

P 0823 Evaluation of EUCAST zone diameter screening breakpoint for high-level streptomycin resistance in enterococci.

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Introduction

Enterococci are intrinsically resistant to aminoglycosides, but for isolates without acquired high-level aminoglycoside resistance, synergy between an aminoglycoside and a cell-wall active agent can be expected. Enterococci with gentamicin MIC >128 mg/L are considered high-level resistant to gentamicin (HLGR) and other aminoglycosides, except streptomycin, which must be tested separately. EUCAST defines high-level streptomycin resistance (HLSR) in enterococci as an MIC >512 mg/L or a zone diameter <19 mm using a 300 µg streptomycin disk. Recent data suggests that the zone diameter breakpoint needs to be adjusted.

Objective

The objective of this study was to evaluate the EUCAST zone diameter screening breakpoint for high-level streptomycin resistance in *Enterococcus* spp.

Methods

Antimicrobial susceptibility testing was performed on 92 clinical isolates of *E. faecalis* (n=43) and *E. faecium* (n=49) from Växjö Central Hospital, Sweden (n=70) and Linköping University Hospital, Sweden (n=22, kindly provided by Lennart E. Nilsson). The collection included 18 *E. faecalis* and 19 *E. faecium* that were HLGR. Disk diffusion tests with streptomycin 300 µg disks from Oxoid, Mast, and BD were performed according to EUCAST methodology. MIC determination was by gradient tests with Etest (bioMérieux) and MIC Test Strip (Liofilchem). Disk diffusion tests and MIC determinations were with Mueller-Hinton agar from two manufacturers (BBL/BD and Oxoid/Thermo Fisher Scientific) in parallel.

Results

Of the 92 isolates tested, 10/43 *E. faecalis* and 24/49 *E. faecium* exhibited streptomycin MICs >512 mg/L and were designated HLSR. Streptomycin MICs with Etest and MIC Test Strip ranged from 16 to ≥1024 mg/L and were within one dilution for all isolates tested, with 88% of MICs being identical. Each isolate was tested with disks, gradient tests and media from several manufacturers, resulting in 12 combinations per isolate and 1104 MIC-zone diameter correlates in total (516 for *E. faecalis* and 588 for *E. faecium*, **Figure 1 a-b**). For *E. faecalis*, HLSR strains were clearly separated from those without high-level resistance, whereas the separation was less distinct for *E. faecium*.

The aggregated data (**Figure 1 c**) suggests a streptomycin zone diameter screening breakpoint of <14 mm for both *E. faecalis* and *E. faecium*. This breakpoint correctly categorized all isolates with HLSR but also reported high-level resistance in 6/33 and 3/25 non-HLSR *E. faecalis* and *E. faecium* isolates, respectively. Discrepant results were mainly related to disks from Mast. Excluding these resulted in only 1 *E. faecalis* and 3 *E. faecium* isolates being reported as false HLSR. As expected, there was no correlation between HLGR and HLSR (**Figure 1 d**).

Conclusions

Disk diffusion with a streptomycin 300 µg disk can be used to screen for high-level streptomycin resistance in enterococci. Our recent data indicate an adjusted streptomycin zone diameter screening breakpoint of <14 mm for *Enterococcus* spp. This breakpoint was published in the EUCAST Clinical Breakpoint Tables v. 6.0, valid from January 2016.

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