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Accuracy for Antibacterial and Antifungal Susceptibility Tests: Report from the College of American Pathologists (CAP) Microbiology Proficiency Survey Program for 2004 - 2005

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ABSTRACT

Background: The CAP has provided external proficiency sample programs (Microbiology Surveys) that monitor participant performance (ca. 3,000 sites) in organism identification and antibacterial (AB)/antifungal (AF) testing. This report summarizes the results for samples processed in 2004 - 2005 (15 organisms).
Methods: One organism/4 mo. was tested (graded) by participants versus AB/AFs and 1 ungraded challenge/4 mo. was added to the AB program in 2005. Most used MIC methods were: Vitek (39%), Vitek 2 (11%) and MicroScan (46%); Etest was most used for fastidious species. Disk diffusion use continued to decline (11 - 12%), 14 - 15% in 2001 - 2003. YeastOne and Etest dominated AF test utilization (59 and 15%). Grading was based on 80% consensus of referees and participants.
Results: AF susceptibility (S) results were first graded in 2004, demonstrating excellent performance (93 - > 99% accuracy) and all methods were acceptable. Wild-type azole-resistant yeast challenges did not diminish AF test accuracy (> 95%). AB test accuracy remained high (DD at 98 - > 99%; MIC at 98 - > 99%), but the results with CLSI QC strains indicated need for re-evaluation of aztreonam, cefepime, clindamycin, oxacillin and vancomycin ranges. Emerging concerns were: 1) correct use of polymyxin test methods; 2) false-S automated system results for VRSA, VISA and piperacillin/tazobactam with *P. aeruginosa* (30% for Vitek 2); and 3) suboptimal MRSA and ESBL detection in commercial systems. Ungraded samples of unusual, fastidious species promoted CAP educational objectives for appropriate testing/reporting (3 challenges in 2005).
Conclusions: The accuracy of AB and AF testing continues to be documented at a high level (> 95%) by the CAP Surveys, but serious reporting errors persist with some automated systems. Manufacturers are urged to rapidly modify systems to minimize false-S errors and to test recently marketed AB and AFs.

INTRODUCTION

The participation of clinical microbiology laboratories in proficiency testing programs is considered an important step in ensuring quality and standardization in the performance of antibacterial and antifungal susceptibility testing. The College of American Pathologists (CAP) Surveys Program for clinical microbiology represents one of the largest comprehensive external proficiency testing programs in the world. An important component 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 overall by participating laboratories, but periodic methods or commercial product deficiencies detected by the CAP 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 2004 - 2005 CAP antibacterial and antifungal surveys are summarized in this presentation including discussion of the most utilized susceptibility testing methods or systems.

MATERIALS AND METHODS

In 2004 - 2005, the CAP Microbiology Surveys (D-series) had nearly 3,000 subscribing laboratories reporting data that were sent unknown challenge organisms for routine susceptibility testing. The organisms included two Gram-positive species (*S. aureus* [two; one MRSA]) and four Gram-negative organisms (*E. coli* [two; one a QC strain], *P. aeruginosa, S. maltophilia*). 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. For example, the clinical infection settings included urinary tract infection caused by *E. coli*, bloodstream infections caused by *S. aureus*, and *P. aeruginosa* lower respiratory tract infection diagnosed by bronchoalveolar lavage. In these specific settings the reporting of antimicrobials that achieved clinically adequate concentrations only in the urine (e.g. cinoxacin, nitrofurantoin, norfloxacin, trimethoprim, etc) was 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. Ungraded, educational specimens included: *R. equi* and *K. kingii*. The final challenge set is pending grading at this time (CAP D-C and F-C, 2005).

Likewise in 2004 - 2005, the CAP Mycology Survey (F-series) had approximately 1,200 subscribing laboratories reporting data that were sent three unknown yeast isolates each year as antifungal susceptibility challenges of which over 100 sites reported antifungal susceptibility results. The quantitative susceptibility test results were monitored, and grading of categorical responses was initiated in 2004. As with the antibacterials, consensus susceptibility categories from ≥ 80% of referees and participants guided the grading process. For purposes of this report the quantitative (MIC) and categorical results were tabulated and discussed.

