

Detection of Antimicrobial Resistance in *Pseudomonas aeruginosa* Using the BD Phoenix Automated Microbiology System.

G. DENYS¹, A. LINSKOTT², S. MIRRETT³, E. PETERSON⁴, L. B. RELLER³, J. SHIGEI⁴, R. SILBERMAN²

¹Clarian Health Partners, Inc., Indianapolis, IN; ²LA State Univ. Health Science Center, Shreveport, LA;

³Duke Univ. Medical Center, Durham, NC; ⁴Univ. of CA Medical Center, Irvine, CA.

ABSTRACT

BACKGROUND: Increased resistance in *Pseudomonas aeruginosa* (PSAE) continues to pose a significant threat to patient care because of limited therapeutic options. The ability to detect resistance in clinical PSAE isolates is critical for appropriate antimicrobial agent selection and eventual patient outcome. In this study, the BD Phoenix™ Automated Microbiology System (BD Diagnostics, Sparks, Maryland, USA), a rapid automated ID/AST system, was compared to the CLSI-recommended standard broth microdilution (SBM) method for performance with seven antimicrobials against PSAE.

METHODS: A total of 271 PSAE clinical and stock isolates, including nine challenge set strains, were tested for AST accuracy against four third-generation cephalosporins (ceftazidime, CAZ; cefoperazone, CFP; ceftizoxime, ZOX; and ceftriaxone, CRO), a fourth-generation cephalosporin (cefepime, FEP), piperacillin (PIP) and piperacillin-tazobactam (TZP). Each isolate was simultaneously tested in Phoenix and the CLSI-recommended SBM reference method. Inocula densities were adjusted equivalent to a 0.5 McFarland standard, and then inoculated into both panel types. Phoenix panels were incubated and read every 20 minutes to completion in the BD-Phoenix instrument, while SBM panels were incubated at 35°C for 18 – 20 hours in ambient air and read manually for MIC endpoint determination. Breakpoints and QC strains were those recommended in the current CLSI standard (M100-S15) for each antimicrobial.²

RESULTS: Essential agreement (EA) between Phoenix and the SBM was between 92% and 98% for the seven antimicrobials. Exact categorical agreement (CA) was between 76 and 97%, while these rates improved to between 95 and 98% when agreement within +/- one dilution was considered. The very major error (VME) rate ranged from 0% for CRO to 7.6% for TZP, though 4/5 TZP VMEs were within EA. Major error rates were all less than 2.8%, except for CRO at 6% (2/33).

CONCLUSIONS: The BD Phoenix System provides a satisfactory level of agreement to the reference method with PSAE and the seven antimicrobial agents tested in this study. The combined rate of resistance detection for these antimicrobial against PSAE was 97.9%.

INTRODUCTION

Pseudomonas aeruginosa (PSAE) is an opportunistic pathogen characterized by an innate resistance to multiple classes of antimicrobials. Accurate *in vitro* susceptibility test methods are important to provide proper therapy and for the detection of newly emerging resistance.

The BD Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) is an automated identification (ID) and antimicrobial susceptibility test (AST) system for both gram-positive and gram-negative organisms. The purpose of this multi-center study (Table 1) was to evaluate the ability of the Phoenix System to detect resistant phenotypes of PSAE from demographically and geographically diverse strains. Seven antimicrobials with indications for PSAE (cefepime, FEP; cefoperazone, CFP; ceftazidime, CAZ; ceftizoxime, ZOX; ceftriaxone, CRO; piperacillin, PIP; and piperacillin-tazobactam, TZP) were assessed for *in vitro* AST performance in Phoenix gram-negative panels, as these agents are often problematic in automated AST systems.¹

Table 1. List of Principle Investigators

Site Name	Site Location	Clinical Investigator
Clarian Health Partners Inc., Methodist Hospital	Indianapolis, IN	Gerald A. Denys, Ph.D.
Duke University Medical Center	Durham, NC	L. Barth Reller, M.D.
Louisiana State University Health Sciences Center	Shreveport, LA	Andrea J. Linscott, Ph.D.
University of California, Irvine Medical Center	Orange, CA	Ellena Peterson, Ph.D.

REFERENCES

- Gilbert, D., R. Moellering Jr., G. Eliopoulos and M. Sande. The Sanford Guide to Antimicrobial Therapy, 34th edition, 2004.
- Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement, Clinical and Laboratory Standards Institute, M100-S15, Vol. 25, No.1, January 2005.

MATERIALS AND METHODS

Organisms: The 271 clinical strains of *PSAE* tested included fresh isolates (< 7 days old and never frozen), recent isolates (>7 days but <60 days old and never frozen), stock strains (>60 days old or frozen), and a challenge set of isolates. No specimens were collected for the sole purpose of this study. Multiple isolates from the same patient and body site were excluded. Isolates were subcultured onto Trypticase® Soy Agar with 5% defibrinated sheep blood (TSA II™, BD Diagnostics, Sparks, MD) and incubated in ambient air at 35°C for 18 – 24 h. Recent and stock strains were subcultured twice before testing.

