The Brucella blood agar for disc diffusion antimicrobial susceptibility testing – reproducibility results for *Clostridium difficile* ATCC 700057

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Introduction and Purpose

The EUCAST disk diffusion antimicrobial susceptibility testing method for fastidious organisms is based on the Mueller-Hinton fastidious agar (MH-F). In a pilot study, most anaerobic bacteria did not grow well enough on MH-F to permit antimicrobial susceptibility testing (Justesen et al.). We decided to investigate whether or not the Brucella Blood Agar supplemented with hemin and vitamin K (BBA), recommended for antimicrobial susceptibility testing of anaerobic bacteria with gradient strips, might also be suited for disk diffusion testing of *C. difficile*.

Methods

C. difficile ATCC 700057 was tested with Etest (bioMérieux, Craponne, France) gradient strips (piperacillin/tazobactam, meropenem, metronidazole, clindamycin, penicillin G, vancomycin, moxifloxacin and tigecycline) on BBA (Becton Dickinson, Heidelberg, Germany) according to the manufacturer's instructions. The corresponding disk (EUCAST disk strength) was included on each plate (Oxoid, Basingstoke, UK). All tests were repeated twelve times. I.e. twelve plates were incubated day 1 at 37°C in an anaerobe environment (10% CO_2, 10% H_2 and 80% N_2) for 24 hours with each antimicrobial agent to assess intra-day variability. This was repeated day 2 to assess inter-day variability. Etest results were compared with the acceptable ranges for ATCC 700057 (reference agar dilution testing, CLSI guideline M11-A7). Twelve plates with disks only were also incubated at 5% CO_2 (10% H_2 and 85% N_2) at 37°C and another twelve plates at 35°C with 10% CO_2, 10% H_2 and 80% N_2. Also; the effect of thioglycollate versus saline 0.85% for inoculum preparation and prereduction of the BBA plates were investigated for vancomycin, metronidazole, moxifloxacin and clindamycin (Figure 1).

Results

Table 1. Zone diameter mean values and ranges are shown in the table.

Antimicrobial agent	Inhibition zone diameters (mm) for *Clostridium difficile* ATCC 700057		
	Day 1 and 2 at 37°C (10% CO_2, 10% H_2 and 80% N_2) (n=12)	5% CO_2 (10% H_2, 85% N_2 and 37°C) (n=12)	35°C (10% CO_2, 10% H_2 and 80% N_2) (n=12)
Piperacillin/tazobactam (30/6 µg)	27.5 (25.7-28.3) 26.7 (25.6-28.2)	26.0 (25.2-26.3)	26.5 (25.7-27.3)
Meropenem (10 µg)	29.7 (28.7-30.1) 29.7 (28.9-30.2)	31.4 (30.5-32.1)	31.5 (30.9-31.8)
Metronidazole (5 µg)	38.7 (37.6-40.9) 35.5 (35.3-36.4)	38.6 (37.6-39.7)	38.9 (38.2-40.6)
Clindamycin (2 µg)	10.3 (10.1-10.5) 10.3 (9.6-10.9)	10.8 (10.4-11.1)	11.8 (10.8-12.3)
Penicillin G (1 U)	6.0 (6.0) NO ZONE 6.0 (6.0) NO ZONE	8.3 (6.0-9.0)	6.4 (6.0-8.2)
Vancomycin (5 µg)	23.1 (22.5-24.0) 25.5 (24.5-26.3)	23.0 (22.6-23.7)	23.5 (23.1-24.3)
Moxifloxacin (5 µg)	23.0 (22.4-23.6) 22.7 (21.8-23.7)	25.4 (24.9-25.8)	25.1 (24.4-25.9)
Tigecycline (15 µg)	34.5 (34.0-34.9) 33.1 (32.2-34.6)	34.5 (33.4-35.1)	35.5 (34.7-36.2)

Figure 1.
Clostridium difficile ATCC 700057, McFarland 1.0 on BBA with clindamycin 2 µg (DA 2), metronidazole 5 µg (MTZ 5), vancomycin 5 µg (VA 5) and moxifloxacin 5 µg (MXF 5) disks. Growth is confluent on the BBA.

All Etest results were within acceptable ranges and the intra- and inter-day variability was <= 1 dilution step. The greatest difference between two mean values was 3.2 mm and the greatest range was 3.3 mm (Table 1). There were small effects of the changes in CO_2 levels and temperature on the inhibition zone diameters.

There were only small differences in zone diameters between thioglycollate or saline 0.85% for inoculum preparation or between prereduced or non-pereduced BBA plates. However, the metronidazole zone diameters were larger for this part of the study 43.0 (42.3-43.9) versus 41.2 (39.8-42.8) with thioglycollate and saline 0.85%, respectively and 44.9 (43.7-46.0) with non-prereduced BBA.

Conclusions

Both intra- and inter-day reproducibility was excellent with disk diffusion on BBA with the eight tested antimicrobial agents. Studies to decide whether this can be repeated with clinical isolates and whether disk diffusion can distinguish resistant isolates from wild type *C. difficile* are in progress. (Abstract and poster 681, ECCMID, London 2012)

References

Justesen US et al. Clin. Microbiol. Infect. 17(Suppl. 4):S174.

Poster 680, ECCMID, London 2012

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This work was supported by ESCMID as part of the development of a EUCAST disk diffusion method