Comparison of BD Phoenix to Vitek 2, MicroScan MICroSTREP, and Etest for Antimicrobial Susceptibility Testing of Streptococcus pneumoniae^{∇}

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The performance of the BD Phoenix Automated Microbiology System (BD Diagnostic Systems) was compared to those of the Vitek 2 (bioMérieux), the MicroScan MICroSTREP *plus* (Siemens), and Etest (bio-Mérieux) for antibiotic susceptibility tests (AST) of 311 clinical isolates of *Streptococcus pneumoniae*. The overall essential agreement (EA) between each test system and the reference microdilution broth reference method for *S. pneumoniae* AST results was >95%. For Phoenix, the EAs of individual antimicrobial agents ranged from 90.4% (clindamycin) to 100% (vancomycin and gatifloxacin). The categorical agreements (CA) of Phoenix, Vitek 2, MicroScan, and Etest for penicillin were 95.5%, 94.2%, 98.7%, and 97.7%, respectively. The overall CA for Phoenix was 99.3% (1 very major error [VME] and 29 minor errors [mEs]), that for Vitek 2 was 98.8% (7 VMEs and 28 mEs), and those for MicroScan and Etest were 99.5% each (19 and 13 mEs, respectively). The average times to results for Phoenix, Vitek 2, and the manual methods were 12.1 h, 9.8 h, and 24 h, respectively. From these data, the Phoenix AST results demonstrated a high degree of agreement with all systems evaluated, although fewer VMEs were observed with the Phoenix than with the Vitek 2. Overall, both automated systems provided reliable AST results for the *S. pneumoniae*-antibiotic combinations in half the time required for the manual methods, rendering them more suitable for the demands of expedited reporting in the clinical setting.

Streptococcus pneumoniae is the leading cause of communityacquired pneumonia in adults and serious respiratory infections in children in the United States. Globally, septicemia is a major cause of infant mortality in developing nations. Penicillin is the antimicrobial agent of choice, and macrolides are the second most common alternative. Within the last two decades, the emergence of strains of *S. pneumoniae* that are resistant to penicillin, macrolides, and other antimicrobial agents has become a serious health care problem (15). As of 2005, 18% of *S. pneumoniae* isolates in the United States were reported as penicillin resistant (9), and internationally they account for up to 60% of isolates (South Africa) (3).

The rise in drug resistance of *S. pneumoniae* underscores the need for clinical microbiology laboratories to accurately determine its antimicrobial susceptibility profile in a timely manner. Rapid reporting of antimicrobial susceptibility test (AST) results has been shown to improve patient outcomes and to reduce hospital costs (2, 10, 19). To this end, automated AST systems offer the promise of shorter turnaround times to results. The literature includes reports evaluating the individual performance of each of the following AST systems for *S. pneumoniae*: Vitek 2, BD Phoenix, MicroScan MICroSTREP, and Etest (1, 13, 14, 16, 20–22). However, to the best of our knowledge a cross-comparative study of all these systems has not been conducted to date. Therefore, the present study was de-

* Corresponding author. Mailing address: CHC3-326, Clinical Microbiology Service, NewYork-Presbyterian Hospital, Columbia University Medical Center, 622 West 168th St., New York, NY 10032. Phone: (212) 305-6237. Fax: (212) 305-8971. E-mail: whittie@nyp.org. signed to evaluate their performance and time to results (TTR).

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MATERIALS AND METHODS

Test isolates. A total of 311 clinical isolates of *S. pneumoniae*, recovered from patients at the NewYork-Presbyterian Hospital, Columbia University Medical Center, were evaluated. All 311 strains were tested using the Phoenix, MicroScan, Etest, and PASCO reference method; 19 strains failed to grow with the Vitek 2, thus limiting the Vitek 2 evaluation to 292 clinical isolates. The specimen sources were predominately respiratory (70%), blood (12%), and eye (7%). Prior to testing, all isolates were subcultured onto BBL Columbia agar with 5% sheep blood and incubated at 35°C with 5% CO₂ for 18 to 24 h. Isolates were tested concurrently on all systems. Manufacturer's guidelines were followed with all commercial AST methods utilized in this study. The hands-on time required for each method was determined for a subset of isolates via a stopwatch controlled by an objective observer.

