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Accuracy of Three Automated Systems for Susceptibility Testing Selected Gram-Negative Bacilli Against Five Broad-Spectrum Beta-Lactam Agents

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AMENDED ABSTRACT

Objectives: To evaluate the accuracy of three automated systems; MicroScan WalkAway (MSWA), Vitek 2 (VT2) and Vitek Legacy (VTL), for susceptibility (S) testing *P. aeruginosa* (PSA) and various Enterobacteriaceae (ENT) species against aztreonam (AZT), cefepime (CPM), ceftazidime (CAZ), imipenem (IMP) and piperacillin/tazobactam (P/T).
Methods: Recent clinical strains (100 PSA and 20 ENT) from hospitals worldwide were selected to over-represent isolates with CPM and P/T MIC values within +/- 1 $\log_2$ dilution of the current NCCLS S and resistant (R) breakpoints for the studied compounds. Categorical results from automated systems were compared to the consensus of 5 reference/standardized methods: broth microdilution (frozen and dry-form panels), agar dilution, Etest (AB BIODISK, Solna, Sweden) and disk diffusion. Categorical disagreements were classified as: in very major (VM, false-S), major error (MA, false- R) and minor errors (MI; involving the intermediate category).
Results: The consensus testing S/R rates (%) among PSA strains were 50/50 for P/T, 44/43 for CAZ, 59/14 for CPM, 71/19 for IMP and 47/27 for AZT. A summary of the categorial disagreements for PSA is shown in the Table.

	Error rates (%)				
System/Error type	Aztreonam	Cefepime	Ceftazidime	Imipenem	Pip/Taz
MSWA					
Very Major	0	0	0	0	19
Major	0	3	0	2	1
Minor	28	32	13	10	-
VTL					
Very Major	2	0	2	0	21
Major	2	0	0	2	0
Minor	28	26	11	11	-
VT2					
Very Major	0	0	1	1	27
Major	1	1	1	2	0
Minor	31	18	9	8	-

All three systems showed a high, unacceptable rate of VM for P/T (19 to 27%). For other drugs VM rates ranged from 0 to only 2% (IMP tested on VT2). MA rates were acceptable for comparisons (0-3%) and MI rates were generally elevated (8-32%), reflecting the high proportion of consensus results within the intermediate category or skewed, more erroneous R results for CPM (VT2 and VTL) and AZT (all systems). Among ENT (20 strains), VM was 5% for AZT (VTL) and 0% for P/T, CAZ, CPM and IMP (100% S) on all 3 systems, and MA was detected mainly on MSWA (0-20%). MI rates were higher for P/T (20 [VT2] to 30% [MSWA and VTL]) and CPM (10 [VT2] and 20% [MSWA]).
Conclusions: The results of this study demonstrates that the automated systems (MSWA, VT2 and VTL) generally failed to accurately detect P/T-R among PSA. The criteria used to select the strains (MIC values close to breakpoints or within R range) increased the sensitivity of detecting significant categorical disagreements, dominated by MI. Re-evaluation of the P/T testing for PSA would be prudent for these systems to minimize adverse therapeutic outcomes worldwide.

INTRODUCTION

Automated or semi-automated systems have been widely used for species identification and susceptibility testing due to the increasing volume of specimens processed by clinical laboratories. These systems have provided clinical laboratories with excellent tools to decrease in-laboratory processing times and possibly turnaround time in order to supply physicians with rapid susceptibility profiles to guide antimicrobial therapy. Unfortunately, not all systems produce universally accurate results and reporting errors by automated systems can have serious implications on the clinical outcome of patients. Several studies have evaluated the accuracy of the automated systems for testing several organism/antimicrobial combinations. The most frequently reported errors involve *Pseudomonas aeruginosa* and select Enterobacteriaceae, especially when these organisms are tested against ß-lactams. Manufacturers are continuously updating software and issuing product notices recommending alternative testing methods for certain organism/antimicrobial combinations.

