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Evaluation of the Dade MicroScan MICroSTREP Antimicrobial Susceptibility Testing Panel with Selected *Streptococcus pneumoniae* Challenge Strains and Recent Clinical Isolates

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The MicroScan MICroSTREP panel is a recently marketed frozen broth microdilution panel for susceptibility testing of various streptococci, including *Streptococcus pneumoniae*. The panel contains 10 antimicrobial agents in cation-adjusted Mueller-Hinton broth supplemented with 3% lysed horse blood, similar in concept to the National Committee for Clinical Laboratory Standards (NCCLS) reference broth microdilution method for testing streptococci. A group of 210 isolates of *S. pneumoniae* were selected to include isolates with previously documented resistance to agents incorporated in the MICroSTREP panel, as well as recent invasive clinical isolates. All isolates were tested simultaneously with the MICroSTREP panel and an NCCLS reference panel whose drug concentrations were prepared to coincide with those of the MICroSTREP panel. Of the 210 isolates, 5 failed to grow in the MICroSTREP panel; 3 of those also did not grow in the reference panel. Essential agreement of MICs determined by the two methods (test MIC ± one dilution of the reference MIC) was 99.6% overall and ranged from 98.0% with chloramphenicol to 100% with penicillin, ceftriaxone, erythromycin, tetracycline, and vancomycin. There were no very major or major interpretive category errors resulting from the MICroSTREP panel tests. Minor interpretive category errors ranged from 12.2% with cefotaxime and 9.8% with ceftriaxone (due mainly to clustering of MICs for the selected strains near the breakpoints) to 0% with chloramphenicol and vancomycin. These results indicate that the MicroScan MICroSTREP frozen panels provide susceptibility results with pneumococci that are essentially equivalent to results derived by the NCCLS reference broth microdilution procedure.

Streptococcus pneumoniae is now the most common causative agent of bacterial meningitis, otitis media, and sinusitis in the United States, as well as being a common cause of pneumonia and bacteremia (3, 21). Antibiotic resistance among *S. pneumoniae* clinical isolates has been documented worldwide (5, 13, 17, 22) and has risen sharply in the United States since approximately 1990 (2, 3, 5–8). In some areas of the United States, more than 50% of pneumococcal strains are no longer susceptible to penicillin (10). Pneumococci may also be resistant to other classes of drugs, including chloramphenicol (by production of the inactivating enzyme chloramphenicol acetyltransferase), and to macrolides, clindamycin, tetracyclines, rifampin, or trimethoprim-sulfamethoxazole (17). The extended-spectrum cephalosporins have been widely used for empiric therapy of serious pneumococcal infections during the era of increasing penicillin resistance (7). However, the alteration of certain of the penicillin-binding protein targets that results in penicillin resistance in pneumococci can also give rise to high-level resistance to extended-spectrum cephalosporins (18). Specific modifications to the penicillin-binding proteins dictate the degree of resistance to penicillin and may result in some strains being more resistant to the extended-spectrum cephalosporins than to penicillin. Indeed, pneumococcal meningitis treatment failures have been reported due to strains highly resistant to cefotaxime and ceftriaxone (4, 7, 12, 23).

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The rapid emergence of antimicrobial resistance in pneumococci has heightened the importance of reliable and convenient susceptibility testing methods for use by clinical laboratories. The National Committee for Clinical Laboratory Standards (NCCLS) has published guidelines for performance of broth microdilution and agar disk diffusion tests of streptococci with a number of antimicrobial agents (19, 20). The present study has evaluated the MicroScan MICroSTREP frozen antimicrobial susceptibility testing panel, a recently marketed broth microdilution product that is patterned after the NCCLS reference MIC method.

MATERIALS AND METHODS

Test organisms. Two hundred ten unique isolates of *S. pneumoniae* of clinical origin were selected for use in this evaluation. These included 115 strains with previously documented resistance to one or more antimicrobial agents that were intended to challenge the ability of the MICroSTREP panels to detect resistant pneumococci. The remaining 95 strains were recent clinical isolates undergoing susceptibility testing as part of a large North American surveillance program.