All clinical isolates were simultaneously tested in the Phoenix System and CLSI-recommended broth microdilution method. Challenge isolates were compared to a previously established (i.e., expected) MIC result. Both the reference and Phoenix System contained the same dilution range for the seven antimicrobials being evaluated (Table 2), and at least three lots of Phoenix and reference panels were used during the testing. Both the Phoenix and the CLSI methods were setup on the same day using the same inoculum suspension adjusted to a McFarland 0.5 Standard.

Phoenix Method: The standardized inoculum was prepared in Phoenix ID Broth according to the manufacturer's instructions. Five hundred microliters of this standardized ID broth inoculum was aseptically transferred to a tube of sterile reference diluent. Twenty-five microliters of ID broth inoculum was aseptically transferred to Phoenix AST Broth containing one drop of Phoenix AST Indicator. The AST broth suspension was gently mixed several times to insure an even organism distribution and then poured into the AST side of the Phoenix panel. The remaining ID broth was poured into the ID side of the panel. Panels were inoculated and loaded into the Phoenix instrument according to the manufacturer's instructions. All incubation, readings, and results were performed automatically within the instrument.

Reference Method: Frozen panels were thawed at room temperature for two hours. The inoculated reference diluent was gently mixed several times to insure an even organism suspension. Panels were inoculated within 15 minutes of the Phoenix ID Broth being standardized and according to the methods outlined in the CLSI document M7-A6 (Methods for

Table 2. Antimicrobials, Ranges and Breakpoints

Antimicrobial	Code	Test Range (mcg/ml)	CLSI* Breakpoints for <i>P. aeruginosa</i>		
			S	I	R
Ceftazidime	CAZ	0.5–64	< 8	16	> 32
Cefoperazone	CFP	0.5–64	≤ 16	32	≥ 64
Ceftriaxone	CRO	0.5–64	≤ 8	16–32	≥ 64
Cefepime	FEP	0.5–64	≤ 8	16	≥ 32
Ceftizoxime	ZOX	0.5–64	≤ 8	16–32	≥ 64
Piperacillin	PIP	0.5–128	≤ 64	–	≥ 128
Piperacillin / Tazobactam	TZP	0.5/4–128/4	≤ 64/4	–	≥ 128/4

* Clinical and Laboratory Standards Institute (formerly NCCLS), M100-S15, January 2005

Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard — Sixth Edition). Reference panels were incubated 16 – 20 hr at 35°C in ambient air. MIC results were read and recorded manually. Breakpoints used for the evaluation of each antimicrobial were those published in CLSI M100-S15, January 2005 (Table 2).

Discrepancy Testing: Clinical isolates which yielded major (Phoenix R, Reference S) or very major (Phoenix S, Reference R) errors were repeated in duplicate in both the Phoenix and reference methods, though only initial test results are represented in this study. Errors produced with challenge isolates were not repeated.

Quality Control: For each day of testing (initial and repeat), the recommended CLSI QC strains as well as additional on-scale manufacturer QC strains were tested in both the Phoenix and reference methods. Reference quality control results were used to determine the acceptability of the test results for each day of testing.

RESULTS

The number of *PSAE* compliant strains for each antimicrobial agent ranged from 235 to 271 for the cephalosporins, while over 400 for PIP and TZP. All SIR interpretations assigned for the total 2,163 isolate/antimicrobial combinations were based on reference MICs. The level of resistance demonstrated by the *PSAE* isolates to these antimicrobials ranged from 14.3% of the strains tested with TZP to 71.3% with ZOX (Figure 1 & Table 4).

Essential agreement (EA), categorical agreement (CA) and discrepancies were calculated using the initial Phoenix result and the reference result for each of the 7 antimicrobials (Tables 3 & 4). Repeat test results are included for information purposes only under 'Comments' in Table 4. For the 7 antimicrobials, EA ranged from 91.4% (FEP) to 98.1% (ZOX). Evaluable EA, which considers only strains with on-scale reference results and is a more accurate measure of performance, ranged from 92.6% to 96.4% for all 7 antimicrobials.

