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Detection of Antimicrobial Susceptibility Testing Errors by Automated Systems: College of American Pathologists Microbiology Surveys Results for 2003-2006

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ABSTRACT

Background:

College of American Pathologists (CAP) Microbiology Surveys provide proficiency sample challenges for thousands of laboratories, many using automated antimicrobial susceptibility testing (AST) systems (MicroScan [MS], Vitek [V], Vitek 2 [V2]). The most significant testing errors by these AST systems for the last four CAP survey years are summarized.

Methods:

The CAP Microbiology Survey Program distributes three graded AST challenges/year, each processed by approximately 2,500 participants. Methods used (last mailing; DA, 2007) were MS (39%), V (24%), V2 (21%), Disk Diffusion (DD) (12%) and other MIC methods (4%). Grading was based on 80% consensus of referees and participants by antimicrobial agent and resistance genotype by reference laboratories.

Results:

Numerous false-susceptible (FS) or false-resistant (FR) organism/antimicrobial test error events were noted for each antimicrobial AST system: V2 (9 total; 6 FS, 2 FR) > MS (6 total; 3 FS, 2 FR) = V (6 total; 3 FS, 3 FR). The most serious discords were V2 FS with carbapenems when testing a SME-1-enzyme-producing *S. marcescens*, four drugs (piperacillin tazobactam, amikacin, imipenem) when testing a multidrug-R *P. aeruginosa*, and linezolid tested against a G2576T mutant *S. aureus*. MS and V had slightly fewer FS errors, but all systems produced false (+) ESBL results for a CMY-2 AmpC-producing strain due to faulty "expert' rules that also compromised tests with *S. lugdunensis* (V only).

	Distribution (type) of significant errors:		
Challenge year	MicroScan	Vitek	Vitek 2
2003	Cefoxitin (FR)	Imipenem (FS) Meropenem (FS) Cefoxitin (FR)	Meropenem (FS) Cefoxitin (FR)
2004	-	-	P/T (FS) Amikacin (FS) Imipenem (FS) Piperacillin (FS)
2005	Linezolid (FS) Cefepime (FR)	T/S (FS) Levofloxacin (FR)	Linezolid (FS)
2006	A/C (FS) P/T (FS)	Penicillin (FR)	Cefoxitin (FR)

Abbreviations. P/T = piperacillin/tazobactam, A/C = amoxicillin/clavulanate, T/S = trimethoprim/sulfamethoxazole.

Conclusions:

Automated AST testing products have several established systematic errors requiring corrective action as documented by the CAP proficiency sample program. Some of these erroneous results (FS) may contribute to adverse clinical outcomes while others (FR) could deny patients valuable treatment options.

INTRODUCTION

The participation of clinical microbiology laboratories in external proficiency testing programs is considered an important component of ensuring quality and standardization in the performance of antimicrobial susceptibility testing. The College of American Pathologists (CAP) Surveys Program for clinical microbiology represents the largest comprehensive proficiency testing program in the world. An important part of the CAP Bacteriology and Mycology Surveys has been the graded antibacterial/antifungal susceptibility testing challenges (three organisms per year).

Previous reports by this program dating from 1982 have documented consistent high-quality performance by participating laboratories in the United States, but periodic methods or commercial product deficiencies have led to technical or methods modifications by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS]) and/or product changes by various commercial manufacturers.

The results of the 2003 - 2006 CAP antibacterial surveys are summarized in this presentation including a specific discussion of the problems noted among the most utilized susceptibility testing methods or systems (MicroScan, Vitek, Vitek 2, BD Phoenix, etc.).

MATERIALS AND METHODS

In 2003 - 2006, the CAP Microbiology Surveys (D-series) had 2,500-3,000 subscribing laboratories reporting data derived from organisms sent as unknown challenges for routine susceptibility testing. The organisms included:

