Accuracy of Beta-Lactam Susceptibility Testing Results from Vitek and Vitek 2 Systems when Testing *Pseudomonas aeruginosa*

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ECCMID 2007
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ABSTRACT

Objectives: To critically assess Vitek and Vitek 2 accuracy for determining beta-lactam susceptibility (S) when testing *P. aeruginosa* (PSA). Recent publications have questioned the interpretative and quantitative agreement of various automated systems especially for piperacillin/tazobactam (P/T), cefepime (FEP), and aztreonam (AZM). A collaborative multi-site study tested local (LOC) clinical strains and selected challenge (CH) strains.
Methods: This study was performed in 3 sites (Emory University/CDC, Atlanta, GA; St. Vincent's Hospital [STV], New York, NY; Loyola University Medical Center [LUMC], Maywood, IL) with each site testing strains by one or more automated methods e.g. Vitek by CDC and LUMC; and Vitek 2 by CDC and STV. Each processed 30 PSA (15 LOC and 15 CH) that included an equal distribution of isolates S and resistant (R) to beta-lactams. The tested agents were AZM, FEP, ceftazidime (CAZ), imipenem (IPM), piperacillin (PIP) and P/T. Utilized cards/software were: for Vitek (GNS-122/WSVTK-R10.01) and for Vitek 2 (AST-6N09/WSVT2-R04.02). Reference methods were reference frozen-form panels, Etest and disk diffusion methods using standardized CLSI procedural details or manufacturer instructions. Quality Control (QC) was assured via concurrent testing, and all presented data were associated with acceptable QC results.
Results: Analyses compared Vitek and Vitek 2 results to those produced by the reference broth microdilution test, and consensus categorical results from all reference methods. A significant bias toward S or R was defined as a shift of ≥10% for S rate when using the commercial product. Unacceptable levels of intermethod error were found using both applied analyses. Elevated minor error (mE; limit 10%) was noted for both systems with AZM (18.3-33.3%), FEP (13.3-36.7%), CAZ (16.7-23.3%) and IPM (13.3-26.7%). Serious, very major error (VME; false-S) several-fold greater than acceptable limit (1.5%) was detected for P/T with lesser degrees of ME and VME for PIP. Error rates by each system were distributed among LOC and CH strains and test sites.

Table. Errors produced when testing 30 *P. aeruginosa* against 6 beta-lactam agents by Vitek and Vitek 2 automated systems in two laboratoies each.

System/Antimicrobial agent	Percentage of error: Compared to BMD result			Compared to consensus result		
(no. tested)	VME	ME	mE	VME	ME	mE
Vitek						
Aztreonam (60)	0.0	3.3	18.3	0.0	5.0	31.7
Cefepime (60)	1.7	0.0	36.7	1.7	0.0	36.7
Ceftazidime (60)	1.7	0.0	20.0	1.7	3.3	16.7
Imipenem (60)	8.3	0.0	13.3	6.7	0.0	10.0
Piperacillin (60)	0.0	8.3	NA	0.0	6.7	NA
Piperacillin/tazobactam (60)	15.0	5.0	NA	15.0	5.0	NA
Vitek 2						
Aztreonam (60)	1.7	0.0	28.3	0.0	0.0	33.3
Cefepime (60)	0.0	0.0	13.3	1.7	0.0	16.7
Ceftazidime (60)	3.3	0.0	23.3	1.7	0.0	21.7
Imipenem (60)	6.7	0.0	25.0	5.0	0.0	26.7
Piperacillin (60)	5.0	0.0	NA	6.7	0.0	NA
Piperacillin/tazobactam (60)	21.7	1.7	NA	20.0	0.0	NA

a. Unacceptable levels of error are underlined; NA = not applicable.

Conclusions: PSA were tested by Vitek and Vitek 2 and results were observed as unacceptable for all 6 agents. VME was common with IPM, PIP and P/T (15.0-21.7%), and mE with significant skewing toward false R (11.0-21.0%) was noted for AZM, FEP and CAZ. These systematic errors adversely affect local antibiograms (empiric choices), individual patient care, and require corrective action by the manufacturer.

INTRODUCTION

Concern about the accuracy of commercial automated systems when testing *Pseudomonas aeruginosa* has been long-standing and featured in two contemporary presentations (J.H. Jorgensen, S.A. Crawford, M.K. Mansell, M.L. McElmeel, and L.C. Fulcher, Abstr. 106th ASM General Meeting, abstr. C-118, 2006) [1]. Highly elevated rates of error have been reported for ß-lactam agents tested by the MicroScan WalkAway, Vitek and Vitek 2 instruments [1-8] compared to results with reference methods [9, 10] ranging from false-resistant (major error) to false-susceptible (very major error) results. The most recent comprehensive study [1] also described unacceptable levels [11] of minor interpretive errors for aztreonam (28-31%) and cefepime (18-32%) when testing the three commonly used commercial automated systems (MicroScan WalkAway, Vitek and Vitek 2). The most serious very major errors were detected for piperacillin/tazobactam (19-27%) [1] confirmed by results (10.0% very major errors) reported by Jorgensen et al. (J.H. Jorgensen, S.A. Crawford, M.K. Mansell, M.L. McElmeel, and L.C. Fulcher, Abstr. 106th ASM General Meeting, abstr. C-118, 2006), which also noted a minor error rate of 23.6% for cefepime from testing 55 *P. aeruginosa* isolates.

