Disk diffusion antimicrobial susceptibility testing of the Bacteroides fragilis group using EUCAST clinical MIC breakpoints

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Abstract

Consecutive clinical B. fragilis group isolates from blood cultures (n=88) from Odense University Hospital and resistant isolates (n=16) from the R. M. Alden Research Laboratory (Los Angeles, USA), were included in the study. The isolates were tested with agar dilution (standard EUCAST for anaerobic bacteria, EUCAST), 24-hour disk diffusion testing, EUCAST disk potencies and microdilution (Table 1). Disk diffusion was performed on Brucella blood agar supplemented with hemin and vitamin K (Becton Dickinson, Heidelberg, Germany). The plates had been preincubated 24 hours before use. A 1 McFarland suspension was prepared in thioglycolate broth and plates were incubated at 37°C (complying with the EUCAST 15-15 rule) in an anaerobic environment for 24 hours (cindamycin 48 hours). The zone diameters were read at 100% inhibition. Zone diameter breakpoints were chosen to minimize very major discrepancies, VMD, major discrepancies, MD, and minor discrepancies, mD, according to the ISO guideline 20776-2:2007.

Results: The 104 isolates were categorized as resistant, intermediate or susceptible by agar dilution as follows: piperacilli (94 isolates), metronidazole 30/6 µg disks, meropenem 10/6 µg disk and piperacillin-tazobactam 30/6 µg disks. The correlation between inhibition zones and MICs are shown in Figure 2. None of the isolates showed evidence of inducible clindamycin resistance as judged by the D-test.

Conclusions

There was good agreement between susceptibility categorization using MICs and zone diameters. Disk diffusion was able to detect resistance with an acceptable level of VMD, according to ISO guideline 20776-2:2007. However, 9.6% of minor discrepancies were observed with piperacillin-tazobactam. Disk diffusion could be an option for antimicrobial susceptibility testing of the B. fragilis group. Our results indicated that resistance and susceptibility to clindamycin was accurately predicted using 24 hour disk diffusion testing. However, we cannot be sure that the method will work with inducible clindamycin resistant isolates.

References


Figure 1. Bacteroides fragilis ATCC 25285 McFarland 1 on supplemented Brucella blood agar with clindamycin 2 µg, metronidazole 5 µg, meropenem 10 µg and piperacillin-tazobactam 30/6 µg disks.

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Introduction and Purpose

Bacteria with Bacteroides fragilis group species is associated with high mortality rates if appropriate antimicrobial therapy is not administered [1]. Increasing resistance in the Bacteroides fragilis group have been reported worldwide, especially towards clindamycin and piperacillin-tazobactam [2]. Lately, resistance towards the carbapenems and metronidazole has also been reported. The clinical significance of increasing levels of antimicrobial resistance in the B. fragilis group emphasises the need for a simple susceptibility test method for the routine laboratory. The aim of our study was to calibrate disk diffusion zone diameter breakpoints against MICs obtained with gold standard agar dilution and to suggest tentative correlate zone diameter breakpoints for EUCAST clinical MIC breakpoints [3].

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References


