

# An Evaluation of the BD Phoenix™ Automated Microbiology System to Detect Penicillins and Cephalosporins Resistance in *Streptococcus pneumoniae*\*

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## ABSTRACT

**BACKGROUND:** Penicillin and cephalosporin resistance in *Streptococcus pneumoniae* remains an important worldwide problem with the increasing spread of multi-drug resistance clones. Using the BD Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD), a new STREP Antimicrobial Susceptibility Test (AST) Panel plus a specially formulated non-blood containing AST-S Broth were tested for the detection of penicillin/cephalosporin resistance in *S. pneumoniae*.

**METHODS:** A challenge set consisting of 70 *S. pneumoniae* strains, including 22 penicillin resistant (MIC  $\geq 2$   $\mu\text{g/mL}$ ), 21 penicillin intermediate (MIC 0.125–1  $\mu\text{g/mL}$ ), and other representative clonal multi-drug resistant strains were evaluated for the following 6 drugs: penicillin (P), amoxicillin (AMX), cefotaxime (CTX), ceftriaxone (CRO), cefepime (FEP) and cefuroxime (CXM). All testing was performed in parallel and repeated on three separate days with the Phoenix System and NCCLS standard broth microdilution (SBM) method. Inoculum was prepared simultaneously for Phoenix and SBM for each test strain to achieve the NCCLS recommended density of approximately  $5 \times 10^5$  cfu/mL. Results for Phoenix and SBM were compared and analyzed for Essential Accord (EA) and Categorical Agreement (CA) based on NCCLS established breakpoints (M100-S12). For CTX, CRO, and FEP the meningitis breakpoints were used for CA calculations.

**RESULTS:** EA for P, AMX, CTX, CRO, FEP, and CXM were between 94 – 100%. CA for penicillins (P and AMX) and cephalosporins (CTX, CRO, FEP, and CXM) were 99–100%. No Very Major (VME) or Major Errors were detected.

**CONCLUSIONS:** These results suggest that the Phoenix Strep AST test showed good correlation with the NCCLS recommended SBM procedure for the detection of penicillins and cephalosporins resistance in *S. pneumoniae*.



Figure 1.  
Phoenix™ Automated  
Microbiology System

## INTRODUCTION

The emergence of antimicrobial resistance in *Streptococcus pneumoniae* during the past 10 years has resulted in a dramatic worldwide increase in the incidence of pneumococcal strains resistant to penicillin G and other  $\beta$ -lactam and non- $\beta$ -lactam antibiotics including macrolides, tetracycline, chloramphenicol, clindamycin, and trimethoprim-sulfamethoxazole. This problem has been amplified by the tendency of these resistant clonal populations to spread from region to region and from continent to continent. Survey results in the USA for 2000 (CDC Active Bacterial Core Surveillance [ABC's] Report) has shown 72.5% of *S. pneumoniae* had penicillin minimum inhibitory concentration (MICs) of  $<0.125$   $\mu\text{g/mL}$ , 9.9% of strains with penicillin intermediate MIC (0.125–1.0  $\mu\text{g/mL}$ ) and 17.6% of strains with penicillin resistant MIC ( $\geq 2.0$   $\mu\text{g/mL}$ ). The significant increase in antimicrobial resistance of *S. pneumoniae* has placed greater importance on accurate and rapid testing of these isolates in clinical laboratories.

The Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) is currently under development for rapid ID/AST of *Streptococcus* (including *S. pneumoniae*, viridans group *Streptococcus*, and Beta-hemolytic *Streptococcus*). In this study, the Phoenix System was compared to NCCLS standard broth microdilution (SBM) method to evaluate the MIC results for *S. pneumoniae* with penicillin G (P), amoxicillin (AMX), cefotaxime (CTX), ceftriaxone (CRO), cefepime (FEP), and cefuroxime (CXM).

## METHODS

**BACTERIAL STRAINS.** A total of 70 *S. pneumoniae* isolates (including 22 penicillin resistant and 21 penicillin intermediate) were tested. These geographically diverse challenge strains were obtained from the Centers for Disease Control and Prevention (CDC), University of Iowa Health Care (Iowa City, Iowa), National Public Health Institute (Turku, Finland), and BD Diagnostic Systems internal culture collection. TABLE 1 summarizes the phenotype information for these strains and TABLE 2 describes the American Type Culture Collection (ATCC) reference strains representing different multi-drug resistant clones used in this study.

The total number of susceptible (S), intermediate (I), and resistant (R) strains for each drug based on SBM are summarized in TABLE 3. The slightly lower total results for the 70 strains tested were from reading failures due to no growth wells or skipped wells. The National Committee for Clinical Laboratory Standards (NCCLS) recommended QC strain *S. pneumoniae* ATCC 49619 was also included in each experiment performed. All test strains and NCCLS recommended QC strains were tested with the Phoenix and SBM method in parallel.