For these reasons and concerns, a multicenter investigation was organized to evaluate the accuracy of two automated susceptibility testing methods (Vitek and Vitek 2) for testing *P. aeruginosa*. The study was performed in three laboratories as follows: for Vitek (Emory University-CDC and Loyola University Medical Center), and for Vitek 2 (St. Vincent's Hospital-Manhattan and Emory University-CDC).

MATERIALS AND METHODS

The participating laboratories processed 30 strains of *P. aeruginosa* representing local contemporary clinical isolates (15 strains) and a selected challenge set (15 strains) that included approximately equal distributions of isolates that were susceptible and resistant to the anti-pseudomonal ß-lactams [1]. The antimicrobial agents tested included aztreonam, cefepime, ceftazidime, imipenem, piperacillin and piperacillin/tazobactam. The organisms were tested at the laboratories using their routine automated system by procedures and reporting protocols recommended by the manufacturer (bioMerieux, Hazelwood, MO). The specific utilized product cards/software programs were: for Vitek (GNS-122/WSVTK-R10.01) and for Vitek 2 (AST-GN09/WSVT2-R04.02).

Comparison methods, also tested concurrently at each participant location, were reference frozen-form panels produced under GMP by TREK Diagnostics (Cleveland, OH), Etest (AB BIODISK, Solna, Sweden) and the disk diffusion method (BD, Sparks, MD) using the CLSI reference methods or the method recommended by the manufacturer [9-12]. The agar diffusion methods (disk diffusion and Etest) results have previously been validated for testing *P. aeruginosa* by Burns et al [13]. Quality control (QC) was assured via concurrent testing of CLSI-recommended strains, and all presented results were associated with acceptable QC test results [12].

Data were analyzed by comparing the results from each automated system to those produced by the reference broth microdilution test [9, 11, 12], as well as to the consensus categorical results of the reference broth microdilution and agar diffusion (Etest and disk diffusion) methods [9-14]. The origin of the errors (laboratory or organism subset) was also assessed, and acceptable performance was defined by intermethod error criteria found in NCCLS M23-A2 [11]. A significant bias toward susceptibility or resistance was defined as a shift of ≥10% in the perceived rate of susceptibility of the entire population (60 results/method or system) when using the commercial product compared to results from the categorical consensus [1].

RESULTS

- Table 1 lists the results (error rates) after comparing the automated system categorical test result to the CLSI reference test [10] and the consensus result of three validated methods [9, 10, 12, 13]. Unacceptable levels of intermethod error [11] were encountered using both applied comparative analyses.

- High minor error levels (limit of ≤10%) were noted for both automated systems when testing aztreonam (18.3-33.3%), cefepime (13.3-36.7%), ceftazidime (16.7-23.3%) and imipenem (13.3-26.7%). More serious, very major error (false-susceptible) rates several-fold greater than the acceptable limit (≤1.5%) were detected for piperacillin/tazobactam when using the Vitek (15.0%) and Vitek 2 (20.0-21.7%), while lesser rates of unacceptable serious error (false-susceptible or -resistant) were encountered with Vitek and Vitek 2 when testing piperacillin alone (5.0-8.3%).

- Unacceptable very major error rates were also encountered for both Vitek Systems when testing imipenem (5.0-8.3%).

- The error rates by each Vitek System were comparably distributed between tested organism populations and testing centers.

- Table 2 shows the trends toward greater susceptibility or resistance for Vitek or Vitek 2 categorical interpretations among the strains having intermethod categorical errors. Clear trending toward false resistance was observed for Vitek (aztreonam, cefepime, ceftazidime) and Vitek 2 (aztreonam); this was most marked for nearly one-third of aztreonam and cefepime results. In contrast, a false susceptibility bias for ≥10% of the tested strains was noted with imipenem (eight to 18 occurrences) and piperacillin/tazobactam (nine to 12 occurrences) using both systems.

Table 1. Types of intermethod errors produced when testing 30 *P. aeruginosa* isolates by Vitek and Vitek 2 automated systems in three laboratories[a].

