

Improved screen for beta-lactam resistance in *Streptococcus pneumoniae* with the oxacillin 1- μ g disk using the EUCAST disk diffusion method

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Introduction

EUCAST recommends screening with the oxacillin 1- μ g disk to detect beta-lactam non-susceptibility in *Streptococcus pneumoniae*. The disk provides sensitive screening for all degrees of beta-lactam non-susceptibility (oxacillin zone diameter <20 mm), but has hitherto been used only to distinguish between isolates belonging to the wild type (WT) and those with any acquired beta-lactam resistance mechanisms.

Objective

The objective of this study was to investigate if the oxacillin screen could be further optimised to reduce additional laboratory testing for non-wild type *S. pneumoniae*.

Methods

Clinical isolates of *S. pneumoniae* (n=100) with a variety of beta-lactam resistance mechanisms were selected from the SENTRY collection (JMI Laboratories, USA). MIC values were determined for beta-lactam agents by broth microdilution (BMD) at JMI Laboratories according to the ISO standard 20776-1, 2006. The Mueller-Hinton broth was supplemented with 5% lysed horse blood (by repeated freezing and thawing) and 20 mg/L β -NAD (MH-F broth). Disk diffusion was performed at the EUCAST Laboratory according to EUCAST methodology for fastidious organisms on in-house prepared MH-F plates (Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β -NAD) using agar from two manufacturers (BD and Oxoid/Thermo Fisher Scientific). Data analysis was performed by EUCAST.

