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Multicenter Evaluation of the BD Phoenix Automated Microbiology System for Antimicrobial Susceptibility Testing of *Streptococcus* Species▽

Sandra S. Richter,[1]* Wanita J. Howard,[1] Melvin P. Weinstein,[2] David A. Bruckner,[3] Janet F. Hindler,[3] Michael Saubolle,[4] and Gary V. Doern[1]

Department of Pathology, University of Iowa Carver College of Medicine, Iowa City, Iowa[1]; Robert Wood Johnson Medical School, New Brunswick, New Jersey[2]; UCLA Healthcare, Los Angeles, California[3]; and Lab Science of Arizona, Tempe, Arizona[4]

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This multicenter study evaluated the BD Phoenix Automated Microbiology System STREP panel (BD Diagnostic Systems). Antimicrobial susceptibility testing (AST) with 13 agents was performed on 2,013 streptococci (938 *Streptococcus pneumoniae* isolates; 396 group B streptococci [GBS]; 369 viridans group streptococci [VGS]; 290 beta-hemolytic streptococcus groups A, C, and G; and 20 other streptococci) with the Phoenix system and a broth microdilution reference method. Clinical and challenge isolates were tested against cefepime, cefotaxime (CTX), ceftriaxone (CTR), clindamycin (CLI), erythromycin (ERY), gatifloxacin, levofloxacin, linezolid, meropenem, penicillin (PEN), tetracycline (TET), trimethoprim-sulfamethoxazole, and vancomycin. Clinical isolates with major errors or very major errors (VMEs) were retested in duplicate by both methods. The final results for clinical isolates showed the following trends. For all of the organism-antimicrobial agent combinations tested, categorical agreement (CA) was 92 to 100%, with one exception—VGS-PEN (87% CA; all errors were minor). For *S. pneumoniae*, there was one major error with CLI (0.1%) and one or two VMEs with CTX (4%), CTR (4.5%), ERY (0.9%), and TET (0.7%). For groups A, C, and G, the CA was 97 to 100% and the only VMEs were resolved by additional reference laboratory testing. For GBS, there was only one VME (TET, 0.3%) and D-zone testing of 23 isolates with CLI major errors (one isolate unavailable) revealed inducible CLI resistance. For VGS, the major error rates were 0 to 3% and VMEs occurred with seven agents (3.5 to 7.1%). The mean times required for organism groups to generate results ranged from 8.4 to 9.4 h. The Phoenix system provided reliable and rapid AST results for most of the organism-antimicrobial agent combinations tested.

Since the early 1990s, antimicrobial resistance among *Streptococcus pneumoniae* and other streptococci has been increasing. Currently, ca. 18% of the pneumococcal isolates in the United States are penicillin resistant (MIC, ≥2 μg/ml) and the overall rate of multidrug resistance (i.e., resistance to at least three classes of antimicrobial agents) is 22% (6). International surveillance of *S. pyogenes* in 2002 and 2003 found an overall macrolide resistance rate of 11% with higher rates in Spain (23%) and Italy (25%) (1), while U.S. rates approached 7% (12). For *S. agalactiae*, the reported rates of resistance to erythromycin and clindamycin in the United States during the previous decade were 16 to 20% and 7 to 9%, respectively (10, 11). U.S. surveillance of viridans group streptococcal bloodstream isolates in the 1990s reported that 32 to 56% were not susceptible to penicillin and 38 to 46% were resistant to erythromycin (4, 5).

Because of the emergence of antimicrobial resistance among streptococci, the availability of accurate and convenient antimicrobial susceptibility testing (AST) methods in clinical microbiology laboratories is essential. This study evaluated a new BD Phoenix Automated Microbiology System STREP AST panel (BD Diagnostic Systems, Sparks, MD) in four clinical microbiology laboratories by comparing its performance to the Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution method for the susceptibility testing of streptococci.

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MATERIALS AND METHODS

Isolate characteristics. Susceptibility testing was performed in parallel on 2,013 streptococcal isolates (938 *S. pneumoniae* isolates; 396 group B streptococci [*S. agalactiae*]; 290 beta-hemolytic streptococcus groups A, C, and G; 369 viridans group streptococci [VGS]; and 20 other streptococci) with the BD Phoenix Automated Microbiology System STREP AST panel and the CLSI broth microdilution reference method. The Phoenix system identification of the isolates included in this study is presented in Table 1. A diverse group of 1,878 clinical isolates (35% fresh [stored for ≤7 days, never frozen], 25% recent [stored for 8 to 59 days, never frozen], and 40% stock) were collected and tested at one of the four clinical laboratory sites (University of Iowa Health Care, Iowa City; Robert Wood Johnson University Hospital, New Brunswick, NJ; UCLA Healthcare, Los Angeles, CA; or Lab Science of Arizona, Tempe). In addition, 135 challenge isolates from BD representing a wide range of resistance phenotypes were divided among three study sites for testing. All organisms were tested following subculture on Trypticase soy agar with 5% sheep blood and 18 to 24 h of incubation at 35°C in an atmosphere of 5 to 7% CO_2.

Phoenix susceptibility testing. The Phoenix instrument measures colorimetric change and turbidity to determine growth. Organism growth causes the Phoenix AST-S indicator to change from blue (oxidized) to pink (reduced form). Each isolate was tested on three prototype Phoenix panels (one ID-AST combination

* Corresponding author. Mailing address: Department of Pathology, University of Iowa Roy J. and Lucille A. Carver College of Medicine, 200 Hawkins Drive, C606 GH, Iowa City, IA 52242-1009. Phone: (319) 356-2990. Fax: (319) 356-4916. E-mail: sandra-richter@uiowa.edu.

