaroline disks from two manufacturers (Mast Diagnostics and Oxoid/Thermo Fisher Scientific) and Mueller-Hinton (MH) agar from four manufacturers (BD, bioMérieux, Bio-Rad and Oxoid/Thermo Fisher Scientific). Data from five additional European laboratories were used to validate the tentative QC range.

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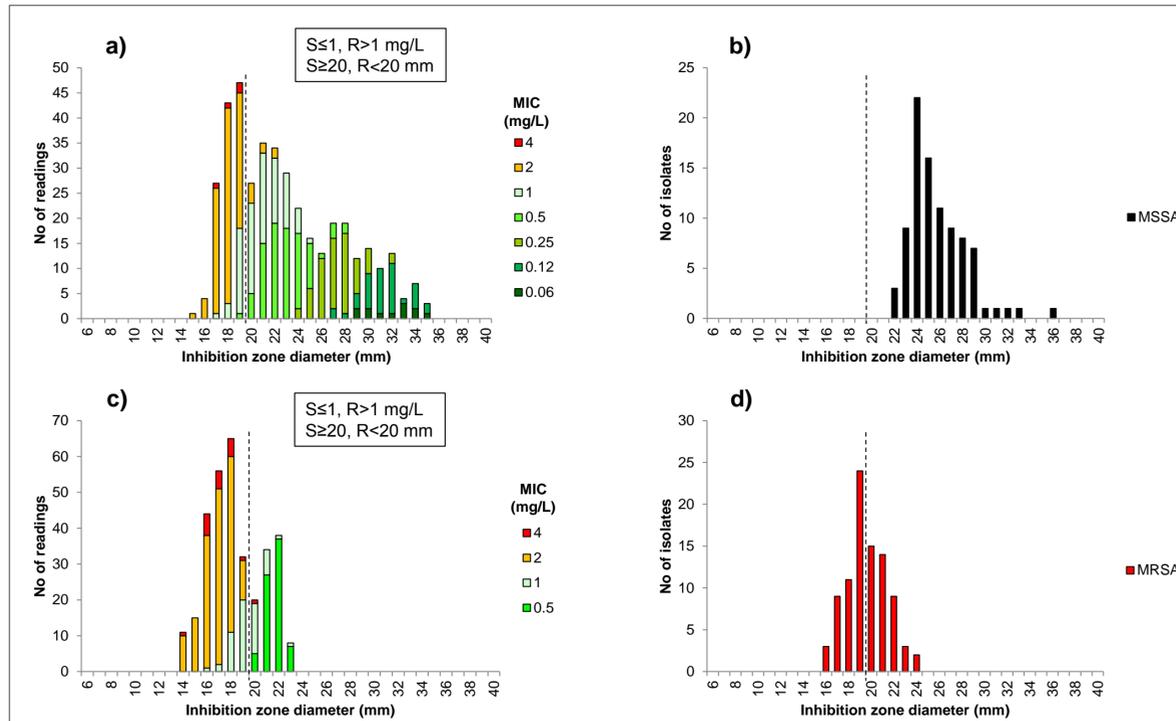


Figure 1. Inhibition zone diameter distributions for *S. aureus* with ceftaroline 5 μ g. MIC values or resistance mechanisms are shown as coloured bars. EUCAST zone diameter breakpoints are shown as dotted lines. S=Susceptible, R=Resistant.

- A challenge set of 100 clinical isolates tested at two sites using MH agar from two manufacturers.
- Zone diameter distribution for MSSA (n=90).
- Repeated testing of 17 *mecA*-positive isolates (disks from two manufacturers, MH agar from three manufacturers, two sites and five readers).
- Zone diameter distribution for MRSA (n=90).

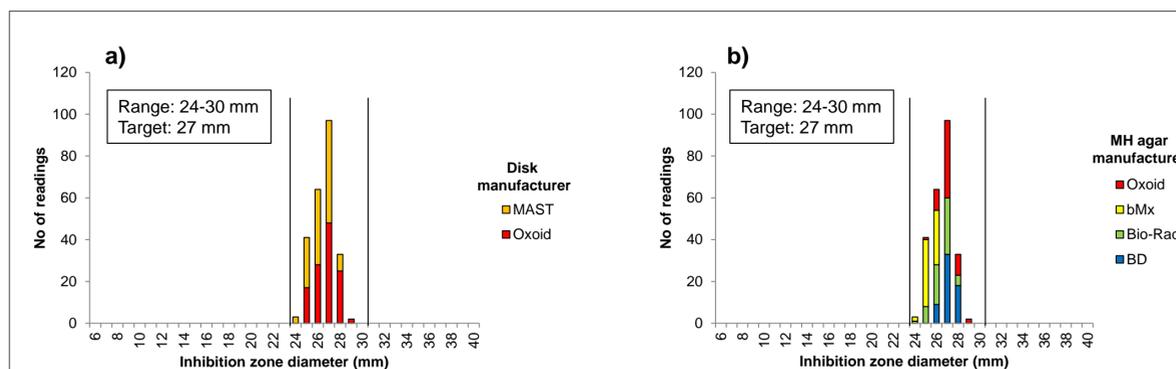


Figure 2. Inhibition zone diameter distributions for *S. aureus* ATCC 29213 with ceftaroline 5 μ g from the initial two-site study (240 readings in total). The EUCAST QC range is shown as black lines.

- with manufacturer of disks shown as coloured bars.
- with manufacturer of MH agar shown as coloured bars. bMx = bioMérieux.

Results

Zone diameter breakpoints

Ceftaroline MICs ranged from 0.12 to 4 mg/L. The ceftaroline 5- μ g disk provided better separation between susceptible (\leq 1 mg/L) and resistant ($>$ 1 mg/L) isolates than the other disk concentrations (data not shown) and supported zone diameter breakpoints of S \geq 20 mm, R $<$ 20 mm (Figure 1a). Discrepant results on 21 and 22 mm belonged to one single isolate. The suggested zone diameter breakpoints were further supported by data on wild-type isolates (MSSA, Figure 1b) and data from repeated testing (MIC and zone diameter) of *mecA*-positive isolates with MICs close to the breakpoint using disks and MH agar from several manufacturers (Figure 1c). However, the breakpoints bisect the MIC distribution of MRSA (Figure 1d) and we suggest that susceptibility is confirmed with an MIC determination for isolates with zone diameters of 19-21 mm.

Quality control criteria

Data from two sites on *S. aureus* ATCC 29213 with ceftaroline 5 μ g corresponded well, and a tentative range of 24-30 mm (target 27 mm) was established. Differences between disks and MH agar from different manufacturers were small (Figure 2). Readings at the five additional laboratories on local Mueller-Hinton plates were normally distributed with a mean of 27 mm (=EUCAST target), and only 3/50 readings were out of range (all 23 mm).

Conclusions

Following the analysis of MIC-inhibition zone correlates, the 5 μ g disk was chosen for EUCAST disk diffusion testing of ceftaroline. Zone diameter breakpoints for *S. aureus* with ceftaroline were set at S \geq 20 mm, R $<$ 20 mm. The QC range for *S. aureus* ATCC 29213 was set at 24-30 mm with a target of 27 mm. Clinical breakpoints QC criteria for ceftaroline are now available in the EUCAST Tables on www.eucast.org.

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