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**Temocillin disc diffusion susceptibility testing by EUCAST methodology**

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Sir,

Temocillin is a semi-synthetic β-lactam antibiotic which is the 6-α-methoxy derivative of ticarcillin. It is a narrow-spectrum agent and its activity is almost exclusively limited to the Enterobacteriaceae—it is not effective against Gram-positive organisms, anaerobes or Pseudomonas aeruginosa.1 As temocillin is highly stable against hydrolysis by β-lactamases, including extended-spectrum and AmpC β-lactamases, it provides a therapeutic option in the management of infections caused by multiresistant Enterobacteriaceae. The clinical efficacy of temocillin in this setting has recently been demonstrated.2

Temocillin MIC breakpoints for Enterobacteriaceae were first proposed by Fuchs et al.3 (susceptible if ≤16 mg/L and resistant if ≥32 mg/L). Currently, the BSAC defines temocillin MIC breakpoints for Enterobacteriaceae as susceptible if ≤8 mg/L in systemic infections and ≤32 mg/L in urinary tract infections, which correspond to zone diameter breakpoints of ≥20 mm and ≥12 mm, respectively.4 Zone diameter breakpoints remain to be established using EUCAST methodology. Many clinical laboratories in the UK, Belgium and Luxembourg (where temocillin is currently licensed) use EUCAST methodology for susceptibility testing and consequently cannot accurately define temocillin susceptibility by disc diffusion. As susceptibility testing by broth dilution is relatively laborious and time-consuming for a routine clinical service, these laboratories currently have to use either automated or Etest (AB Biodisk, Sweden) methodology. Automated susceptibility testing for temocillin by both the Phoenix (Becton-Dickinson, USA) and Vitek 2 (bioMérieux, France) systems has been shown to be inaccurate, and Etest methodology is relatively expensive.5,6

In this study, the susceptibility to temocillin of consecutive, non-duplicate Enterobacteriaceae isolates received at the Department of Medical Microbiology, the Royal Free Hampstead NHS Trust between April and July 2011 and from November 2011 to March 2012 was determined using broth microdilution and disc diffusion methods, in order to establish temocillin disc diffusion interpretative criteria for EUCAST guidelines. The 519 isolates included 320 *Escherichia coli*, 80 *Klebsiella* spp., 42 *Enterobacter* spp., 26 *Proteus* spp., 25 *Citrobacter* spp., 13 *Serratia* spp., 6 *Morganella* morgagni, 3 *Pantoea* spp., 2 *Providencia* spp., 1 *Raoultella* sp. and 1 *Klyvera* sp. These included 47 with extended-spectrum β-lactamase (ESBL), 36 with inducible AmpC (IaMPC), 10 with derepressed AmpC (dAmpC) and 3 *Klebsiella pneumoniae* carbapenemase (KPC)-producing isolates.

Temocillin MICs were determined for all isolates by broth microdilution with geometric 2-fold serial dilutions in cation-adjusted Mueller–Hinton broth following the standard ISO 20776-1:2006.7 Control *E. coli* strains ATCC 25922 and ATCC 35218 (MIC 8 mg/L and 4 mg/L, respectively) were included in every batch of broth microdilutions, and results for clinical isolates accepted only if MICs of the control isolates were within one doubling dilution of these values.

Temocillin zone diameters were determined for all isolates using a 30 μg temocillin disc (Thermostifer) on pre-poured Mueller–Hinton agar plates (Oxoid, UK) following EUCAST guidelines.8 Acceptable limits for the zone sizes for the control strains *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were determined by testing the isolates 25 times and were shown to be 20±1 mm and 25±1 mm, respectively. To establish potential zone size breakpoints for temocillin disc susceptibility testing using EUCAST methodology, broth microdilution MICs and zone sizes for the 519 clinical isolates were compared (Figure 1).

Considering a breakpoint of 8 mg/L, a zone diameter of 20 mm was shown to be the most accurate to determine temocillin susceptibility, with 492/519 (94.8%) of all isolates tested being assigned the correct susceptibility. Of the 10 (1.9%) isolates that were incorrectly determined as susceptible, 8 were *E. coli* (1 ESBL producer), 1 was *K. pneumoniae* and 1 was *Enterobacter cloacae*. The MICs of 9/10 (90%) of the isolates falsely classified as susceptible were within one doubling dilution of the breakpoint of 8 mg/L. Seventeen isolates (3.3%) were incorrectly determined as resistant using a zone size of 20 mm, of which 15 were *E. coli* (4 ESBL producers), 1 was *K. pneumoniae* (ESBL producer) and 1 was *Serratia marcescens* (IaMPC producer).

Considering a breakpoint of 32 mg/L, a zone diameter of 12 mm was shown to be the most accurate, with 513/519 (98.8%) of isolates tested being assigned the correct susceptibility. Of the 6 (1.2%) isolates that were incorrectly determined as resistant, 5 were *E. coli* (1 ESBL producer and 1 dAmpC producer) and 1 was *E. cloacae*.

This study shows that, using EUCAST methodology, zone diameters of ≥20 mm and ≥12 mm correspond to MIC breakpoints of ≤8 mg/L and ≤32 mg/L, respectively. These values can therefore be adopted by EUCAST as zone diameter breakpoints for disc diffusion susceptibility testing using 30 μg temocillin discs in the settings of systemic and urinary tract infections, respectively. These are the same zone diameter breakpoints recommended for the susceptibility testing of temocillin by BSAC methodology.9

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References

Figure 1. Scattergram for a 30 µg temocillin disc for 519 clinical isolates of Enterobacteriaceae using EUCAST methodology.

Evaluation of vancomycin and daptomycin MIC trends for methicillin-resistant Staphylococcus aureus blood isolates over an 11 year period

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Sir,
There are many reports concerning an increase in vancomycin MICs for Staphylococcus aureus isolates over time, which has been described as MIC creep.1–5 Considering the limitations and effectiveness of vancomycin, alternative agents are needed for the treatment of S. aureus infections. Daptomycin is one of the new drugs that is active against various Gram-positive bacteria.5–7

The aim of this study was to determine the in vitro activity of vancomycin and daptomycin against methicillin-resistant S. aureus (MRSA) strains, to investigate the agreement between the