Phoenix system. The identification (ID) and AST combination panels (SMIC/ID-100) for the Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) (software version 5.02H/4.11B) were used in this study. They contain antimicrobial agents on one side of the panel and ID substrates on the other side. Each bacterial culture was adjusted to a 0.5 to 0.6 McFarland standard using the CrystalSpec nephelometer (BD), and 25 μ l was transferred to AST broth to obtain a final inoculum density of approximately 5 × 10⁵ CFU/ml. The contents of the AST broth tubes were mixed, poured into the AST side of the panel, and loaded into the Phoenix carousel within 30 min of inoculation.

Vitek 2. Isolates were adjusted to a McFarland standard of 0.5 to 0.63 in 0.45% sodium chloride using the Vitek DensiChek densitometer. The Vitek 2 (software version 4.02) AST-GP62 cards (bioMérieux, Marcy l'Etoile, France) were inoculated from the suspension vial using the Smart Carrier Station and loaded into the Vitek 2 automated reader-incubator.

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Etest. Etest strips (bioMérieux, Marcy l'Etoile, France) were placed on 49 50 mm Mueller Hinton agar plates with 5% sheep blood (BD Diagnostics Systems), which had been inoculated with a 0.5 McFarland standard suspension of test isolates. All plates were incubated at 35°C with 5% CO₂ for 20 to 24 h before being examined. The MIC was determined to be the value at which the elliptical growth margin intersected the Etest strip.

MicroScan. MicroScan (Siemens Healthcare Diagnostics, Deerfield, IL) MICroSTREP *plus* panels were inoculated with the Renok hydrator/inoculator, which delivered 115 μ l of Mueller-Hinton broth with 2 to 5% (vol/vol) lysed horse blood to each well. After inoculation with a 0.5 McFarland standard bacterial suspension, the panels were incubated at 35°C in ambient air for 20 to 24 h prior to visual determination of MICs.

Reference method. Frozen BD Pasco Panels (BD, Sparks, MD) microdilution trays containing cation-adjusted Mueller-Hinton broth supplemented with 2 to 5% lysed horse blood prepared by Pasco laboratories (BD Diagnostics) were used in this study in accordance with the Clinical Laboratory Standards Institute (CLSI) 2007 guidelines (7).

Antimicrobial agents. Results for antimicrobial agents were organized in accordance with CLSI test/report groups (7) as follows: group A, appropriate for primary testing panel; group B, may warrant primary testing but selectively reported; group C, alternative or supplemental agents; and group O, generally not routinely tested and reported in the United States. The antimicrobial agents within each group were as follows: A, erythromycin, penicillin, and trimethoprim-sulfamethoxazole (T/S); B, cefepime, cefotaxime, ceftriaxone, clindamycin, levofloxacin, meropenem, tetracycline, and vancomycin; C, linezolid; and O, gatifloxacin. Of the 13 agents tested, ceftriaxone, erythromycin, levofloxacin, linezolid, penicillin, T/S, and vancomycin were common to all methods. Clindamycin and meropenem were shared by all test methods except Vitek 2, cefotaxime and tetracycline were shared by all but Etest, and cefepime and gatifloxacin were shared only by Phoenix and MicroScan.

Interpretation of results and resolution of discrepancies. To avoid unnecessary variability in the visual determination of MICs, MicroScan and Pasco results were read by the same individual. Discordant results were resolved by repeat testing of all AST methods, including the reference method. Analyses were performed using discordant data that persisted through secondary testing. Isolates were classified as susceptible, intermediate, or resistant according to their categorical susceptibility using CLSI 2007 breakpoint values (7). Our isolate collection did not include any cerebrospinal fluid sources; therefore, nonmeningitis CLSI breakpoints for penicillin (susceptible, MIC of $\leq 0.06 \mu g/ml$; intermediate, MIC of 0.125 to 1.0 $\mu g/ml$; and resistant, MIC of $\geq 2 \mu g/ml$) were utilized.

