

Performance Accuracy of Antibacterial and Antifungal Susceptibility Test Methods: Report from the College of American Pathologists (CAP) Microbiology Surveys Program (2001 - 2003)

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ABSTRACT

Background: CAP provides external proficiency samples, Microbiology Surveys Program, that monitor the performance of nearly 3,000 laboratories that perform and report susceptibility tests on antimicrobial agents. This report summarizes challenge samples in 2001 - 2003 (18 organisms).

Methods: One organism/4 months were tested by Surveys participants versus antibacterials/antifungals by routinely used methods. The most common tests were Vitek (38 - 43%) and MicroScan (39 - 43%), although Etest was most used for fastidious species. Disk diffusion (DD) tests were utilized by 14 - 15% of laboratories. YeastOne was the dominant antifungal test (50 - 55%). Reported tests were graded by qualitative, category (S, I, R) based on a 80% consensus of referees and participants. Nearly one-half million reported results were analyzed.

Results: Antifungal results remained ungraded, but mock grading (2003) showed > 90% categorical accuracy across 5 agents, highest for YeastOne and broth microdilution (BMD) tests. Antibacterial test accuracy was consistently > 97% vs Gram-positive challenges for DD and MIC tests. For Gram-negative (GN) strains (4) with characterized resistant mechanisms (ESBL, SME-1), the accuracy was DD > BMD > automated systems. Problems identified: 1) QC ranges require re-evaluation for *H. influenzae*; 2) overuse of β -lactamase tests; 3) discords of *E. faecium* vs penicillins (Vitek 2, MicroScan); 4) false-S results with TMP/SMX vs CoNS (MicroScan); 5) MLS_B false-S vs β -streptococcus (MicroScan); 6) DD/MIC discord for amikacin vs GNS; and 7) flawed reporting of drugs not active at the site of infection ("reporting error").

Conclusions: Susceptibility tests, generally commercial, are performing well as measured by the CAP Survey, but serious errors were identified with some drug/bug combinations. The testing problems of most concern involve fastidious species that may require action by the NCCLS and FDA.

INTRODUCTION

The College of American Pathologists (CAP) Surveys Program for clinical microbiology represents one of the largest comprehensive external quality assurance programs in the world. A broad variety of Surveys are available; they include two (mycobacteriology) to three (bacteriology and mycology) clinical challenge mailings of specimens covering six topics, as well as focused programs on molecular diagnostics and epidemiology of infectious diseases. An important component of the CAP Bacteriology and Mycology Surveys has been the antimicrobial/antifungal susceptibility testing challenges (three organisms/year). Previous reports by this program dating from 1982 have documented consistent high-quality performance overall (see selected references), but periodic methods or commercial product deficiencies detected by the CAP have led to technical or methods modifications by the National Committee for Clinical Laboratory Standards (NCCLS) and/or product changes by various commercial systems. The results of the 2001 - 2003 CAP Surveys are summarized in this presentation, including the ranking of the most utilized antimicrobial susceptibility testing methods or systems.

MATERIALS AND METHODS

In 2001 - 2003, the CAP Microbiology Surveys (D-series) had 2,685 - 2,979 subscribing laboratories reporting data that were sent three unknown challenge organisms for routine susceptibility testing each year. The organisms were: five Gram-positive species (*E. faecium*, *E. faecalis*, *S. epidermidis*, *S. dysgalactiae* and *S. pneumoniae*) and four Gram-negative organisms (*A. baumannii*, *H. influenzae*, *K. pneumoniae* and *S. marcescens*). Each specimen was to be processed by identification and susceptibility 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. For example, the clinical infection settings for each pathogen were meningitis caused by *S. pneumoniae*, bloodstream infection caused by *E. coli*, and a *P. aeruginosa* lower respiratory tract infection diagnosed by a sputum bronchoalveolar lavage. The reporting of antimicrobials that achieved clinically adequate concentrations only in the urine (cinoxacin, nitrofurantoin, norfloxacin, trimethoprim, etc.) was considered unacceptable performance for these three samples. Acceptable graded categorical results were those achieved by \geq 80% of referees and participants.