RESULTS

- Table 1 shows the continued high level of antifungal susceptibility testing accuracy with recent categorical grading varying from 92 to 99% accuracy across five challenge samples. One organism (*C. albicans* ATCC 24433) - antifungal (5FC) combination may require re-evlauation of the reference MIC control range.
- Gram-positive organism challenges exhibited very high participant accuracy (Table 2), but two serious issues emerged:
  - Three to seven antimicrobials may require re-evaluation of disk diffusion QC ranges for *S. aureus* ATCC 25923 (Table 3); and
  - False-susceptible results for linezolid were unacceptably high for MicroScan WalkAway (21.6%) and Vitek 2 (38.2%), see Table 4.
- Gram-negative challenges graded-to-date (three) showed several troublesome items (Table 5):
  - Disk diffusion QC ranges for *E. coli* ATCC 25922 may require re-evaluation by the CLSI for aztreonam, cefepime, ceftriaxone, ciprofloxacin, piperacillin and tetracycline (Table 6);
  - Unacceptable levels of false-susceptible results (≥ 3.0%) were detected for a multidrug-resistant *P. aeruginosa* strain when testing some agents (Table 7), especially piperacillin and piperacillin/tazobactam tested by Vitek 2;
  - Current CLSI M100-S15 recommendations for testing non-*P. aeruginosa*, non-Enterobacteriaceae appear to be confusing to some document users. The challenge with a trimethoprim/sulfamethoxazole-resistant *S. maltophilia* was, however, not as accurately processed as other 2004 - 2005 samples (Table 5) with the reporting of unvalidated (CLSI) disk diffusion results (other than TMP/SMX, levofloxacin and minocycline) and MIC results contraindicated by some commercial system (Vitek) manufacturers.

Table 1. Quantitative and categorical accuracy of antifungal susceptibility testing for five CAP-Mycology Surveys (F-series) samples in 2004 - 2005.

Challenge organisms/ antifungal agent (no. reports)	Participant MIC (µg/ml) Median	Mode	Target MIC or range (µg/ml)[a]	% of responses in QC range	% categorical accuracy
F series (2004)					
C. krusei ATCC 6258					
Amphotericin B (53)	0.5	0.5	0.12-4	98	83
5FC (64)[b]	8	8	4-32	94	94
Fluconazole (67)	32	32	8-256	100	96
Itraconazole (73)	0.25	0.25	0.12-1	96	94
Ketoconazole (29)	0.25	0.25	0.12-1	93	U[b]
C. tropicalis ATCC 750					
Amphotericin B (60)	0.5	1	0.25-2	92	100
5FC (65)	≤0.06	≤0.06	≤0.06-0.5	97	100
Fluconazole (84)	1	1	0.5-4	99	99
Itraconazole (68)	0.25	0.25	≤0.06-0.5	100	95
Ketoconazole (64)	≤0.06	≤0.06	≤0.06-0.25	100	100
C. parapsilosis ATCC 22019					
Amphotericin B (68)	0.5	0.5	0.25-4	96	97
5FC (79)	0.25	0.25	≤0.06-0.5	97	100
Fluconazole (99)	4	4	0.5-4	88	100
Itraconazole (79)	0.25	0.25	0.12-0.5	85	99
Ketoconazole (36)	≤0.06	≤0.06	≤0.06-0.5	100	100
F-series (2005)					
C. albicans ATCC 90028					
Amphotericin B (71)	0.5	0.5	0.25-2	90	100
5FC (77)	0.5	0.5	0.25-2	91	100
Fluconazole (64)	0.5	0.5	0.12-1	98	99
Itraconazole (82)	≤0.06	≤0.06	NA[c]	NA	99
Ketoconazole (36)	≤0.06	≤0.06	NA	NA	100
C. albicans ATCC 24433					
Amphotericin B (71)	0.5	0.5	0.25-2	90	100
5FC (77)	0.5	0.5	1-4	26[b]	100
Fluconazole (104)	0.5	0.5	0.25-1	98	99
Itraconazole (82)	≤0.06	≤0.06	NA	NA	94
Ketoconazole (36)	≤0.06	≤0.06	NA	NA	100

a. Target quality control or reference MIC ranges were derived from M27-A2 and peer-reviewed references. Also ranges used for calculating accuracy of wild-type strains where only a mode or target single concentration was available, were derived from that concentration ± one $\log_2$ dilution step e.g. a three $\log_2$ dilution range or a four $\log_2$ dilution range if the mode and median differed.
b. 5FC = 5-flucytosine; U = ungraded; and NA = not applicable.
c. CLSI QC range should be re-evaluated.

- CAP Surveys participants continue to report results in clinical infection types where the agent maybe contraindicated due to limited concentrations in vivo or metabolism to an inactive product; also reporting of tests where the CLSI has no interpretive criteria remains worrisome. Penalties will be imposed in future survey samples (2006).

Table 2. "Categorical accuracy" of antimicrobial susceptibility test methods for CAP Surveys Gram-positive challenge strains in 2004 - 2005 (D-10, *S. aureus* ATCC 25923 [2004] and D-05, *S. aureus* [2005]).