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TABLE 1. Phoenix identification of the 2,013 streptococci evaluated in this study

Organism(s)	No. of isolates	No. of isolates of the following type:			
		Challenge	Clinical		
			Fresh	Recent	Stock
Streptococcus pneumoniae	938	102	171	149	516
S. agalactiae	396	2	189	153	52
Beta-hemolytic streptococcus groups A, C, and G	290	20	153	88	29
S. pyogenes	240	11	134	78	17
S. dysgalactiae	12	3	5	4	0
S. dysgalactiae/canis	38	6	14	6	12
Viridans group streptococci	369	11	152	67	139
S. anginosus	28	2	6	10	10
S. constellatus	12	0	2	5	5
S. cristatus	4	0	1	0	3
S. equinus	1	0	1	0	0
S. gordonii	15	1	1	2	11
S. intermedius	12	0	2	4	6
S. mitis	85	0	58	6	21
S. mitis group	46	2	30	6	8
S. mitis/pneumoniae	21	1	5	4	11
S. mutans	3	1	0	1	1
S. oralis	83	1	26	17	39
S. parasanguinis	36	1	15	8	12
S. salivarius	11	1	4	2	4
S. sanguinis	7	0	1	2	4
S. sobrinus	1	1	0	0	0
S. vestibularis	4	0	0	0	4
Other streptococci	20	0	4	8	8
S. acidominimus	7	0	3	1	3
S. bovis	13	0	1	7	5
All isolates	2,013	135	669	465	744

and two AST-only panels). One drop of Phoenix AST-S indicator was added to Phoenix AST-S broth tubes and used within 2 h (if stored on a bench top) or 8 h (if stored in a dark place). Well-isolated growth from a subculture plate was suspended in a Phoenix ID broth tube and adjusted to the turbidity equivalent of a 0.5 McFarland standard with a CrystalSpec nephelometer. Within 60 min, a sterile pipette was used to transfer 25-μl aliquots of the ID broth to five Phoenix AST-S broth tubes (inoculum density of $\sim 5 \times 10^5$ CFU/ml per tube) and onto Trypticase soy agar with 5% sheep blood as a purity check. A 1.5-ml aliquot of the ID broth was transferred to 12.5 ml of sterile diluent for inoculation of reference broth microdilution trays. Within 30 min, each side of the two Phoenix AST panels and the right side of the combination panels were inoculated with a tube of AST-S broth. The left side of the combination panel was inoculated with the remaining Phoenix ID broth. Panels were placed in the Phoenix instrument within 30 min of inoculation.

Reference method. The CLSI broth microdilution method (2) was performed with frozen microdilution trays containing cation-adjusted Mueller-Hinton broth with 2 to 5% lysed horse blood prepared by PASCO Laboratories (Wheat Ridge, CO). The reference panels were thawed at room temperature and used within 2 h. Within 15 min of standardization of the Phoenix ID broth, 1.5 ml was transferred to 12.5 ml of sterile diluent. After mixing, the diluted suspension was poured into a seed tray and a new disposable multipronged device was used to inoculate each panel. The final concentration of bacteria in the microdilution tray was ca. 5×10^5 CFU/ml. A purity check was performed with the remaining seed tray inoculum. The panels were incubated in ambient air for 20 to 24 h at 35°C prior to the visual reading of endpoints.

Quality control. Four quality control (QC) strains were tested daily with the reference method and the Phoenix system. These strains were *S. pneumoniae* ATCC 49619, *S. pneumoniae* BD 3951, *S. pneumoniae* BD 4218, and *S. mitis* BD 3992.

Antimicrobials tested. The antimicrobial agents (and dilutions) tested in the Phoenix panels were cefepime (0.03 to 4 μg/ml), cefotaxime (0.015 to 4 μg/ml), ceftriaxone (0.015 to 4 μg/ml), clindamycin (0.015 to 4 μg/ml), erythromycin (0.015 to 16 μg/ml), gatifloxacin (0.06 to 8 μg/ml), levofloxacin (0.25 to 16 μg/ml), linezolid (0.25 to 16 μg/ml), meropenem (0.03 to 2 μg/ml), penicillin (0.015 to 32 μg/ml), tetracycline (0.06 to 16 μg/ml), trimethoprim-sulfamethoxazole (TMP-SMX; 0.06 to 16 μg/ml), and vancomycin (0.06 to 32 μg/ml). The broth microdilution reference panels included the same antimicrobial dilutions, with the exception that the linezolid range extended to 32 μg/ml. The clindamycin wells of the Phoenix panels included a low level of inducer (erythromycin).

Evaluation of results. CLSI interpretive criteria were used to interpret each MIC as susceptible (S), intermediate (I), or resistant (R) (nonmeningitis breakpoints were used for pneumococci) (3). For each antimicrobial agent tested, the Phoenix system MIC and Phoenix interpretative category of susceptible (S), intermediate (I), or resistant (R) was compared to the broth microdilution results to determine rates of essential agreement (EA; MIC within 1 $\log_2$ dilution), categorical agreement (CA; interpretative category of S, I, or R agreement), very major (VM) errors (false susceptible with rates determined by using the number of resistant organisms as the denominator), major errors (false resistance with rates determined by using the number of susceptible isolates as the denominator), and minor errors (I by the reference method and S or R by the Phoenix system or I by the Phoenix system and S or R by the reference method). The Phoenix MICs used for comparison were taken directly from the instrument without expert system (BDExpert) interpretation.

