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Evaluation of CLSI Agar Dilution Method and Trek Sensititre Broth Microdilution Panel for Determining Antimicrobial Susceptibility of *Streptococcus pneumoniae*▽

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Both the CLSI agar dilution method and Trek Sensititre broth microdilution panel for *Streptococcus pneumoniae* antimicrobial susceptibility testing were evaluated against the reference CLSI broth microdilution method using the most recently published CLSI breakpoints. While agar dilution was not an optimal method, the commercial panel appeared to be an acceptable method, with minor errors encountered for ceftriaxone, penicillin, and meropenem.

Broth dilution is the reference method of choice recommended by the CLSI (Clinical and Laboratory Standards Institute) for *Streptococcus pneumoniae* antimicrobial susceptibility testing (AST) (3). Agar dilution is considered an acceptable method for *S. pneumoniae* AST and has been widely used by large-scale laboratories due to high-volume suitability and cost effectiveness; however, the performance of agar dilution using the CLSI method for *S. pneumoniae* AST has not been previously reported in comparison to that of the broth dilution method. A commercial broth microdilution panel (part no. STP5F, Sensititre; Trek Diagnostic Systems, Cleveland, OH) is available for *S. pneumoniae* AST. Recently, the CLSI published new penicillin breakpoints and interpretive criteria for *S. pneumoniae* for the following: oral penicillin, parenteral penicillin (nonmeningitis), and parenteral penicillin (meningitis) (3). The purpose of this study was to evaluate the CLSI agar dilution method and the Trek Sensititre broth microdilution panel for *S. pneumoniae* AST with the most recent CLSI interpretive breakpoints.

A total of 132 *S. pneumoniae* clinical isolates, consisting of a selection of isolates with MICs that span the CLSI criteria, were collected for the evaluation. The CLSI broth microdilution method was chosen as the reference method for this study. The broth microdilution panels were made by following the CLSI broth dilution protocol (2) and were tested against CLSI quality control (QC) strains (*S. pneumoniae* ATCC 49619, *Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 25922) before they were used in the study. Their MIC results were all within the acceptable CLSI QC ranges (3). During the study, *S. pneumoniae* ATCC 49619 was used as a QC strain, and the MIC results of the broth microdilution plates were acceptable if the QC results were within the acceptable ranges. The CLSI agar dilution method was performed by following the CLSI agar dilution procedure, using Mueller-Hinton agar with 5% sheep blood (2). The method for the Trek Sensititre broth microdilution panel was followed according to the manufacturer's instructions. The Sensititre panels were read by a Sensititre SensiTouch reader (Trek Diagnostic Systems, Cleveland, OH). Both the agar dilution plates and the Trek Sensititre panels were run in parallel with the CLSI broth microdilution panels. The MIC was interpreted by the recently published CLSI breakpoints (3). The performance of the two testing methods (agar dilution and the Trek Sensititre panel) was determined using essential agreement (the MIC result of the test method is within ±1 doubling dilution of the MIC result of the reference method) and category agreement (agreement of interpretative results between the test method and the reference method). Error assessment was categorized as "very major error" (VME) (the test method indicates a susceptible response while the reference method indicates a resistant response), "major error" (ME) (the test method indicates a resistant response while the reference method indicates a susceptible response), and "minor error" (MiE) (the test method indicates an intermediate response while the reference method indicates a susceptible or resistant response or the test method indicates a susceptible or resistant response while the reference method indicates an intermediate response). Acceptable performance rates were measured as follows: ≥90% for essential agreement or category agreement, ≤3% for very major error or major error, and ≤7% for major error plus minor error (1, 5).

Eleven antimicrobial agents were evaluated using the agar dilution method, of which five had unacceptable results compared to those of the reference method (Table 1). For ceftriaxone, a very low level of category agreement (53.8 to 68.2%) was observed as a result of 6 VMEs (13.6%) and 42 to 55 MiEs (31.8 to 41.7%); the essential agreement rate (86.6 to 88.9%) was also lower than the acceptable rate (≥90%). MiE rates for penicillin (12.2 to 14.5%), meropenem (16%), and telithromycin (15.2%) were unacceptable. Three VMEs (3.2%) for par-

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TABLE 1. Evaluation of the performance of the CLSI agar dilution method for *S. pneumoniae* AST[c]

