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Variability of β-lactam susceptibility testing for *Streptococcus pneumoniae* using 4 commercial test methods and broth microdilution

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ABSTRACT

Limited data are available that verify the performance of commercial susceptibility methods for *Streptococcus pneumoniae* following the 2008 Clinical and Laboratory Standards Institute revision of the β-lactam breakpoints. We compared the performance of Etest, M.I.C. Evaluator (M.I.C.E), Vitek, and Sensititre systems to broth microdilution for *S. pneumoniae* susceptibility testing of penicillin, ceftriaxone, meropenem, and amoxicillin. Essential agreement was ≥90% for the majority of the β-lactams and methods tested, particularly for penicillin and ceftriaxone. Categorical agreements (CAs) for penicillin using meningeal and nonmeningeal breakpoints were ≥90%; CAs using penicillin oral breakpoints were 84–89%. Ceftriaxone CAs using nonmeningeal and meningeal breakpoints were 68–88% for Etest, M.I.C.E., and Vitek2 with 6–12% very major errors (VMEs) using meningeal breakpoints. Sensititre CAs for ceftriaxone, amoxicillin, and meropenem were ≥90% with no VMEs. In the context of the current guidelines, there exists considerable method-dependent variability in the susceptibility of *S. pneumoniae* to β-lactams.

1. Introduction

In response to a rise in penicillin MICs with a corresponding increase in isolates labeled as resistant, the Clinical and Laboratory Standards Institute (CLSI) revised β-lactam interpretive breakpoints for *Streptococcus pneumoniae* in 2008 to better reflect clinical and pharmacological data (Weinstein et al., 2009). This change encouraged the use of penicillin monotherapy for nonmeningitis *S. pneumoniae* infection and reduced the incidence of β-lactam resistance (Goossens et al., 2013; Reingold et al., 2008). Currently, CLSI and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) recommend that β-lactam MIC confirmation be done on *S. pneumoniae* isolates that exhibit an oxacillin (1 μg) disk zone diameter ≤19 mm or a penicillin MIC >0.06 μg/mL (CLSI, 2015a; European Committee on Antimicrobial Susceptibility Testing, 2014).

Broth microdilution (BMD), as described by CLSI and EUCAST, is considered the reference standard but is an unlikely method of choice for many clinical laboratories based on the challenges of manufacturing BMD panels in-house. Commercially available methods include the Sensititre® (Thermo Fisher Scientific) BMD panel, Etest® (bioMérieux, Marcy l'Étoile, France) and MIC Evaluator™ (M.I.C.E) (Thermo Fisher Scientific, Basingstoke, United Kingdom) gradient endpoint diffusion methods, and the Vitek®2 AST-STO1 (bioMérieux).

In our laboratory, *S. pneumoniae* susceptibility to penicillin and other β-lactams is determined by a gradient endpoint diffusion test, and isolates with penicillin MIC values ≥0.06 μg/mL are confirmed using a commercial BMD panel. This approach led to the observation of MIC discordance between the β-lactam results of the 2 test methods. Such performance differences have not been reported in previous pneumococcal susceptibility evaluation studies, but those reports applied pre-2008 CLSI β-lactam clinical breakpoints (Jacobs et al., 1992; Jorgensen et al., 1994; Kelly et al., 1999; Kiska et al., 1995; Mittman et al., 2009; Skulnick et al., 1995; Zhang et al., 2011). *S. pneumoniae* with higher MICs to β-lactams are now more frequent (Jones et al., 2013), and the need for additional test method performance data is necessary to assure the accuracy of the pneumococcal β-lactam MICs. The objective of this study was to determine the optimal method of determining β-lactam MICs in clinical isolates of *S. pneumoniae* using BMD as the reference standard and applying the current CLSI susceptibility breakpoints.

