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

Emma Jonasson[1], Erika Matuschek[2], Martin Sundqvist[3] and Gunnar Kahlmeter[1,2]

[1]Department of Clinical Microbiology, Central Hospital, Växjö, Sweden; [2]EUCAST Development Laboratory, Växjö, Sweden

[3]Department of Laboratory Medicine, Clinical Microbiology, Örebro University Hospital, Örebro, Sweden

Introduction

The outcome of therapy in patients with septic shock is dependent on rapid administration of appropriate antibiotic therapy. Increasing resistance calls for rapid diagnosis and antimicrobial susceptibility testing. We have previously shown promising results for early reading (6 and/or 8 hours of incubation) of disk diffusion tests (1,2).

Objectives

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.

Methods

This study includes i) previously shown data (1,2) and ii) data for additional isolates with known resistance mechanisms, low-level resistance and/or zones close to the breakpoints in the first test. A high number of resistant and difficult isolates were intentionally chosen to provoke the system. The following organisms were included: *Escherichia coli* (n=180, of which 55 ESBL and 17 carbapenemase producing (CPE)), *Klebsiella pneumoniae* (n=129, of which 23 ESBL and 18 CPE), *Staphylococcus aureus* (n=155, of which 52 MRSA) and *Streptococcus pneumoniae* (n=117, of which 65 penicillin resistant).

Disk diffusion was performed on over-night cultures according to EUCAST methodology but with shorter incubation times. *E. coli* and *K. pneumoniae* were read after 6 and 8 h but *S. aureus* and *S. pneumoniae* 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. This resulted in 660, 449, 490 and 470 readings for *E. coli*, *K. pneumoniae*, *S. aureus* and *S. pneumoniae*, respectively. After aggregating data, tentative breakpoints for early reading were set to ensure correct categorization into susceptibility and resistance. Results between these breakpoints were defined as uncertain.

For more information, please contact:
Emma.k.jonasson@kronoberg.se

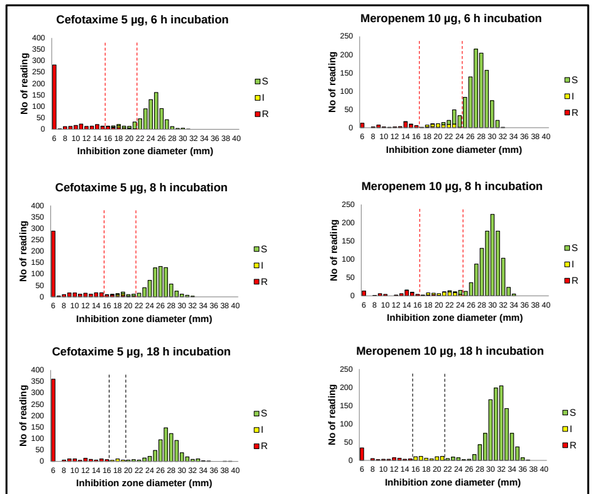

Figure1 a: *Escherichia coli* and *Klebsiella pneumoniae* aggregated

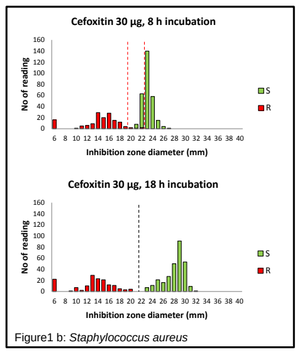

Figure1 b: *Staphylococcus aureus*

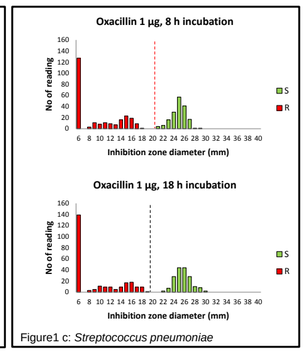

Figure1 c: *Streptococcus pneumoniae*

Table 1. Tentative breakpoints for early reading (6 and/or 8 h) with disk diffusion. For *E. coli* and *K. pneumoniae* zones can be interpreted after either 6 or 8 h incubation and for *S. aureus* and *S. pneumoniae* only after 8 h incubation.

