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Critical Assessment of *P. aeruginosa* (PSA) Susceptibility Testing Results for Six Beta-Lactams Using the BD Phoenix System

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ABSTRACT

Objectives: To study the accuracy of commercial automated systems when testing PSA due to escalating and recent reports of interpretive error. Documented high error rates were recorded for beta-lactams tested by MicroScan WalkAway, Vitek and Vitek 2 when compared to CLSI methods ranging from false-resistant (ME; major) to false-susceptible (VME; very major) results. Although in depth studies comparing automated systems have been reported, the BD Phoenix System has limited intermethod data for PSA, with susceptibility (S) test results often interpreted by non-CLSI criteria; thus, a 2-laboratory study was designed to validate Phoenix PSA results.

Methods: 60 tests were produced by local processing of 15 recent clinical strains (RCS) from each site (Arkansas Children's Hospital and St. Luke's Regional Laboratories) and 15 challenge strains (CS) representing equal numbers of PSA that were S and resistant (R) to the tested beta-lactams (aztreonam [AZM], cefepime [FEP], ceftazidime [CAZ], imipenem [IPM], piperacillin [PIP] and piperacillin/tazobactam [P/T]). Each strain was tested by Phoenix (panel NMIC-112; software V5.15A/04.11B) and compared to CLSI broth microdilution (BMD) results or to the consensus result from BMD, disk diffusion test (DD) and Etest. Errors were defined as VME, ME and minor (mE; intermediate by one method) guided by M23-A2.

Results: The table compares Phoenix categorical results and the consensus results from 3 validated methods. Unacceptable rates of mE (16.7-36.7%) occurred with AZM, FEP and CAZ, regardless of reference result utilized for analysis. Results were consistent between sites, RCS or CS subsets, and with prior publications that showed compromised categorical agreement. For AZM, the mE level was associated with systematic bias toward false-R. In contrast, IPM, PIP and P/T had generally acceptable error rates or were only marginally elevated.

Table. Types of intermethod errors when testing 30 *P. aeruginosa* isolates by the automated Phoenix system in two laboratories.[a]

Antimicrobial agent (no. tested)	% error types compared to consensus[b] Very Major	Major	Minor
Aztreonam (60)	0.0	1.7	36.7
Cefepime (60)	0.0	1.7	18.3
Ceftazidime (60)	1.7	0.0	16.7
Imipenem (60)	0.0	0.0	1.7
Piperacillin (30)[c]	0.0	3.3	NA
Piperacillin/tazobactam (60)	1.7	5.0	NA

a. Results from two laboratories (Arkansas Children's Hospital and St. Luke's Regional Laboratories).
b. Consensus of broth microdilution, disk diffusion and Etest categorical results. Unacceptable levels of error are underlined and mE was associated with a systematic trend toward false-R. NA = not applicable.
c. Results from one laboratory only.

Conclusions: This rigorous challenge of the Phoenix to assess the PSA susceptibility versus key beta-lactams displayed error rates indicating a modest need for re-evaluation (AZM). Automated systems have been documented to produce inaccurate results with trending toward false S as well as R. Clinical laboratories should be aware of these interpretive problems for PSA testing and seek alternative, validated methods such as simple agar diffusion tests (DD and Etest). Among the automated systems evaluated to date, the Phoenix appears to possess the fewest beta-lactam/PSA testing discords.

INTRODUCTION

Concern about the accuracy of commercial automated systems when testing *Pseudomonas aeruginosa* has been long-standing and featured in two recent presentations [1, 2]. The most elevated rates of error were reported for ß-lactam agents tested by the MicroScan WalkAway, Vitek and Vitek 2 [1, 8] instruments with discords compared to reference methods [9, 10] ranging from false-resistant (major error) to false-susceptible (very major error) results. [2] The most recent comprehensive study also showed unacceptable levels [11] of minor interpretive errors for aztreonam (28-31%) and cefepime (18-32%) when using the three most used commercial products (MicroScan, Vitek and Vitek 2). The most serious very major errors were detected for piperacillin/tazobactam (19-27%) [2] confirmed by results (10.0%) reported by Jorgensen et al. [1] that also noted a minor error rate of 23.6% for cefepime when testing 55 *P. aeruginosa* isolates.

Although in depth studies compared to all reference methods have been reported for *P. aeruginosa* tested by MicroScan, Vitek and Vitek 2 , the studies of BD Phoenix System (BD Diagnostic Systems, Sparks, MO, USA) have had limited testing for *P. aeruginosa* (only 63 strains), with susceptibility test results often interpreted by criteria other than the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards [NCCLS]) [12-15]. For these reasons, a two-laboratory protocol was designed to validate BD Phoenix System results for six ß-lactam agents compared to reference broth microdilution (BMD) [10] or a consensus of BMD and validated agar diffusion methods [16].

