

Evaluation of the BD Phoenix™ Automated Microbiology System for Antibiotic Susceptibility Testing of *Streptococcus pneumoniae*

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REVISED ABSTRACT

We examined the performance of the BD Phoenix™ Strep panel (BD Diagnostics, Sparks, MD) compared to Etest and Pasco for 154 clinical isolates of *Streptococcus pneumoniae*. This Strep panel is designed to rapidly identify and determine antimicrobial susceptibilities for streptococci. Overall, 149 of 154 (96.8%) Phoenix identifications were in agreement with traditional biochemical testing. Five discordant results between Phoenix and routine biochemicals were observed and these were confirmed as *S. pneumoniae* by 16S rRNA sequencing. Twelve of 13 antimicrobial agents included in the Phoenix Strep panel were compared to the Pasco microbroth dilution method (BD Diagnostics, Sparks, MD), which served as the reference standard. Categorical agreement of both Phoenix and Etest with Pasco averaged 99.7%. Categorical agreement of Phoenix compared to Pasco for penicillin was 98.1%; no very major or major errors were encountered and a minor error rate of 1.9% was observed. The median time for Phoenix AST reporting was 12.3 hours as compared to 20 - 24 hours for Etest and Pasco. The Phoenix System was easy to use, allowed for efficient workflow well-suited for a high volume clinical laboratory, and demonstrated outstanding performance for *S. pneumoniae* identification and AST determination.

INTRODUCTION

Streptococcus pneumoniae is a leading cause of morbidity and mortality (i.e., community-acquired pneumonia, meningitis, otitis media, bacteremia). Resistance to penicillin, the therapeutic agent of choice, remains high; therefore, expeditious identification (ID) and antimicrobial susceptibility test (AST) results are essential. The BD Phoenix Automated Microbiology System (BD Diagnostics, Sparks, MD. [BD]) is an automated instrument that, in concert with the SMIC-ID/100 combination Strep panel, can determine the identification of 32 species of streptococci and the susceptibility to 13 antimicrobial agents.

We present a performance evaluation of the Phoenix combined ID/AST Strep panel using clinical isolates of *S. pneumoniae* recovered from patients at our medical center. Phoenix IDs were compared to those obtained by routine biochemical tests; discordants were resolved using 16S rRNA technology. The Phoenix AST was compared to Etest and the Pasco microdilution system, which served as the reference method.

METHODS

Bacterial Isolates

A total of 154 frozen and fresh clinical isolates of *S. pneumoniae* recovered from Columbia University Medical Center patients were used to evaluate the Phoenix system. Respiratory (76%) and blood (13%) were the predominant sites from which isolates were obtained.

Phoenix Identification

The Phoenix system offers a combination Strep panel (SMIC-ID/100) ID and AST, with the identification substrates on one side and antimicrobial agents on the other side of the panel. Isolates were sub-cultured onto Columbia agar supplemented with 5% sheep blood (BD). The Phoenix ID broth was inoculated with several bacterial colonies from a pure culture adjusted to 0.5 to 0.6 McFarland standard (5×10^5 cfu/mL) using a PhoenixSpec Nephelometer (BD Diagnostics). After the transfer of 25 μ L of the ID broth suspension to the Phoenix AST-S broth, the remaining suspension was poured into the ID side of the panel. Once inoculated the panel was labeled, logged and loaded into the instrument and incubated at 35°C. Purity plates were prepared for all isolates.

Traditional Biochemical Identification.

Routine biochemical tests for ID of *S. pneumoniae* study isolates included bile solubility (BD, BBL, Sparks, MD), co-agglutination (Phadebact, Remel, Lenexa KS) and optochin susceptibility (BD, BBL, Sparks, MD).

ID Resolution

Isolates, for which there were discordant IDs, were identified by 16S rRNA sequencing (1539 bp DNA fragment). The isolate sequence was aligned with a known *S. pneumoniae* sequence (strain TIGR4, accession #AE005672) for calculating sequence homology.

ANTIBIOTIC SUSCEPTIBILITY TESTING**Phoenix AST**

Preparation of the Phoenix AST-S broth requires adding a drop of Phoenix AST indicator (resazurin based dye acting as the terminal electron acceptor) before inoculation of 25 µL of the broth aliquot from the standardized ID suspension. After addition of the ID broth suspension, the tube was mixed by inverting several times. The broth was then poured into the AST side of the panel. As described previously, the panel was loaded into the Phoenix apparatus.

Etest

A sterile cotton swab saturated with a 0.5 McFarland standard suspension of the test isolate was used to inoculate a 150 mm Mueller-Hinton agar plate containing 5% sheep blood (BD). The media were incubated at 35°C in 5% CO₂ for 20 to 24 h before being examined. The MIC was determined to be the point at which the elliptical growth margin intersected the Etest strip.

Pasco (reference AST)

For the Pasco MIC Supplemental III panels, organisms were suspended in 6 mL of 0.85% saline to the turbidity of a 1.0 McFarland standard. A 1.5 mL aliquot of this suspension was transferred to 12.5 mL of SP Blood Supplement. The suspension was inverted 8 to 10 times, and the inoculum was poured into the inoculum tray and incubated in ambient air at 35°C for 20 to 24 h. All MIC values were interpreted using CLSI breakpoints (2006). The MIC breakpoint values for penicillin were ≤ 0.06 (susceptible), 0.12-1.0 (intermediate) and ≥ 2.0 (resistant).

Quality Control

For QC, *S. agalactiae* ATCC 13813 and *S. pneumoniae* ATCC 49619 were tested weekly with all systems, in accordance with manufacturers' guidelines.