Antimicrobial agent	% accuracy (good performance): Disk diffusion D-10 (2004)	Disk diffusion D-05 (2005)	MIC methods D-10 (2004)	MIC methods D-05 (2005)
Amoxicillin/Clavulanate	100.0	97.8	99.4	99.2
Ampicillin	86.8[a]	100.0	98.4	97.6
Ampicillin/Sulbactam	100.0	100.0	99.5	99.1
Azithromycin	100.0	100.0	96.8	100.0
Cefazolin	100.0	100.0	99.5	99.6
Cefotaxime	100.0	100.0	98.6	100.0
Ceftriaxone	100.0	96.2	100.0	97.9
Cephalothin	100.0	100.0	100.0	98.2
Chloramphenicol	100.0	92.6	98.7	U[b]
Ciprofloxacin	97.1	100.0	99.9	98.2
Clindamycin	99.1	100.0	99.6	99.7
Erythromycin	97.4	100.0	99.6	99.6
Gatifloxacin	100.0	U	100.0	U
Gentamicin	100.0	99.4	99.9	98.9
Imipenem	100.0	100.0	100.0	95.3
Levofloxacin	100.0	98.2	99.7	98.9
Linezolid	100.0	81.1	100.0	81.3
Oxacillin	99.6	99.6	99.7	99.9
Penicillin	93.0	100.0	95.2	99.7
Quinupristin/Dalfopristin	100.0	100.0	100.0	100.0
Rifampin	100.0	100.0	99.7	99.2
Tetracycline	98.6	98.7	99.8	93.5
Trimethoprim/Sulfamethoxazole	99.5	99.2	99.7	96.1
Vancomycin	99.6	100.0	100.0	99.7

a. Underlined value is the lowest graded accuracy for that challenge and method.
b. U=Ungraded because of too few participant reports or not achieving ≥ 80% consensus.

Table 3. Comparisons of mean zone diameters from CAP samples D-03 (1997) and D-10 (2004) of *S. aureus* ATCC 25923 for seven antimicrobials having concerns about currently published QC zone diameter ranges.[a]

Antimicrobial agent	Mean reported zone (mm) D-03 (1997)	Mean reported zone (mm) D-10 (2004)	CLSI QC guidelines (mm) Midpoint	CLSI QC guidelines (mm) Range (% in range)
Ciprofloxacin	24.0	25.1	26.0	22-30 (89)[a]
Clindamycin	24.6	26.1[b]	27.0	24-30 (71)[a]
Erythromycin	25.0	26.0	26.0	22-30 (94)
Oxacillin	20.3	20.4[b]	21.0	18-24 (92)
Penicillin	32.3	32.4	31.5	26-37 (88)[a]
Trimethoprim/Sulfamethoxazole	26.8	27.6	28.0	24-32 (92)
Vancomycin	17.6	18.5[b]	19.0	17-21 (79)[a]

a. Results with < 90% of reported zones in the CLSI range.
b. This table lists the seven drugs where the greatest number of participant laboratories reported "out-of-control" range results in 1997. From that sample to 2004, the mean zones for ciprofloxacin, clindamycin, erythromycin, trimethoprim/sulfamethoxazole and vancomycin increased to near the mid-point of the published CLSI ranges. However, some drug zone diameters remain skewed toward the lower end of the range (small zones) such as vancomycin, oxacillin and clindamycin that have mean zones only 1.5 to 2.4 mm above the lower zone extreme.

Table 4. Occurrence of linezolid false-susceptible errors for specimen D-05 (2005), a multidrug-resistant MRSA.

Type of error (no. occurrences)	Errors by method (no./%)
False-susceptible (44)	MicroScan (30/21.6)[a] Vitek (1/1.4) Vitek 2 (13/38.2)[a]
False-intermediate (2)	MicroScan (1/0.7) Vitek (1/1.4)
None (199)	MicroScan (108/77.7) Vitek (70/97.2) Vitek 2 (21/61.8)

a. High-level error rate.

Table 5. "Categorical accuracy" of antimicrobial susceptibility test methods for CAP Surveys Gram-negative challenge strains in 2004 - 2005 (D-03, *E. coli* ATCC 25922 [2004]; D-17, *P. aeruginosa* [2004]; and D-12, *S. maltophilia* [2005]).

