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Proposed breakpoints for rapid antimicrobial susceptibility testing with disk-diffusion tests direct from positive blood cultures for Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Streptococcus pneumoniae

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Background: With increasing antimicrobial resistance, rapid antibiotic susceptibility testing becomes important, especially in patients with blood stream infections. We have previously shown good results with early reading after 6 and 8 hours incubation (Poster 850, ECCMID 2016) with a standard inoculum from an overnight culture. The objective of this study was to shorten the time further by inoculating disk diffusion plates directly from warm blood culture bottles and to read when visual growth was seen.

Material/methods: Blood culture bottles, BACTEC™ Plus Aerobic (BD), were inoculated with pure cultures of *Escherichia coli* (n=61), *Klebsiella pneumoniae* (n=52), *Staphylococcus aureus* (n=54) and *Streptococcus pneumoniae* (n=56) together with 5 mL defibrinated horse blood and placed in the BACTEC FX (BD). Isolates with varying levels of non-suscetibilty were included. Disk diffusion was performed with EUCAST methodology, but with modified inoculum and incubation time. Approximately 150 µL (3 drops from a syringe) from the positive bottle was added to each Muller-Hinton agar plate and evenly distributed. Clinically relevant antibiotics for sepsis treatment 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. Broth microdilution according to ISO 20776-1 (EUCAST MH-F broth for *S. pneumoniae*) was used as a reference, MICs were interpreted according to EUCAST breakpoints. Suggested zone diameter breakpoints were set to avoid any false susceptibility, whereas occasional false resistance was accepted.

Results: Using direct inoculation from positive and warm blood cultures bottles shortened the time to result (defined as a zone which can be comfortably read) compared to when a “cold” inoculum of McFarland 0.5 was utilized. Many zones could be read already after 4 hours and almost all after 6 and 8 (Table 1). The table also shows proposed breakpoints for 4, 6 and 8 hour reading for the 25 organism-antibiotic combinations. Breakpoints were set to avoid false susceptibility. Therefore, results between S and R were deemed an uncertain result and not reported. None of the reported S were later reported as R. Depending on incubation time, species and antibiotic 0-30% (mean 12%) were “uncertain”.

Conclusions: Disk diffusion following direct inoculation of susceptibility plates from positive blood cultures with attempted reading after 4, 6 and 8 hours is possible. The number of isolates categorised as “uncertain” is depending on the local resistance rate, in consecutive clinical isolates. When zones were uncategorised after 4h, the isolates should be re-incubated and read again after 6 and 8 h. Results that cannot be interpreted S or R even after 8 hours incubation must be retested with standard methodology. We will further evaluate the proposed method and breakpoints for more clinical isolates and more species.

Table 1, Proposed breakpoints for disk diffusion from positive blood cultures with reading after 4, 6 and/or 8 hours incubation.

E. coli **Antimicrobial agent and disk content**	**Proposed breakpoints (mm)**									**Zones not possible to read (%)**		
	4h			**6h**			**8h**					
	S≥	**Uncertain**	**R<**	**S≥**	**Uncertain**	**R<**	**S≥**	**Uncertain**	**R<**	**4h**	**6h**	**8h**
Piperacillin-tazobactam 30-6 µg	-	≥12	12	-	≥12	12	-	≥12	12	15	0	0
Cefotaxime 5 µg	15	13-14	13	18	14-17	14	18	14-17	14	12	0	0
Ceftazidime 10 µg	15	12-14	12	16	14-15	14	17	14-16	14	14	0	0
Meropenem 10 µg	17	12-16	12	17	14-16	14	17	14-16	14	5	0	0
Ciprofloxacin 5 µg	16	13-15	13	19	15-18	15	20	15-19	15	8	0	0
Gentamicin 10 µg	14	12-13	12	14	12-13	12	14	12-13	12	2	0	0
Tobramycin 10 µg	14	12-13	12	15	12-14	12	15	12-14	12	2	0	0
Amikacin 30 µg	15	13-14	13	15	13-14	13	15	13-14	13	2	0	0

K. pneumoniae **Antimicrobial agent and disk content**	**Proposed breakpoints (mm)**									**Zones not possible to read (%)**		
	4h			**6h**			**8h**					
	S≥	**Uncertain**	**R<**	**S≥**	**Uncertain**	**R<**	**S≥**	**Uncertain**	**R<**	**4h**	**6h**	**8h**
Piperacillin-tazobactam 30-6 µg	-	≥12	12	-	≥12	12	-	≥12	12	1	0	0
Cefotaxime 5 µg	15	12-14	12	18	14-17	14	18	14-17	14	2	0	0
Ceftazidime 10 µg	15	11-14	11	16	12-15	12	17	11-16	11	2	0	0
Meropenem 10 µg	15	12-14	12	17	13-16	13	17	13-16	13	4	0	0
Ciprofloxacin 5 µg	18	15-17	15	18	15-17	15	19	16-18	16	3	0	0
Gentamicin 10 µg	14	12-13	12	14	12-13	12	13	12	12	0	0	0
Tobramycin 10 µg	14	12-13	12	13	11-12	11	13	11-12	11	0	0	0
Amikacin 30 µg	15	13-14	13	14	11-13	11	13	10-12	12	0	0	0

Staphylococcus aureus **Antimicrobial agent and disk content**	**Proposed breakpoints (mm)**									**Zones not possible to read (%)**		
	4h			**6h**			**8h**					
	S≥	**Uncertain**	**R<**	**S≥**	**Uncertain**	**R<**	**S≥**	**Uncertain**	**R<**	**4h**	**6h**	**8h**
Cefoxitin 30 µg screen for beta-lactam resistance	17	15-16	15	19	17-18	17	20	18-19	18	33	9	9
Norfloxacin 10 µg screen for fluoroquinolone resistance	12	<12	-	13	<13	-	14	<14	-	45	11	9
Gentamicin 10 µg	15	13-14	13	16	14-15	15	16	14-15	14	46	9	9
Erythromycin 15 µg	18	16-17	16	18	16-17	16	18	16-17	16	56	14	10
Clindamycin 2 µg	-	-	-	-	-	-	-	-	-	57	15	11

Streptococcus pneumoniae **Antimicrobial agent and disk content**	**Proposed breakpoints (mm)**									**Zones not possible to read (%)**		
	4h			**6h**			**8h**					
	S≥	**Uncertain**	**R<**	**S≥**	**Uncertain**	**R<**	**S≥**	**Uncertain**	**R<**	**4h**	**6h**	**8h**
Oxacillin 1 µg screen for beta-lactam resistance	14	<14	-	17	<17	-	18	<18	-	7	2	0
Norfloxacin 10 µg screen for fluoroquinolone resistance	10	<10	-	11	<11	-	11	<11	-	21	2	0
Erythromycin 15 µg	19	17-18	17	19	17-18	17	19	17-18	17	19	2	0
Clindamycin 2 µg	-	≥14	14	-	≥14	14	18	14-17	14	18	2	1
Trimethoprim-sulfamethoxazole 25 µg	14	10-13	10	14	10-13	10	14	10-13	10	15	2	0