D-A (2003) - *S. marcescens* (SME-1 enzyme producer; graded)
D-B (2003) - *S. pneumoniae* (multidrug-resistant [MDR]; graded)
D-C (2003) - *K. pneumoniae* (CTX-M series ESBL; graded)
D-A (2004) - *E. coli* (QC strain; graded)
D-B (2004) - *S. aureus* (QC strain; graded)
D-C (2004) - *P. aeruginosa* (MDR strain; graded)
D-A (2005) - *R. equi* (ungraded)
- *S. aureus* (linezolid-resistant MRSA; graded)
D-B (2005) - *K. kingae* (ungraded)
- *S. maltophilia* (trimethoprim/ sulfamethoxazole-resistant; graded)
D-C (2005) - *E. coli* (CMY-2 enzyme producer; graded)
D-A (2006) - *H. influenzae* (fluoroquinolone-resistant; ungraded)
- *E. faecalis* (VRE; graded)
D-B (2006) - *B. cepacia* (wildtype; ungraded)
- *S. pneumoniae* (fluoroquinolone-resistant; graded)
D-C (2006) - *S. lugdunensis* (wildtype; ungraded)
- *K. pneumoniae* (CTX-M series ESBL; graded)

Each specimen was to be processed by identification and susceptibility testing methods routinely used in the participating laboratory, with the reporting of only those "antimicrobial agents considered appropriate" for the clinical settings stated on the CAP survey form. As examples: the clinical infection settings included urinary tract infections, bloodstream infections and lower respiratory tract infection diagnosed by sputum or invasive sampling. In these specific settings the reporting of antimicrobials that achieved clinically adequate concentrations only in the urine (e.g. cinoxacin, nitrofurantoin, norfloxacin, trimethoprim, etc) were considered unacceptable performance for pulmonary and bloodstream infections.

Acceptable graded categorical results were those achieved by ≥ 80% (2003 onward) of referees and participants; allowing grading of all samples during this period. Ungraded specimens were introduced in 2005 as educational exercises to introduce new susceptibility testing guidelines or to correct potential practice errors (see above list).

Subsets of each sample were analyzed by the method used or the commercial product applied to the challenge organisms. Where elevated levels of error occurred for these products, those results were listed with the rates of unacceptable performance in Survey critiques (see Tables).

RESULTS

- The automated systems' performance for 17 CAP challenge strains were reviewed (2003-2006) and significant accuracy issues were identified in eight events (Table 1).
- Occurrences of unacceptable, generally false-susceptible, performance were found across all commonly used systems (MicroScan [6], Vitek [7] and Vitek 2 [9]).
- Table 1 summarizes details of each challenge organism and the type of erroneous test results. Most errors were noted for organisms having an uncommon but clinically important resistance phenotype or genotype (examples: serine carbapenemases, ESBLs and plasmidic Amp-C enzymes).

Table 1. Listing of elevated error rates for automated susceptibility testing systems observed in 8 of 17 CAP Survey challenge samples (2003-2006).

Year	Sample number	Organism	System	Antibiotic(s)	Error type[a]	Error rates	Comment
2003	D-03	*S. marcescens*	Vitek	Carbapenems	FS, FI	13-50%	Bush group 2f serine carbapenemase producer with resistance to carbapenems
			Vitek 2	Meropenem	FS	>50%	
	D-17	*K. pneumoniae*	MicroScan	Cephamycins	FR	6-7%	CTX-M ESBL-producing isolate with reference MIC values in susceptible
			Vitek	Cephamycins	FR	5-11%	range for cefoxitin and cefotetan
			Vitek 2	Cephamycins	FR	9-78%	
2004	D-17	*P. aeruginosa*	MicroScan	P/T[b]	FS	3%	MDR strain with markedly elevated MIC results (see Table 2)
			Vitek	P/T	FS	2%	
			Vitek 2	P/T	FS	30%	
				Amikacin	FS	4%	
				Imipenem	FS	6%	
				Piperacillin	FS	10%	
2005	D-05	*S. aureus*	MicroScan	Linezolid	FS	22%	G2576T mutant with high-level linezolid resistance
			Vitek 2	Linezolid	FS	38%	
	D-12	*S. maltophilia*	Vitek	T/S[b]	FS	31%	Drug of choice (T/S)-resistant *Stenotrophomonas*
				Levofloxacin	FR	12%	
	D-19	*E. coli*	MicroScan	-	F(+) ESBL	14%	Amp-C plasmidic CMY-2 enzyme-producer (not ESBL; see Table 3),
			Vitek	-	F(+) ESBL	2%	also clear trend toward falsely elevated cefepime MICs (see Figure 1)
			Vitek 2	-	F(+) ESBL	9%	
2006	D-15	*S. lugdunensis*	Vitek	ß-lactams	FR	85%	Extrapolated CoNS oxacillin-breakpoint error to all *Staphylococcus*-active ß-lactams (possible "expert system" error)
	D-17	*K. pneumoniae*	MicroScan	A/C[b]	FS	63%	CTX-M ESBL-producing isolate (see above; D-17, 2003),
			P/T		FS	43%	errors were not corrected
			Vitek 2	Cefoxitin	FR, FI	53%	

a. FS - false-susceptible; FI = false-intermediate; and F(+) ESBL = false-positive ESBL test.
b. P/T = piperacillin/tazobactam, T/S = trimethoprim/sulfamethoxazole and A/C = amoxicillin/clavulanate.