Antimicrobial agents. The antimicrobial agents (and concentration ranges) included in both the MICroSTREP and the reference panels were penicillin (0.03 to 4 μg/ml), ampicillin (0.03 to 8 μg/ml), cefotaxime (0.03 to 2 μg/ml), ceftriaxone (0.03 to 2 μg/ml), erythromycin (0.03 to 1 μg/ml), clindamycin (0.03 to 1 μg/ml), chloramphenicol (0.25 to 16 μg/ml), tetracycline (0.06 to 8 μg/ml), trimethoprim-sulfamethoxazole (0.06 to 4 μg/ml; based on the trimethoprim component in a 1:19 ratio), and vancomycin (0.03 to 4 μg/ml).

Broth microdilution reference susceptibility tests. For comparison with MICs generated with the MICroSTREP panels, the MIC of each isolate was determined by the broth microdilution procedure recommended by the NCCLS (19). This testing involved preparation of microdilution panels for the purposes of this study that contained the same concentration ranges of antimicrobial agents included in the MICroSTREP panels. The medium for the reference panels was cation-adjusted Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with 3% lysed horse blood. Inocula of the test organisms were pre-

TABLE 1. Comparison of MICs determined by the MICroSREP panel with MICs determined by the NCCLS reference method for 205 isolates of *S. pneumoniae*

Antimicrobial agent	No. of MicroStrep MICs within indicated $\log_2$ of reference MICs						% Agreement within ± $\log_2$ dilution
	Less than −2	−2	−1	Same	+1	+2	
Ampicillin			12	176	17		100
Penicillin			24	175	6		100
Cefotaxime			3	170	31	1	99.5
Ceftriaxone			4	177	24		100
Erythromycin			68	133	4		100
Clindamycin	1		49	145	10		99.5
Chloramphenicol	1	3	59	136	6		98.0
Tetracycline			77	123	5		100
Trimeth-sulfa[a]			17	162	25	1	99.5
Vancomycin			169	36			100

[a] Trimethroprim-sulfamethoxazole tested in a 1:19 ratio.

pared from colonies grown on sheep blood agar plates (Becton Dickinson Microbiology Systems, Cockeysville, Md.) that had been incubated for 20 to 24 h in 5% CO_2. Colonies were suspended in 0.9% saline to obtain a suspension with a turbidity equivalent to that of a 0.5 McFarland standard, and the suspension was further diluted 1:10 in 0.9% saline within 15 min. This process provided a final inoculum density of approximately 5×10^5 CFU/ml in the wells of the microdilution panels following transfer with disposable 96-prong plastic inoculators (Dynatech Laboratories, Inc., Chantilly, Va.). Colony counts of positive control wells were performed periodically to verify the desired inoculum concentrations. The reference microdilution panels were incubated at 35°C in ambient air for 20 to 24 h prior to visual determination of MICs.

MICroSTREP susceptibility tests. The MICroSTREP panels were inoculated and read according to the manufacturer's recommendations. Briefly, inoculum suspensions with turbidity equivalent to that of the 0.5 McFarland standard were prepared in 3 ml of MicroScan inoculum water. Two milliliters of the adjusted suspension was added to 25 ml of MicroScan inoculum water with Pluronic-F, the tube was inverted, and then its contents were transferred to a MicroScan disposable inoculum transfer device. This process provided a final inoculum density of approximately 5×10^5 CFU/ml in the wells of the MICroSTREP panels. The inoculated panels were then incubated at 35°C in ambient air for 20 to 24 h prior to visual determination of MICs.

Quality control organism. The NCCLS control strain, *S. pneumoniae* ATCC 49619 (19), was included each day in both the reference and MICroSTREP panels to ensure the adequacy of the reagents and procedures.