**MEDIA.** Bacterial suspensions were prepared from 18–24 hour cultures on Trypticase™ Soy Agar with 5% sheep blood (TSAII,™ BD Diagnostic Systems) incubated at 35°C in 5% CO<sub>2</sub>.

**ANTIMICROBIAL AGENTS IN PHOENIX AND SBM.** The SBM panels were manufactured and stored according to NCCLS recommended guidelines (M7-A5). Prior to use, the Phoenix panels were stored at room temperature. The following drug concentrations were tested in both methods: P (0.015 – 32 µg/mL), AMX (0.015–32 µg/mL), CRO (0.015–4 µg/mL), CTX (0.015–4 µg/mL), CXM (0.125–4 µg/mL), and FEP (0.03–4 µg/mL).

**PHOENIX AST METHOD.** All Phoenix testing was performed per manufacturer recommended procedures. Bacterial suspensions from an overnight culture on TSAII™ were prepared and adjusted to a 0.5 McFarland standard in Phoenix ID broth. The Phoenix AST-S Broth, a proprietary non-blood containing broth specifically formulated for rapid susceptibility testing for streptococci was inoculated and used in the Phoenix panel. A final inoculum density of 5 x 10<sup>5</sup> cfu/mL, equivalent to the NCCLS recommendations was used for both Phoenix and SBM method. The inoculated Phoenix panels were then placed into the Phoenix instrument for incubation (ambient air) and continuous reading.

**REFERENCE MICRODILUTION (SBM) METHOD.** The SBM panels were prepared and inoculated according to NCCLS recommended guidelines (M7-A5) using the same inoculum prepared for Phoenix testing. After 20–24 hours of incubation at 35±1°C in ambient air, the SBM panels were interpreted visually by trained technologists. The NCCLS (M100-S12) was used for categorical interpretation (TABLE 4).

Table 1. Summary of Phenotype/Genotype Information for the well-characterized strains used in this study.

| Phenotype/Genotype  | Number of Strains Included |
|---|----------------------------|
| High Level Penicillin Resistance (MIC ≥ 4)                  | 14                         |
| MLSb Phenotype — erythromycin ribosome methylase (erm gene) | 16                         |
| M Phenotype — macrolide efflux (mef gene)                   | 11                         |

Table 2. Summary of the pneumococcal resistant clones used in this study.

| Reference Strain | Serotype | Clone Description    |
|------------------|----------|----------------------|
| ATCC 51916       | 23F      | Tennessee 23F-4      |
| ATCC 700670      | 6B       | Spain 6B-2           |
| ATCC 700673      | 19A      | Hungary 19A-6        |
| ATCC 700676      | 14       | England 14-9         |
| ATCC 700677      | 14       | Czech Republic 14-10 |
| ATCC 700678      | 19A      | Slovakia 19A         |
| ATCC 700903      | 6B       | Finland 6B-12        |
| ATCC 700905      | 19F      | Taiwan 19F-14        |

Table 3. Number of susceptible (S), intermediate (I), or resistant (R) *S. pneumoniae* for each antibiotic tested determined by SBM.

| Drug | Number of <i>S. pneumoniae</i> strains by SBM <sup>1</sup> |    |    | Total |
|------|--|----|----|-------|
|      | S  | I  | R  |       |
| AMX  | 61   | 3  | 3  | 67    |
| CRO  | 39   | 12 | 15 | 66    |
| CTX  | 40   | 9  | 17 | 66    |
| CXM  | 40   | 2  | 27 | 69    |
| FEP  | 42   | 10 | 14 | 66    |
| P    | 24   | 21 | 22 | 67    |

<sup>1</sup> For drugs with meningitis and nonmeningitis breakpoints (CRO, CTX and FEP), the NCCLS Standard M100-S12 (Jan, 2002) breakpoints for meningitis were applied

Table 4. MIC Interpretative Standards\* (µg/mL) for *Streptococcus pneumoniae*.

| Drug        | Source        | S      | I        | R   |
|-------------|---------------|--------|----------|-----|
| AMX         | Nonmeningitis | ≤ 2    | 4        | ≥ 8 |
| CRO/CTX/FEP | Meningitis    | ≤ 0.5  | 1        | ≥ 2 |
| CRO/CTX/FEP | Nonmeningitis | ≤ 1    | 2        | ≥ 4 |
| CXM         | N/A           | ≤ 1    | 2        | ≥ 4 |
| P           | N/A           | ≤ 0.06 | 0.12 – 1 | ≥ 2 |

\* NCCLS Standard M100-S12 (Jan, 2002)

## RESULTS

This study evaluated the performance of the Phoenix System for rapid susceptibility testing of *S. pneumoniae* with 6 drugs. The MICs for Phoenix compared favorably (97.5% overall agreement within +/- 1 dilution) with the MICs determined by the reference method. Overall Categorical Agreement (calculated for errors at >1 dilution) for Phoenix was 99.7%. No difference in Categorical Agreement for CRO, CTX, and FEP was observed using either the meningitis or the nonmeningitis breakpoints. These results are summarized in TABLE 5.

