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Influence of Errors Caused by Automated Systems in the Antimicrobial Susceptibility Rates of *Pseudomonas aeruginosa* against Selected ß-Lactam Antimicrobials

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AMENDED ABSTRACT

Objectives: To evaluate the accuracy of automated susceptibility (S) testing systems for *P. aeruginosa* (PSA) against ß-lactams and the influence of those errors on the S rates.

Methods: The antimicrobial S of 397 PSA strains from North American hospitals (SENTRY Program; 2003) were tested against selected ß-lactams by broth microdilution (BMD) methods. In addition, a challenge collection of 100 PSA strains selected to over-represent isolates with cefepime (CPM) and piperacillin/tazobactam (P/T) MIC values within +/- 1 log_2 dilution of the current NCCLS S and/or resistant (R) breakpoints was used to evaluate MicroScan Walkaway (MSWA), Vitek 1 (VT1) and 2 (VT2) systems. Categorical results from automated systems were compared to the consensus of 4 reference/standardized methods: BMD (dry and frozen form), agar dilution, Etest and disk diffusion. Categorial discords were classified as: very major error (VM, false-S), major error (MA, false-R) and minor error (MI; involving the intermediate [I] category).

Results: The S and R rates of 397 PSA strains by reference tests are shown in table:

Antimicrobials	%S	%R
Aztreonam (AZT)	74.1	14.1
Cefepime (CPM)	88.4	3.5
Ceftazidime (CAZ)	87.2	8.3
Imipenem (IMI)	89.4	6.5
Piperacillin/tazobactam (P/T)	91.7	8.3

Among the 397 PSA isolates, the lowest R rates were detected for CPM and IMI, while the highest S rates were detected for P/T and IMI. All 3 systems showed an unacceptable rate of VM for P/T (19 to 27%). For other drugs, VM rates ranged from 0 to 2%. MA rates were acceptable (0-3%) and MI rates were generally elevated (range, 8-32%), reflecting the high proportion of consensus results within the I category. Skewed (erroneously high) MIC results were noted for CPM (MSWA and VT1). All VT2 results trended towards lower MIC values for the five drugs.

Conclusions: MSWA, VT1 and VT2 routinely fail to accurately detect P/T-R among PSA strains (1/3 false-S). The ß-lactam choice for empirical treatment of PSA infections currently appears to be driven by inappropriate local antibiograms in hospitals that use these automated systems for routine antimicrobial susceptibility testing of PSA. Re-evaluations are urgently required.

INTRODUCTION

Several mechanisms may lead to ß-lactam resistance among gram-negative bacteria, including 1) hyper-production of AmpC ß-lactamase or other broad- and extended-spectrum ß-lactamases such as carbapenemases; 2) decreased outer membrane permeability; and 3) active efflux. Some of these mechanisms may affect one ß-lactam compound more than others and some automated systems may have difficulty to accurately categorize the susceptibility profile of the isolates.

Automated or semi-automated systems have been widely used for species identification and susceptibility testing due to the increasing volume of clinical specimens processed by clinical laboratories. These systems have provided clinical laboratories with excellent tools to decrease in-laboratory turnaround time in order to supply physicians with timely 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 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-lactam agents. Manufacturers are continuously updating software and issuing product notices which recommend alternative testing methods for certain organism/antimicrobial combinations.

We evaluated the accuracy of automated susceptibility testing systems (3) for *P. aeruginosa* against ß-lactams and the influence of those errors on the S rates.

MATERIALS AND METHODS

SENTRY Program Collection. All *P. aeruginosa* strains collected from North American Hospitals through the SENTRY Antimicrobial Surveillance Program in 2003 (n=397) were included in the study. Only one isolate per patient was included. The isolates were tested for susceptibility in the Program by reference broth microdilution methods according to Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) recommendations against aztreonam, cefepime, ceftazidime, imipenem and piperacillin/tazobactam.