For discrepancy resolution of clinical isolates with major or VM errors, the organism-drug combination was retested in duplicate by both methods. All three results (initial and two repeat results) were used to determine the majority result. The majority result became the final result. Results were excluded from analysis when Phoenix or reference test results were not available, when duplicate isolates of the same species had been tested from the same patient and the same body site, when isolate purity was questioned, when there was no growth in either the Phoenix or reference panel, and when QC results were out of control.

Macrolide resistance phenotype. The double-disk diffusion test was performed on erythromycin-resistant and clindamycin-susceptible *S. agalactiae* clinical isolates (D-zone test) with 15-μg erythromycin and 2-μg clindamycin disks placed 12 mm apart (3). Isolates with blunting of the inhibition zone around the clindamycin disk adjacent to the erythromycin disk (D-zone positive) were considered to have inducible clindamycin resistance.

RESULTS

For *S. pneumoniae*, the EA of the Phoenix system with the reference method was >96% for all of the agents tested except the TMP-SMX challenge isolate results (93.4% EA) (Table 2). CAs of <90% occurred among challenge isolates primarily because of minor error rates of 14.7% for meropenem, 15.8% for ceftriaxone, and 21.7% for cefepime and levofloxacin. Discrepancy testing of clinical isolates resolved 8 of 13 VM errors and 12 of 13 major errors. The final CA for clinical isolates was 91.8 to 100%, with a single major error for clindamycin (0.1%), only two VM errors for erythromycin (0.9%), and one VM error for cefotaxime (4%), ceftriaxone (4.5%), and tetracycline (0.7%). The Phoenix MICs of clindamycin, erythromycin, gatifloxacin, levofloxacin, linezolid, TMP-SMX, and vancomycin for clinical isolates were often 1 dilution lower than those obtained by the reference method. Most of the discordant Phoenix penicillin MICs were 1 dilution higher than those obtained by the reference method.

The final Phoenix results for beta-hemolytic streptococcus groups A, C, and G compared favorably to those obtained by the reference method, with CAs of 97 to 100% for clinical isolates (Table 3). Discrepancy testing of clinical isolates resolved 13 of 15 VM errors and 6 of 12 major errors. The remaining major errors were for clindamycin (2.3%) and tetracycline (0.5%). The VM errors remaining after discrepancy analysis were attributed to two *S. pyogenes* clinical isolates that were linezolid resistant by the reference method only. However, additional testing of the banked isolates at a reference laboratory indicated that the isolates were linezolid susceptible. The only clinical isolate EA of <90% was obtained with gatifloxacin (85% EA), with most Phoenix MICs falling 1 or 2 dilutions higher than the reference broth microdilution MICs, yet the CA was 98.9% (only three minor errors). The Phoenix MICs of clindamycin and levofloxacin were also often 1 dilution higher than those obtained by the reference method. The EA was <90% for challenge isolate results with clindamycin (88.2%), erythromycin (82.4%), gatifloxacin (89.5%), and tetracycline (85%), but this caused only two minor errors.

The Phoenix results were accurate for the *S. agalactiae* clinical isolates, except for the overcalling of clindamycin resistance (24 major errors, 7.3%) (Table 4). The CLSI D-zone test (5) was performed on the 50 available isolates that were erythromycin resistant and clindamycin susceptible by the reference method (one isolate that also represented a major error was unavailable for D-zone testing). The 23 available *S. agalactiae* isolates representing the major errors were all positive for inducible clindamycin resistance. Two erythromycin-resistant *S. agalactiae* isolates with minor errors for clindamycin (Phoenix method, I; reference method, S) were also D-zone positive. Only 1 of the 25 *S. agalactiae* isolates that were erythromycin resistant and clindamycin susceptible by both the Phoenix and reference methods was positive for inducible clindamycin resistance by D-zone testing.

There was also a tendency for *S. agalactiae* penicillin MICs reported by the Phoenix system to be 1 or 2 dilutions lower than those obtained by the reference method (88.3% EA) (Table 4). For the two *S. agalactiae* isolates included in the challenge set, the Phoenix system showed 100% CA and EA with the reference method (susceptible to all agents; data not shown).

For the VGS clinical isolates (Table 5), the CA was below 90% for only one antimicrobial agent (penicillin, 13.1% minor errors). Discrepancy testing of VGS clinical isolates resolved 9 of the 27 VM errors, but the final VM error rate for 7 agents exceeded 1.5%. The major error rate for VGS was 0 to 3% after discrepancy testing resolved 6 of the 21 major errors. Among the 19 clinical isolates identified as *S. bovis* and *S. acidominimus* (other streptococci, Table 4), there was a single VM error (100%, meropenem) and one major error (5.6%, clindamycin).

The mean time necessary to generate all of the AST results was 9.0 h. The mean times necessary to generate results for the organism groups were as follows: *S. pneumoniae*, 9.0 h; beta-hemolytic streptococcus groups A, C, and G, 8.4 h; *S. agalactiae*, 8.7 h; viridans group streptococci, 9.4 h; other streptococci, 9.0 h (Table 6).