Data analysis. Assessment of method variability included calculation of essential agreement (EA), defined as the percentage of MIC results within a single doubling dilution of MICs as determined by the reference method for each antibiotic tested. Categorical agreement (CA) was defined as test MICs interpreted within the same susceptibility category as the reference method. The derivation of CA involved the interpretation of all MIC data in accordance with CLSI criteria and compared against the reference method. The equality of agreement (EA and CA) between methods (the null hypothesis was that the agreements were equivalent $[H_0: P_1 = P_2]$) was examined by proportion analysis using the two-proportion Z-test using 99% as a confidence level (P = 0.01). AST error rates were calculated and reported according to the following definitions: very major error (VME), false resistant result; and minor error (mE), intermediate result reported as either susceptible or resistant.

Quality control. All AST systems recommended weekly testing of strains *S. pneumoniae* ATCC 49619 and *Streptococcus agalactiae* ATCC 13813 for quality control purpose. The AST results from the reference strains were consistently within the acceptable MIC range (data not shown).

RESULTS

The 311 test isolates of *S. pneumoniae* were categorized based on susceptibility to penicillin, as determined by the reference method using CLSI 2007 criteria (7). There were 204 (65.6%) penicillin-susceptible, 67 (21.5%) penicillin-intermediate, and 40 (12.9%) penicillin-resistant strains. The categorical proportion of isolates used in this study was representative of all *S. pneumoniae* isolates at our medical center.

With regard to the AST results reported by the Phoenix system for each individual antimicrobial agent, compared to

TABLE 1. EA and CA of AST compared to the reference method

	% Agreement									
Antimicrobial	A	utomat	ed AS	Т	Manual AST					
agent	Phoenix		Vitek 2		MicroScan		Etest			
	EA	CA	EA	CA	EA	CA	EA	CA		
Penicillin	90.7	95.5	91.8	94.2	95.8	98.7	96.1	97.7		
Erythromycin	92.0	99.7	94.2	97.3	96.8	99.7	97.1	100		
T/Š	92.6	98.1	92.4	97.9	96.1	97.7	96.1	98.4		
Cefepime	95.5	99.7	NA ^a	NA	99.7	99.7	NA	NA		
Cefotaxime	94.5	100	97.9	100	99.0	100	NA	NA		
Ceftriaxone	97.1	99.7	96.9	100	99.7	99.7	100	100		
Clindamycin	90.4	100	NA	NA	98.4	99.4	95.5	100		
Levofloxacin	96.5	100	94.8	100	100.0	100	100	100		
Meropenem	94.2	98.4	NA	NA	98.7	99.4	98.4	99.7		
Tetracycline	95.5	99.4	93.1	98.6	99.4	99.7	NA	NA		
Vancomycin	100	100	98.3	100	100	100	100	100		
Linezolid	98.4	100	95.9	100	100	100	100	100		
Gatifloxacin	100	100	NA	NA	100	100	NA	NA		
	agent Penicillin Erythromycin T/S Cefepime Cefotaxime Ceftriaxone Clindamycin Levofloxacin Meropenem Tetracycline Vancomycin Linezolid	Anumicrooral agent Pho agent Pho EA Penicillin Perythromycin 90.7 T/S 92.0 Cefepime 95.5 Cefotaxime 94.5 Ceftriaxone 97.1 Clindamycin 90.4 Levofloxacin 96.5 Meropenem 94.2 Tetracycline 95.5 Vancomycin 100 Linezolid 98.4	Antumicrobial agentPhoenixEACAPenicillin Erythromycin T/S 90.7 95.5 92.0 99.7 $7/5$ 92.6 98.1 Cefepime Cefotaxime Ceftriaxone Levofloxacin 95.5 99.7 Clindamycin Levofloxacin 90.4 100 Levofloxacin Meropenem Vancomycin 94.2 98.4 Vancomycin Linezolid 98.4 100	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		

^a NA, not applicable.