MATERIALS AND METHODS

A total of 60 tests were produced by the protocol that included local processing of 15 routine clinical strains from each study site (Arkansas Children's Hospital and St. Luke's Regional Laboratories) and a common challenge set of 15 strains representing approximately equal numbers of *P. aeruginosa* that were susceptible and resistant to the tested ß-lactams (aztreonam, cefepime, ceftazidime, imipenem, piperacillin and piperacillin/tazobactam); the latter provided by Dr. H.S. Sader. Each strain was tested by the BD Phoenix System (panel, NMIC-112; software V5.15A/04.11B) and compared to the CLSI [10] test results or to the consensus categorical result from the BMD test [10], disk diffusion test [9] and the Etest (AB BIODISK, Solna, Sweden). All MIC or zone diameter results were interpreted by current CLSI [12] published breakpoints.

Errors were defined as very major (false-susceptible by the BD Phoenix System), major (false-resistant by the BD Phoenix System) and minor (intermediate result by one of the compared tests). Acceptance levels of error were those listed by the NCCLS M23-A2 guideline [11].

RESULTS

- Tables 1 and 2 compare the BD Phoenix System MIC categorical results to those of the reference BMD [10] or the consensus of three validated procedures [5, 10]. One participant did not test piperacillin, therefore only 30 of 60 test results were recorded for that penicillin; 60 results were available for analysis for piperacillin/tazobactam, a carbapenem, two cephalosporins and the one monobactam tested.

- Unacceptable elevated rates of minor errors (16.7-36.7%) were identified with aztreonam, cefepime and ceftazidime, regardless of the reference result utilized for analysis. These results were consistent between laboratories and the organism subsets (recent clinical or challenge strains).

- Prior publications have recorded very high minor error rates for the ß-lactams and low overall "categorical agreement" (75.8-84.8%) when tested against *P. aeruginosa* [13, 14].

- For aztreonam and cefepime, the minor error level was combined with a systematic bias toward false resistance (16.7-38.3%; Table 2). In contrast, imipenem and piperacillin ± tazobactam had error rates that were generally acceptable or were only marginally elevated (>5% major errors [false-resistant]).

Table 1. Types of intermethod errors when testing 30 *P. aeruginosa* isolates by the automated BD Phoenix System in two laboratories.[a]

Antimicrobial agent (no. tested)	% error types comparable to BMD[b] Very major	Major	Minor	% error types compared to consensus[c] Very Major	Major	Minor
Aztreonam (60)	0.0	1.7	33.3[d]	0.0	1.7	36.7[d]
Cefepime (60)	0.0	1.7	18.3[d]	0.0	1.7	18.3[d]
Ceftazidime (60)	1.7[d]	0.0	18.3[d]	1.7[d]	0.0	16.7[d]
Imipenem (60)	0.0	0.0	3.3	0.0	0.0	1.7
Piperacillin (30)[e]	0.0	6.7[d]	NA[f]	0.0	3.3	NA[f]
Piperacillin/tazobactam (60)	1.7[d]	6.7[d]	NA[f]	1.7[d]	5.0	NA[f]

a. Results from two laboratories (Arkansas Children's Hospital, Little Rock, AR; and St. Luke's Regional Laboratories, Kansas City, MO).
b. BMD = broth microdilution reference method results [10].
c. Consensus of BMD, disk diffusion [9] and Etest categorical results
d. Unacceptable levels of error [11].
e. One laboratory only.
f. NA = not applicable because of no CLSI [12] intermediate category criteria.

Table 2. Assessment of systemic bias toward false-susceptible or -resistant categorical results for the BD Phoenix System.

System/Antimicrobial agent (no. errors)	Automated system error result (categorical trend; no.) More susceptible	More resistant	Net Trend (%)
Aztreonam (23)	0	23	38.3[a]
Cefepime (12)	1	11	16.7[a]
Ceftazidime (11)	7	4	5.0
Imipenem (1)	0	1	1.7
Piperacillin (1)	0	1	3.3[b]
Piperacillin/tazobactam (4)	1	3	3.3

a. Underlined results with significant testing bias as defined by a ≥ 10% net trend (≥6 occurrences) toward susceptibility or resistance when compared to consensus results (broth microdilution, disk diffusion and Etest categories) [9-12].
b. Based upon 30 test results.

CONCLUSIONS

- This rigorous challenge of the ability of the BD Phoenix System to assess the susceptibility of *P. aeruginosa* versus key ß-lactams displayed some error rates indicating a need for system re-evaluation, especially the results from aztreonam and cefepime.

- Some errors (false-susceptible) with other automated systems [1, 2] have been so severe that a highly resistant *P. aeruginosa* quality assurance challenge strain (MIC, ≥1024 mg/L) was interpreted as susceptible (MIC, ≤64 mg/L) to piperacillin/tazobactam by nearly one-third of Vitek 2 participants [3].

CONCLUSIONS continued

- Clinical laboratories should be aware of these interpretive problems for the automated system tests of *P. aeruginosa* and seek alternative, validated methods for routine use [5, 16]. Simple agar diffusion methods (disk diffusion and Etest) [9, 16] appear more accurate compared to the commercial automated options and equal to the results generated by the CLSI [10] reference methods having MIC endpoints read manually [17].

- Among the automated test systems evaluated to date, the BD Phoenix System appears to possess the fewest "drug/bug" testing discords for the most widely used broad-spectrum ß-lactam agents.

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