# 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

**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<sub>B</sub> 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 simulated 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 ≥ 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
- 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).

Ranges used for calculating a

step e.g. a three log<sub>2</sub> dilution i

5FC = 5-flucytosine.

- 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 effecting 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 ceftriaxone), 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.

Quantitative accuracy of antifungal susceptibility testing for nine CAP-Mycology Surveys (F-series) samples in 2001-2003.

	Participant	t MIC (µg/ml)		
Challenge organisms/	Median	Mode	Target MIC or	% of responses
antifungal agent (no. reports)			range (µg/ml)ª	in QC range
<u>F series (2001)</u>				
C. albicans 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) Ketoconazole (34)	≤0.06 ≤0.06	≤0.06 ≤0.06	≤0.06 <sup>b</sup> ≤0.06 <sup>b</sup>	87 97
	≥0.06	≥0.00	≥0.00	91
C. parapsilosis 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 <sup>b</sup>	98
Ketoconazole (30)	≤0.06	_ ≤0.06	_ ≤0.06 <sup>b</sup>	97
C. tropicalis ATCC 750				
Amphotericin B (64)	0.5	1	0.5-2	77
5FC (47)	≤0.06	≤0.06	≤0.12-0.25	96
Fluconazole (66)	2	2	1-4	88
Itraconazole (52)	0.25	0.25	0.25 <sup>b</sup>	83
Ketoconazole (34)	≤0.06	≤0.06	≤0.06 <sup>b</sup>	91
F-series (2002)				
C. parapsilosis ATCC 22019				
Amphotericin B (60)	0.5	0.5	0.25-1	70
5FC (50)	0.25	0.25	0.12-0.5	90
Fluconazole (67)	2	2	2-8	91
Itraconazole (54)	0.12 0.12	0.25 ≤0.06	0.06-0.25 0.06-0.25	72 97
Ketoconazole (35)	0.12	≥0.00	0.00-0.25	91
C. albicans ATCC 24433 Amphotericin B (62)	0.5	0.5	0.25-1	85
5FC (55)	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 <sup>b</sup>	100
Ketoconazole (31)	≤0.06	_5.06 ≤0.06	_0.06 <sup>b</sup>	97
C. tropicalis 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 (81)	2	2	1-4	85
Itraconazole (66)	0.25	0.25	0.25 <sup>b</sup>	94
Ketoconazole (34)	≤0.06	≤0.06	≤0.06 <sup>b</sup>	97
F - series (2003)				
C. albicans 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 (78)	≤0.06 <0.06	≤0.06 <0.06	≤0.06-0.12 <0.06-0.12	81
Ketoconazole (40)	≤0.06	≤0.06	≤0.06-0.12	95
C. parapsilosis ATCC 90018 Amphotericin B (60)	0.5	0.5	0.25-2	93
5FC (58)	0.25	0.25	0.25-2 ≤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
C. krusei 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

ence MIC ranges were derived from M27-A2 and peer-reviewed references.	
accuracy where only a mode or target single concentration was available, were derived from that concentration ± one log₂ dilution	
range or a four log₂ dilution range if the mode and median differed.	

					% accuracy (goo	od performance):				
			Disk diffusion					MIC methods		
Antimicrobial agent	E. faecium	E. faecalis	S. epidermidis	S. dysgalactiae	S. pneumoniae	E. faecium	E. faecalis	S. epidermidis	S. dysgalactiae	S. pneumoniae
Amikacin	Uª	U	100.0	U	U	U	U	94.5	U	U
Amoxicillin/Clavulanate	U	U	U	U	U	U	U	96.2	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	U	96.1	U	U
Azithromycin	U	U	U	100.0	U	U	U	U	98.2	U
Cefazolin	U	U	U	98.2	U	U	U	97.2	98.1	U
Cefepime	U	U	Ü	100.0	U	U	U	U	96.5	U
Cefotaxime	U	U	U	97.2	U	U	U	95.9	99.8	U
Ceftriaxone	U	U	U	93.0	U	U	U	91.2 <sup>b</sup>	99.6	U
Cefuroxime	U	U	U	U	U	U	U	93.3	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	U	98.6	U	94.4
Erythromycin	99.3	U	99.5	99.2	100.0	99.4	U	97.8	91.6	95.0
Gatifloxacin	U	U	U	91.7	U	U	U	U	100.0	100.0
Gentamicin	100.0°	U°	99.7	U	U	98.5°	98.2°	99.5	U	U
Imipenem	U	U	U	U	U	U	U	91.4	U	U
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	<u>93.6</u>	U	U	100.0	U
Ofloxacin	U	U	U	93.8	U	U	U	97.1	100.0	U
Oxacillin	U	U	<u>93.4</u>	U	100.0	U	U	98.5	U	U
Penicillin	97.5	97.7	99.5	96.9	<u>89.6</u>	U	99.2	99.6	99.7	<u>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°	90.0°	U	U	U	94.2°	93.9°	U	U	U
Tetracycline	100.0	94.3	98.8	97.1	90.4	99.5	U	99.6	98.1	91.3
Trimethoprim/Sulfamethoxazole	U	U	98.7	U	100.0	U	U	U	U	93.9
Trovafloxacin	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