Similarly, the CAP Mycology Surveys (F-series) forwarded yeast isolates for susceptibility testing and identification also on the three occasions each year. The susceptibility tests were monitored, but grading of responses was not initiated until 2004. As with the antimicrobials, consensus susceptibility categorical responses from \geq 80% of referees and participants will guide acceptable grading in the future. Only the quantitative (MIC) information is presented for the antifungal susceptibility testing (see Table 1).

RESULTS

- Nine yeast challenge samples were tested for susceptibility to antifungal agents by CAP subscribers between 2001 - 2003 (Table 1). Results were quantitatively compared to NCCLS or published QC ranges with accuracies of: amphotericin (49 - 98%), 5FC (36 - 98%), fluconazole (71 - 100%), itraconazole (56 - 100%) and ketoconazole (91 - 100%).

- The most commonly used methods for antifungal testing were: YeastOne > reference broth microdilution > Etest. For antibacterial susceptibility testing, the most used methods were: Vitek (includes Vitek 2) > MicroScan > disk diffusion > Etest, however when fastidious challenge strains were tested, Etest was the most often applied method (data not shown).

- Table 2 shows the graded categorical accuracy for five Gram-positive challenge strains tested by disk diffusion and MIC methods. Among 126 graded organism-drug pairs, only two pairs had an accuracy level of < 90%, one for each test method. Three of four graded responses had an accuracy of \geq 95%.

- For the Gram-negative organisms, Table 3 summarizes the test accuracy when used for four challenge strains. The number of graded organism-drug pairs was markedly increased and the overall accuracy was also improved. Both methods performed extremely well with overall rates of accuracy exceeding 98%.

- Table 4 illustrates the accuracy of β -lactamase tests for a *H. influenzae* isolate. Rank order of test quality remains unchanged over the last two decades at: chromogenic cephalosporins > iodometric > acidometric tests.

- Product-based, high rates of testing error were observed:
 - Ampicillin false-resistance by Vitek 2 (Table 5).
 - Penicillin false-susceptible by MicroScan (Table 5).
 - Trimethoprim/Sulfamethoxazole false-susceptibility by Vitek 2 and MicroScan (Table 6).
 - False-susceptibility for erythromycin and/or clindamycin for MicroScan and Vitek (Table 7).

Each of these problems requires method modification to prevent serious reporting errors from adversely affecting patient care.

- A chronic problem of reported zone diameters for the *H. influenzae* QC strain at the lower limits (small zones) of published ranges was noted again in 2002 (Table 8). The best examples of this phenomenon are the cephalosporins (cefotaxime, ceftazidime and ceftioxime), chloramphenicol and the quinolones (ciprofloxacin and levofloxacin). HTM QC ranges for several agents may require re-evaluation using the latest M23-A2 guidelines and contemporary medium lots.

