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O0254 Multi-laboratory evaluation in southern Europe of the EUCAST method for rapid antimicrobial susceptibility testing directly from positive blood cultures

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Background: Time to susceptibility test results can be shortened for phenotypic methods. EUCAST has developed a standardised rapid antimicrobial susceptibility testing (RAST) method directly from positive blood cultures (BC) (Poster 165, ECCMID 2017) that has been evaluated at 40 clinical laboratories in Nordic countries (Poster O0747, ECCMID 2018). The purpose of this study was to evaluate the RAST method in laboratories in southern Europe with presumably higher resistance levels.

Materials/methods: During two months, the participating laboratories (n=15) performed the EUCAST RAST method, with readings after 4, 6 and 8h, on consecutive positive clinical BCs. Antimicrobial agents commonly used in the treatment of blood stream infections were tested. Locally used manufacturers of antimicrobial disks (n=6), Mueller-Hinton media (n=6) and BC systems (n=2) were used in the study. All isolates were sent to the EUCAST development laboratory were disk diffusion according to EUCAST standard methodology was performed. Categorical agreement vs. EUCAST standard disk diffusion were calculated for *Escherichia coli* (n=150), *Klebsiella pneumoniae* (n=66), *Pseudomonas aeruginosa* (n=32) and *Staphylococcus aureus* (n=70) using two sets of RAST breakpoints: the preliminary breakpoints used to conduct the two multi-laboratory studies and the EUCAST final RAST breakpoints based on the aggregated material from all studies performed, accepted by EUCAST November 2018. The proportion of inhibition zones which could be read at the respective times was also calculated.

Results: Almost all zone diameters could be read already after 6h and for *E. coli* and *K. pneumoniae* already after 4h. The number of categorical errors was low (Table 1) with both preliminary and final RAST breakpoints. The proportion of results in the Area of technical uncertainty (ATU) was highest at 4h. With the final breakpoints, fewer results were categorised as ATU, resulting in more agents with a final report.

Conclusions: This study shows that the EUCAST RAST method are appropriate also in a clinical setting with a high resistance levels (11- 43% depending on species). The results in this study were used together with all previous results to fine tune the preliminary EUCAST RAST breakpoints. The first version of the EUCAST RAST methodology and breakpoints are published on www.eucast.org.

Table 1. RAST categorical agreement vs. EUCAST standard disk diffusion and proportion of readable zones per species and incuabtion time.
Categorical agreements are presented for preliminary and final RAST breakpoints, with results for final breakpoints within parenthesis.

	***E. coli* (150 isolates)**			***K. pneumoniae* (66 isolates)**			***P. aeruginosa* (32 isolates)**		***S. aureus* (70 isolates)**		
	Piperacillin-tazobactam, cefotaxime, ceftazidime, meropenem, ciprofloxacin, amikacin, gentamicin and tobramycin			Piperacillin-tazobactam, cefotaxime, ceftazidime, meropenem, ciprofloxacin, amikacin, gentamicin and tobramycin			Piperacillin-tazobactam, ceftazidime, imipenem, meropenem, ciprofloxacin, gentamicin and tobramycin		Cefoxitin, norfloxacin*, gentamicin and clincamycin**		
Incubation time	**4h**	**6h**	**8h**	**4h**	**6h**	**8h**	**6h**	**8h**	**4h**	**6h**	**8h**
Tests for which a zone could be measured (%)	90	98	100	89	96	96	63	99	46	95	99
	Categorical agreement in % of measured zones, with preliminary and final (in parenthesis) breakpoints										
Correct	77 (79)	81 (89)	82 (91)	81 (86)	83 (88)	83 (89)	84 (88)	88 (91)	63 (61)	81 (81)	91 (86)
mE	0.9 (0.6)	1.0 (1.4)	1.0 (1.5)	2.2 (2.6)	2.2 (2.4)	2.4 (3.3)	0 (0)	0.5 (0.5)	0 (0)	0 (0.4)	0 (0.4)
ME	1.7 (1.7)	0.4 (0.8)	0.4 (0.7)	0.9 (0.9)	0.6 (0.8)	0.2 (0.4)	2.8 (3.4)	1.4 (1.8)	20 (8.0)	4.6 (6.9)	2.5 (4.4)
VME	0.4 (0.4)	0.3 (0.3)	0.3 (0.4)	0.7 (0.9)	0.8 (1.0)	1.6 (1.4)	1.4 (1.4)	0.9 (0.5)	0 (0.8)	3.1 (2.3)	3 (2.2)
ATU	20 (18)	17 (9)	16 (7)	16 (10)	13 (8)	12 (6)	12 (8)	10 (6)	17 (30)	11 (10)	3.5 (7)

*There are no preliminary breakpoints for norfloxacin at 4 h incuabtion.

**There are preliminary breakpoints for clindamycin.

mE (minor Error) = Categorised as susceptible (S) or resistant (R) with RAST when intermediate (I) with standard method.

ME (Major Error) = False resistant.

VME (Very Major Error) = False susceptible.

ATU (Area of technical uncertainty) = Results in an area where interpretation is uncertain. The proportion in the ATU decreases when plates are read after 6 and 8h of incubation or occasionally retested with the standard method.