The most common reason for exclusion from analysis was the availability of a Phoenix or reference result. Eight isolate exclusions were duplicates of the same species from the same patient and the same body site. The overall growth failure rate was 1.1%—well below the FDA requirement of <10% (7). The growth failure rates for each antimicrobial agent ranged from 0.8% to 1.2%. The number of result exclusions due to growth failure varied among organism-agent combinations. For example, 7 of 29 viridans group streptococcus-penicillin result exclusions, 8 of 21 *S. pneumoniae*-ceftriaxone exclusions, 11 of 14 *S. agalactiae*-erythromycin exclusions, and all 4 beta-hemolytic streptococcus group A, C, and G-erythromycin exclusions were due to growth failure. Exclusions due to a mixed-purity plate or QC results out of range were infrequent.

DISCUSSION

The U.S. Food and Drug Administration requires AST systems to generate results that are comparable to those of the CLSI reference method for each antimicrobial agent reported (≥90% CA, ≥90% EA, ≤1.5% VM errors, ≤3% major errors) (7). In this multicenter study, the Phoenix system generated accurate susceptibility test results for most of the streptococcal species tested. The advantages of using the Phoenix instrument rather than a manual method for testing streptococci in a clinical laboratory are the generation of results in a shorter time period and labor savings associated with automated reading and interpretation of MICs. The BDExpert system analyzes susceptibility profiles for unusual results, and potentially erroneous results are flagged for verification. Reporting of results is predicated on CLSI guidelines (3). BDExpert interpretation was not evaluated in this study.

Of the *Streptococcus* species included in this study, pneumococci are the most frequently tested in clinical microbiology laboratories because of the significant rate of resistance to multiple antimicrobial agents now recognized with this pathogen. The provision of accurate susceptibility results in a timely

TABLE 2. Comparison of Phoenix to reference method MICs and interpretive category errors for *S. pneumoniae*

Isolates and antimicrobial agent(s)	No. of isolates	Initial/resolved							No. of Phoenix MICs within specified $\log_2$ no. of dilutions of reference method MIC						
		% CA	No. of resistant isolates[a]	No. (%) of VM errors[b]	No. of susceptible isolates[a]	No. (%) of major errors[c]	No. (%) of minor errors[d]	% EA[e]	>−2	−2	−1	Same	+1	+2	>+2
Clinical															
Cefepime	813	92.4	17	0 (0)	708	1 (0.1)/0 (0)	61 (7.5)/62 (7.6)	98.8/99	0	6	50	669/670	84/85	3/1	1
Cefotaxime	815	96.4	25	1 (4.0)	758	0 (0)	28 (3.4)	99	0	7	64	712	31	1	0
Ceftriaxone	815	97.8	22	1 (4.5)	777	0 (0)	17 (2.1)	99.4	0	5	46	706	58	0	0
Clindamycin	828	99.5	82	0 (0)	745	2 (0.3)/1 (0.1)	2 (0.2)/3 (0.4)	96.4	1	23	252	489	57	4	2
Erythromycin	820	98.7/99.4	235/234	4 (1.7)/2 (0.9)	582/583	4 (0.7)/0 (0)	3 (0.4)	96.3/97.1	6/4	14	349/355	398	43	6	4/0
Gatifloxacin	820	99.3/99.8	3/2	1 (33.3)/0 (0)	815/816	1 (0.1)/0 (0)	4 (0.5)/2 (2.4)	98.4/98.8	2	7	199/200	485/487	123	3/1	1/0
Levofloxacin	821	99.4/99.6	2	0 (0)	817	2 (0.2)/0 (0)	3 (0.4)	99.4/99.8	0	3/2	240/242	552/553	24	0	2/0
Linezolid	821	99.6/100	2/0	2 (100)/0 (0)	819/821	1 (0.1)/0 (0)		98.5/98.8	1/0	10/9	313/315	456	40	1	0
Meropenem	817	91.6/91.8	95/94	0 (0)	622/621	1 (0.2)/0 (0)	68 (8.3)/67 (8.2)	99.0/99.3	2	3	47	684/685	78/79	1/0	2/1
Penicillin	823	93.8	189	0 (0)	508	0 (0)	51 (6.2)	97.7	4	4	96	470	238	11	0
Tetracycline	809	97.9/98.0	151/150	2 (1.3)/1 (0.7)	648	1 (0.2)/0 (0)	14 (1.7)/15 (1.9)	96.5/96.7	13/12	2	81	617/618	83	11	2
TMP-SMX	815	95.6/95.7	243/242	1 (0.4)/0 (0)	493/494	0 (0)	35 (4.3)	96.1/96.2	3/2	27	283	444	56/57	1	1
Vancomycin	822	99.9/100	1/0	1 (100)/0 (0)	821/822	0 (0)		98.2/98.3	1/0	13	307/308	491	9	1	0
Challenge															
Cefepime	92	78.3	7	0 (0)	62	0 (0)	20 (21.7)	100	0	0	6	56	30	0	0
Cefotaxime	92	92.4	14	0 (0)	60	0 (0)	7 (7.6)	97.8	0	2	6	76	8	0	0
Ceftriaxone	95	84.2	13	0 (0)	66	0 (0)	15 (15.8)	98.9	0	1	14	68	12	0	0
Clindamycin	91	98.9	28	0 (0)	63	1 (1.6)	0 (0)	98.9	0	0	14	69	7	0	1
Erythromycin	89	98.9	56	1 (1.8)	33	0 (0)	0 (0)	98.9	1	0	16	64	8	0	0
Gatifloxacin	87	100	7	0 (0)	80	0 (0)	0 (0)	97.7	0	2	16	59	10	0	0
Levofloxacin	100	78.3	7	0 (0)	62	0 (0)	20 (21.7)	99	0	1	28	67	4	0	0
Linezolid	88	100	0	0 (0)	88	0 (0)		98.9	0	0	14	60	13	1	0
Meropenem	95	85.3	30	0 (0)	49	0 (0)	14 (14.7)	98.9	0	0	3	67	24	1	0
Penicillin	93	95.7	50	0 (0)	23	0 (0)	4 (4.3)	97.8	0	2	5	48	38	0	0
Tetracycline	94	98.9	47	1 (2.1)	47	0 (0)	0 (0)	97.9	1	0	11	74	7	1	0
TMP-SMX	91	92.3	60	1 (1.7)	19	0 (0)	6 (6.6)	93.4	1	5	12	66	7	0	0
Vancomycin	92	100	0	0 (0)	92	0 (0)		98.9	0	1	9	81	1	0	0