Challenge Collection. One hundred recent clinical strains of *P. aeruginosa* were collected from hospitals worldwide and were selected primarily according to the cefepime MIC results and secondarily according to the piperacillin/tazobactam MIC values to over-represent values that fell within ± two log_2 dilutions of current CLSI susceptible and resistant breakpoints for those compounds.

Evaluation of Automated Systems. Isolates from the Challenge collection were susceptibility tested using five reference/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 the categorical result for each organism/antimicrobial combination.

The MicroScan WalkAway tests were performed at Weland Clinical Laboratories, P.C. (Cedar Rapids, IA, USA) 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.

Consensus categorical results were obtained by comparing results of the frozen broth microdilution and agar dilution methods. When results of these two methods agreed, this value was considered the "consensus result". If these test methods did not agree, discords were resolved by the disk diffusion method. Consensus results were 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 susceptibility), major error (false resistance) and minor error (involving the intermediate category).

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 examined the disk diffusion result. For example, if ceftazidime results were 16 and 32 µg/ml and the disk diffusion provided a resistant result, the consensus MIC would be 32 µg/ml. If a consensus MIC could not be achieved with these three methods, we then considered the dry form broth microdilution and Etest results; this was rare.

RESULTS

- Table 1 shows the antimicrobial susceptibility patterns of both collections analyzed in this study. Against the SENTRY Program collection, piperacillin/tazobactam showed the highest rate of susceptibility (91.7%) but a relatively high rate of resistance (8.3%) due to no intermediate category. The lowest rate of resistance was exhibited by cefepime (3.5%).
- Resistance rates to piperacillin/tazobactam were six-fold higher in the challenge collection (50.0%) when compared to SENTRY Program results for North America (8.6%), but in both collections the majority of isolates resistant to piperacillin/tazobactam had MIC results at or one log_2 dilution higher than the resistant breakpoint. Isolates with piperacillin/tazobactam MIC results of 128 or 256 µg/ml represented 73% (24/33) and 80% (40/50) of the resistant strains in the SENTRY Program and challenge collections respectively.
- The percentage of piperacillin/tazobactam MIC results within ± one log_2 dilution of the breakpoints was 59.0% among strains of the challenge collection but only 14.0% among strains of the SENTRY Program collection (Table 2). This allows a precise estimation of test accuracy.
- When testing the challenge collection with the automated systems, the highest numbers of discrepancies were detected with piperacillin/tazobactam (Table 3). All three automated systems showed a clear tendency toward false-susceptible results (very major errors). Piperacillin/tazobactam susceptibility rates raised 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 testing cefepime (Table 3). 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).

Table 1. Antimicrobial activity of selected ß-lactams against *P. aeruginosa* strains evaluated in the present study.

Collection/antimicrobial agent (no. tested)	MIC (µg/ml) 50%	MIC (µg/ml) 90%	MIC (µg/ml) Range	% category Susceptible	% category Resistant
SENTRY Program (397)					
Aztreonam	8	>16	0.5->16	74.1	14.1
Cefepime	4	16	0.25->16	88.4	3.5
Ceftazidime	2	16	≤1->16	87.2	8.3
Imipenem	1	8	≤0.5->8	89.4	6.5
Piperacillin/Tazobactam	8	32	0.5->256	91.7	8.3
Challenge (100)					
Aztreonam	16	32	2->256	45.0	27.0
Cefepime	8	32	1->256	59.0	14.0
Ceftazidime	16	>16	1->16	44.0	43.0
Imipenem	2	>8	≤0.5->8	71.0	19.0
Piperacillin/Tazobactam	64	256	4->256	50.0	50.0

Table 2. Distribution of MIC results among isolates from the SENTRY Program and the challenge collection (Consensus MIC values[a]).

