P1441

Evaluation of a ready-to-use metronidazole agar dilution panel for antimicrobial susceptibility testing of anaerobic bacteria

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Background

Metronidazole is the drug of choice for treatment of anaerobic infections. For antimicrobial susceptibility testing (AST) EUCAST have developed a disk diffusion method for selected fast growing anaerobic bacteria. The metronidazole disk diffusion zone diameter breakpoints are conservative, which means that isolates might be susceptible although just below the disk diffusion breakpoint. This discrepancy could ideally be determined with a reference method. The EUCAST reference method for anaerobic bacteria is agar dilution based on FAA-HB and ready-to-use 12-well agar dilution panels (Figure) have been developed for different antimicrobial agents. In this study, we tested a 12-well metronidazole agar dilution panel (Liofilchem, Abruzzi, Italy) for AST of anaerobic bacteria.

Methods

Bacteroides fragilis ATCC 25285 (metronidazole MIC: 0.25-1 mg/L, susceptible), *Clostridium perfringens* ATCC 13124 (metronidazole MIC: 1-4 mg/L, susceptible) and two clinical *Bacteroides* isolates with MICs of ≈4 (susceptible, but just on the breakpoint) and >32 mg/L (resistant) were tested to evaluate precision, and intra and inter-day variation. All isolates were tested six times on day 1 and then again one week later (day 2). The 12-well agar dilution panel had one negative and one positive control well and ten wells with metronidazole, range 0.064-32 mg/L. According to the EUCAST reference method, a 0.5 McFarland suspension was prepared and 2 µl of the suspension was transferred to each well, except for the negative control, which was inoculated with the 0.85% saline solution used for inoculum preparation. Panels were incubated in an anaerobic atmosphere and read after 42-48 hours.

Results

Results are shown in the Table:

Metronidazole MICs from the four tested strains with intra- and inter-day variation

Strain	MICs (mg/L) day 1 (n=6) with median and (range)	MICs (mg/L) day 2 (n=6) with median and (range)
***Bacteroides fragilis* ATCC 25285**	0.25 (0.25)	0.25 (0.25)
***Bacteroides fragilis* (MIC ≈4 mg/L)**	4 (4)	4 (4)
***Bacteroides fragilis* (MIC >32 mg/L)**	>32 (>32)	>32 (>32)
***Clostridium perfringens* ATCC 13124**	0.25 (0.25)	0.25 (0.25)

Results

Figure: Metronidazole agar dilution panels in an anaerobic jar (A) and panel with *Bacteroides fragilis* (MIC =4 mg/L (B).

Conclusions

The panels were very easy to work with. There were no intra or inter-day variation in panel performance. The *B. fragilis* ATCC 25285 metronidazole MIC were within the reference interval but one dilution step under the target. The two clinical isolates had MICs as expected. The *C. perfringens* ATCC 13124 MICs were two dilution steps under the range. The panel worked well over a range of MICs, from susceptible to resistant, for *B. fragilis* but not for *C. perfringens* ATCC 13124.

Disclosure

The panels were delivered free of charge by Liofilchem, Abruzzi, Italy

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