Challenge organisms/ antifungal agent (no. reports)	Participant MIC (μ g/ml)		Target MIC or range (μ g/ml) ^a	% of responses in QC range
	Median	Mode		
F-series (2001)				
<i>C. albicans</i> ATCC 90028				
Amphotericin B (59)	0.5	0.5	0.5-2	61
5FC (47)	0.5	0.5	0.5-2	66
Fluconazole (61)	0.5	0.5	0.25-1	89
Itraconazole (47)	<0.06	<0.06	<0.06 ^b	87
Ketoconazole (34)	<0.06	<0.06	<0.06 ^b	97
<i>C. parapsilosis</i> ATCC 90018				
Amphotericin B (53)	0.5	0.5	0.5-2	64
5FC (43)	0.12	0.12	<0.12-0.25	91
Fluconazole (56)	0.5	0.5	0.25-1	77
Itraconazole (45)	0.12	<0.06	<0.06 ^b	98
Ketoconazole (30)	<0.06	<0.06	<0.06 ^b	97
<i>C. tropicalis</i> ATCC 750				
Amphotericin B (64)	0.5	1	0.5-2	77
5FC (47)		<0.06	<0.12-0.25	96
Fluconazole (66)	2	2	1-4	88
Itraconazole (52)	0.25	0.25	0.25	83
Ketoconazole (34)	<0.06	<0.06	<0.06 ^b	91
F-series (2002)				
<i>C. parapsilosis</i> ATCC 22019				
Amphotericin B (60)	0.5	0.5	0.25-1	70
5FC (53)	0.25	0.25	0.12-0.5	90
Fluconazole (67)	2	2	2-8	91
Itraconazole (54)	0.12	0.25	0.06-0.25	72
Ketoconazole (35)	0.12	<0.06	0.06-0.25	97
<i>C. albicans</i> ATCC 24433				
Amphotericin B (62)	0.5	0.5	0.25-1	85
5FC (65)	0.5	0.5	1-4	36
Fluconazole (74)	0.5	0.5	0.25-1	91
Itraconazole (58)	0.12	<0.06	<0.06 ^b	100
Ketoconazole (31)	<0.06	<0.06	<0.06 ^b	97
<i>C. tropicalis</i> ATCC 750				
Amphotericin B (62)	0.5	0.5	0.5-2	69
5FC (64)	<0.06	<0.06	<0.12-0.25	95
Fluconazole (61)	2	2	1-4	85
Itraconazole (66)	0.25	0.25	0.25	94
Ketoconazole (34)	<0.06	<0.06	<0.06 ^b	97
F-series (2003)				
<i>C. albicans</i> ATCC 90028				
Amphotericin B (70)	0.25	0.25	0.25-2	49
5FC (75)	0.25	0.5	0.25-2	91
Fluconazole (96)	0.5	0.5	0.12-1	94
Itraconazole (96)	<0.06	<0.06	<0.06-0.12	91
Ketoconazole (40)	<0.06	<0.06	<0.06-0.12	85
<i>C. parapsilosis</i> ATCC 90018				
Amphotericin B (60)	0.5	0.5	0.25-2	93
5FC (68)	0.25	0.25	<0.12-0.5	98
Fluconazole (78)	4	4	0.5-4	91
Itraconazole (63)	0.25	0.25	<0.06-1	56
Ketoconazole (32)	<0.06	<0.06	<0.06-0.12	94
<i>C. krusei</i> ATCC 6258				
Amphotericin B (61)	0.5	1	0.25-4	98
5FC (61)	8	8	4-32	97
Fluconazole (68)	32	32	8-128	100
Itraconazole (72)	0.25	0.25	0.12-1	97
Ketoconazole (34)	0.25	0.25	0.25-1	97
Voriconazole (25)	0.25	0.25	<0.06-1	100
a. Target quality control or reference MIC ranges were derived from M27-A2 and peer-reviewed references.				
b. Ranges used for calculating accuracy when only a mode or target single concentration was available, were derived from that concentration \pm one log, dilution step e.g., a three log, dilution range or a four log, dilution range if the mode and median differed.				
c. 5FC = 5-fluorouracil.				

