Multicenter comparison of different commercial antimicrobial susceptibility testing methods compared to broth microdilution for beta-lactam susceptibility testing of *Streptococcus pneumoniae*

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BACKGROUND

- Treatment of pneumococcal infections is most often based on the use of penicillins or cephalosporins. More than 13% of Belgian invasive *S. pneumoniae* were non-wild type for penicillin (BEN) (MIC >0.06 mg/L) in 2023.
- EUCAST issued a warning against the use of gradient test for BEN MIC determination in *S. pneumoniae* in 2019. No recent performance evaluation of commercial automated broth dilution methods has been described in literature.

OBJECTIVE

To assess performance of Etest®, Vitek®2 and BD Phoenix™ to determine the susceptibility of *Streptococcus pneumoniae* strains to penicillin, ampicillin and cefotaxime.

METHODS

- Sixty unique *S. pneumoniae* strains were selected to cover a wide range of penicillin, ampicillin and cefotaxime minimal inhibitory concentrations (MICs) (table 1). Most *S. pneumoniae* strains however had MICs close to the various breakpoints (“challenge”).
- Strains were analyzed in four different Belgian laboratories. Etest® benzylpenicillin (BEN), ampicillin/amoxicillin (AMP) and cefotaxime (CTA) (bioMérieux), Vitek®2 AST-ST03 (bioMérieux) and BD Phoenix™ SMIC/ID-11 testing were each performed in two different labs. Etest® was performed on two different plates.
- Results were compared to Sensititre® broth microdilution (BMD) (Thermo Fisher Scientific) results. MIC results were interpreted using EUCAST non-meningitis breakpoints (v 13.0).

RESULTS

Antimicrobial	MIC (mg/L)										
	≤ 0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16
BEN	0	1	11	4	6	7	7	7	10	7	0
AMP	0	5	8	5	7	6	8	7	3	4	7
CTA	1	4	9	8	6	6	12	10	1	3	0

Table 1: MIC distribution for beta-lactam antibiotics of 60 *S. pneumoniae* strains based on broth microdilution testing results. The vertical lines indicate the EUCAST clinical breakpoints for non-meningitis. BEN: penicillin; AMP: amoxicillin/ampicillin; CTA: cefotaxime/ceftriaxone.

	BEN						AMP						CTA					
Testing method	EA (%)	CA (%)	VME (n)	ME (n)	mE (n)	bias (%)	EA (%)	CA (%)	VME (n)	ME (n)	mE (n)	bias (%)	EA (%)	CA (%)	VME (n)	ME (n)	mE (n)	bias (%)
BD Phoenix™	**90.8**	82.5	1	15	5	**+19.9**	**99.2**	88.3	0	12	2	**+7.1**	**100.0**	87.5	3	0	12	**-24.8**
Vitek®2	**96.6**	**90.0**	6	0	6	**-8.7**	**91.7**	86.7	0	16	0	**+18.3**	**99.2**	**90.0**	0	5	7	**+7.5**
Etest on Oxoid plate	58.3	74.2	31	0	10	-73.0	65.8	75.8	19	2	8	-72.0	**90.8**	79.2	4	0	21	-35.1
Etest on BD BBL plate	**94.2**	84.2	12	1	6	**-20.4**	84.2	82.5	7	10	4	**-27.7**	**95.0**	87.5	1	2	12	**+7.9**

Table 2: Performance of BD Phoenix™, Vitek®2 and Etest® compared to broth microdilution for the determination of susceptibility to penicillin, amoxicillin and cefotaxime of 60 *S. pneumoniae* strains. Each testing method was performed in 2 different labs (n=120). EA and bias were calculated and evaluated using ISO 20776-2:2021. CA and VME/ME were calculated and evaluated using CLSI M52. Results within ISO or CLSI acceptance criteria (EA and CA ≥ 90%, difference bias ±30%) are in bold and green.

- Essential agreement (EA) was ≥90% for all methods compared to BMD, except for Etest® BEN on Oxoid plate (58.3%) and Etest® AMP (both on Oxoid (65.8%) and BD BBL plate (84.2%)) (Table 2).
- Categorical agreement (CA) for BEN was only ≥90% for Vitek®2, for other methods CA ranged between 74.2-84.2%.
- CA for AMP was for all methods <90% (range 75.8-88.3%) and CA for CTA was between 87.5-90% for all methods except for Etest® on Oxoid plate (79.2%).

CONCLUSION

- Vitek®2 and BD Phoenix™ are reliable for providing accurate pneumococcal susceptibility results for BEN, AMP and CTA.
- Using Etest® BEN or AMP on Oxoid plate carries a risk of underestimating the MIC and should be interpreted with caution, especially when the obtained MIC is 1 or 2 doubling dilutions below the S or R clinical breakpoint.
- The low CA (≤90%) for all methods might be explained by the selection of challenge strains with MICs close to the clinical breakpoints.