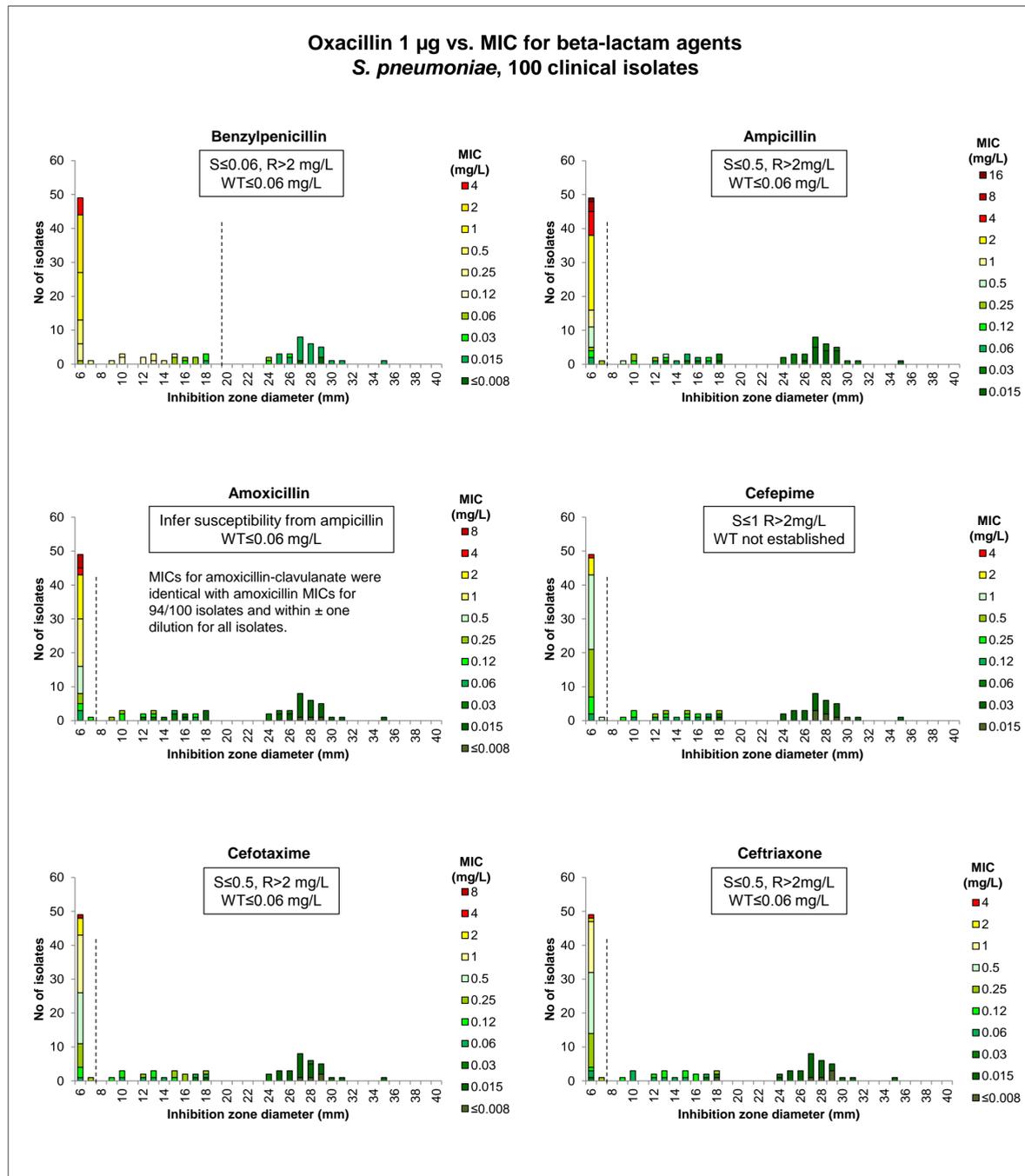


Figure 1. Inhibition zone diameter distributions for *S. pneumoniae* with oxacillin 1 μ g. MIC values for different beta-lactam agents are shown as coloured bars. EUCAST improved oxacillin 1- μ g screen breakpoints (<20 mm for benzylpenicillin and <8 mm for the other agents) are shown as dotted lines. S=Susceptible, R=Resistant, WT=Wild Type.

Results

Benzylpenicillin MICs for *S. pneumoniae* ranged from ≤ 0.008 to 4 mg/L (Table 1).

Table 1. Benzylpenicillin susceptibility vs. oxacillin 1- μ g zone diameter

Benzylpenicillin susceptibility	MIC (mg/L)	No of isolates		
		6-7 mm	8-19 mm	≥ 20 mm
Susceptible (n=40)	≤ 0.008 -0.06	1	9	30
Intermediate (n=55)	0.12-2	44	11	0
Resistant (n=5)	4	5	0	0

All isolates with oxacillin zones ≥ 20 mm (n=30) were wild type and categorised as susceptible to all investigated beta-lactam agents. Furthermore, isolates with oxacillin zones ≥ 8 mm (n=50) were all fully susceptible to ampicillin, amoxicillin, amoxicillin-clavulanate, cefepime, cefotaxime and ceftriaxone (Figure 1). The suggested algorithm for the oxacillin screen test is presented in Table 2.

Table 2. Improved screen for beta-lactam resistance in *S. pneumoniae* with the oxacillin 1- μ g disk.

Oxacillin 1 μ g disk zone diameter	Antimicrobial agent	Further testing and/or interpretation
≥ 20 mm	All beta-lactam agents for which clinical breakpoints are listed (including those with "Note")	Report susceptible irrespective of clinical indication.
< 20 mm	Benzylpenicillin (meningitis) and phenoxymethylpenicillin (all indications)	Report resistant.
	Benzylpenicillin (for infections other than meningitis)	Determine the MIC and interpret according to the clinical breakpoints.
	Ampicillin and amoxicillin (without and with beta-lactamase inhibitor), cefepime, cefotaxime and ceftriaxone	Oxacillin zone diameter ≥ 8 mm: Report susceptible. Oxacillin zone diameter < 8 mm: Determine the MIC of the beta-lactam agent intended for clinical use but for ampicillin, amoxicillin and piperacillin (without and with beta-lactamase inhibitor) infer susceptibility from the MIC of ampicillin.
	Other beta-lactam agents	Determine the MIC of the agent considered for clinical use and interpret according to the clinical breakpoints.

Conclusions

Based on the oxacillin 1- μ g disk screen, we present an improved algorithm to predict susceptibility to clinically important beta-lactam agents in non-wild type *S. pneumoniae*. This reduces laboratory work and allows susceptibility test results to be released earlier. The new screening criteria are available in the EUCAST Clinical Breakpoint Table v 3.1, 2013.