P0281
Poster Session I
EUCAST antimicrobial susceptibility testing

EVALUATION OF RETAPAMULIN ETEST: VALIDATION AGAINST BROTH MICRODILUTION AND INTER-LABORATORY VARIATION USING EUCAST MEDIA

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Objectives

Retapamulin (RET) has *in vitro* activity against *Staphylococcus aureus* and beta-haemolytic streptococci. There are no clinical breakpoints for RET, but the epidemiological cut-off (ECOFF) values can be used to distinguish between wild-type isolates and isolates with acquired resistance mechanisms (non-wild type isolates). The objectives of this study were to evaluate RET Etest by validation against reference methodology and by investigation of inter-laboratory variation.

Methods

Etest MIC determination was performed on EUCAST media, un-supplemented Mueller-Hinton (MH) agar for *S. aureus* and MH with 5% defibrinated horse blood and 20 mg/L beta-NAD (MH-F) for *Streptococcus pyogenes*. Preparation of inoculum, inoculation and incubation of plates were performed according to EUCAST disk diffusion methodology. Broth microdilution (BMD) was performed on custom Sensititre plates (TREK Diagnostics/Thermo Fisher Scientific) according to ISO standard 20776-1 using cation-adjusted MH broth for *S. aureus* and EUCAST MH-F broth for *S. pyogenes*. Evaluation of RET Etest against BMD was performed at the EUCAST Laboratory for 100 methicillin-susceptible (MSSA), 100 methicillin-resistant (MRSA) *S. aureus* and 100 *S. pyogenes*. The inter-laboratory variation for RET Etest was investigated by 5 additional European laboratories using local clinical isolates (100 MSSA, 100 MRSA and 100 *S. pyogenes* per site if available) on common lots of MH and MH-F commercial plates (Oxoid/Thermo Fisher Scientific). Quality control was performed with *S. aureus* ATCC 29213 and *S. pneumoniae* ATCC 49619.

Results

For *S. aureus*, the correlation between RET Etest and BMD was excellent with 99 and 100% of Etest MICs within ± 1 dilution of BMD MICs for MSSA and MRSA, respectively. For *S. pyogenes*, Etest MICs tended to be higher than BMD MICs, 39% at +1 dilution and 11% at +2 dilutions. However, 89% of the Etest MICs were still within ± 1 dilution of BMD MICs. RET MIC distributions from 5 sites showed little variation for MSSA and MRSA with medians ranging from 0.06 to 0.12 mg/L (see Table 1). The aggregated distribution correlated well with the EUCAST reference distribution (www.eucast.org). Thirteen MRSA from one site had MICs of 64 mg/L and 12 of these belonged to the same clonal complex, CC398. For *S. pyogenes*, the MIC distributions were wider and the variation between sites was larger than for *S. aureus*, but the aggregated distribution correlated well with the EUCAST reference distribution. Medians for *S. pyogenes* varied from 0.015-0.06 mg/L.

Conclusions

The MIC distributions for MSSA, MRSA and *S. pyogenes* produced with retapamulin Etest in this study correlated well with EUCAST reference MIC distributions. Etest MICs performed at the EUCAST laboratory correlated well with reference BMD. We therefore conclude that retapamulin Etest can be used to categorise clinical isolates of *S. aureus* and *S. pyogenes* as wild-type and non-wild type, respectively.

Table 1
Retapamulin MIC distributions (no of isolates)

MSSA (ECOFF: WT ≤ 0.5 mg/L)

Test and site	No of isolates	MIC (mg/L)																		
		≤0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
BMD EUCAST Lab	100					1	67	31								1				
Etest EUCAST Lab	100					4	60	35									1			
Etest Lab A	100					3	34	63												
Etest Lab B	100					4	48	48												
Etest Lab C	100						47	52	1											
Etest Lab D	99						2	91	6											
Etest Lab E	100				1	14	62	22	1											

MRSA (ECOFF: WT ≤ 0.5 mg/L)

Test and site	No of isolates	MIC (mg/L)																		
		≤0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
BMD EUCAST Lab	100					9	81	10												
Etest EUCAST Lab	100					1	79	20												
Etest Lab A	102					3	27	70	2											
Etest Lab B	100					9	70	21												
Etest Lab C	100						7	93												
Etest Lab D	87						1	61	24			1					13*			
Etest Lab E	100				1	20	69	8		2										

S. pyogenes (ECOFF: WT ≤ 0.12 mg/L)

Test and site	No of isolates	MIC (mg/L)																		
		≤0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
BMD EUCAST Lab	100				37	55	8													
Etest EUCAST Lab	100			6	78	16														
Etest Lab A	98			7	19	43	26	2	1											
Etest Lab B	38					7	19	12												
Etest Lab C	90			1	10	66	8	5												
Etest Lab D	99					16	81	1	1											
Etest Lab E	99			12	37	41	9													