the reference method, the EAs ranged from 90.4% (clindamycin) to 100% (vancomycin and gatifloxacin); the penicillin EA was 90.7% (Table 1). The CAs for the Phoenix system ranged from 95.5% (penicillin) to 100% (cefotaxime, clindamycin, levofloxacin, vancomycin, linezolid, and gatifloxacin). By comparison, the Vitek 2 EA ranges were 91.8% (penicillin) to 98.3% (vancomycin), and the CA ranged from 94.2% (penicillin) to 100% (cefotaxime, ceftriaxone, vancomycin, and linezolid). The MicroScan EA ranges were 95.8% (penicillin) to 100% (levofloxacin, vancomycin, linezolid, and gatifloxacin), and the CA ranges were 97.7% (T/S) to 100% (cefotaxime, levofloxacin, vancomycin, linezolid, and gatifloxacin). For Etest, the EA ranges were 95.5% (clindamycin) to 100% (ceftriaxone, levofloxacin, vancomycin, and linezolid), and the CA ranged from 97.7% (penicillin) to 100% (erythromycin, ceftriaxone, clindamycin, levofloxacin, vancomycin, and linezolid).

In terms of interpretive errors (Table 2) compared to the reference method, no MEs were observed during the course of the study with the two automated systems. Twenty-three isolates resulted in a total of 30 discrepancies (29 mEs and 1 VME) with Phoenix. Vitek 2 resulted in 35 discrepancies (28 mEs and 7 VMEs) associated with 31 isolates. MicroScan and Etest generated only mEs (19 and 13, respectively). The largest proportion of total mEs were observed with penicillin and T/S, combined, for all test methods (Phoenix, 69%; Vitek 2, 79%; MicroScan, 58%; and Etest, 92%) (Table 2). Of the 42 isolates yielding penicillin and T/S mEs, 18 of these isolates also produced two or more errors for other antimicrobials between methods (data not shown). Ten isolates were responsible for more than one error within an AST method, and 20 isolates were the source of multiple errors across methods (data not shown). The single Phoenix VME occurred when the system failed to detect erythromycin resistance. The same isolate resulted in a Vitek 2 erythromycin VME. In total, Vitek 2 produced seven VMEs, six of which were with group A antimicrobial agents (five with erythromycin and one with penicillin) and one of which was with tetracycline.

An analysis of mEs with respect to penicillin susceptibility

	Antimicrobial agent					No. (%) of interpretative errors						
CLSI group		No. in category ^a :		Automated systems				Manual methods				
					Phoenix		Vitek 2					
		S	Ι	R	mEs	VMEs	mEs	VMEs	MicroScan, mEs	Etest, mEs		
А	Penicillin Erythromycin T/S	204 236 242	67 1 15	40 74 54	$ \begin{array}{c} 14 (4.5) \\ 0 \\ 6 (1.9) \end{array} $		16 (5.5) 3 (1.0) 6 (2.1)	1 (0.3) 5 (1.7) 0	4 (1.3) 1 (0.3) 7 (2.3)	7 (2.3) 0 5 (1.6)		
В	Cefepime Cefotaxime Ceftriaxone Clindamycin Levofloxacin Meropenem Tetracycline Vancomycin	281 294 297 283 307 265 262 311	25 11 5 1 1 19 3 0	5 6 9 27 3 27 46 0	$ \begin{array}{c} 1 (0.3) \\ 0 \\ 1 (0.3) \\ 0 \\ 5 (1.6) \\ 2 (0.6) \\ 0 \end{array} $	0 0 0 0 0 0 0 0	${{\rm NA}^b \atop 0} \\ 0 \\ {\rm NA} \\ 0 \\ {\rm NA} \\ 3 (1.0) \\ 0$	NA 0 NA 0 NA 1 (0.3) 0	$1 (0.3) \\ 0 \\ 1 (0.3) \\ 2 (0.6) \\ 0 \\ 2 (0.6) \\ 1 (0.3) \\ 0$	NA NA 0 0 1 (0.3) NA 0		
С	Linezolid	311	0	0	0	0	0	0	0	0		
0	Gatifloxacin	307	0	4	0	0	NA	NA	0	NA		
Total					29 (9.3)	1 (0.3)	28 (9.6)	7 (2.4)	19 (6.2)	13 (4.2)		

TABLE 2. Interpretative errors for all AST compared to the reference method

^a S, susceptible; I, intermediate; R, resistant.