Antimicrobial agent	Disk diffusion D-03 (2004)	Disk diffusion D-17 (2004)	Disk diffusion D-12 (2005)	MIC methods D-03 (2004)	MIC methods D-17 (2004)	MIC methods D-12 (2005)
Amikacin	100.0	97.8	100.0	100.0	96.4	98.4
Amoxicillin/Clavulanate	98.8	100.0	U	99.5	100.0	U
Ampicillin	96.0	100.0	98.2	99.3	99.5	97.7
Ampicillin/Sulbactam	100.0	100.0	U	100.0	99.5	U
Aztreonam	100.0	97.4[a]	100.0	100.0	99.1	98.9
Carbenicillin	94.3	U[b]	U	100.0	U	U
Cefazolin	99.4	100.0	100.0	99.7	98.6	97.2
Cefepime	100.0	97.8	81.0	100.0	98.8	93.1
Cefotaxime	100.0	100.0	100.0	100.0	100.0	97.3
Cefotetan	100.0	100.0	U	99.7	98.6	U
Cefoxitin	100.0	U	U	100.0	U	U
Ceftazidime	100.0	100.0	86.2	99.6	99.8	87.8
Ceftriaxone	100.0	100.0	96.0	99.8	99.9	97.3
Cefuroxime	96.9	100.0	U	99.8	100.0	U
Cephalothin	96.0	U	U	98.7	U	U
Ciprofloxacin	100.0	100.0	94.4	100.0	99.8	89.4
Gatifloxacin	100.0	U	U	99.4	U	U
Gentamicin	99.6	99.7	100.0	99.9	99.5	98.7
Imipenem	100.0	98.6	98.4	100.0	92.8	98.3
Levofloxacin	100.0	100.0	99.2[c]	99.8	100.0	99.0
Meropenem	U	100.0	U	U	94.9	U
Minocycline	U	U	99.0[c]	U	U	91.2
Nitrofurantoin	100.0	U	98.2	99.7	U	88.5
Norfloxacin	U	U	83.3	U	U	93.5
Piperacillin	100.0	100.0	84.4	99.8	98.8	U
Piperacillin/Tazobactam	100.0	99.3	U	100.0	93.3	U
Tetracycline	100.0	U	U	99.4	U	U
Ticarcillin/Clavulanate	100.0	100.0	U	100.0	99.8	U
Tobramycin	100.0	100.0	100.0	99.8	99.6	99.2
Trimethoprim/Sulfamethoxazole	99.6	100.0	98.9[c]	100.0	100.0	97.9

a. Underlined value is the lowest graded accuracy for that challenge and method.
b. U = ungraded because of too few participant reports or not achieving ≥ 80% consensus.
c. Only agents to be reported [CLSI, 2005].

Table 6. Comparisons of mean zone diameters from CAP specimens D-09 (2000) and D-03 (2004), *E. coli* ATCC 25922, to the currently recommended CLSI QC ranges and midpoints.

Antimicrobial agent	Mean reported zone (mm) D-09 (2000)	Mean reported zone (mm) D-03 (2004)	CLSI QC guidelines (mm) Midpoint	CLSI QC guidelines (mm) Range
Aztreonam	30.9	30.3[a]	32.0	28-36
Cefepime	32.5	32.3[a]	34.0	31-37
Ceftriaxone	29.7	30.6[a]	32.0	29-35
Ciprofloxacin	32.6	33.4[a]	35.0	30-40
Piperacillin	25.8	25.9[a]	27.0	24-30
Tetracycline	22.7	23.4[a]	21.5	18-25

a. Variation of > 1.0 mm (usually smaller) between CAP Surveys mean zone diameter and the midpoint of CLSI QC range.

Table 7. Distributions of incorrect responses and very major (false-susceptible) results for a *P. aeruginosa* strain listed by susceptibility test method/system for the four antimicrobial agents having the greatest frequency of serious errors (D-17, 2004).

Method	Total responses	False-susceptible responses	% false susceptible responses	Total incorrect responses[a]	% of total incorrect responses
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 results.

CONCLUSIONS

- Antimicrobial and antifungal test accuracy remains high during the period from 2004 - 2005 as monitored by the CAP Microbiology Surveys Program. However, several serious organism-method errors of false-susceptibility and -resistance were detected during the studied interval, most of which involved automated methods.
- Disk diffusion testing, although very accurate and cost-effective, continues to decline to <15% of participating laboratories (data not shown), replaced by commercial, automated methods having a convenient laboratory information system (LIS) interface. As noted before, these automated and/or semi-automated systems do make mistakes and thus cannot be treated as some infallible "black box".
- Survey's participants need to be familiar with the current interpretive tables of the CLSI, published annually in January of each year.
- The CAP Microbiology Surveys will continue to monitor and grade (by category) the participant responses for antibacterial and antifungal agents as a major component of assuring laboratory quality assurance.

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