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Antibiotic susceptibility in *Streptococcus pneumoniae* can be predicted after 8h incubation

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Objectives

The increasing antimicrobial resistance prompts the need for rapid susceptibility reports. The "Eurostar Rapid Disk project" has validated early reading (6-8h) with Disk diffusion for non-fastidious bacteria and *Haemophilus influenzae* using EUCAST clinical breakpoints (1,2). This study evaluated early reading of disk diffusion in *Streptococcus pneumoniae*.

Methods

Disk diffusion was performed on 55 well defined isolates of *Streptococcus pneumoniae* (PNC) on Mueller-Hinton Fastidious agar (MH-F, Mueller-Hinton agar supplemented with 5% defibrinated horse blood and 20 mg/L β-NAD) with an inoculum of 0.5-0.6 McF. MH-F plates were incubated in 35°C with 4-6% CO_2 in air for 8 h. All isolates were also tested using EUCAST methodology with standard incubation, all of the isolates had previously been tested using broth micro dilution (BMD). Seven antibiotics were tested using disk contents and SIR-breakpoints according to the EUCAST. Zone measurements were independently performed by two experienced technicians. Zone/zone correlation and categorical agreement was evaluated for each antibiotic. Errors in relation to SIR-category (based on BMD) were assigned minor Errors (mE), major Errors (ME) or very major Errors (VME).

Results

52 out of 55 isolates were possible to read after 8 h incubation for all antibiotics, for 1 strain no zones were measurable and for the other 2 only a few zones were possible to read.
The oxacillin result was fully consistent with 18h disk diffusion leading to detection of all isolates with non-susceptibility to Benzylpenicillin (figure 1). The results for norfloxacin was in line with 18h disk diffusion results in all but one isolate. However in comparison with BMD data this resulted in 8 cases categorized as mE. For the other antibiotics 6 VME (for clindamycin), 1 ME (for trimethoprim-sulfamethoxazole), 7mE (for trimethoprim-sulfamethoxazole n=4 and erythromycin n=3) were found. All isolates were correctly assigned to susceptible category for rifampicin and tetracycline. As seen in the figures below breakpoints adjusted for the shorter incubation time could improve the SIR-categorization after 8h incubation for some antibiotics.

Figure 1. Zone/zone correlation for oxacillin 1 µg (n=53), 8 hours incubation to standard EUCAST incubation. Clinical breakpoint S≥ 20 mm.

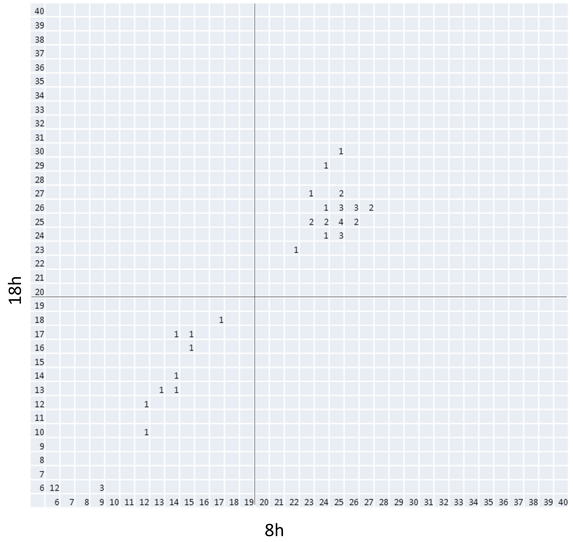
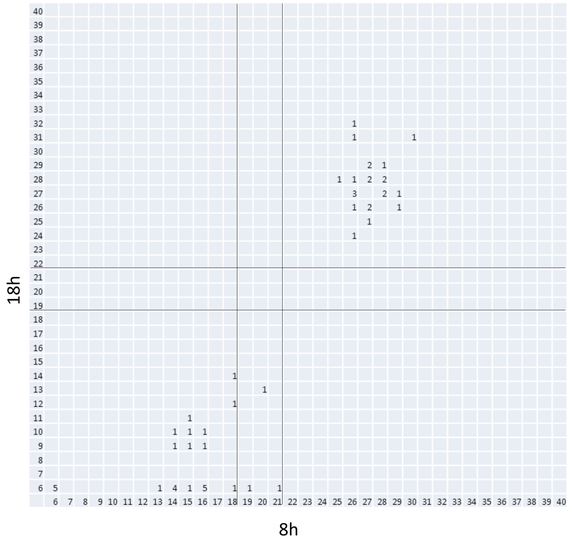


Figure 2. Zone/zone correlation for erythromycin 15 µg (n=53), 8 hours incubation to standard EUCAST incubation. Clinical breakpoint S ≥ 22 mm R< 19.



Conclusion

- This study shows the feasibility of predicting susceptibility to penicillins and 5 more antibiotics in PNC using EUCAST breakpoints already after 8h incubation.
- Zones close to the breakpoint should be verified with standardised AST and breakpoints adjusted for the shorter incubation time could possibly further improve the SIR-categorization.
- The errors observed for clindamycin showed large differences in zone-diameters and an adjustment of the breakpoint could thus not resolve the problem. So far we discourage from interpreting clindamycin after 8h.
- This study highlights the importance of evaluating each antibiotic/bacteria combination for early reading before applying it in routine clinical microbiology.

Acknowledgements

All members of the EuroStar Disk consortium.
JMI Laboratories, USA, for usage of BMD data.

References

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