[a] Based on reference method result.
[b] VM error, resistant by the reference method but susceptible by Phoenix; percent based on the number of resistant isolates.
[c] Major error, susceptible by the reference method but resistant by Phoenix; percent based on the number of susceptible isolates.
[d] Minor error, intermediate by the reference method but resistant or susceptible by Phoenix or intermediate by Phoenix but susceptible or resistant by the reference method.
[e] EA, Phoenix MIC ± 1 $\log_2$ dilution of reference method MIC.

TABLE 3. Comparison of Phoenix to reference method MICs and interpretive category errors for beta-hemolytic streptococcus groups A, C, and G

Isolates and antimicrobial agent	No. of isolates	Initial/resolved							No. of Phoenix MICs within specified log_2 no. of dilutions of reference method MIC						
		% CA	No. of resistant isolates[a]	No. (%) of VM errors[b]	No. of susceptible isolates[a]	No. (%) of major errors[c]	No. (%) of minor errors[d]	% EA[e]	>−2	−2	−1	Same	+1	+2	>+2
Clinical															
Cefepime	266	100	0	0 (0)	266	0 (0)	0 (0)	98.9/99.6	0	3/1	2/3	261/262	0	0	0
Cefotaxime	267	100	0	0 (0)	267	0 (0)	0 (0)	99.3/100	1/0	1/0	0/1	265/266	0	0	0
Ceftriaxone	266	99.6/100	1/0	1 (100)/0 (0)	265/266	0 (0)	0 (0)	99.2/100	1/0	1/0	2/3	262/263	0	0	0
Clindamycin	268	96.6/97.0	3	0 (0)	264	7 (2.7)/6 (2.3)	2 (0.7)	93.3/93.7	0	2	24	87/88	139	8	8/7
Erythromycin	266	97.7/99.2	25	0 (0)	241	4 (1.7)/0 (0)	2 (0.8)	92.9/94.4	0	0	30	145/147	72/74	15/14	4/1
Gatifloxacin	268	98.9	0	0 (0)	268	0 (0)	3 (1.1)	85.1/85.4	0	1	4	33	191/192	33/32	6
Levofloxacin	268	99.3/99.6	0	0 (0)	267/268	0 (0)	2 (0.7)/1 (0.4)	95.9/96.3	0	2/1	13	108	136/137	8	1
Linezolid	268	96.3/99.3	10/2	10 (100)/2 (100)	258/266	0 (0)		95.9/98.5	1	9/2	145/152	103	9	0	1
Meropenem	267	100	0	0 (0)	267	0 (0)	0 (0)	99.6/100	0	1/0	1	264/265	1	0	0
Penicillin	267	98.9/100	3/0	3 (100)/0 (0)	264/267	0 (0)	0 (0)	97.8/98.5	3/1	3	8	251/253	2	0	0
Tetracycline	247	98.0/98.4	31/30	1 (3.2)/0 (0)	212/213	1 (0.5)	3 (1.2)	91.5/91.9	1/0	1	24	140/141	62	14	5
Vancomycin	267	100	0	0 (0)	267	0 (0)		99.6	0	1	69	190	7	0	0
Challenge															
Cefepime	20	100	0	0 (0)	20	0 (0)	0 (0)	100	0	0	0	20	0	0	0
Cefotaxime	20	100	0	0 (0)	20	0 (0)	0 (0)	100	0	0	0	20	0	0	0
Ceftriaxone	19	100	0	0 (0)	19	0 (0)	0 (0)	100	0	0	0	19	0	0	0
Clindamycin	17	94.1	3	0 (0)	14	0 (0)	1 (5.9)	88.2	0	0	1	5	9	1	1
Erythromycin	17	94.1	12	0 (0)	5	0 (0)	1 (5.9)	82.4	0	0	0	11	3	2	1
Gatifloxacin	19	100	0	0 (0)	19	0 (0)	0 (0)	89.5	0	0	0	0	17	2	0
Levofloxacin	20	100	0	0 (0)	20	0 (0)	0 (0)	100	0	0	0	9	11	0	0
Linezolid	19	100	0	0 (0)	19	0 (0)		100	0	0	5	14	0	0	0
Meropenem	20	100	0	0 (0)	20	0 (0)	0 (0)	100	0	0	0	20	0	0	0
Penicillin	20	100	0	0 (0)	20	0 (0)	0 (0)	100	0	0	0	20	0	0	0
Tetracycline	20	100	6	0 (0)	14	0 (0)	0 (0)	85	0	2	0	10	7	1	0
Vancomycin	20	100	0	0 (0)	20	0 (0)		100	0	0	0	18	2	0	0

[a] Based on reference method result.
[b] VM error, resistant by the reference method but susceptible by Phoenix; percent based on the number of resistant isolates.
[c] Major error, susceptible by the reference method but resistant by Phoenix; percent based on the number of susceptible isolates.
[d] Minor error, intermediate by the reference method but resistant or susceptible by Phoenix or intermediate by Phoenix but susceptible or resistant by the reference method.
[e] EA, Phoenix MIC ± 1 log_2 dilution of reference method MIC.