	% correct by susceptibility use	er group (no. reports)
Method (no.)	Disk diffusion (1,911)	MIC (760)
Chromogenic cephalosporins		
Nitrocefin (2,295)	98.0	98.9
Cefinase-2 (154)	96.5	100.0
Acidometric (208)	94.9	84.7
lodometric (31)	95.2	90.0

c. Gentamicin and streptomycin results for enterococci indicate tests for the detection of high-level resistance negating potential synergistic bactericidal action between aminogl

Table 5.	Method or system results varying from the consensus categorical determination for ampicillin (susceptible) and penicillin (resistant); ≥ 25 responses from <i>E. faecium</i> D-03 (2001).					
Antimicrobial	Variable result	Method	% error			
Ampicillin	Resistant	Etest	6.8%			
·		MicroScan	3.4%			
		Vitek	2.2%			
		Vitek-2	22.2%			
Penicillin	Susceptible	Etest	9.1%			
	·	MicroScan	54.7%			
		Vitek	2.3%			
		Vitek-2	8.7%			

		Reports (no.) by category:		
Method or system	Susceptible	Intermediate	Resistant	% error <sup>a</sup>
MicroScan	233	0	479	32.7
Vitek <sup>⁵</sup>	29	2	705	4.2
All methods	264	2	1,215	21.9

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	Wife metricus		
faecalis	S. epidermidis	S. dysgalactiae	S. pneumoniae
U	94.5	U	U
U	96.2	U	U
99.4	99.5	100.0	U
U	96.1	U	U
U	U	98.2	U
U	97.2	98.1	U
U	U	96.5	U
U	95.9	99.8	U
U	<u>91.2⁵</u>	99.6	U
U	93.3	U	89.9
99.4	99.7	94.1	93.2
99.2	98.0	<u>90.3</u>	U
U	98.6	U	94.4
U	97.8	91.6	95.0
U	U	100.0	100.0
98.2°	99.5	U	U
U	91.4	U	U
99.6	96.4	99.4	100.0
U	U	100.0	U
U	97.1	100.0	U
U	98.5	U	U
99.2	99.6	99.7	<u>89.4</u>
U	U	U	U
99.7	99.7	U	U
<u>93.9°</u>	U	U	U
U	99.6	98.1	91.3
U	U	U	93.9
100.0	U	U	U
99.5	99.6	99.7	100.0
lycosides ar	nd cell-wall active age	ents.	

	Error rates for erythromycin and clindamycin by testing method when applied to serogroup G β-haemolytic streptococcus (D-15, 2002; ≥ 25 responses).						
	No. (%) error						
Antimicrobial/method (no. reports)	Susceptible	Intermediate	Total % error				
<u>Erythromycin</u>							
Etest (118)	0 (0.0)	0 (0.0)	0.0				
MicroScan (453)	43 (9.5)	2 (0.4)	9.9				
PASCO (38)	1 (2.6)	2 (5.3)	7.9				
Vitek (40)	4 (10.0)	2 (5.0)	15.0				
Clindamycin							
Etest (64)	3 (4.7)	1 (1.6)	6.3				
MicroScan (552)	177 (32.1)	8 (1.4)	33.5				
PASCO (35)	3 (8.6)	1 (2.9)	11.4				
Vitek (38)	26 (68.4)	2 (5.3)	73.7				

Disk diffusion	on zone (mm)	MIC (µg/ml)		
Mean	NCCLS range	Mode	NCCLS	
16.6 (18.1) <sup>a</sup>	13-21	4	2-8	
16.6 (18.2)	14-22	4	2-8	
18.1	13-21	4	1-4	
31.1 (31.3)	31-39	≤0.25	0.12-0.5	
28.9 (28.6)	27-35	≤0.5	0.12-1	
30.5 (31.4)	31-39	≤0.12	0.06-0.25	
32.3 (31.8)	31-40	0.5	0.25-1	
33.3 (33.0)	34-42	≤0.25	0.004-0.03	
32.3	32-40	≤0.06	0.008-0.03	
22.0 (23.4)	21-29	1	-	
22.9	20-28	0.25	-	
15.5 (16.3)	14-22	8	4-32	
	Mean  16.6 (18.1) <sup>a</sup> 16.6 (18.2)  18.1  31.1 (31.3)  28.9 (28.6)  30.5 (31.4)  32.3 (31.8)  33.3 (33.0)  32.3  22.0 (23.4)  22.9	16.6 (18.1) <sup>a</sup> 13-21         16.6 (18.2)       14-22         18.1       13-21         31.1 (31.3)       31-39         28.9 (28.6)       27-35         30.5 (31.4)       31-39         32.3 (31.8)       31-40         33.3 (33.0)       34-42         32.3       32-40         22.0 (23.4)       21-29         22.9       20-28	Mean       NCCLS range       Mode         16.6 (18.1) <sup>a</sup> 13-21       4         16.6 (18.2)       14-22       4         18.1       13-21       4         31.1 (31.3)       31-39       ≤0.25         28.9 (28.6)       27-35       ≤0.5         30.5 (31.4)       31-39       ≤0.12         32.3 (31.8)       31-40       0.5         33.3 (33.0)       34-42       ≤0.25         32.3       32-40       ≤0.06         22.0 (23.4)       21-29       1         22.9       20-28       0.25	