Drug	No. of isolates				% EA (no. of isolates that agree/total no. of isolates)[a]	% CA (no. of isolates that agree)	% error rate (no. of isolates that disagree)		
	Total	S	I	R			VME	ME	MiE
Ceftriaxone									
Meningitis	132	60	28	44	88.9 (72/81)	53.8 (71)	13.6 (6)	0	41.7 (55)
Nonmeningitis	132	87	39	6	86.6 (71/82)	68.2 (90)	0	0	31.8 (42)
Clindamycin	131	82	2	47	N/A[b]	93.1 (122)	2.1 (1)	3.7 (3)	3.8 (5)
Erythromycin	131	48	1	82	N/A	97.7 (128)	2.4 (2)	0	0.8 (1)
Levofloxacin	131	104	6	21	N/A	98.5 (129)	0	0	1.5 (2)
Meropenem	131	60	26	45	N/A	84.0 (110)	0	0	16.0 (21)
Penicillin									
Oral penicillin V	131	38	26	67	98.0 (99/101)	87.8 (115)	0	0	12.2 (16)
Parenteral									
Meningitis	131	38	N/A	93	98.0 (99/101)	96.9 (127)	3.2 (3)	2.6 (1)	N/A
Nonmeningitis	131	98	32	1	98.0 (99/101)	85.5 (112)	0	0	14.5 (19)
Chloramphenicol	131	120	N/A	11	90.7 (88/97)	99.2 (130)	0	0.8 (1)	N/A
Tetracycline	132	62	2	68	N/A	97.7 (129)	0	0	2.3 (3)
Telithromycin	132	131	1	0	N/A	84.8 (112)	N/A	0	15.2 (20)
TMP-SMX	129	30	20	79	N/A	97.7 (126)	0	0	2.3 (3)
Vancomycin	132	132	N/A	N/A	N/A	100 (132)	N/A	0	N/A

[a] The denominators of the essential agreement (EA) were less than the total number of the isolates tested because MIC results of some isolates were out of the drug concentration ranges, and thus, the EA cannot be determined.
[b] The EA cannot be determined because the MIC results were out of the drug concentration ranges.
[c] S, susceptible; I, intermediate, R, resistant; EA, essential agreement, CA, category agreement; VME, very major error, ME, major error, MiE, minor error; N/A, not available; TMP-SMX, trimethoprim-sulfamethoxazole.

enteral penicillin (meningitis) and three MEs (3.7%) for clindamycin also occurred. The agar dilution method was unable to reliably determine the susceptibility of the five antimicrobial agents tested for *S. pneumoniae*, particularly ceftriaxone and penicillin, considered first-line agents for the treatment of *S. pneumoniae* infection. In contrast to our study, a British study reported that the agar dilution method using BSAC (British Society for Antimicrobial Chemotherapy) protocols was found to have good agreement with the CLSI (formerly NCCLS) broth microdilution method (5a), although the antimicrobial agents tested in the British study were different from those tested in our study, e.g., ceftriaxone was not assessed in this study, and testing parameters were different (6).

Overall, the CLSI agar dilution method tended to result in lower MICs than the reference method (possibly due to the stability of some drugs, such as ceftriaxone in agar media, and some other factors), resulting in six VMEs (falsely susceptible) for ceftriaxone and three VMEs for penicillin. Moreover, growth of *S. pneumoniae* on agar media may not be optimal due to its fastidious nature (2). This assumption may be supported by the notion that special formulations and supplements are needed for testing fastidious organisms using agar media; for example, GC agar base and 1% defined growth supplement are required for testing *Neisseria gonorrhoeae* (2).

Fourteen antimicrobial agents were evaluated using the Trek Sensititre broth microdilution panel. Three antimicrobial agents had MiEs compared to those evaluated by the reference method (Table 2): ceftriaxone (11.5 to 13%), penicillin (10 to 17.7%), and meropenem (9.2%). Two MEs (5.3%) occurred with parenteral penicillin (meningitis) breakpoints. Almost all the MICs accounting for the discrepant results were clustered at the breakpoints. For example, all MICs causing MiEs for ceftriaxone were at 0.5, 1, and 2 μg/ml, the interpretive breakpoints for *S. pneumoniae* susceptibility. The two parenteral penicillin (meningitis) MICs that were MEs were at 0.12 μg/ml (resistant breakpoint), and the reference MICs were at 0.06 μg/ml (susceptible breakpoint). Due to the lack of an intermediate interpretation category, a one-dilution difference resulted in two MEs (falsely resistant).

