

EUCAST fluoroquinolone breakpoints in enterococci in urinary tract infections – determination of disk diffusion criteria for susceptibility testing.

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Introduction and objective

Treatment of systemic enterococcal infections with fluoroquinolones has been shown to carry a significant risk of treatment failure. However, both *in vitro* and clinical data support that enterococci should be considered possible targets for fluoroquinolone therapy in urinary tract infection (UTI).¹⁻² EUCAST has therefore recently published clinical breakpoints for ciprofloxacin and levofloxacin vs. enterococci valid for **uncomplicated UTI only** (now available in the EUCAST Breakpoint Table v. 4.0).³ The clinical breakpoints equal the epidemiological cut-off values for respective agent and species (4 mg/L for both species).

The objective of this study was to evaluate the EUCAST recommendation to screen for fluoroquinolone resistance in enterococci using the norfloxacin 10 µg disk and a screening breakpoint of < 12 mm. This procedure is recommended for several other grampositive bacteria.

Methods

Antimicrobial susceptibility testing was performed on clinical isolates of *Enterococcus faecalis* (n=50) and *E. faecium* (n=50). Disk diffusion against norfloxacin 10 µg and ciprofloxacin 5 µg was performed according to EUCAST methodology on Mueller-Hinton agar from three manufacturers (BBL/BD, Bio-Rad and Oxoid/Thermo Fisher Scientific). Ciprofloxacin MIC values were determined by broth microdilution (BMD) on custom Sensititre plates (TREK Diagnostics/Thermo Fisher Scientific), in accordance with ISO standard 20776-1.

Results

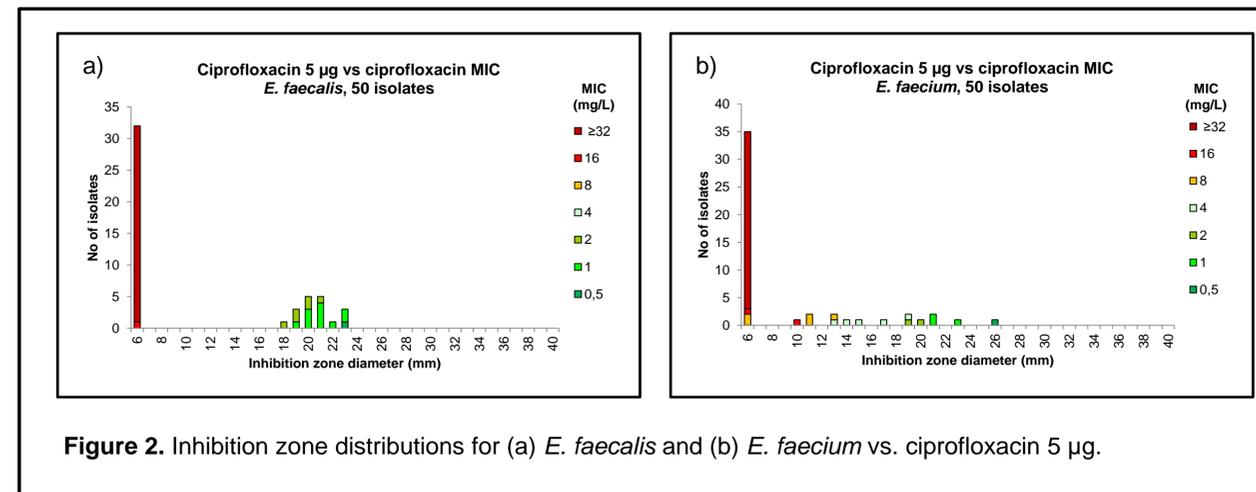
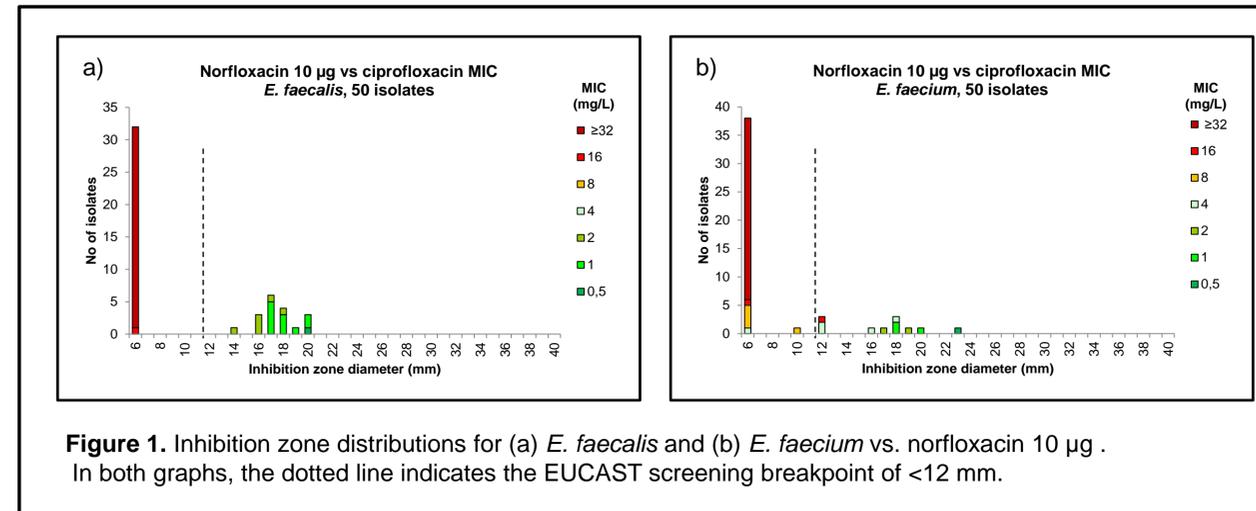
Screening with the norfloxacin 10 µg disk clearly separated ciprofloxacin wild-type from non-wild type isolates of *E. faecalis* (Figure 1a). All ciprofloxacin-resistant isolates had a norfloxacin 10 µg zone diameter of 6 mm. For *E. faecium*, a similar distribution was seen, with 48/50 isolates classified correctly according to ciprofloxacin susceptibility (Figure 1b). However two isolates of *E. faecium* were incorrectly classified, one as ciprofloxacin susceptible and one as resistant. This was independent of the Mueller-Hinton agar used. The ciprofloxacin 5 µg disk also reliably separated ciprofloxacin wild-type from non-wild type isolates of *E. faecalis*, while in *E. faecium* an overlap in zone diameter was seen for isolates with MIC values close to the breakpoint (Figure 2).

Conclusions

Disk diffusion using norfloxacin 10 µg and a screening breakpoint of < 12 mm is a reliable method for detecting ciprofloxacin resistance in *E. faecalis* and *E. faecium*. Our data suggest that the ciprofloxacin 5 µg disk would work well in *E. faecalis* but not in *E. faecium*.

REFERENCES

1. Talan DA, Klimberg IW, Nicolle LE et al. *J Urol* 2004; 171: 734-739.
2. Peterson J, Kaul S, Khashab M et al. *Urology* 2008; 71: 17-22.
3. EUCAST Breakpoint table 4.0 (2014); www.eucast.org.



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