^b NA, not applicable.

groups shows that the penicillin-intermediate group accounted for the highest frequency (n = 44) of mEs from all antimicrobial agents, followed by the penicillin-resistant (n = 26) and penicillin-susceptible (n = 18) groups (Table 3). The penicillin-intermediate and -resistant groups generated more sporadic mEs across the other individual antimicrobials than the susceptible groups.

Final TTRs for the automated systems demonstrated decreased TTRs, with Phoenix completing all tests at an average of 12.1 h and Vitek 2 at 9.8 h. For penicillin-resistant strains,

 TABLE 3. mEs for category A antimicrobials with

 S. pneumoniae isolates

Penicillin	AST	No. of	No. (%) of mEs with:				
susceptibility (no. of isolates)	method ^a	mEs	Penicillin	T/S	Erythromycin		
Susceptible (204)	Phoenix	2	0	1 (0.5)	0		
	Vitek 2*	8	3 (1.6)	2(1.0)			
	MicroScan	4	0	2(1.0)	1(0.5)		
	Etest	4	3 (1.5)	1(0.5)	0		
	Total	18					
Intermediate (67)	Phoenix	18	12 (17.9)	2 (3.0)	0		
Internetiate (07)	Vitek 2**	10	7(10.9)	2 (3.0) 3 (4.7)			
	MicroScan	9	2(3.0)		$0^{1}(1.0)$		
	Etest	6	2(3.0) 2(3.0)	3 (4.5)	0		
	Total	44					
Desistant (40)	Phoenix	9	2(50)	2 (7 5)	0		
Resistant (40)	Vitek 2***	8	2(5.0)	3(7.5)			
	MicroScan	8 6	6 (16.7) 2 (5.0)	1(2.8) 1(2.0)	$ \begin{array}{c} 1 \\ 0 \end{array} $ 1 (2.8)		
	Etest	3	2(5.0) 2(5.0)	1(2.0) 1(2.0)	0		
	Total	26					

 a^{a} , n = 192; **, n = 64; ***, n = 36.

the TTR increased by 1.95 h for Phoenix and 1.3 h for Vitek 2 compared to those for penicillin-susceptible strains. The manual methods required 20 to 24 h for final results.

The *S. pneumoniae* AST performance summary for all AST methods is shown in Table 4. Results from the Phoenix automated system produced an overall EA and CA of 95.2% and 99.3%, respectively, when all 4,043 MICs from every isolate/ antibiotic test combination were examined. Vitek 2, Micro-Scan, and Etest demonstrated similar respective EAs of 95%, 98.5%, and 98.1% and CAs of 98.8%, 99.5%, and 99.5%.

DISCUSSION

Timely and appropriate decisions in choosing antibiotic therapy are predicated upon the accuracy and efficiency of the clinical microbiology laboratory's AST capability. Highlighting the critical role of AST reports is the increasing prevalence of *S. pneumoniae* resistance to penicillin, the antimicrobial agent of choice, as well as other antimicrobials that may be used to treat pneumococcal infections. In general, increasing resistance has led to a focus on the need for testing methods that are accurate, reproducible, and easy to perform and have a short TTR. Of particular concern is the recognition that testing accuracy and reproducibility are largely dependent on the learned skills and experience of the laboratory staff. For ex-

TABLE 4. AST summary

AST method	ģ	%	No. of:				
	EA	CA	Tests performed	mEs	VMEs		
Phoenix	95.2	99.3	4,043	29	1		
Vitek 2	95.0	98.8	2,628	28	7		
MicroScan	98.5	99.5	4,043	19	0		
Etest	98.1	99.5	2,799	13	0		

ample, while disk diffusion methods for *S. pneumoniae* AST may be easy to perform, the literature recognizes the large number of errors (major and minor) reported with this method (17). In addition, certain antimicrobials that may be used to treat pneumococcal infections cannot be reliably tested using disk diffusion (i.e., amoxicillin [amoxicilline], ampicillin, cefepime, cefotaxime, ceftriaxone, cefuroxime, ertapenem, imipenem, and meropenem) (6). Automated systems with built-in expert systems can potentially increase the reproducibility and the reliability of test results and, as a consequence, are expected to improve the quality of patient care.

