P0850
Paper Poster Session
Rapid susceptibility testing and resistance detection

Tentative breakpoints for early reading of disk diffusion tests for Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Streptococcus pneumoniae

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Background: The outcome of therapy in patients with septic shock is dependent on rapid administration of appropriate antibiotic therapy. Increasing resistance calls for rapid antibiotic susceptibility testing. We have previously shown promising results for early reading (6 and/or 8 hours of incubation) of disk diffusion tests. The objectives of this study were to i) further investigate the correlation between early reading and standard incubation in disk diffusion, in particular the expression of known resistance mechanisms, and ii) to determine tentative breakpoints for a selection of clinically relevant antibiotics and organisms important in blood stream infections.

Material/methods: This study includes 1) previously shown data (Sundqvist ECCMID 2013 and Åkerlund ECCMID 2014) and 2) data for additional isolates with known resistance mechanisms, low-level resistance and/or zones close to the breakpoints in the first test to further provoke the test system. Disk diffusion was performed according to EUCAST methodology but with shorter incubation times. *Escherichia coli* (n=180, 692 readings) and *Klebsiella pneumoniae* (n=129, 487 readings) were read after 6 and 8 h, respectively, but *Staphylococcus aureus* (n=141, 434 readings) and *Streptococcus pneumoniae* (n=111, 474 readings) only after 8 h due to in-sufficient growth after 6 h. Disk diffusion with standard incubation (16-20 h) was performed in parallel and used as reference. Mueller-Hinton agar from two manufacturers (BBL/BD and Oxoid/Thermo Fisher Scientific) was used and inhibition zones were read by two technicians. All data was aggregated, and tentative breakpoints for early reading were set to guarantee susceptibility and resistance. Results between these breakpoints were defined as uncertain.

Results: For all organism-antibiotic combinations, separation between wild-type and non-wild type isolates was poorer with short compared to standard incubation. With short incubation, zones for non-wild type isolates were larger whereas zones for wild-type isolates were smaller than with standard incubation. It was possible to establish tentative breakpoints for early reading for 24 organism-agent combinations (Table 1). With these breakpoints, no isolate with a known resistance mechanism was categorised as susceptible after short incubation.

Conclusions: Due to poorer separation between wild-type and non-wild type isolates with shortened incubation, we propose breakpoints for early reading that ensure susceptibility and resistance. We also introduce a category of uncertainty to prevent errors. The high number of test results in the uncertain category in this study was expected as a high number of difficult isolates were included to provoke the test system. In a clinical situation isolates with results classified as uncertain must be

reanalysed with standard incubation. By using this method, the time to the susceptibility test result will be considerably shortened for a number of important organism-antibiotic combinations.

Table 1, Tentative breakpoints for early reading (6 and/or 8 h) with disk diffusion and categorisation of isolates with defined resistance mechanisms using these breakpoints.

Antimicrobial agent and disk content	Tentative breakpoints (mm)			*Klebsiella pneumoniae* (n=487)[1,2]						*Escherichia coli* (n=692)[3,4]					
				Results 6 h (%)			Results 8 h (%)			Results 6 h (%)			Results 8 h (%)		
	S≥	Uncertain	R<	S≥	Uncertain	R<	S≥	Uncertain	R<	S≥	Uncertain	R<	S≥	Uncertain	R<
Piperacillin-tazobactam 30-6 µg	22	14-21	14	10	65	25	15	61	24	33	57	10	53	38	9
Cefotaxime 5 µg	23	15-22	15	45	20	34	48	19	34	48	14	38	56	7	37
Ceftazidime 10 µg	24	17-24	17	20	43	37	28	36	35	26	40	33	47	22	32
Meropenem 10 µg	25	18-24	18	72	13	14	81	4	14	86	13	1	91	8	1
Ciprofloxacin 5 µg	23	15-22	15	56	18	26	60	14	26	52	13	35	55	10	34
Gentamicin 10 µg	19	12-18	12	35	43	23	36	41	23	44	34	22	50	27	23
Tobramycin 10 µg	18	13-17	13	52	20	28	49	22	29	50	28	22	55	22	23

1. Of which 210 ESBL-producing. No of tests for ESBL-producing *K. pneumoniae* categorised as uncertain (6h/8h): 15/23.
2. A screening breakpoint of <27 mm for meropenem can be used to detect carbapemase production. No of tests incorectly categorised as screen positive (6h/8h): 166/30.
3. Of which 289 ESBL-producing. No of tests for ESBL-producing *E. coli* categorised as uncertain (6h/8h): 6/6.
4. A meropenem screening breakpoint of <27 mm for 6 and/or 8 h incubation detected all carbapemase-producing isolates. No of tests incorectly categorised as screen positive (6h/8h): 130/16.

Antimicrobial agent and disk content	Tentative breakpoints (mm)			*Staphylococcus aureus* (n=434)[1]		
				Results 8 h (%)		
	S≥	Uncertain	R<	S≥	Uncertain	R<
Cefoxitin 30 µg screen for beta-lactam resistance	23	20-22	20	52[2]	17[3]	31[4]
Norfloxacin 10 µg screen for fluoroquinolone resistance	17	14-16	14	74	4	22
Erythromycin 15 µg	21	18-20	18	75	2	23

1. Of which 140 MRSA.
2. Of which no MRSA.
3. 7 MRSA of totally 175 *S. aureus.*
4. All MRSA.

Antimicrobial agent and disk content	Tentative breakpoints (mm)			*Streptococcus pneumoniae* (n=455)[1]		
				Results 8 h (%)		
	S≥	Uncertain	R<	S≥	Uncertain	R<
Oxacillin 1 µg screen for beta-lactam resistance	20	-	20	41[2]	-	59[3]
Norfloxacin 10 µg screen for fluoroquinolone resistance	17	14-16	14	63	20	16
Erythromycin 15 µg	23	21-22	21	46	2	51
Tetracycline 30 µg	25	19-24	19	53	10	37
Trimethoprim-sulfamethoxazole 25 µg	19	14-18	14	54	12	33

1. Of which 265 penicillin non susceptible.
2. Of which no penicillin non susceptible.
3. Of which all penicillin non susceptible.