Antimicrobial agent	% accuracy (good performance):									
	Disk diffusion					MIC methods				
	<i>E. faecium</i>	<i>E. faecalis</i>	<i>S. epidermidis</i>	<i>S. dysgalactiae</i>	<i>S. pneumoniae</i>	<i>E. faecium</i>	<i>E. faecalis</i>	<i>S. epidermidis</i>	<i>S. dysgalactiae</i>	<i>S. pneumoniae</i>
Amikacin	U ^a	U	100.0	U	U	U	94.5	U	U	U
Amoxicillin/Clavulanate	U	U	U	U	U	U	96.2	U	U	U
Ampicillin	U	99.6	98.5	94.7	U	95.6	99.4	99.5	100.0	U
Ampicillin/Sulbactam	U	U	U	U	U	U	96.1	U	U	U
Azithromycin	U	U	U	100.0	U	U	98.2	U	U	U
Cefazolin	U	U	U	98.2	U	U	97.2	98.1	U	U
Cefepime	U	U	U	100.0	U	U	U	96.5	U	U
Cefotaxime	U	U	U	97.2	U	U	95.9	99.8	U	U
Ceftazidime	U	U	U	93.0	U	U	91.2	99.6	U	U
Cefturoxime	U	U	U	U	U	U	93.3	U	U	89.9
Chloramphenicol	U	90.9	98.3	U	95.2	94.1	99.4	99.7	94.1	93.2
Ciprofloxacin	U	U	94.3	U	U	98.4	99.2	98.0	90.3	U
Clindamycin	U	U	94.9	91.4	100.0	U	98.6	U	94.4	U
Erythromycin	99.3	U	99.5	99.2	100.0	99.4	U	97.8	91.6	95.0
Gatifloxacin	U	U	U	U	91.7	U	U	100.0	100.0	U
Gentamicin	100.0 ^b	U	U	99.7	U	98.5 ^c	98.2 ^c	99.5	U	U
Impipenem	U	U	U	U	U	U	91.4	U	U	94.4
Levofloxacin	98.0	97.7	93.7	97.9	97.1	99.2	99.6	96.4	99.4	100.0
Linezolid	94.8	U	U	100.0	U	93.6	U	100.0	U	U
Oxloxacillin	U	U	U	U	93.8	U	U	97.1	100.0	U
Oxacillin	U	U	93.4	U	U	100.0	U	96.5	U	U
Penicillin	97.5	97.7	99.5	96.9	99.6	99.2	99.6	99.7	89.4	U
Quinupristin/Dalfopristin	98.4	U	U	U	U	99.2	U	U	U	U
Rifampin	100.0	U	100.0	U	U	99.0	99.7	99.7	U	U
Streptomycin	100.0 ^b	90.2 ^c	U	U	U	94.2 ^c	93.3 ^c	U	U	U
Tetracycline	100.0	94.3	98.8	97.1	100.0	99.4	99.5	U	91.3	U
Trimethoprim/Sulfamethoxazole	U	U	98.7	U	100.0	U	U	93.9	U	U
Trovafoxacin	100.0	U	U	U	U	99.0	100.0	U	U	U
Vancomycin	99.7	94.3	99.8	98.2	99.6	99.4	99.5	99.6	99.7	100.0

a. U = ungraded because of too few participant reports or not achieving \geq 80% consensus.
b. Underlined value is the lowest graded accuracy for that challenge and method.
c. Gentamicin and streptomycin results for enterococci indicate tests for the detection of high-level resistance negating potential synergistic bactericidal action between aminoglycosides and cell-wall active agents.

Method (no.)	% correct by susceptibility user group (no. reports)	
	Disk diffusion (1,911)	MIC (760)
Chromogenic cephalosporins		
Nitrocefin (2,235)	98.0	98.9
Cefinase-2 (154)	96.5	100.0
Acidometric (208)	94.9	84.7
Iodometric (31)	95.2	90.0

Antimicrobial	Variable result	Method	% error
Ampicillin	Resistant	Etest	6.8%
		MicroScan	3.4%
		Vitek	2.2%
Penicillin	Susceptible	Vitek-2	22.2%
		Etest	9.1%
MicroScan	Susceptible	Vitek	54.7%
		Vitek-2	8.7%

Method or system	Reports (no.) by category:			% error ^a
	Susceptible	Intermediate	Resistant	
MicroScan	233	0	479	32.7
Vitek ^b	29	2	705	4.2
All methods	264	2	1,215	21.9

a. Correct results = resistant.
b. Excludes Vitek-2 which had 40.0% error, but only five responses.

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Antimicrobial agent	Disk diffusion				MIC methods			
	<i>A. baumannii</i>	<i>H. influenzae</i>	<i>S. marcescens</i>	<i>K. pneumoniae</i>	<i>A. baumannii</i>	<i>H. influenzae</i>	<i>S. marcescens</i>	<i>K. pneumoniae</i>
	Amikacin	100.0	U ^a	100.0	U	99.5	U	99.0
Amoxicillin/Clavulanate	100.0	U	99.9	100.0	U	U	98.3	98.9
Ampicillin	100.0	U	99.7	100.0	99.9	U	99.7	99.9
Ampicillin/Sulbactam	U	U	96.7	99.0	U	U	98.9	99.8
Azithromycin	U	100.0	U	U	U	U	U	U
Aztre								