	Frequency of occurrence (%) at each MIC (µg/ml) ≤1	2	4	8	16	32	64	128	256	≥512
Piperacillin/Tazobactam										
SENTRY Program (397)	5(1.6)	25(6.3)	159(40.1)	90(22.7)	56(14.1)	24(6.0)	5(1.3)[c]	11(2.8)[d]	13(3.3)	9(2.3)
Challenge (100)	NT[b]	NT	11(11.0)	14(14.0)	6(6.0)	9(9.0)	10(10.0)[c]	23(23.0)[d]	17(17.0)	10(10.0)
Aztreonam										
SENTRY Program (397)	5(1.3)	21(5.3)	152(38.3)	116(29.2)[c]	47(11.8)	45(14.1)[c,e]	NT	NT	NT	NT
Challenge (100)	NT	4(4.0)	21(21.0)	22(22.0)[c]	26(26.0)	18(18.0)[c]	3(3.0)	3(3.0)	3(3.0)	NT
Cefepime										
SENTRY Program (397)	44(11.2)	149(37.5)	86(21.7)	72(18.1)[c]	32(8.1)	14(3.5)[c,e]	NT	NT	NT	NT
Challenge (100)	6(6.0)	15(15.0)	13(13.0)	25(25.0)[c]	27(27.0)	9(9.0)[c]	1(1.0)	2(2.0)	2(2.0)	NT

a. Consensus MIC results 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.
e. Isolates with MICs, results ≥ 32 µg/ml.

Table 3. Evaluation of the accuracy of automated system for susceptibility testing 100 *P. aeruginosa* strains (challenge collection) against ß-lactam antimicrobial agents.

Antimicrobial	No. of isolates 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.
b. The system did not provide a result for one strain.

- High rates of minor errors were detected when testing aztreonam. A tendency towards less susceptible results was detected only with the Vitek 2 (a shift from susceptible to intermediate), while no significant trend was observed with MicroScan or Vitek Legacy (Table 3 and 4).
- A tendency towards more resistant results (shift from intermediate to resistant) was noted when imipenem was tested in the Vitek Legacy system (Table 3 and 4).
- Unacceptable high rates of very major errors (false-susceptible) were detected when piperacillin/tazobactam was tested in all three systems (19 - 27%) as shown in Table 4.

Table 4. Evaluation of the accuracy of automated system for susceptibility testing a challenge collection of 100 *P. aeruginosa* strains against ß-lactam antimicrobial agents.

Antimicrobial / Error type[a]	No. of isolates MicroScan	Vitek 2	Vitek Legacy
Aztreonam			
Very Major	0	0	2
Major	0	1	2
Minor	28	31	28
Cefepime			
Very Major	0	0[b]	0
Major	3	1[b]	0
Minor	32	18[b]	26
Ceftazidime			
Very Major	0	1	2
Major	0	1	0
Minor	13	9	11
Imipenem			
Very Major	0	1[b]	0
Major	2	2[b]	2
Minor	10	8[b]	11
Piperacillin/tazobactam			
Very Major	19	27[b]	21
Major	1	0[b]	0

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

CONCLUSIONS

- The automated systems (MicroScan WalkAway, Vitek 2 and Vitek Legacy) generally failed to accurately detect piperacillin/tazobactam resistance among *P. aeruginosa*. All three systems showed a high, unacceptable rate of very major errors (false-susceptible results, 19 to 27%).
- In contrast, all three automated systems showed a tendency of more resistant results for cefepime when compared to reference methods.
- The majority of piperacillin/tazobactam resistant strains collected by the SENTRY Program in North America in 2003 had piperacillin/tazobactam MIC results at or only one dilution higher than the resistant breakpoints (128-256 µg/ml). Thus, the high rates of false-susceptible results provided by the automated systems when testing this compound may have a great impact in the local hospital antibiograms and guidelines for empiric treatment of *P. aeruginosa* infections in North American medical centers.
- Re-evaluation of the piperacillin/tazobactam testing for *P. aeruginosa* would be prudent for these systems to minimize adverse therapeutic outcomes worldwide. Several other ß-lactams also appear to need re-evaluations to reduce false-resistant errors.

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