TABLE 4. Comparison of Phoenix to reference method MICs and interpretive category errors for *S. agalactiae* and other streptococci

Isolates and antimicrobial agent	No. of isolates	Initial/resolved													
		% CA	No. of resistant isolates[a]	No. (%) of VM errors[b]	No. of susceptible isolates[a]	No. (%) of major errors[c]	No. (%) of minor errors[d]	% EA[e]	No. of Phoenix MICs within specified $\log_2$ no. of dilutions of reference method MIC						
									>−2	−2	−1	Same	+1	+2	>+2
S. agalactiae															
Cefepime	373	99.2/100	0	0 (0)	373	3 (0.8)/0 (0)	0 (0)	98.4/98.9	0	3	145/147	202	20	0	3/1
Cefotaxime	374	99.7/100	0	0 (0)	374	1 (0.3)/0 (0)	0 (0)	99.2/99.7	0	1/0	1	369/371	1	1	1/0
Ceftriaxone	375	100	0	0 (0)	375	0 (0)	0 (0)	100	0	0	0	370	5	0	0
Clindamycin	382	91.4/91.9	51/53	0 (0)	331/329	24 (7.3)/21 (6.4)	9 (2.4)/10 (2.6)	89.0/89.5	2	0	15	232/234	93	13	27/25
Erythromycin	380	96.8/98.2	98/99	0 (0)	281/280	8 (2.8)/3 (1.1)	4 (1.1)	90.3/91.8	2	9	90	211/215	51/52	7	10/5
Gatifloxacin	381	99.7	1	0 (0)	380	0 (0)	1 (0.3)	97.9	0	6/7	70	248	55	2/1	0
Levofloxacin	381	99.7	1	0 (0)	380	0 (0)	1 (0.3)	96.1	0	14	177	180	9	0	1
Linezolid	380	96.8/99.5	5/0	5 (100)/0 (0)	375/380	7 (1.9)/2 (0.5)		92.4/94.5	1/0	22/19	229/234	116/117	6/8	4/1	2/1
Meropenem	376	100	0	0 (0)	376	0 (0)	0 (0)	98.4/98.7	0	2	152/153	168	50	2	2/1
Penicillin	377	100	0	0 (0)	377	0 (0)	0 (0)	88.3	2	41	179	122	32	1	0
Tetracycline	377	97.3/98.9	333/339	1 (0.3)	43/37	6 (14.0)/0 (0)	3 (0.8)	95.5/97.1	1	0	1	341/347	18	9	7/1
Vancomycin	378	99.5/100	0	0 (0)	378	2 (0.5)/0 (0)		98.9/99.5	0	2	101/102	268/269	5	0	2/0
Other streptococci[f]															
Cefepime	19	100	0	0 (0)	19	0 (0)	0 (0)	100	0	0	1	15	3	0	0
Cefotaxime	19	100	0	0 (0)	19	0 (0)	0 (0)	100	0	0	2	15	2	0	0
Ceftriaxone	19	100	0	0 (0)	19	0 (0)	0 (0)	100	0	0	0	14	5	0	0
Clindamycin	18	88.9	0	0 (0)	18	1 (5.6)	1 (5.6)	77.8	0	0	1	8	5	3	1
Erythromycin	19	100	6	0 (0)	13	0 (0)	0 (0)	100	0	0	2	15	2	0	0
Gatifloxacin	19	94.7	0	0 (0)	19	0 (0)	1 (5.3)	78.9	1	0	3	4	8	2	1
Levofloxacin	19	100	0	0 (0)	19	0 (0)	0 (0)	84.2	0	1	2	12	2	0	2
Linezolid	19	100	0	0 (0)	19	0 (0)		100	0	0	11	8	0	0	0
Meropenem	19	94.7	1	1 (100)	18	0 (0)	0 (0)	100	0	0	2	17	0	0	0
Penicillin	19	100	1	0 (0)	17	0 (0)	0 (0)	94.7	0	0	2	7	9	1	0
Tetracycline	19	94.7/100	10/11	0 (0)	9/8	0 (0)	0 (0)	73.7/78.9	1	0	1	11/12	2	3	1/0
Vancomycin	19	100	0	0 (0)	19	0 (0)		100	0	0	2	14	3	0	0

[a] Based on reference method result.
[b] VM error, resistant by the reference method but susceptible by Phoenix; percent based on the number of resistant isolates.
[c] Major error, susceptible by the reference method but resistant by Phoenix; percent based on the number of susceptible isolates.
[d] Minor error, intermediate by the reference method but resistant or susceptible by Phoenix or intermediate by Phoenix but susceptible or resistant by the reference method.
[e] EA, Phoenix MIC ± 1 $\log_2$ dilution of reference method MIC.
[f] Other streptococci: *S. acidominimus* and *S. bovis*.