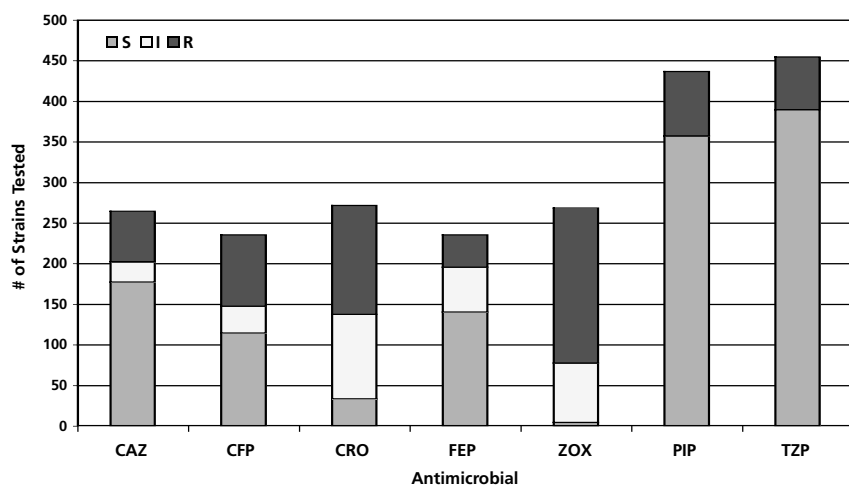
Table 3. Level of Agreement Between Phoenix and the Reference Method

Antimicrobial	CODE	n	EA* (%)	Ev. EA (%)	CA (%)
Ceftazidime	CAZ	264	94.7	95.1	96.6
Cefoperazone	CFP	235	96.6	96.2	87.7
Ceftriaxone	CRO	271	94.1	92.7	94.5
Cefepime	FEP	235	91.9	92.6	96.9
Ceftizoxime	ZOX	268	98.1	96.4	98.1
Piperacillin	PIP	436	94.0	93.2	97.0
Piperacillin/ Tazobactam	TZP	454	94.5	94.2	98.0

* Essential agreement (EA): Phoenix result within +/- 1 dilution of the reference result. Evaluable Essential agreement (Ev. EA): Phoenix on-scale result within +/- 1 dilution of the reference result.

Categorical agreement (CA): Agreement of Phoenix and reference interpretation within +/- 1 dilution of the reference result

Figure 1. Categorical Totals for *P. aeruginosa* Isolates by Antimicrobial Agent



The level of CA ranged from 94.1% for CRO, to 98.1% for TZP and ZOX, except for CFP at 87.7%. The lower than expected CA for CFP was due to a 10.6% (25/235) rate for minor errors (Phoenix = I, Reference = S or R, or Phoenix = S or R, Reference = I).

Categorical disagreements are listed in Table 4 for each antimicrobial. There were no very major errors (VME) for CRO, only one VME for FEP and ZOX and two for CFP. One of the two VME for CAZ resolved to Intermediate (I) when retested twice in the reference method. Two of the three VME for PIP, and 4/5 VME for TZP, were within EA, with neither of these drugs having an 'I' category.

There were no major errors (ME) for ZOX, and ≤ 3 ME for the other four cephalosporins. Both PIP and TZP yielded an ME rate of 2.8% for susceptible strains, with several of these also within EA. One of the two ME for CRO did resolve to Intermediate (I) when it was retested twice in the reference method.

Table 4. Major and Very Major Categorical Disagreements and Discussion

Antimicrobial	Code	Resistant (n)	% R of total n	VME* (n)	VME (%)	Susceptible (n)	ME** (n)	ME (%)	Comments
Ceftazidime	CAZ	62	23.4	2	3.2	177	3	1.7	1/2 VME changed in reference to 'I' (minor) on retest (x2)
Cefoperazone	CFP	88	37.4	2	2.2	114	2	1.8	
Ceftriaxone	CRO	134	49.4	0	0.0	33	2	6.0	1/2 ME changed in reference to 'I' (minor) on retest (x2)
Cefepime	FEP	40	17.0	1	2.5	140	3	2.1	
Ceftizoxime	ZOX	191	71.3	1	0.5	4	0	0.0	
Piperacillin	PIP	79	18.1	3	3.8	357	10	2.8	2/3 VME and 2/10 ME within EA (no I category)
Piperacillin / Tazobactam	TZP	65	14.3	5	7.6	389	11	2.8	4/5 VME and 3/11 ME within EA (no I category)

* Very Major Error (VME): Phoenix result = susceptible, reference result = resistant.

** Major Error (VME): Phoenix result = resistant, reference result = susceptible.

CONCLUSIONS

- Piperacillin, piperacillin/tazobactam, cefepime and the four third-generation cephalosporins tested in Phoenix gram-negative panels demonstrated satisfactory performance against clinical and challenge strains of *P. aeruginosa*.
- The Evaluable EA for all seven antimicrobials was > 92% demonstrating excellent comparability of Phoenix to the reference method.
- Detection of resistance to these antimicrobials in PSAE was also demonstrated by an acceptable number of VMEs (≤ 2) for the five cephalosporins, and only 1 VME each for PIP and TZP that was not within +/- one dilution of the reference result.
- The BD Phoenix System can be reliably used to detect antimicrobial resistance in *P. aeruginosa*.