- Error rates cited were all in need of correction and could misdirect chemotherapy possibly leading to suboptimal clinical outcomes. D-17 (2004) is an example where a MDR *P. aeruginosa* was reported as susceptible to several ß-lactams and amikacin (Table 2).
- False resistances were also detected, example D-19 (2005) where significant differences in the cefepime MIC results were observed for the three most used commercial products. MicroScan produced significantly elevated MIC results compared to Vitek and Vitek 2 (≥two-fold) and was at least two- to four-fold greater than reference MIC values. The organism tested was a cefepime-susceptible CMY-2 enzyme-producer (Figure 1).

Table 2. Distributions of all incorrect participant responses and very major (false-susceptible) errors listed by commercial susceptibility test system when testing a MDR *P. aeruginosa* against eight antimicrobial agents (D-17, 2004; drugs with ≥10 incorrect interpretations and ≥10 participant users).

Antimicrobial/system	Total responses	False-susceptible response (no./%)	Total incorrect responses[a] (no./%)
Amikacin			
MicroScan	742	22 / 3.0	32 / 58.2
Vitek	542	10 / 1.8	14 / 25.5
Vitek 2	184	7 / 3.8	7 / 12.7
Imipenem			
MicroScan	790	4 / 0.5	15 / 12.3
Vitek	691	16 / 2.3	56 / 45.9
Vitek 2	183	11 / 6.0	49 / 40.2
Piperacillin			
MicroScan	549	2 / 0.4	2 / 11.8
Vitek 2	146	15 / 10.3	15 / 88.2
Piperacillin/tazobactam			
MicroScan	579	17 / 2.9	23 / 26.7
Vitek	537	12 / 2.2	13 / 15.1
Vitek 2	156	46 / 29.5	48 / 55.8

a. Includes false-intermediate responses.

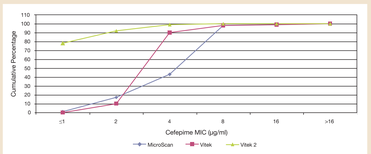
- Some screening tests were also suspect for accuracy (Table 3). False-positive ESBL screening/ confirmation test results were noted for all methods (manual and automated) when testing sample D-19 (2005). Highest error rates were observed for the CLSI disk test using clavulanic acid (32.0%), and the errors for the automated systems ranged from only 1.9% (Vitek) to 14.0% (MicroScan).

Table 3. Distributions of false-positive ESBL confirmation test (CMY-2 enzyme) results for six automated or manual methods (1,133 reported results from D-19, 2005).

	Results		
Method or test system (no.)	Positive	Negative	% Error
Vitek (262)	5	257	1.9
Vitek 2 (90)	8	82	8.9
MicroScan (221)	31	190	14.0
Disk approximation (76)[a]	16	60	21.1
Etest (31)	8	23	25.8
CLSI, disk ± CA (453)[b]	145	308	32.0

a. Also known as the double disk method.
b. CLSI method found in M100-S17 and CA = clavulanic acid.

Figure 1. Cumulative percentage cefepime MIC distributions produced by the three (3) most utilized automated susceptibility testing systems (D-19, 2005) when testing a cefepime-susceptible CMY-2 enzyme-producing *E.coli*.



CONCLUSIONS

- Susceptibility testing categorical errors as detected in the CAP Survey were most common among automated systems users (2003-2006).
- Errors that were detected require corrections by manufacturers to prevent adverse impact on patient care.
- Errors were systematic within an antimicrobial class or could also be limited to a single species and antimicrobial agent.
- Performance errors were found among all currently available automated systems, therefore requiring continued focused monitoring by the CAP Surveys and local validation of products by medical center laboratories.

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