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

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Abstract

Objectives: 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 zone diameter breakpoints from disk diffusion to gold standard agar dilution MICs and to suggest tentative zone diameter breakpoints, using the EUCAST clinical MIC breakpoints.

Methods: 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 (piperacillin-tazobactam, meropenem, metronidazole and clindamycin) according to the CLSI guideline M11-A8 and disk diffusion (standard EUCAST potency and metronidazole 5 microgram from Oxoid, Basingstoke, UK). Disk diffusion was performed on Brucella blood agar supplemented with hemin and vitamin K (Becton Dickinson, Heidelberg, Germany). The plates had been prereduced 18-24 hours before use. A 1 McFarland suspension was prepared in thioglycolate broth and plates were incubated at 37°C (complying with the EUCAST 15-15-15 rule) in an anaerobe environment for 24 hours (clindamycin 48 hours). The zone diameter was read at 100% inhibition. Zone diameter breakpoints were chosen to minimise very major discrepancies, VMD, major discrepancies, MD, and minor discrepancies, mD, according to the ISO guideline 20776-2:2007.

Results: the 104 isolates were categorised as resistant, intermediate or susceptible by agar dilution as follows: piperacillin-tazobactam 11, 6 and 87, meropenem 9, 10 and 85, metronidazole 3, 0 and 101 and clindamycin 26, 0 and 78. Tentative zone diameter breakpoints with VMD, MD and mD are presented in Table 1.

Antimicrobial agent	Tentative zone diameter breakpoints (mm)		EUCAST clinical MIC breakpoints (mg/L)		Discrepancies (%)		
	S≥	R<	S≤	R>	VMD	MD	mD
Piperacillin-tazobactam	23	17	8	16	1	0	9.6
Meropenem	29	19	2	8	0	0	4.8
Metronidazole	25	25	4	4	0	1	-
Clindamycin 24 hours	8	8	4	4	1	1.9	-
Clindamycin 48 hours	8	8	4	4	0	6.7	-

Conclusion: 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. 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.

Introduction and Purpose

Bacteremia 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].

Methods

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 (piperacillin-tazobactam, meropenem, metronidazole and clindamycin) according to the CLSI guideline M11-A8 [4] and disk diffusion (standard EUCAST disk potencies and metronidazole 5 microgram from Oxoid, Basingstoke, UK) [3]. Disk diffusion was performed on Brucella blood agar supplemented with hemin and vitamin K (Becton Dickinson, Heidelberg, Germany). The plates had been pre-reduced 18-24 hours before use. A 1 McFarland suspension was prepared in thioglycolate broth and plates were incubated at 37°C (complying with the EUCAST 15-15-15 rule) in an anaerobe environment for 24 hours (clindamycin 48 hours). All isolates with a clindamicin MIC ≥4 mg/L were screened for inducible clindamycin resistance with a D-test using a 15 µg erythromycin disk. The zone diameter was read at 100% inhibition (Figure 1). Zone diameter breakpoints were chosen to minimise very major discrepancies, VMD, major discrepancies, MD, and minor discrepancies, mD, according to the ISO guideline 20776-2:2007 [5].

Results

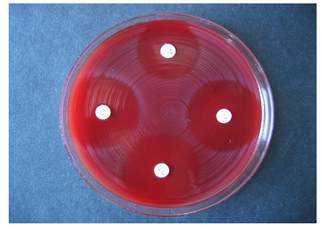

Figure 1.
Bacteroides fragilis ATCC 25285 McFarland 1 on supplemented Brucella blood agar with clindamycin 2 µg, metronidazole 5 µg, meropenem 10 µg and piperacillin-tazobactam 30/6 µg disks.

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.

Results

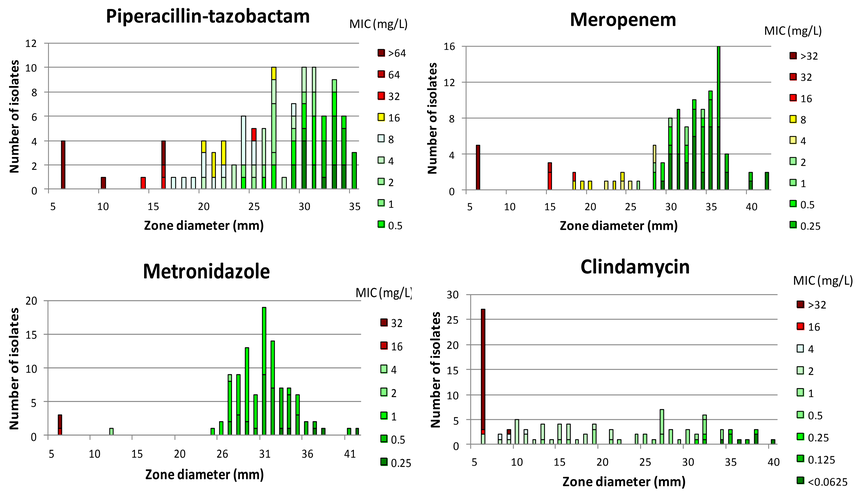

Figure 2. Distribution of zone diameters and MICs for 104 isolates of the *Bacteroides fragilis* group tested against piperacillin-tazobactam (30/6 µg disk), meropenem (10 µg disk), metronidazole (5 µg disk) and clindamycin 24 hours (2 µg disk). Each isolate in the zone diameter histogram is also represented by its MIC value (colours from green to dark red).

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 (Table 1 in abstract). 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

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