E. coli* and *K. pneumoniae Antimicrobial agent and disk content	Tentative breakpoints (mm)		
	S≥	Uncertain	R<
Piperacillin-tazobactam 30-6 µg	21	15-20	15
Cefotaxime 5 µg	22	16-21	16
Ceftazidime 10 µg	22	17-21	17
Meropenem 10 µg	25	17-24	17
Meropenem 10 µg screening bp	28	-	28
Ciprofloxacin 5 µg	22	16-21	16
Gentamicin 10 µg	18	13-17	13
Tobramycin 10 µg	18	14-17	14

Streptococcus pneumoniae Antimicrobial agent and disk content	Tentative breakpoints (mm)		
	S≥	Uncertain	R<
Oxacillin 1 µg screen for beta-lactam resistance	21	-	21
Norfloxacin 10 µg screen for fluoroquinolone resistance	17	14-16	14
Erythromycin 15 µg	24	22-23	22
Tetracycline 30 µg	26	21-25	21
Rifampicin 5µg	22	19-21	19
Trimethoprim-sulfamethoxazole 25 µg	19	15-18	15

Staphylococcus aureus Antimicrobial agent and disk content	Tentative breakpoints (mm)		
	S≥	Uncertain	R<
Cefoxitin 30 µg screen for beta-lactam resistance	23	20-22	20
Norfloxacin 10 µg screen for fluoroquinolone resistance	17	14-16	14
Erythromycin 15 µg	22	18-21	18

Figure 1. Inhibition zone distributions for a) aggregated data on *E. coli* and *K. pneumoniae* for cefotaxmine 5 µg and meropenem 10 µg after 6, 8 and 18 h incubation
b) *S. aureus* for cefoxitin 30 µg after 8 and 18h incubation and
c) *S. pneumoniae* for oxacillin 1 µg after 8 and 18h incubation.
SIR categorization based on results from standard disk diffusion (16-20h incubation). Breakpoints are shown as dotted lines (EUCAST standard breakpoints in black and tentative breakpoints for 6 and 8 h incubation in red).

Results

For all organism-antibiotic combinations, the separation between wild-type and non-wild type isolates was poorer with short compared to standard incubation (Figure 1). With short incubation, zones for non-wild type isolates were larger whereas zones for wild-type isolates were smaller than with standard incubation. Results for *E. coli* and *K. pneumoniae* were similar and therefore aggregated. For *E. coli* and *K. pneumoniae*, the separation was better after 8 compared to 6 h of incubation. For about 10% of the *S. aureus* and *S. pneumoniae* (n=17 and 11, respectively), zones could not be read due to no or insufficient growth after 8 h incubation. For Enterobacteriaceae, only 1% (n=4) of the isolates had insufficient growth after 6 h incubation, and zones could be read for all isolates after 8 h incubation. Distinct and clearly visible zone edges were crucial to get reliable results.

It was possible to establish tentative breakpoints for early reading for 17 organism-agent combinations (Table 1). With these breakpoints, isolates were correctly interpreted as susceptible and resistant for the vast majority of the tests, with less than 1% being misinterpreted. All isolates with a known resistance mechanism were correctly categorised after short incubation. The width of the uncertain category ranged from 2 to 8 mm. For piperacillin-tazobactam vs. Enterobacteriaceae, the separation after 6 and 8 h incubation was poor and a large proportion of the results were classified as uncertain with the suggested breakpoints.

Conclusions

Based on the results in this study, we propose breakpoints for early reading that as far as possible ensure correct categorization into susceptibility and resistance. Isolates in between these categories (uncertain) may either be tentatively categorized as resistant or not categorized until after a standardized test has been performed. The number of test results in the uncertain category in consecutive clinical isolates will depend on local resistance rates. Comparing 8 and 6 hour incubation for *E. coli* and *K. pneumoniae*, the number of isolates categorized as uncertain is lower with the longer incubation. By using this method, the time to the susceptibility test result will be considerably shortened for a number of important organism-antibiotic combinations, but the breakpoints must be validated for direct tests from positive blood cultures.

References

1. Sundqvist et al., ECCMID 2013 Berlin, Germany, P1541
2. Åkerlund et al., ECCMID 2014 Barcelona, Spain, P0334

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