The high EA and CA values yielded by Phoenix exceeded the criteria suggested by the Food and Drug Administration (FDA) (11), which sets the standard for the minimal performance requirements ($\geq 90\%$ CA, $\geq 90\%$ EA, $\leq 1.5\%$ VMEs, and $\leq 3\%$ MEs). Furthermore, the Phoenix compares favorably with the other methods, as there was no statistically significant difference between the methods. Although the different methods were characterized by similar amounts of variability, certain antibiotics for some methods contributed more errors than in other AST assays.

The analysis of the MIC data from all methods showed that 78% of all errors were observed with CLSI group A antibiotics (penicillin, T/S, and erythromycin), which are considered appropriate primary therapy. For Phoenix, 47% of all errors were seen with penicillin, a tendency reported by Kanemitsu et al. (18), where penicillin was responsible for the highest mE rate of the agents tested. These mEs can, in large part, be attributed to the fact that many of the MICs translated by the automated instrument fell close to the interpretative breakpoints. Etest presented the lowest mE rates of the study, perhaps owing to the fact that the method relies on manual reading of strips that contain a continuous gradient, allowing for more precise determinations of MICs in and around the interpretative breakpoints.

Efforts to make meaningful comparisons between the two automated systems must take into account that there were 19 fewer sets of AST results for Vitek 2, resulting in a smaller denominator for Vitek 2 across all analyses. Vitek 2 was unable to generate AST results for 12 (5.9%) penicillin-susceptible isolates, 3 (4.5%) penicillin-intermediate isolates, and 4 (10%) penicillin-resistant isolates due to insufficient growth in the control well. While initial testing gave a larger number of AST resistant results (n = 24; data not shown), subsequent testing found more success by increasing the turbidity toward the upper limit of the protocol (0.63 McFarland standard) in the Vitek 2 card setup. While strategies to enhance growth were considered, including increasing the bacterial suspension to a 0.65 to 0.7 McFarland standard and growing mucoid isolates in broth culture prior to inoculation, these modifications of the manufacturer's recommendations were beyond the scope of this investigation.

In terms of grouping errors by penicillin susceptibility with Phoenix and the other methods, the penicillin-intermediate population accounted for the bulk of the penicillin and T/S errors (Table 3). In fact, the penicillin-intermediate population had the highest mE rates for all methods except Vitek 2, which had the highest number of mEs in the penicillin-resistant population. This aberration is likely the result of Vitek 2's smaller population of resistant isolates that produced AST results, thereby lowering the denominator of resistant isolates (from 40 to 36). Despite the elevated frequency of mEs, the high EA values with all methods for penicillin and T/S suggest that these errors can, in large part, be attributed to the MICs for these isolates falling close to the interpretative breakpoints. Some single isolates generated multiple mEs when the reference MIC was close to a breakpoint value. As has been observed elsewhere (12), several (n = 11) isolates were responsible for generating more than one mE within a method. These cases were observed with eight isolates that resulted in two errors each (Phoenix, five instances; Vitek 2, twice; and MicroScan, once), and three isolates were responsible for three errors each (Vitek 2, twice; Phoenix, once) (data not shown).

For most antibiotics, the Phoenix MICs tended to be one twofold dilution lower than the reference. The notable exceptions were the MIC results for penicillin, where the Phoenix tended to report a higher MIC, especially in the penicillinintermediate and -resistant populations (data not shown), which contributed heavily to the increased number of penicillin mEs. Overall, no MEs were observed for any test method. Based on the analyses thus far described, in consideration of the aforementioned FDA standard and applications of CLSI 2007 (7) and CLSI 2008 (6) interpretive criteria on the MICs for all methods, each method was found to meet or exceed the minimum requirements.