Several mechanisms may lead to ß-lactam resistance among Gram-negative bacteria, including 1) hyper-production of AmpC ß-lactamase or other broad-spectrum ß-lactamases such as various carbapenemases; 2) decreased outer membrane permeability; and 3) active drug efflux. Some of these mechanisms may affect one ß-lactam compound more than others and some automated systems may have problems categorizing these isolates. The inoculum concentrations and incubation time may also affect the detection of resistance due to ß-lactamase production. In summary, some commercial susceptibility testing systems may have difficulty in detecting resistance to some ß-lactam compounds due to various reasons, including the resistance mechanisms, the method employed by the system and faulty categorical/quantitative calculations.

The purpose of this study was to evaluate the accuracy of three widely used, automated systems, MicroScan WalkAway (Dade Behring, Deerfield, IL, USA), Vitek 2 and Vitek Legacy (bioMerieux, Hazelwood, MO, USA) for susceptibility testing of *P. aeruginosa* and various Enterobacteriaceae against aztreonam, cefepime, ceftazidime, imipenem and piperacillin/tazobactam.

MATERIALS AND METHODS

Bacterial isolates. A total of 120 recent clinical strains from hospitals worldwide were evaluated in the present study. The collection included *P. aeruginosa* (n=100) and Enterobacteriaceae (n=20). The isolates were selected primarily according to cefepime and piperacillin/tazobactam MIC values to over represent the values within ± one $\log_2$ dilution of current Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) susceptibility and resistant breakpoints for these compounds. Such a population of strains would amplify any systemic testing error among the systems (3) as used against the five frequently used antipseudomonal ß-lactams

Susceptibility testing. All isolates were susceptibility tested using five reference/reference-quality/standardized methods and three automated systems. Broth microdilution (frozen panels and commercially prepared dry-form panels [TREK Diagnostics, Cleveland, OH, USA], agar dilution, disk diffusion and Etest (AB BIODISK, Solna, Sweden) methods were used as the reference methods to establish a consensus MIC value and categorical result for each organism/antimicrobial combination. The MicroScan WalkAway tests were performed at Weland Clinical Laboratories, P.C. (Cedar Rapids, IA, USA) by using a Gram-negative MIC panel type 30 (B1017-308; Dade Behring). The Vitek 2 tests were performed at the University of Washington (Seattle, WA, USA) with the GN09 (bioMerieux) susceptibility cards and the results were analyzed by an advanced expert system (AES) software version WSVT2-R03.01. The Vitek Legacy and all reference/standardized methods were performed at JMI Laboratories (North Liberty, IA, USA). GNS-122 (bioMerieux) susceptibility cards were used in the Vitek Legacy and data was interpreted using software version WSVTK-R09.01. All reference/standardized and automated system susceptibility testing was performed in compliance with current NCCLS methods (M7-A6 and M7-A8) and/or according to manufacturer's recommendations. Quality control was monitored using the following organisms: *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 and 35218.

Data analysis. Consensus categorical results were obtained initially by comparing the results of the frozen broth microdilution and agar dilution methods. When the results of these two methods agreed, this value was considered the "consensus result". If these test methods did not agree (rare), discords were resolved by the disk diffusion method. A clear consensus result was obtainable in 100.0% of the strains using these three methods. Categorical results from each automated system were then compared to the consensus results. Categorical disagreements were classified as very major error (false-susceptible), major error (false-resistant) and minor error (involving the intermediate category produced by one method or system).

The consensus MIC results were also calculated initially using the frozen broth microdilution and agar dilution methods. If the MIC results of these methods were different, we first look at the disk diffusion result. For example, if we had ceftazidime results of 16 and 32 mg/L and the disk diffusion provided a resistant result, the consensus MIC would be 32 mg/L. If a consensus MIC could not be achieved with these three methods, the dry-form broth microdilution and Etest results were assessed.