Data Analysis

The Phoenix, Etest and Pasco data were interpreted using CLSI MIC breakpoints for susceptible, intermediate, and resistant (SIR) categories (CLSI document M100-16, 2006). Errors were classified as very major (VME), i.e. false susceptible result; major (ME), i.e. false resistant result; and minor (mE), i.e., one system reporting an intermediate result and the other reporting a susceptible or resistant result.

RESULTS**IDENTIFICATION**

TABLE 1. Of the 154 *S. pneumoniae* clinical isolates identified using the Phoenix Strep panel, 96.8% (149) were in complete agreement with traditional biochemicals. The 5 discordant Phoenix IDs were verified as *S. pneumoniae* by 16S rRNA sequencing. Of these 5 isolates, Phoenix was unable to identify 2 isolates, 2 were misidentified as *S. oralis* and *S. mitis* and one was reported as *S. mitis/pneumoniae*, requiring bile solubility for resolution.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

When Phoenix and Etest results were compared against Pasco as the reference method, categorical agreements for ceftriaxone, clindamycin, erythromycin, levofloxacin, linezolid, tetracycline, and vancomycin were 100%, (results not shown). Cefepime and cefotaxime were 100% concordant with Phoenix and Pasco only (Etest not evaluated).

TABLE 2. The results of Phoenix penicillin susceptibility tests demonstrated 98.1% categorical agreement with Pasco. There were no very major or major errors and 3 minor errors (1.9%).

TABLE 3. The results of Etest for penicillin when compared to Pasco showed a 98.8% categorical agreement with Pasco. There were no very major errors, one major error (0.6%), and one minor error (0.6%).

TABLE 4. Of 12 antimicrobial agents evaluated in this study, only penicillin, meropenem and trimethoprim/sulfamethoxazole demonstrated errors. There were 9 minor errors and one major error detected in 8 isolates. Etest showed one major error for penicillin and minor errors for penicillin (n=1) and trimethoprim/sulfamethoxazole (n=2). Phoenix gave minor errors for penicillin (n=3), meropenem (n=2) and trimethoprim/sulfamethoxazole (n=1).

TURNAROUND TIME TO RESULTS

The average Phoenix turnaround time (TAT) required to obtain both ID and AST was 12 h 18 min. However, the TAT for Etest averages 20-24 hr. Although traditional biochemical batteries for *S. pneumoniae* are rapid and reliable, ID to the species level for the streptococci viridans group have lengthy TAT and results are problematic. We plan to evaluate the Phoenix system for the accurate identification of this group of streptococci.

Table 1. Resolution of 5 Discordant Identifications

TRADITIONAL BIOCHEMICALS			PHOENIX	MOLECULAR (16S rRNA)
Bile Solubility	Co-Agglutination	Optochin		
Pos	Pos	Suscept	No Identification	<i>S. pneumoniae</i>
Pos	Equiv	Suscept	<i>S. mitis</i>	<i>S. pneumoniae</i>
Pos	Equiv	Resist	<i>S. oralis</i>	<i>S. pneumoniae</i>
Pos	Pos	Resist	No Identification	<i>S. pneumoniae</i>
Pos	Pos	Resist	<i>S. mitis/pneumoniae</i>	<i>S. pneumoniae</i>

Table 2. Categorical Agreement for Phoenix vs. Pasco

Penicillin	Total Number of Isolates	Very Major Errors (VME)	Major Errors (ME)	Minor Errors
Susceptible (MIC \leq 0.06)	97	0	0	0
Intermediate (MIC 0.12 - 1.0)	38	0	0	3
Resistant (MIC \geq 2.0)	19	0	0	0
TOTAL	154	0	0	3 (1.9%)

Table 3. Categorical Agreement for Etest vs. Pasco

Penicillin	Total Number of Isolates	Very Major Errors (VME)	Major Errors (ME)	Minor Errors
Susceptible (MIC \leq 0.06)	97	1	0	0
Intermediate (MIC 0.12 - 1.0)	38	0	0	1
Resistant (MIC \geq 2.0)	19	0	0	0
TOTAL	154	1 (0.6%)	0	1 (0.6%)

Table 4. Data for 10 Discordant Susceptibility Results*

Isolates	Assay	MICs (Interpretation)		
		Penicillin	Meropenem	Trimeth/Sulfa
1	Phoenix	\leq 0.03 (S)		
	Etest	2 (R)		
	Pasco	\leq 0.03 (S)		
2	Phoenix			0.5/9.5 (S)
	Etest			1/19 (I)
	Pasco			1/19 (I)
3	Phoenix	0.12 (I)		\leq 0.25/4.75 (S)
	Etest	0.064 (S)		0.75/14.25 (I)
	Pasco	0.12 (I)		\leq 0.5/9.5 (S)
4	Phoenix	2 (R)		
	Etest	0.38 (I)		
	Pasco	1 (I)		
5	Phoenix	2 (R)	1 (R)	
	Etest	0.38 (I)	0.5 (I)	
	Pasco	1 (I)	0.5 (I)	
6	Phoenix			> 2/38 (R)
	Etest			2/38 (I)
	Pasco			> 2/38 (R)
7	Phoenix	2 (R)		
	Etest	1 (I)		
	Pasco	1 (I)		
8	Phoenix		1 (R)	
	Etest		0.5 (I)	
	Pasco		0.5 (I)	

* gray = discordants

CONCLUSIONS

- This study demonstrated that the Phoenix Strep ID/AST panel compares favorably to comparator assays for the identification and antimicrobial susceptibility testing of clinical isolates of *S. pneumoniae*.
- Only 3 minor errors were detected using the Phoenix relative to the Pasco reference assay for penicillin.
- The turnaround time for AST results is nearly twice as fast with Phoenix (average of 12.3 hours) as Etest and Pasco (20 – 24 hours).
- Studies are ongoing to compare the ID/AST combination Strep panel results to those from other automated systems.