Comparison of results. MICs of each drug determined by the MICroSTREP panel for each strain were compared to the MICs determined by the NCCLS reference procedure. A susceptibility category was assigned to each MIC based on the current NCCLS breakpoint criteria (19). Interpretive errors were assessed with each drug based on the following definitions: a very major error indicated that a strain was shown to be susceptible by MICroSTREP but resistant by the reference method; a major error indicated that a strain was shown to be resistant by MICroSTREP but susceptible by the reference method; and a minor error indicated that a strain was shown to be intermediate by either the MICroSTREP or reference method and either susceptible or resistant by the other method.

RESULTS

This study has evaluated the MicroScan MICroSTREP frozen broth microdilution panel for susceptibility testing of pneumococci. Only 5 of the 210 test isolates failed to grow in the MICroSTREP panel, and 3 of those also failed to grow sufficiently in the NCCLS reference panel used for comparative purposes in this study. The MICs of 10 antimicrobial agents generated with the MICroSTREP panel agreed very closely with the reference MICs for this collection of selected stock strains and recent clinical isolates. Table 1 depicts a comparison of the MICs of each agent determined by MICroSTREP and those determined by the reference panel. Essential agreement of MICs (MICroSTREP MIC the same as or within one dilution increment of the reference MIC) was 99.6% overall. The lowest degree of agreement was 98.0% with chloramphenicol; the highest degree of agreement between methods was 100% with penicillin, ceftriaxone, erythromycin, tetracycline, and vancomycin. As depicted in Table 2, there were no very major or major interpretive category errors resulting from the MICroSTREP panel tests with the nine agents for which NCCLS interpretive criteria exist. Minor interpretive category errors ranged from highs of 12.2% with cefotaxime and 9.8% with ceftriaxone (due mainly to clustering of MICs for the selected strains near the breakpoints) to 0% with chloramphenicol and vancomycin.

DISCUSSION

The rapid emergence of resistant strains has placed considerable importance on accurate antimicrobial susceptibility methods for routine use with pneumococci. Resistance to all currently available agents used to treat pneumococcal infections, with the notable exception of vancomycin has been recognized (3, 5, 7, 11, 17). Most recently, this has included the recognition of resistance to several members of the quinolone class of antibiotics (14, 24). In response to this problem, collaborative studies organized through the NCCLS have established generic broth dilution and disk diffusion susceptibility testing methods, quality control values, and interpretive break-

TABLE 2. Interpretive category errors determined from a comparison of MICroSTREP and reference MICs

Antimicrobial agent	Category[a]	No. of reference MICs	No. (%) of minor interpretive errors[b]
Penicillin	S	113	1 (0.5)
	I	19	
	R	73	
Cefotaxime	S	135	25 (12.2)
	I	35	
	R	35	
Ceftriaxone	S	130	20 (9.8)
	I	41	
	R	34	
Erythromycin	S	151	1 (0.5)
	I	1	
	R	53	
Clindamycin	S	192	5 (2.4)
	I	4	
	R	9	
Chloramphenicol	S	194	0
	I	0	
	R	11	
Tetracycline	S	180	2 (0.9)
	I	1	
	R	24	
Trimeth-sulfa[c]	S	101	4 (1.9)
	I	10	
	R	94	
Vancomycin	S	205	0
	I	0	
	R	0	

[a] Strains shown to be susceptible (S), intermediately susceptible (I), or resistant (R) to the indicated agent at the reference MIC(s).

[b] There were no major or very major errors (see the text for definitions).

[c] Trimethoprim-sulfamethoxazole tested in a 1:19 ratio.

point criteria specific for pneumococci (15, 16, 19, 20). While many laboratories may have chosen in the past to screen pneumococci only from blood, cerebrospinal fluid, or other sterile body fluids for penicillin resistance, the rapid spread of multidrug-resistant strains requires a more aggressive approach today. The NCCLS now recommends that all pneumococcal isolates from patients with meningitis be tested for susceptibility to penicillin, either cefotaxime or ceftriaxone, and vancomycin upon initial isolation (19, 20). With cephalosporin-resistant strains, it may be helpful to test chloramphenicol and rifampin as well (11, 12). Non-central nervous system isolates should be tested routinely with erythromycin and trimethoprim-sulfamethoxazole, in addition to penicillin (3, 11, 19, 20). Other agents that may be active against multiply resistant pneumococci include clindamycin and certain newer quinolones (1, 3, 11, 15).