RESULTS

- Table 1 shows the consensus MIC results distribution for *P. aeruginosa* strains. The percentage of MIC results within ± one $\log_2$ dilution of the breakpoints were 90.0% for aztreonam, 75.0% for cefepime and 59.0% for piperacillin/tazobactam.
- Based upon the consensus results for *P. aeruginosa*, susceptible and resistance rates were as follows: 47 and 27% for aztreonam, 59 and 14% for cefepime, 44 and 43% for ceftazidime, 71 and 19% for imipenem, and 50 and 50% for piperacillin/tazobactam (Table 1).
- The highest numbers of discrepancies were detected when testing *P. aeruginosa* against piperacillin/tazobactam, with the automated systems showing a disturbing tendency toward false-susceptible results (very major errors). Piperacillin/tazobactam susceptibility rates changed from 50% (consensus results) to 68% (MicroScan), 71% (Vitek Legacy) and 77% (Vitek 2).
- On the other hand, a tendency towards more resistant results was detected when cefepime was tested against *P. aeruginosa*. Susceptibility rates dropped from 59% (consensus) to 39% (MicroScan), 44% (Vitek Legacy) and 56% (Vitek 2). In addition, resistance rates increased from 14% (consensus) to 32% (MicroScan) and 21% (Vitek Legacy and Vitek 2).
- High rates of minor errors were detected when testing aztreonam against *P. aeruginosa*. A tendency towards less susceptible results was detected with the Vitek 2 (a shift from susceptible to intermediate), and Vitek Legacy (a shift from intermediate to resistant), while no significant trend was noticed with MicroScan (Table 2).

Table 1. Challenge collection of *P. aeruginosa* (100 strains) used in evaluation of commercial MIC products: Consensus MIC values.[a]

	Frequency of occurrence (number and %) at each MIC (mg/L)									
	1	2	4	8	16	32	64	128	256	≥512
Piperacillin/Tazobactam	NT[b]	NT	11	14	6	9	10[c]	23[d]	17	10
Aztreonam	NT	4	21	22[c]	26	18[d]	3	3	3	NT
Cefepime	6	15	13	25[c]	27	9[d]	1	2	2	NT

a. Consensus MICs defined as identical reference agar dilution and broth microdilution MIC results; discords resolved using dry-form broth microdilution panels and Etest.
b. NT; not tested.
c. Susceptible breakpoint.
d. Resistant breakpoint.

Table 2. Evaluation of the accuracy of automated systems when susceptibility testing 100 *P. aeruginosa* strains against ß-lactam antimicrobial agents.

	No. of isolates (%)			
Antimicrobial	Consensus[a]	MicroScan	Vitek 2	Vitek Legacy
Aztreonam				
Susceptible	47	41	33	49
Intermediate	26	32	41	14
Resistant	27	27	26	37
Cefepime				
Susceptible	59	39	56[b]	44
Intermediate	27	29	22	35
Resistant	14	32	21	21
Ceftazidime				
Susceptible	44	43	44	42
Intermediate	13	16	12	20
Resistant	43	41	44	38
Imipenem				
Susceptible	71	68	71[b]	69
Intermediate	10	9	14	1
Resistant	19	23	14	30
Piperacillin/Tazobactam				
Susceptible	50	68	77	71
Resistant	50	32	22[b]	29

a. Consensus result among broth microdilution (frozen form panels), agar dilution, and disk diffusion methods; see Materials and Methods.
b. The system did not provide result for one strain.

- A tendency towards more resistant results (shift from intermediate to resistant) was noticed when imipenem was tested against *P. aeruginosa* in the Vitek Legacy system (Table 2).
- Unacceptable high rates of very major (false-susceptible) errors were detected when piperacillin/tazobactam was tested against *P. aeruginosa* in all three commercial systems (19 - 27%) as shown in Table 3.
- Minor errors were also extremely high with all three systems when cefepime and aztreonam were tested against *P. aeruginosa* (Table 3).
- Based upon the consensus results for Enterobacteriaceae, susceptible and resistance rates were as follows: 35 and 45% for piperacillin/tazobactam, 15 and 80% for ceftazidime, 90 and 5% for cefepime, 100 and 0% for imipenem and 15 and 80% for aztreonam.

Table 3. Evaluation of the accuracy of automated systems when susceptibility testing 100 *P. aeruginosa* strains against ß-lactam antimicrobial agents.