TABLE 5. Comparison of Phoenix to reference method MICs and interpretive category errors for viridans group streptococci

Isolates and antimicrobial agent	No. of isolates	Initial/resolved													
		% CA	No. of resistant isolates[a]	No. (%) of VM errors[b]	No. of susceptible isolates[a]	No. (%) of major errors[c]	No. (%) of minor errors[d]	% EA[e]	No. of Phoenix MICs within specified $\log_2$ no. of dilutions of reference method MIC						
									>−2	−2	−1	Same	+1	+2	>+2
Clinical															
Cefepime	329	92.7	14	1 (7.1)	294	0 (0)	23 (7.0)	90.9/91.2	1	16/15	82/83	192	25	9	4
Cefotaxime	329	96.7	17	1 (5.9)	304	0 (0)	10 (3.0)	91.5	2	18	73	207	21	6	2
Ceftriaxone	330	96.7	15	0 (0)	307	0 (0)	11 (3.3)	92.4	1	11	62	214	29	9	4
Clindamycin	327	99.1	22	1 (4.5)	302	0 (0)	2 (0.6)	96.3	1	2	55	214	46	7	2
Erythromycin	333	94/95.5	199/198	10 (5.0)/7 (3.5)	133/134	7 (5.3)/4 (3.0)	3 (0.9)/4 (1.2)	84.7/85.9	13/12	20/19	86/89	171	25/26	9	9/7
Gatifloxacin	333	98.2/98.5	15	0 (0)	315/317	2 (0.6)	4 (1.2)	91.6/92.2	2	7/6	71	163/165	71	14/13	5
Levofloxacin	334	98.5	17	1 (5.9)	316	2 (0.6)	2 (0.6)/3 (0.9)	96.4/96.7	1/0	4	98	189/190	35	4	3
Linezolid	328	99.4	0	0 (0)	328	2 (0.6)		97.9/98.2	0	3/2	124/125	169	28	2	2
Meropenem	330	98.2/98.8	38/37	4 (10.5)/2 (5.4)	292/293	2 (0.7)	0 (0)	89.1/89.4	5/4	18	77/78	180	37	11	2
Penicillin	329	86.9	30	0 (0)	188	0 (0)	43 (13.1)	86.9	10	14	54	165	68	10	8
Tetracycline	323	90.4/92.0	102/101	9 (8.8)/5 (5.0)	207	6 (2.9)/3 (1.4)	16 (5.0)/18 (5.6)	83.3/85.1	15/11	12/11	55/56	172/176	42/43	13	14/13
Vancomycin	329	100	0	0 (0)	329	0 (0)		96.0	1	8	155	147	14	1	3
Challenge															
Cefepime	11	90.9	0	0 (0)	11	0 (0)	1 (9.1)	100	0	0	2	7	2	0	0
Cefotaxime	10	90	0	0 (0)	10	0 (0)	1 (10.0)	100	0	0	0	7	3	0	0
Ceftriaxone	11	90.9	0	0 (0)	11	0 (0)	1 (9.1)	100	0	0	0	8	3	0	0
Clindamycin	9	100	0	0 (0)	9	0 (0)	0 (0)	88.9	0	0	0	7	1	1	0
Erythromycin	8	87.5	3	1 (33.3)	5	0 (0)	0 (0)	75	0	1	0	4	2	1	0
Gatifloxacin	10	100	1	0 (0)	9	0 (0)	0 (0)	100	0	0	1	5	4	0	0
Levofloxacin	11	100	1	0 (0)	10	0 (0)	0 (0)	100	0	0	3	7	1	0	0
Linezolid	9	100	0	0 (0)	9	0 (0)		100	0	0	3	5	1	0	0
Meropenem	9	100	0	0 (0)	9	0 (0)	0 (0)	88.9	0	0	1	6	1	1	0
Penicillin	11	90.9	1	0 (0)	8	0 (0)	1 (9.1)	90.9	0	0	1	4	5	1	0
Tetracycline	10	80	1	0 (0)	8	0 (0)	2 (20.0)	80	1	0	2	3	3	0	1
Vancomycin	10	100	0	0 (0)	10	0 (0)		100	0	0	3	6	1	0	0

[a] Based on reference method result.
[b] VM error, resistant by the reference method but susceptible by Phoenix; percent based on the number of resistant isolates.
[c] Major error, susceptible by the reference method but resistant by Phoenix; percent based on the number of susceptible isolates.
[d] Minor error, intermediate by the reference method but resistant or susceptible by Phoenix or intermediate by Phoenix but susceptible or resistant by the reference method.
[e] EA, Phoenix MIC ± 1 $\log_2$ dilution of reference method MIC.

TABLE 6. Times required to obtain results for 2,013 streptococcal clinical and challenge isolates

Antimicrobial agent(s)	Mean time (h) required to obtain results				
	S. pneumoniae (n = 938)	Beta-hemolytic streptococcus groups A, C, and G (n = 290)	*S. agalactiae* (n = 396)	Viridans group streptococci (n = 369)	Other streptococci (n = 20)
Cefepime	9.4	8.7	9.1	9.6	9.2
Cefotaxime	9.1	8.7	9.0	9.3	9.2
Ceftriaxone	9.2	8.7	9.0	9.4	9.2
Clindamycin	10.8	10.6	10.6	10.6	12.1
Erythromycin	9.9	10.2	10.4	12.0	10.6
Gatifloxacin	9.4	7.9	8.6	9.7	9.1
Levofloxacin	9.4	7.9	8.5	9.6	8.9
Linezolid	7.8	7.5	8.0	8.1	7.9
Meropenem	9.0	7.5	7.9	8.9	8.1
Penicillin	8.5	7.6	7.9	8.7	8.1
Tetracycline	8.8	8.8	7.1	9.7	8.5
TMP-SMX	7.9				
Vancomycin	7.7	7.5	8.0	8.3	8.1
All	9.0	8.4	8.7	9.4	9.0

manner can minimize the time patients receive inappropriate therapy and limit the use of needlessly broad-spectrum agents. The Phoenix is the second automated AST instrument to offer a panel for *S. pneumoniae* testing that may be reported after a relatively short incubation period (<16 h).

