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Abstract (poster session)

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

U.S. Justesen, L.T. Erikstrup\*, T.K. Danielsen, E. Matuschek, G. Kahlmeter (Odense, Aarhus, DK; Växjö, SE)

**Objectives:** 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. 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<sub>2</sub>, 10% H<sub>2</sub> and 80% N<sub>2</sub>) 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<sub>2</sub> (10% H<sub>2</sub> and 85% N<sub>2</sub>) at 37°C and another twelve plates at 35°C with 10% CO<sub>2</sub>, 10% H<sub>2</sub> and 80% N<sub>2</sub>. **Results:** All Etest results were within acceptable ranges and the intra- and inter-day variability was ≤ 1 dilution step. Zone diameter mean values and ranges are shown in the table. The greatest difference between two mean values was 3.2 mm and the greatest range was 3.3 mm. There were small effects of the changes in CO<sub>2</sub> levels and temperature on the inhibition zone diameters. **Conclusion:** 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.

Antimicrobial agent	Inhibition zone diameters (mm) for <i>Clostridium difficile</i> ATCC 700057		
	Day 1 and 2 at 37°C	5% CO <sub>2</sub> (10% H <sub>2</sub> and 85% N <sub>2</sub> )	35°C
	(10% CO <sub>2</sub> , 10% H <sub>2</sub> and 80% N <sub>2</sub> ) (n=12)	(37°C) (n=12)	(10% CO <sub>2</sub> , 10% H <sub>2</sub> and 80% N <sub>2</sub> ) (n=12)
Piperacillin/tazobactam (30/6 microg)	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 microg)	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 microg)	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 microg)	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 microg)	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 microg)	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 microg)	34.5 (34.0-34.9) 33.1 (32.2-34.6)	34.5 (33.4-35.1)	35.5 (34.7-36.2)