2. Methods

2.1. Isolates

Clinical isolates submitted to the Alberta Provincial Laboratory for Public Health (Edmonton, AB, Canada) from 2011 to 2013 with a previously tested penicillin MIC of 0.06–4 μg/mL were selected for

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study. A total of 91 isolates were chosen so that there was an even distribution of penicillin MICs and individual patients were represented once. *S. pneumoniae* strains ATCC 49619 (penicillin MIC 0.25–1 μg/mL) and ATCC 51915 (penicillin MIC 4 μg/mL) were used for quality control, precision, and reproducibility assessments (Clinical and Laboratory Standards Institute, 2015a; Coffey et al., 1995). Clinical isolates were stored in skim milk at −70 °C.

2.2. Sample preparation

As per CLSI guideline M07-A10 (CLSI, 2015b), all isolates were subcultured twice on blood agar 5% (v/v) sheep blood agar plates (Oxoid, Nepean, ON, Canada) in preparation for susceptibility testing. A single 0.5 McFarland suspension in normal saline was used to inoculate each isolate to BMD and all test methods.

BMD was performed as described in CLSI M07-A10 using 4% lysed horse blood in Mueller–Hinton broth (BD, Mississauga, ON, Canada), and trays were incubated at 35 °C for 20–24 h in ambient air. Serial 2-fold dilutions were set up from 0.016 to 8 μg/mL for amoxicillin, penicillin, ceftriaxone, and meropenem. All commercial tests were performed according to the manufacturer's instructions. For Sensititre, the manufacturer's Mueller–Hinton broth with 5% laked horse blood was added as per instructions to the dried antimicrobial panels and incubated in ambient air. For gradient endpoint diffusion methods, 150-mm plates using Mueller–Hinton agar (Thermo Fisher Scientific; Oxoid) with 5% sheep blood were inoculated as per CLSI M07-A10 and incubated at 35 °C for 20–24 h in 5% CO_2. The concentration range of antimicrobials for the Sensititre panel were amoxicillin-clavulanate 2–16 μg/mL (amoxicillin component with a 2:1 ratio amoxicillin:clavulanate), penicillin 0.03–4 μg/mL, ceftriaxone 0.25–2 μg/mL, and meropenem 0.25–2 μg/mL. The calling ranges for the Vitek2 AST-STO1 card were ≤0.12 to ≥8 μg/mL for ceftriaxone and ≤0.06 to ≥8 μg/mL for penicillin.

The quality control strain ATCC 49619 was run every day that testing was performed (n = 17). ATCC 51915 was used to control for variability in measurements at higher MICs and was repeated 11 times for each test method.

Breakpoints were defined as follows (in μg/mL) for each antimicrobial agent according to CLSI M100-S25 (CLSI, 2015a): penicillin (meningitis)—susceptible (S) ≤0.06 and resistant (R) ≥0.12; penicillin (nonmeningitis, parenteral)—S ≤2, intermediate (I) 4, R ≥8; penicillin (oral)—S ≤0.06, I 0.12–1, R ≥2; ceftriaxone (meningitis)—S ≤0.5, I = 1, R ≥2; ceftriaxone (nonmeningitis)—S ≤1, I = 2, R ≥4; amoxicillin—S ≤2, I = 4, R ≥8 (same breakpoints for amoxicillin-clavulanate); meropenem—S ≤0.25, I = 0.5, R ≥1.

2.3. Determining error rate

The performance of the 4 test methods was determined using essential agreement (EA) and categorical agreement (CA). EA was defined as the percentage of isolates with a test method MIC within ±1 doubling dilution of the reference method MIC result. CA was defined as the percentage of isolates with identical breakpoint interpretations of S, I, or R between the challenge and reference test methods. Error rates were calculated as per Food and Drug Administration (FDA) Antimicrobial Susceptibility Test Systems guidance document, CDRH 2009 (US Department of Health and Human Services et al., 2009). Acceptable performance rates were measured as follows: ≥90% for EA or CA, ≤3% for very major errors (VMEs) or major errors (MEs), and ≤10% for minor errors (MiEs). VMEs were defined as the number of VMEs based on interpretation divided by the total number of resistant strains; MEs were defined as the number of MEs based on interpretation divided by the total number of susceptible strains.