The NCCLS disk diffusion test is convenient and results are reproducible with various non-beta-lactam antibiotics (15, 16), but it has the recognized shortcoming of not providing acceptable accuracy with pneumococci and some of the most important drugs, e.g., the penicillins and cephalosporins. Excessive major and minor errors have been recognized when various beta-lactams have been tested by the disk diffusion method (16). The 1-μg-oxacillin disk can be used to screen for penicillin susceptibility, indicated by a zone of inhibition with a diameter of ≥20 mm. Such strains are also predictably susceptible to other beta-lactam antibiotics that normally have activity against pneumococci, including most cephalosporins (20). However, if the oxacillin zone is <20 mm in diameter, it is necessary to determine the penicillin MIC to clarify whether an isolate is resistant, intermediate, or borderline susceptible to penicillin (20). Two large studies have shown 11 to 14% major interpretive errors (false resistance) with the oxacillin disk screen test (9, 16). Similar minor error rates of more than 15% also compromised the diagnostic usefulness of the disk test for cefotaxime and ceftriaxone (16). Because screening first with an oxacillin disk may delay appropriate therapy, the NCCLS encourages laboratories to examine pneumococcal isolates from patients with meningitis by an MIC method with the antibiotics listed above as soon as colonies become available for testing (20).

The MicroScan MICroSTREP frozen panel appears to yield results essentially comparable to those obtained by the NCCLS reference broth microdilution MIC method. The frozen panels incorporate the use of 3% lysed horse blood-supplemented Mueller-Hinton broth, along with 10 antimicrobial agents useful in the therapy of pneumococcal infections. The results obtained in this study indicate very close agreement with those obtained with a reference panel that followed the NCCLS methodology explicitly. There were no very major or major interpretive errors, and relatively few minor errors, resulting from these tests. The exception to that statement was a seemingly large percentage of minor errors with cefotaxime and ceftriaxone. However, it is important to note that those compounds have an intermediate category at only a single concentration (19) and that the minor errors resulted primarily from the frozen challenge strains for which MICs clustered at the breakpoints separating the three interpretive categories. Of greater importance was the agreement of MICroSTREP MICs (± one dilution) with the reference MICs in 98% or more of tests.

Potential shortcomings of the MICroSTREP panel include possible difficulties in visualizing the MIC endpoints in the panel if a suitable viewing device is not employed. We preferred to use a simple parabolic magnifying mirror incorporated in an aluminum stand that allowed clear visualization of the bottoms of the panel wells. In many instances, the "browning" of the lysed horse blood supplement assisted us in recognizing the growth endpoints. However, it is important to examine the wells carefully for the presence of buttons of growth, irrespective of any color change of the medium. Reliable determinations of MICs with pneumococci require that the wells of a panel be clearly visualized.

The MICroSTREP panel incorporates ampicillin, which is intended by the manufacturer for testing of streptococci other than *S. pneumoniae*. NCCLS interpretive criteria have been developed for pneumococci with amoxicillin but not ampicillin. The usefulness of the MICroSTREP panel may be improved by addition of amoxicillin, cefuroxime, meropenem, levofloxacin, and perhaps sparfloxacin, all of which have been approved by the U.S. Food and Drug Administration for therapy of pneumococcal infections and for which NCCLS interpretive breakpoints have been published (19, 20). Perhaps still newer agents for treating multidrug-resistant pneumococci will become available for clinical use in a few years. It will be imperative for manufacturers of commercial systems for susceptibility testing of pneumococci to endeavor to provide the ability to test accurately the most relevant agents available for therapy of infections due to *S. pneumoniae.*

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