

O0746 Proposed breakpoints for EUCAST rapid antimicrobial susceptibility testing with disk-diffusion tests direct from positive blood cultures for *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Enterococcus faecalis* and *Enterococcus faecium*

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Background: EUCAST is preparing to publish a standardised method for rapid antimicrobial susceptibility testing (RAST) using disk diffusion direct from positive blood cultures (BC) with reading after 4, 6 and 8 hours incubation. Good results have been shown for *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus pneumoniae* (Poster 165, ECCMID 2017). The objective of this study was to validate the method for *Pseudomonas aeruginosa*, *Haemophilus influenzae* and enterococci.

Materials/methods: BC bottles, BACTEC™ Plus Aerobic (BD), were inoculated with isolates of *Pseudomonas aeruginosa* (n=53), *Haemophilus influenzae* (n=93), *Enterococcus faecalis* (n=23) and *Enterococcus faecium* (n=34) together with 5 mL defibrinated horse blood and placed in the BACTEC FX system (BD). Isolates with varying susceptibility patterns were included. Disk diffusion was performed according to the EUCAST RAST methodology, as previously presented. Antibiotic agents important for treatment of septicaemia were included (Table 1). Inhibition zones were read after 4, 6 and 8 hours incubation, on the same re-incubated plate. Mueller-Hinton agar from two manufacturers (BBL/BD and Oxoid/Thermo Fisher Scientific) was used. MIC values, used as reference, were determined with broth microdilution according to ISO 20776-1 (EUCAST MH-F broth for *H. influenzae*) and interpreted according to EUCAST breakpoints v. 7.1 (2017). Tentative breakpoints were set to avoid false susceptibility. An “Area of technical uncertainty” (ATU) was used to avoid reporting false susceptible or false resistant results.

Results: It was possible to read zones for almost all isolates after 6h incubation and for *E. faecalis* also after 4h incubation. Reading zones for *H. influenzae* was sometimes difficult, but benzylpenicillin screening test correctly detected all beta-lactam resistant isolates. Tentative breakpoint were suggest for the majority of species-antibiotic combinations tested (Table 1). With these breakpoints and short incubation, only two resistant isolates were reported as susceptible (*P. aeruginosa* vs piperacillin-tazobactam at 6h and *H. influenzae* vs. cefotaxime at 8h).

Conclusions: Inhibition zones for the four species could be reliably read after 6 hours, and for *E. faecalis* after 4 hours, using the EUCAST RAST methodology. On the basis of these results we have proposed tentative zone diameter breakpoints for 4, 6 and 8 hour incubation to EUCAST.

Table 1, Tentative breakpoints for EUCAST RAST of *Ps. aeruginosa*, *H. influenzae*, *E. faecalis* and *E. faecium* directly from positive blood cultures with reading after 4, 6 and 8 hours incubation.

<i>Ps. aeruginosa</i> Antimicrobial agent	Disk content (µg)	Proposed breakpoints (mm)						Zones not possible to read, (%)	
		6h			8h			6h	8h
		S≥	ATU ^A	R<	S≥	ATU ^A	R<		
Piperacillin-tazobactam	30-6	16	12-15	12	18	13-17	13	11	2
Ceftazidime	10	15	12-14	12	16	13-15	13	8	2
Imipenem	10	16	12-15	12	16	12-15	12	8	2
Meropenem	10	15	12-14	12	15	12-14	12	10	2
Ciprofloxacin	5	20	16-19	16	23	20-22	20	6	2
Gentamicin	10	15	9-14	9	15	10-14	10	8	2
Tobramycin	10	14	12-13	12	15	13-14	13	8	2

<i>H. influenzae</i> Antimicrobial agent	Disk content (µg)	Proposed breakpoints (mm)						Zones not possible to read, (%)	
		6h			8h			6h	8h
		S≥	ATU ^A	R<	S≥	ATU ^A	R<		
Benzylpenicillin (screen)	1 unit	13 ^B	Note ^B	Note ^B	12 ^B	Note ^B	Note ^B	3	0
Cefotaxime	5	21	≤20	-	22	≤21	-	6	1
Nalidixic acid (screen)	30	18	16-17	16	18	16-17	16	3	0
Tetracycline	30	19	17-18	17	21	19-20	19	3	0
Trimethoprim-sulfamethoxazole	1.25-23.75	17	10-16	10	19	12-18	12	6	0

<i>E. faecium</i> Antimicrobial agent	Disk content (µg)	Proposed breakpoints (mm)						Zones not possible to read, (%)	
		6h			8h			6h	8h
		S≥	ATU ^A	R<	S≥	ATU ^A	R<		
Ampicillin	2	-	≥8	8	-	≥8	8	5	0
Imipenem	10	-	≥10	10	-	≥10	10	7	3
Gentamicin (high-level aminoglycoside resistance test)	30	Note ^C	11-12	Note ^C	Note ^C	10-13	Note ^C	5	0
Vancomycin	5	-	≥12 ^D	12 ^D	-	≥12 ^D	12 ^D	9	3
Linezolid	10	20	17-19	17	19	17-18	17	11	1

<i>E. faecalis</i> Antimicrobial agent	Disk content (µg)	Proposed breakpoints (mm)									Zones not possible to read, (%)		
		4h			6h			8h			4h	6h	8h
		S≥	ATU ^A	R<	S≥	ATU ^A	R<	S≥	ATU ^A	R<			
Ampicillin	2	9	≤8	-	10	≤9	-	10	≤9	-	8	0	0
Imipenem	10	14	≤13	-	15	≤14	-	16	≤15	-	6	0	0
Gentamicin (high-level aminoglycoside resistance test)	30	Note ^C	13-15	Note ^C	Note ^C	12-15	Note ^C	Note ^C	13-15	Note ^C	4	2	0
Vancomycin	5	-	≥8 ^D	8 ^D	-	≥9 ^D	9 ^D	-	≥9 ^D	9 ^D	6	0	0
Linezolid	10	17	12-16	12	17	12-16	12	17	12-16	12	12	0	0

A: ATU = Area of Technical Uncertainty

B: Screening for beta-lactam resistance in *H. influenzae*.

C: Screen for high-level aminoglycoside resistance (HLAR). S = non HLAR, R = HLAR

D: It was not possible to distinguish between fuzzy and sharp zone edges for vancomycin with short incubation.