The only observed exceptions to the performance standards occurred with VMEs. As described in the FDA guidance document (11), an instrument's AST results may not be considered acceptable when the instrument's VME rates exceed 1.5%. As shown in Table 3, there was one antibiotic (erythromycin) for which that measure was exceeded for Vitek 2. Based on the accepted definition of VME rate, which is a "risk-corrected error rate" (17), by definition the denominator represents the actual number of those isolates that are at risk of generating a VME (i.e., a resistant isolate) (4). Therefore, VME rate calculations have questionable value as a statistic when using individual drug-resistant populations that result in denominators that are relatively small. In order for a VME rate to not exceed the standard, where there is only a single observed VME, that resistant population will need to be comprised by no fewer than 67 isolates. The more underrepresented a resistant isolate population is, the greater the possibility of presenting an inaccurate reflection of the performance characteristics of the testing instrument. Our collection of sequential clinical isolates was similar in distribution to those percentages reported in national surveillance studies (5, 8). Therefore, using our penicillin susceptibility category distribution as an example, it was unlikely that our isolate testing pool would be anything similar to the FDA-suggested 50% susceptible and 50% resistant distribution that would be desired to generate meaningful statistics regarding the performance characteristics of a method or system (a distribution goal that the FDA recognizes as being "rare" in a sequential collection) (11). This constraint becomes even more acute given the new 2008 CLSI criteria, which raised the parenteral nonmeningitis penicillin breakpoints high enough (the susceptible category breakpoint increased by five doubling dilutions and the resistant breakpoint by four doubling dilutions) that our penicillin-resistant population of 40 (based on the 2007 CLSI criteria) decreased

to 8 strains. As a result, the probability of gathering a meaningfully large penicillin-resistant ($\geq 8 \mu g/ml$) population would be very low by using anything other than a collection of frozen isolates gathered over a considerable time period. Therefore, the calculated measures of VME rate performance in this study should be interpreted accordingly. Nonetheless, it is noteworthy that Vitek 2 produced considerably more VMEs than the other methods. If shortened incubation times can be linked to errors that involved a failure to detect resistance, it may be relevant that Vitek 2 produced the fastest average TTR (9.8 h) in this study, reflecting an incubation period that was 19% shorter than that of the next fastest method (12.1 h for Phoenix).

Unlike the automated methods, the two manual comparator methods, MicroScan and Etest, did not result in any VMEs. Overall, both of these manual methods produced high EA and CA values. Similar to the Phoenix and Vitek 2, these two manual methods demonstrated the greatest frequency of mEs with penicillin and T/S. A high frequency of mEs with penicillin and T/S with MicroScan has been noted previously (13), and similarly, these were explained to result from MICs for penicillin close to the breakpoint. Guthrie et al. (13) attributed the rate of errors for T/S to the well-known trailing effect of the drug in broth microdilution methods. Despite producing relatively few observed discrepancies in this Phoenix evaluation, the MicroScan reliance upon a visual assessment of a color change was ostensibly a potential source of errors, which includes difficulties in making a reproducibly accurate assessment of the MIC endpoints in the panel. In terms of effort, MicroScan, along with Pasco, required the highest level of effort in setup due to the multistep inoculation procedure.

Compared with three commercially available systems and one reference method, the performance of the Phoenix system affirms the capability of the instrument as a reliable and efficient diagnostic tool for determining appropriate antimicrobial agents for use against *S. pneumoniae* infections. Overall, this instrument was able to provide a short TTR primarily because of the shortened incubation period and also because of the reduced hands-on time that the system requires in comparison to conventional laboratory methods. In short, the Phoenix provides a measure of needed efficiency for the clinical microbiology laboratory and has the potential to reduce the time to when a patient receives appropriate therapy based on actual microbe susceptibility data.

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