We also conducted a 3-month prospective study to test the reproducibility of the commercial panel and to rule out any technical error attributable to the above-described discrepancies. The prospective study was done using the new isolates prospectively collected during the 3-month period and with the same batch of the Sensititre plates. Any isolate with a MIC at the breakpoint for ceftriaxone, penicillin, and/or meropenem had repeat AST performed. A total of 159 isolates required repeat testing, of which 20 showed MICs different from those found when the first AST was performed, but all were within ±1 doubling dilution except 1 isolate. Only four repeats (two for ceftriaxone and two for meropenem) resulted in a change in the interpretation from intermediate to resistant and vice versa. Of note, the one repeat (ceftriaxone) with a two-doubling-dilution difference did not result in a change in the interpretation. These results demonstrated that the Trek Sensititre panel was not only found to have acceptable reproducibility (only 1 out of 159 repeat tests [≤1%] had a MIC outside the range by one doubling dilution) but also gave an acceptable level of accuracy because only 2.5% (4/159) of the repeat tests resulted in a different interpretive category.

TABLE 2. Evaluation of the performance of the Trek Sensititre broth microdilution panel for *S. pneumoniae* AST[c]

Drug	No. of isolates				% EA (no. of isolates that agree/ total no. of isolates)[a]	% CA (no. of isolates that agree)	% error rate (no. of isolates that disagree)		
	Total	S	I	R			VME	ME	MiE
Ceftriaxone									
Meningitis	131	59	28	44	98.8 (81/82)	88.5 (116)	0	0	11.5 (15)
Nonmeningitis	131	86	39	6	98.8 (80/81)	87.0 (114)	0	0	13.0 (17)
Clindamycin	130	81	2	47	N/A[b]	98.5 (128)	0	0	1.5 (2)
Erythromycin	129	48	0	81	N/A	99.2 (128)	0	0	0.8 (1)
Levofloxacin	131	104	6	21	99.0 (98/99)	98.5 (129)	0	0	1.5 (2)
Meropenem	131	60	26	45	100 (71/71)	90.8 (119)	0	0	9.2 (12)
Penicillin									
Oral penicillin V	130	38	25	67	98.9 (90/91)	90.0 (117)	0	0	10.0 (13)
Parenteral									
Meningitis	130	38	N/A	92	98.9 (90/91)	98.5 (128)	0	5.3 (2)	N/A
Nonmeningitis	130	97	32	1	98.9 (90/91)	82.3 (107)	0	0	17.7 (23)
Chloramphenicol	131	120	N/A	11	94.4 (117/124)	99.2 (130)	0	0.8 (1)	N/A
Tetracycline	130	61	2	67	N/A	97.7 (127)	0	0	2.3 (3)
Telithromycin	131	130	1	0	N/A	99.2 (130)	N/A	0	0.8 (1)
TMP-SMX	131	32	20	79	91.7 (22/24)	97.7 (128)	0	0	2.3 (3)
Vancomycin	131	131	N/A	N/A	N/A	100 (131)	N/A	0	N/A
Linezolid	132	131	N/A	N/A	92.2 (118/128)	100 (131)	N/A	0	N/A
Cefuroxime (sodium)	130	49	3	78	N/A	97.7 (127)	0	0	2.3 (3)
Moxifloxacin	131	111	5	15	N/A	96.2 (126)	0	0	3.8 (5)

[a] The denominators of the essential agreement (EA) were less than the total number of the isolates tested because MIC results of some isolates were out of the drug concentration ranges, and thus, the EA cannot be determined.
[b] The EA cannot be determined because the MIC results were out of the drug concentration ranges.
[c] S, susceptible; I, intermediate, R, resistant; EA, essential agreement, CA, category agreement; VME, very major error, ME, major error, MiE, minor error; N/A, not available; TMP-SMX, trimethoprim-sulfamethoxazole.

Overall, the Trek Sensititre panel was not found to produce significant VME (falsely susceptible) and ME (falsely resistant) rates, although 9.2 to 17.7% MiE rates for penicillin, ceftriaxone, and meropenem were encountered. Other investigators have also observed minor errors for ceftriaxone and penicillin when they used commercial broth microdilution panels for *S. pneumoniae* AST (4, 7). In our study, most of the MiEs occurred when the reference method classified an isolate as susceptible or intermediate but the Trek Sensititre panel classified it as intermediate or resistant; thus, the Trek Sensititre panel appears to "overcall" resistance, resulting in no resistant isolates being falsely identified as susceptible. Therefore, although the Trek Sensititre panel showed relatively low error rates for the three key antimicrobial agents, it is an acceptable method for *S. pneumoniae* susceptibility testing, in conjunction with its ease of use and reagent storage conditions (at room temperature).

In summary, we found that agar dilution was not an optimal method for *S. pneumoniae* AST. In contrast, the Trek Sensititre broth microdilution panel appears to be an acceptable commercial method for routine *S. pneumoniae* AST, although minor errors may occur for ceftriaxone, penicillin, and meropenem.

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