P 825

EUCAST zone diameter breakpoints and quality control criteria for ceftobiprole 5 µg

Erika Matuschek[1], Jenny Åhman[1], Anne Santerre-Henriksen[2], Gunnar Kahlmeter[1]

[1] EUCAST Development Laboratory, Växjö, Sweden; [2] Basilea Pharmaceutica International Ltd., Basel, Switzerland

Introduction

Ceftobiprole is a novel cephalosporin active against a range of Gram-positive and Gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). In 2014, EUCAST established MIC breakpoints for Enterobacteriaceae, *S. aureus* and *Streptococcus pneumoniae*. For disk diffusion, EUCAST has decided to recommend the ceftobiprole 5 µg disk.

Objectives

The aim of this study was to i) establish EUCAST zone diameter breakpoints and quality control (QC) criteria for ceftobiprole 5 µg vs. Enterobacteriaceae and *S. aureus* and ii) validate the EUCAST oxacillin 1 µg screen for β-lactam resistance in *S. pneumoniae* for ceftobiprole.

Methods

Antimicrobial susceptibility testing was performed for Enterobacteriaceae (n=198) *S. aureus* (n=114, of which 84 MRSA) and *S. pneumoniae* (n=115, of which 87 penicillin non-susceptible). The isolates were of different geographical origin and intentionally biased towards beta-lactam resistance. MIC determination was performed with broth microdilution (BMD) according to the ISO standard 20776-1. For *S. pneumoniae*, the broth was supplemented with 5% lysed horse blood and 20 mg/L β-NAD (MH-F broth). Disk diffusion was performed according to EUCAST methodology with ceftobiprole 5 µg disks from Mast and Bio-Rad on in-house prepared plates using Mueller-Hinton (MH) agar from two manufacturers (BBL/BD and Oxoid/Thermo Fisher Scientific). Disk diffusion and BMD were repeated for isolates with MICs close to the breakpoints. Inter-laboratory variation was examined by disk diffusion testing of local clinical isolates of *E. coli* and *S. aureus* at five additional laboratories (see acknowledgement) using local MH media. QC ranges for *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were established according to EUCAST SOP 9.0 (www.eucast.org) using disks from two and media from four manufacturers.

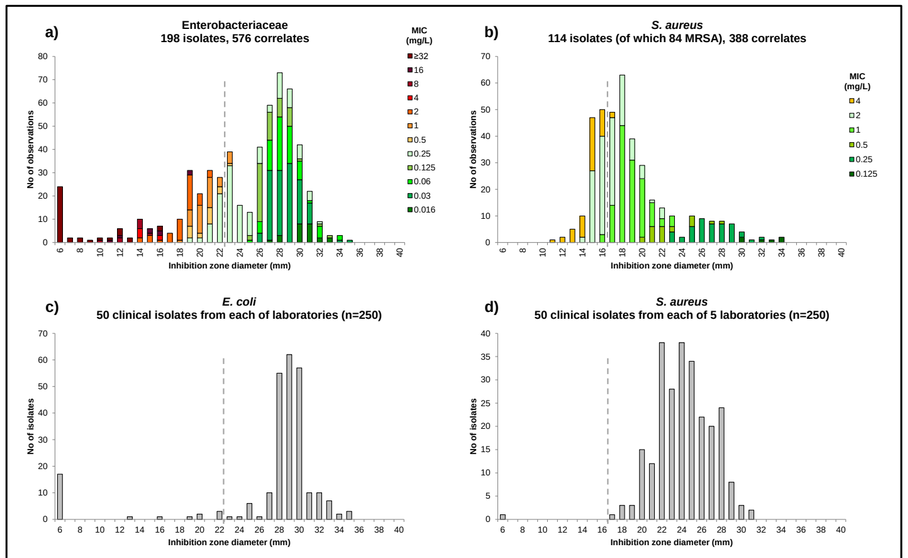

Figure 1. Inhibition zone diameter distributions for ceftobiprole 5 µg with a) Enterobacteriaceae and b) *S. aureus* using disks and media from several manufacturers and for local isolates (50 consecutive isolates from each of 5 laboratories) of c) *E. coli* and d) *S. aureus*.
Corresponding MIC values are shown as coloured bars. Green = Susceptible, Orange and red = Resistant, Grey = No MIC. EUCAST zone diameter breakpoints are shown as dotted lines.

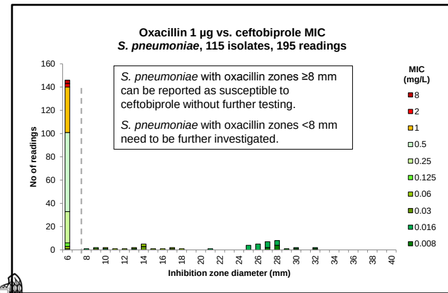

Figure 2. Inhibition zone diameter distribution for oxacillin 1 µg vs. ceftobiprole MIC for *S. pneumoniae*.
Green = Susceptible, Orange and red = Resistant. EUCAST oxacillin 1 µg screening breakpoint for beta-lactam resistance is shown as a dotted line.

Results

Based on the MIC-zone diameter correlates, zone diameter breakpoints for Enterobacteriaceae and *S. aureus* were established to minimize the number of isolates reported as false susceptible (**Figure 1a-b**). The breakpoints were supported by data from testing local consecutive isolates at additional laboratories (**Figure 1c-d**). All methicillin-susceptible *S. aureus* were categorized as susceptible to ceftobiprole by the disk diffusion criteria. For MRSA, the ceftobiprole breakpoint bisects the MIC distribution, resulting in overlapping distributions for isolates with MICs of 2 and 4 mg/L. A corresponding overlap is inevitable also with disk diffusion.

For *S. pneumoniae*, oxacillin zones were ≥8 mm for all ceftobiprole-susceptible isolates and all isolates with varying degrees of reduced susceptibility exhibited zones <8 mm (**Figure 2**). Isolates with oxacillin zones ≥8 mm can be reported susceptible to ceftobiprole without further testing. Isolates with zones <8 mm are not necessarily ceftobiprole resistant and need to be further investigated.

QC criteria for ceftobiprole 5 µg were established for *E. coli* ATCC 25922 (range 25-31 mm, target 28 mm) and *S. aureus* ATCC 29213 (range 22-28 mm, target 25 mm).

Conclusions

Based on these results, EUCAST has published zone diameter breakpoints and QC criteria for ceftobiprole 5 µg for Enterobacteriaceae and *S. aureus* in EUCAST Breakpoint and QC Tables v. 6.0, January 2016. The EUCAST oxacillin 1 µg screen for β-lactam resistance in *S. pneumoniae* has been validated for ceftobiprole.

Acknowledgements for participation in the validation studies:
Andreas Petersen and Nadia Xenia Olsen, Statens Serum Institut, Copenhagen, Denmark
Christiane Müllner, analyse BioLab GmbH, Linz, Austria
Emma Jonasson, Clinical Microbiology, Central Hospital, Växjö, Sweden
Marta Tato Diez, Hospital Universitario Ramón y Cajal, Madrid, Spain
Mandy Wootton, University Hospital of Wales, Cardiff, UK

This study was supported by a grant from Basilea Pharmaceutica International Ltd.
For more information, please contact erika.matuschek@escmid.org