The Phoenix results for the clinical isolates of *S. pneumoniae* compared favorably to those obtained by the reference method, with few exceptions (Table 2). Although the VM error rates of clinical isolates for cefotaxime (4%) and ceftriaxone (4.5%) exceeded 1.5%, these rates represented a failure to detect one resistant isolate; further, the instrument accurately detected resistance for isolates included in the challenge set that were cefotaxime (n = 14) or ceftriaxone (n = 13) resistant. The VM error rates which exceeded 1.5% for challenge isolates (erythromycin, tetracycline, TMP-SMX) were also each due to one isolate. The clinical isolate collection included three- to fourfold more isolates resistant to erythromycin, tetracycline, and TMP-SMX than the challenge set, yet they were detected without difficulty (0 to 0.9% VM error rates). Overcalling of resistance was a rare event, but minor error rates exceeded 10% for challenge isolates with four agents (cefepime, ceftriaxone, levofloxacin, and meropenem). However, the high EAs (98.9 to 100%) for those four drugs suggest that the errors were a consequence of MICs for isolates falling close to the interpretative breakpoints.

An evaluation of the VITEK 2 card for susceptibility testing of *S. pneumoniae* isolates reported major and VM error rates that are similar to the Phoenix error rates in the present study for the six common antimicrobial agents cefotaxime, ceftriaxone, erythromycin, penicillin, tetracycline, TMP-SMX, and vancomycin (8). Minor error rates in the VITEK 2 study obtained with TMP-SMX (16.9 to 19.1%), cefotaxime (11.9 to 19.8%), and penicillin (9.5 to 16.7%) for clinical and challenge pneumococcal strains (8) were higher than those found in this Phoenix evaluation. A more rigorous evaluation of VITEK 2 for detecting fluoroquinolone resistance (challenge set of 196 pneumococci with 66 gatifloxacin resistant or intermediate) reported 13.3% minor errors and one (1.7%) VM error for gatifloxacin (9). There are obvious limitations associated with the comparison of results from studies that tested different isolates; however, head-to-head evaluations of the VITEK 2 and Phoenix instruments with common collections of streptococci have not been described in the literature.

Although *S. pyogenes* and *S. agalactiae* are important pathogens, these species are predictably susceptible to penicillin, the therapeutic agent of choice. The M100-S17 CLSI document states that there is no clinical need to perform susceptibility testing of penicillins and other β-lactams with these organisms (3). However, testing of the susceptibility of these organisms to clindamycin and erythromycin is recommended when the patient is allergic to β-lactam agents (13). The Phoenix system performed well when determining the susceptibility of beta-hemolytic streptococci to erythromycin, with no VM errors, 0 to 1.1% major errors, and 0.7 to 5.9% minor errors. The Phoenix system had no VM errors and acceptable minor error rates (0.7 to 5.9%) when testing clindamycin against beta-hemolytic streptococci.

The detection of inducible clindamycin resistance among beta-hemolytic streptococci by the CLSI reference method requires the performance of the manual D-zone test on erythromycin-resistant, clindamycin-susceptible isolates (3). An evaluation of the VITEK 1 and VITEK 2 systems for the detection of erythromycin and clindamycin resistance among 304 *S. agalactiae* isolates also concluded that the double-disk diffusion method was needed to ensure accurate results (14). In this study, 23 of the 24 *S. agalactiae* clinical isolates with major errors for clindamycin were available for additional testing, and all had positive D-zone test results (inducible clindamycin resistance). Of the 25 *S. agalactiae* isolates that were erythromycin resistant and clindamycin susceptible by both the Phoenix system and the reference method, only 1 was D-zone test positive. Although further evaluation is necessary with more strains, it appears that the Phoenix system's use of an inducer (a small amount of erythromycin in clindamycin wells) reliably defines isolates of *S. agalactiae* with inducible clindamycin resistance as being clindamycin resistant or intermediate, potentially eliminating the need for D-zone testing of erythromycin-resistant and clindamycin-susceptible *S. agalactiae* isolates.

The need to critically evaluate all susceptibility results (not only those generated by an automated instrument) was dem-

onstrated by the VM errors remaining after discrepancy testing for two *S. pyogenes* isolates reported as resistant to linezolid by the reference method only. Since linezolid resistance has not been previously reported in this species, the isolates were sent to a reference laboratory, where testing by the CLSI broth microdilution method in duplicate and Etest revealed linezolid susceptibility.

The lowest rates of concordance of the Phoenix results with the reference method occurred for the VGS isolates (Table 5). Additional studies with more VGS strains are needed to further assess the performance of the Phoenix system for antimicrobial agents with VM error rates above 1.5% or minor error rates exceeding 10%.

Our findings suggest that clinical microbiology laboratories may rely on the Phoenix system for accurate susceptibility testing of *S. pneumoniae* and beta-hemolytic streptococci. The shorter incubation time required by the Phoenix system in comparison to reference methods has the potential to enhance patient care.

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