System/Antimicrobial agent (no. tested)	Percentage of errors: Compared to BMD[b] Very major	Major	Minor	Compared to consensus[c] Very Major	Major	Minor
Vitek						
Aztreonam (60)	0.0	3.3[d]	18.3[d]	0.0	5.0[d]	31.7[d]
Cefepime (60)	1.7[d]	0.0	36.7[d]	1.7[d]	0.0	36.7[d]
Ceftazidime (60)	1.7[d]	0.0	20.0[d]	1.7[d]	3.3[d]	16.7[d]
Imipenem (60)	8.3[d]	0.0	13.3[d]	6.7[d]	0.0	10.0
Piperacillin (60)	0.0	8.3[d]	NA[e]	0.0	6.7[d]	NA[e]
Piperacillin/tazobactam (60)	15.0[d]	5.0[d]	NA[e]	15.0[d]	5.0[d]	NA[e]
Vitek 2						
Aztreonam (60)	1.7[d]	0.0	28.3[d]	0.0	0.0	33.3[d]
Cefepime (60)	0.0	0.0	13.3[d]	1.7[d]	0.0	16.7[d]
Ceftazidime (60)	3.3[d]	0.0	23.3[d]	1.7[d]	0.0	21.7[d]
Imipenem (60)	6.7[d]	0.0	25.0[d]	5.0[d]	0.0	26.7[d]
Piperacillin (60)	5.0[d]	0.0	NA[e]	6.7[d]	0.0	NA[e]
Piperacillin/tazobactam (60)	21.7[d]	1.7	NA[e]	20.0[d]	0.0	NA[e]

a. Vitek 2 results from St. Vincent Hospital-Manhattan (New York, NY) and Emory University-CDC (Atlanta, GA); and the Vitek data contributed by Loyola University Medical Center (Maywood, IL) and Emory University-CDC (Atlanta, GA).
b. BMD = broth microdilution reference method results from CLSI M7-A7 [10].
c. Consensus of BMD, disk diffusion [9] and Etest (AB BIODISK, Solna, Sweden) categorical results.
d. Unacceptable levels of error [11].
e. NA = not applicable because of no CLSI [12] intermediate category criteria.

Table 2. Assessment of systematic bias toward false -susceptible or -resistant results by the Vitek and Vitek 2 Systems when testing *P. aeruginosa* against ß-lactams.

System/Antimicrobial agent (no. errors)	Categorical trend (no.) More susceptible	More resistant	Net Trend (%)
Vitek			
Aztreonam (22)	2	20	30.0[a]
Cefepime (23)	2	21	31.7[a]
Ceftazidime (13)	2	11	15.0[a]
Imipenem (10)	8	2	10.0[a]
Piperacillin (4)	0	4	6.7
Piperacillin/tazobactam (12)	9	3	10.0[a]
Vitek 2			
Aztreonam (20)	3	17	23.3[a]
Cefepime (11)	8	3	8.3
Ceftazidime (14)	9	5	6.7
Imipenem (19)	18	1	28.3[a]
Piperacillin (4)	4	0	6.7
Piperacillin/tazobactam (12)	12	0	20.0[a]

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].

CONCLUSIONS

- This multicenter experiment illustrates the high level of discord between the two challenged automated systems (Vitek and Vitek 2) and the recommended/validated susceptibility methods [10, 13, 14]. These systematic errors of automated system origin result in documented false-susceptible or -resistant trends among the ß-lactam anti-pseudomonal agents.

- Concerns about *P. aeruginosa* antimicrobial susceptibility testing accuracy have been chronic and have recently been highlighted by intermethod comparisons of commercial automated systems (J.H. Jorgensen, S.A. Crawford, M.K. Mansell, M.L. McElmeel, and L.C. Fulcher, Abstr. 106th ASM General Meeting, abstr. C-118, 2006) [1, 8] and the piperacillin/tazobactam false-susceptible results reported to external quality assurance programs such as those of the College of American Pathologists [3]. Automated systems have not performed at acceptable levels of accuracy with some antimicrobial agents (J.H. Jorgensen, S.A. Crawford, M.K. Mansell, M.L. McElmeel, and L.C. Fulcher, Abstr. 106th ASM General Meeting, abstr. C-118, 2006) [2-7], while manually read reference tests (broth microdilution or agar dilution) and agar diffusion methods have functioned as reliable tests producing comparable categorical results [9, 10, 12-14].

- Clinical laboratories should be aware of these interpretive problems for the automated system tests with *P. aeruginosa* and seek alternative, validated methods for routine use [5, 13]. Agar diffusion methods (disk diffusion and Etest) [9, 13] are accurate when compared to the results generated by the CLSI [10] reference methods with MIC endpoints read manually [14]. These intermethod discords for the automated systems may be more widespread and not limited to *P. aeruginosa* as noted by Tenover et al. [15] in the testing of the rapidly emerging, epidemic KPC-enzyme producing *Klebsiella pneumoniae*.

ACKNOWLEDGEMENTS

The co-authors wish to thank the following individuals for technical and organizational contributions or in the provision of the challenge strains: H.S. Sader, D.J. Biedenbach, J. Yates and N. O'Mara-Morrissey. The study was funded by a collaborative educational/research grant from JMI Laboratories (North Liberty, IA) and Elan Pharmaceuticals (San Diego, CA).

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