Disk diffusion					MIC methods			
Antimicrobial agent	A. baumannii	H. influenzae	S. marcescens	K. pneumoniae	A. baumannii	H. influenzae	S. marcescens	K. pneumoniae
Amikacin	100.0	Uª	100.0	U	99.5	U	99.0	90.5
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
Aztreonam	99.2	100.0	U	100.0	98.8	92.9	U	99.9
Carbenicillin	100.0	U	90.0	U	95.5	U	<u>87.5</u> ⁵	U
Cefaclor	U	U	U	U	U	92.9	U	U
Cefazolin	100.0	U	90.0	99.4	99.9	U	99.9	99.8
Cefepime	99.1	U	100.0	100.0	96.8	U	99.3	99.8
Cefixime	U	96.6	U	U	U	100.0	U	U
Cefoperazone	100.0	U	U	U	100.0	U	U	U
Cefotaxime	100.0	96.7	97.3	100.0	94.9	99.2	95.5	99.6
Cefotetan	100.0	U	98.3	88.0	99.4	U	99.6	91.6
Cefoxitin	100.0	U	U	U	99.0	U	U	83.8
Ceftazidime	97.2	93.9	97.8	100.0	U	U	94.8	99.8
Ceftizoxime	100.0	U	U	U	90.2	U	U	U
Ceftriaxone	100.0	96.4	98.1	99.4	99.8	99.5	97.2	99.9
Cefuroxime	100.0	U	96.6	100.0	99.3	U	99.6	99.8
Cephalothin	100.0	U	100.0	98.9	98.9	U	98.9	99.6
Chloramphenicol	100.0	96.8	U	100.0	99.1	100.0	U	100.0
Ciprofloxacin	100.0	99.3	98.5	99.4	99.3	97.0	99.9	99.6
Gentamicin	100.0	U	99.7	99.6	99.8	U	99.8	99.8
Imipenem	99.7	98.2	97.4	100.0	99.2	95.0	93.5	99.5
Levofloxacin	100.0	100.0	99.3	100.0	99.7	100.0	99.9	99.9
Meropenem	90.9	<u>92.8</u>	U	100.0	100.0	100.0	U	99.2
Mezlocillin	100.0	U	U	U	98.9	U	U	U
Ofloxacin	100.0	Ü	100.0	Ū	100.0	Ü	98.1	Ü
Piperacillin	100.0	U	98.4	100.0	99.5	U	93.8	99.8
Piperacillin/Tazobactam	98.2	U	98.5	99.1	U	U	92.8	99.5
Tetracycline	U	96.4	U	90.9	Ü	92.5	U	98.4
Ticarcillin	100.0	U	<u>85.7</u>	U	93.5	U	Ü	U
Tobramycin	97.3	Ü	97.8	100.0	U	Ü	99.6	99.9
Trimethoprim/Sulfamethoxazole	99.2	99.4	98.7	100.0	96.1	99.2	99.8	99.7
<ul><li>a. U = ungraded because of too f</li><li>b. Underlined value is the lowest</li></ul>			sensus.					

"Categorical accuracy" of antimicrobial susceptibility test methods for CAP Surveys Gram-negative challenge strains in 2001-2003 (D-07, A. baumannii [2001]; D-09, H. influenzae ATCC 49247 [2002]; D-03, S.

marcescens [2003]; and D-17, K. pneumoniae [2003]).

#### CONCLUSIONS

- Antimicrobial and antifungal test accuracy remains high (> 95%) as monitored by the CAP Microbiology Surveys Program.
- Serious organism-method (product) errors of false-susceptibility and -resistance have been detected during the 2001 - 2003 interval, most problems involved automated methods.
- The most used tests have changed markedly since the initial published reports of CAP Surveys in the early 1980's. Disk diffusion, although accurate and affordable, has declined to < 15% of laboratories replaced by commercial, automated methods having a LIS interface.
- Also highly accurate niche products have emerged for testing fastidious species (Etest) and antifungal agents (YeastOne and Etest).
- The CAP Microbiology Surveys will continue to monitor and grade (by category) the participant responses for antibacterials and in 2004, the antifungals. Each type of test will be assessed based on regulatory guidelines of ≥ 80% consensus of referees and participants as to susceptibility category, regardless of NCCLS interpretive criteria.

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