	No. of isolates (%)		
Antimicrobial / Error type[a]	MicroScan	Vitek 2	Vitek Legacy
Aztreonam			
Very Major	0 (0.0)	0 (0.0)	2 (2.0)
Major	0 (0.0)	1 (1.0)	2 (2.0)
Minor	28 (28.0)	31 (31.0)	28 (28.0)
Cefepime			
Very Major	0 (0.0)	0 (0.0)[b]	0 (0.0)
Major	3 (3.0)	1 (1.0)[b]	0 (0.0)
Minor	32 (32.0)	18 (18.2)[b]	26 (26.0)
Ceftazidime			
Very Major	0 (0.0)	1 (1.0)	2 (2.0)
Major	0 (0.0)	1 (1.0)	0 (0.0)
Minor	13 (13.0)	9 (9.0)	11 (11.0)
Imipenem			
Very Major	0 (0.0)	1 (1.0)[b]	0 (0.0)
Major	2 (2.0)	2 (2.0)[b]	2 (2.0)
Minor	10 (10.0)	8 (8.1)[b]	11 (11.0)
Piperacillin/Tazobactam			
Very Major	19 (19.0)	27 (27.3)[b]	21 (21.0)
Major	1 (1.0)	0 (0.0)[b]	0 (0.0)

a. Error rates were calculated based on the consensus result among broth microdilution (frozen form panels), agar dilution, and disk diffusion methods; see Materials and Methods.
b. The system did not provide result for one strain.

Table 4. Evaluation of the accuracy of automated systems when susceptibility testing 20 Enterobactereaceae strains against ß-lactam antimicrobial agents.

	No. of isolates (%)		
Antimicrobial / Error type[a]	MicroScan	Vitek 2	Vitek Legacy
Aztreonam			
Very Major	0 (0.0)	0 (0.0)	1 (5.0)
Major	0 (0.0)	0 (0.0)	0 (0.0)
Minor	1 (5.0)	1 (5.0)	5 (25.0)
Cefepime			
Very Major	0 (0.0)	0 (0.0)	0 (0.0)
Major	3 (15.0)	1 (5.0)	0 (0.0)
Minor	4 (20.0)	2 (10.0)	0 (0.0)
Ceftazidime			
Very Major	0 (0.0)	0 (0.0)	0 (0.0)
Major	3 (15.0)	0 (0.0)	0 (0.0)
Minor	1 (5.0)	1 (5.0)	3 (15.0)
Imipenem			
Very Major[b]	0 (0.0)[b]	0 (0.0)[b]	0 (0.0)[b]
Major	0 (0.0)	0 (0.0)	0 (0.0)
Minor	0 (0.0)	1 (5.0)	0 (0.0)
Piperacillin/Tazobactam			
Very Major	0 (0.0)	0 (0.0)	0 (0.0)
Major	4 (20.0)	0 (0.0)	0 (0.0)
Minor	6 (30.0)	4 (20.0)	6 (30.0)

a. Error rates were calculated based on the consensus result among broth microdilution (frozen form panels), agar dilution, and disk diffusion methods; see Materials and Methods.
b. All isolates were susceptible.

- With the Enterobacteriaceae, the highest rates of errors were detected with the MicroScan system. Major error rates were high for piperacillin/tazobactam (20%), cefepime (15%) and ceftazidime (15%); and minor error rates were high for piperacillin/tazobactam (30%) and cefepime (20%). However, only a small number of strains (20) were tested only indicating the urgent need for a comprehensive re-evaluation of these organisms and commercial systems (Table 4).

CONCLUSIONS

- The results of this study demonstrate that the automated systems (MicroScan WalkAway, Vitek 2 and Vitek Legacy) generally failed to accurately detect piperacillin/tazobactam resistance among *P. aeruginosa*. All three systems produced a high, unacceptable rate of very major interpretive errors (false-susceptible results, 19 to 27%).
- The criteria used to select the strains (MIC values close to breakpoints or within resistance range) increased the sensitivity of detecting significant, potentially serious categorical disagreements.
- Re-evaluation of the piperacillin/tazobactam testing for *P. aeruginosa* would be prudent for these systems to minimize adverse therapeutic outcomes worldwide, as well as correcting rates of false resistance for other compounds (aztreonam, cefepime).

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