2.4. Ethics

The work described in this manuscript does not require ethics review as defined in Section 2.4 of the Government of Canada Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (2014).

3. Results

3.1. Performance of antimicrobial gradient endpoint diffusion methods (Etest and M.I.C.E.)

Of the 91 pneumococcal isolates studied, the penicillin BMD MIC distribution ranged from ≤0.03 to 4 μg/mL (Table 1). Penicillin Etest and M.I.C.E. results were in exact agreement with BMD 48.4% of the time, and EAs were 95% and 93%, respectively (Table 2). However, the MIC distributions for the Etest and M.I.C.E. for all 4 β-lactams trended 1 doubling dilution or more below that of the BMD; there were more isolates testing ≤1 doubling dilution below BMD for Etest (36/91) and M.I.C.E. (33/91) than ≥1 doubling dilution above BMD for Etest (11/91) and M.I.C.E. (14/91). When applying the oral penicillin breakpoints, the CA for both tests was 84% with 15 MiEs caused by interpretations of S or I for actual I or R results, respectively (Table 3). Despite a CA of 93% for the penicillin Etest using meningitis breakpoints, 4 MEs and 2 VMEs were recorded. The penicillin M.I.C.E. strip had 7 MEs and no VMEs using the meningitis breakpoint with a CA of 92%.

The BMD MIC distribution for ceftriaxone ranged from ≤0.03 to 2 μg/mL (Table 1). Ceftriaxone EAs for both gradient endpoint diffusion methods were ≥90% with 58.2% and 38.5% in exact agreement for Etest and M.I.C.E., respectively (Table 2). For ceftriaxone, more isolates tested ≤1 doubling dilution below BMD for Etest (20/91) and M.I.C.E. (42/91) than ≥1 doubling dilution above BMD for Etest (16/91) and M.I.C.E. (12/91). Etest and M.I.C.E. CAs were 77% and 68% using meningitis breakpoints and 88% and 84% using nonmeningitis breakpoints, respectively. One VME was detected using the meningitis breakpoint for Etest and M.I.C.E. (different isolates), as well as several MiEs.

Amoxicillin distribution of BMD MICs ranged from ≤0.03 to 8 μg/mL (Table 1). Amoxicillin EA for Etest and M.I.C.E. was 75% and 92% with 25.3% and 41.8% of isolates testing >1 doubling dilution below and above BMD, respectively (Table 2). Etest and M.I.C.E. CAs were 84% and 85% with VME rates of 92% and 50%, respectively (Table 3).

Meropenem BMD MICs ranged from ≤0.03 to 1 μg/mL (Table 1) with EAs of 86% and 78% for Etest and M.I.C.E., respectively. CAs for meropenem were 71% (6% VME) and 69% (24% VME) for Etest and M.I.C.E., respectively.

3.2. Vitek2 AST-ST01

Penicillin on the Vitek ST01 card yielded 41.2% of isolates in exact agreement with an EA of 89%. There were 32 of 91 isolates with MICs ≤1 doubling dilution below the BMD and 15 of 91 isolates with MICs ≥1 doubling dilution above BMD. The Vitek parenteral penicillin CA was ≥90% with 1 VME (1%) and 19% ME using the meningitis breakpoints; using oral penicillin breakpoints, only MiEs were recorded, and the CA was 86% (Table 3).

For ceftriaxone, Vitek produced an EA of 93% and a CA of 85% using the nonmeningitis breakpoint. Similar to gradient endpoint diffusion methods, application of the meningitis breakpoint to Vitek ceftriaxone MICs yielded a CA of 76% with 12% VME. All but 1 of the MiEs for both

Table 1
MIC distribution of 91 isolates of *S. pneumoniae* used in this study for all antimicrobials tested as determined by broth microdilution.

Antimicrobial	MIC (μg/mL)								
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8
Penicillin	3	13	12	16	11	8	19	9	0
Ceftriaxone	3	18	18	10	8	17	17	0	0
Amoxicillin	12	17	7	8	10	11	11	3	12
Meropenem	30	9	11	13	11	17	0	0	0

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