The time to detection of Phoenix MIC result for each drug is summarized in TABLE 6. The average time to result for each drug and cumulative frequency by time for all drugs is graphically depicted in Figure 2 and Figure 3.

Table 5. Essential Accord (EA), Categorical Agreement (CA), and Error Analysis for the six  $\beta$ -lactam drugs using the Phoenix System.

| Drug | No. Of Tests | EA% | CA% <sup>1</sup> | VME% <sup>2</sup> | ME% <sup>2</sup> |
|------|--------------|-----|------------------|-------------------|------------------|
| AMX  | 67           | 100 | 100              | 0.0               | 0.0              |
| CRO  | 68           | 99  | 100              | 0.0               | 0.0              |
| CTX  | 68           | 99  | 99               | 0.0               | 0.0              |
| CXM  | 69           | 99  | 100              | 0.0               | 0.0              |
| FEP  | 66           | 94  | 99               | 0.0               | 0.0              |
| P    | 67           | 94  | 100              | 0.0               | 0.0              |

<sup>1</sup> CA calculated based on +/- 1 dilution categorical difference (using meningitis breakpoints)

<sup>2</sup> VME = Very Major Error, ME = Major Error

Table 6. Time to Result (Hours) for each of the antibiotics to report MIC in the Phoenix System.

| Drug | Average | Minimum | Maximum | Std. Deviation |
|------|---------|---------|---------|----------------|
| AMX  | 8.2     | 6.8     | 11.0    | 1.0            |
| CRO  | 9.0     | 5.6     | 15.8    | 1.3            |
| CTX  | 8.9     | 5.6     | 13.2    | 1.0            |
| CXM  | 8.0     | 5.3     | 10.1    | 1.3            |
| FEP  | 9.1     | 5.6     | 12.3    | 1.1            |
| P    | 8.7     | 6.8     | 12.3    | 1.2            |

Figure 2. Average Time to Result (Hours) in the Phoenix™ System

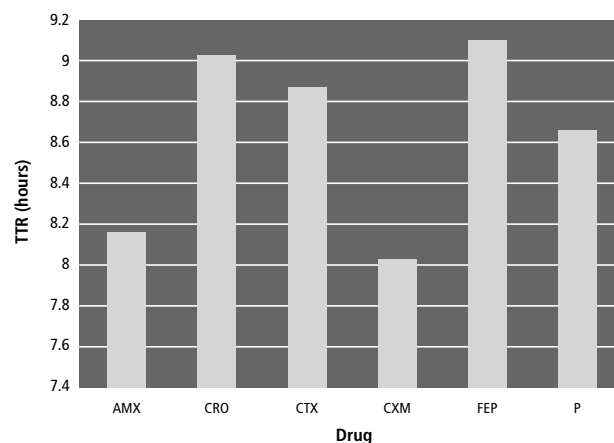
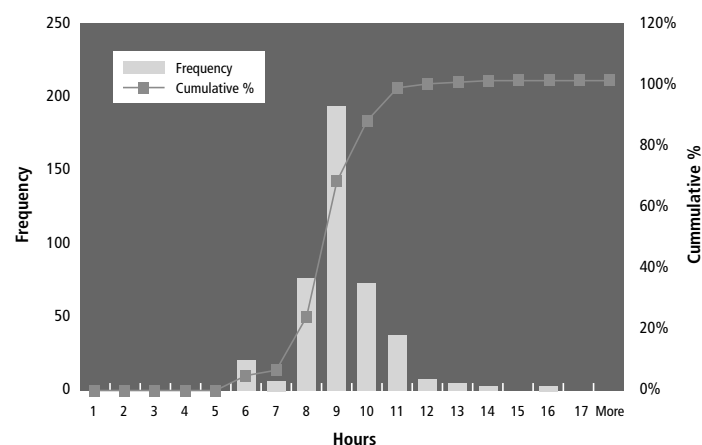


Figure 3. Time to Result for All Drugs in the Phoenix™ System



## CONCLUSIONS

- These preliminary evaluations indicate that the Phoenix System can rapidly and reliably detect resistance to AMX, CRO, CTX, CXM, FEP, and P for *S. pneumoniae*.
- The overall essential accord agreement for the Phoenix System ranged from 94% (penicillin) to 100% (amoxicillin). No Very Major or Major Error was observed for any of the drugs tested during this study.
- The average time to result for the 6 drugs tested with the Phoenix System was 8.6 hours (with a range of 5.3 to 15.8 hours).
- During this study 2 isolates failed to grow in the SBM system (containing 5% lysed horse blood—LHB) and 2 isolates failed to grow in the Phoenix System (containing enhanced broth with no LHB). This supports equivalent performance of the Phoenix AST-S Broth and the SBM method for growth of *S. pneumoniae* for MIC testing.
- Based on this evaluation of challenge strains containing multi-drug resistance as well as intermediate to high level resistance to penicillin, the overall performance of the Phoenix™ System was excellent.

