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Educational Susceptibility Testing as a Critical Component of Laboratory Proficiency Programs: American Proficiency Institute Results for 2007-2010

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ABSTRACT

Background: External laboratory proficiency programs are an important requirement for test quality assurance (QA) and compliance to regulatory guidelines (CLIA and inspections). The American Proficiency Institute (API) regularly (Q4 mo) distributes QA sample challenges (test events) including an Educational Sample (ES) for susceptibility (S) testing.

Methods: Beginning in 2007, API has sent 3 ES samples annually, each a well-characterized (molecular/phenotypic methods) strain having an interesting/emerging mechanism of resistance (R); see Table 3. Hundreds of USA laboratories, usually serving small-medium size hospitals and clinics, participate in ungraded ES test event. Analysis of responses are made and reported electronically as ES critiques addressing contemporary S testing issues that affect therapy.

Results: Six Gram (+) and six Gram (-) ES strains were tested over the 4 years (2007-2010) with organism identification (graded) accuracy of 95.3% (range, 91.0-99.2%) for Gram (-) and 97.0% (range, 94.2-100.0%) from Gram (+) challenges. S testing categorical accuracy was generally greatest for the disk diffusion test (91.0/97.0%) compared to the MIC methods (commercial automated or manual) combined (89.9/96.1%, for Gram [-]/Gram [+]), respectively. The most worrisome observations of these ES samples were: 1.) poor recognition of ESBL- and serine carbapenemase-producing strains (various types) due to delayed application of CLSI guidelines; 2.) overcalling of ESBL in organisms having wildtype non-ESBL enzymes (OXA series; OXA - 1/30 [current 2011 sample]) due to commercial system or participant interpretive error; and 3.) occasional drug-bug discords noted in non-fermentative bacilli.

Conclusions: The API ES series of ungraded S-testing challenges (accuracy was >90%) has been well-received by subscribers and have provided detailed educational critiques to improve laboratory testing performance. ES samples have delivered guidance to enable laboratories to rapidly comply with CLSI document changes in interpretive breakpoints such as those for β-lactams when testing Enterobacteriaceae and *P. aeruginosa*; the program will be sustained into 2012 to document S-test quality.

INTRODUCTION

External Quality Assurance (EQA) programs are important components of laboratory practice that are essential for maintaining test accuracy. These EQA practices complement inspection and accreditation organizations mandated via professional societies (example: College of American Pathologists [CAP]), and government (CLIA). These programs have assessed the implementation and accuracy of antimicrobial susceptibility testing methods over four decades with published summaries dating from 1972 (Jones and Edson, 1985). The initial publications, however, appeared in 1982 covering the CAP Microbiology EQA surveys (Jones, et al., 1982a, 1982b and 1982c), and the most recent summarized testing events through 2005 only (Pfaller and Jones, 2006).

The American Proficiency Institute (API) is a federally approved proficiency testing provider (since 1991) serving over 17,000 hospitals, clinic and physician offices/laboratories. The API initiated a specific educational microbiology objective in 2007 that was designed to monitor and enhance antimicrobial susceptibility testing quality. This Educational Sample (ES) program sends three (3) organisms each year for species identification (graded) and testing of antimicrobials by the routine method applied by the participating laboratory. The susceptibility test results are collected as categories (susceptible [S], intermediate [I], or resistant [R]) and responses are analyzed as an educational, ungraded process to improve laboratory quality. A total of 12 ES challenges were assessed from 2007 through 2010 revealing high levels of test accuracy, yet documenting distinct areas for test improvement (Tables 1-4). Summaries of the API ES Microbiology EQA Program are presented here.

MATERIALS AND METHODS

As a component of a comprehensive Microbiology Proficiency Sample program that offers 43 district microbiology options, API offers the ES antimicrobial susceptibility samples (ungraded) every 4 months (eg. three samples per year). This program was launched in early 2007 to circulate an organism sample, each a well-characterized (molecular/phenotypic level methods) strain having an interesting or an emerging mechanism(s) of resistance (see Table 1).

Nearly 800 USA laboratories, usually serving hospitals and clinics, participated in each microbiology ES test event (>90% from commercial MIC methods or systems). Analysis of responses were made and reported electronically as ES critiques addressing contemporary susceptibility testing issues that affect the quality of contemporary infection therapy.

RESULTS

- Table 1 lists the topics of the ES challenges for Enterobacteriaceae (four species) with SHV-5, KPC-3 (2) and OXY-series β-lactamases; for non-fermentors with MDR patterns; for *S. aureus* of the USA300 and 100 types; for other Gram-positive cocci (viridans group streptococci, *S. haemolyticus*) with MDR patterns; and the *S. pneumoniae* QC strain (ATCC 49619) having a penicillin non-susceptibility.

- Participant laboratories performed very well at the acceptable level of organism identification (Table 2). Identification of the Gram-positive species strains (97.0% acceptable) was slightly greater than Gram-negative species (95.3%), but these rates were comparable to reports from other EQA surveys.

- Table 3 illustrates that the accuracy of susceptibility category results was generally good, but some resistance mechanisms were unrecognized due to various reasons, mainly due to delayed implementation of modified CLSI breakpoints. Also, flaws in "expert software systems" found in some commercial products were noted. However, only five pathogen/method accuracy rates fell below 90%.

- Table 4 lists the most troublesome categorical errors encountered in this survey program (2007-2010):
  - ESBL screening breakpoints were not being uniformly applied to Enterobacteriaceae (false-susceptible rates were noted); ES-01 (2007) and ES-02 (2008).
  - False-susceptible results noted for MRSA (USA 300) with oxacillin and cefoxitin tests.
  - KPC enzymes continue to go undetected (ES-03, 2007; ES-02, 2010) due to delayed application of revised CLSI breakpoints/screening tests.
  - Participants report inappropriate drugs for some infection sites (UTI specific agents for bacteremias).
  - Unusual resistances to linezolid have gone undetected due to limited testing and reporting of this drug by laboratories.

Table 1. API Educational Sample (ES) topics for susceptibility testing (2007-2010) that focused on emerging resistance patterns and susceptibility testing problems/breakpoints.

Year/Sample no.	Species	Resistance educational topic
2007		
ES-01	*E. coli*	Extended spectrum β-lactamase (SHV-5), an ESBL enzyme producing elevated MIC results for ceftazidime and monobactams. Monitored accuracy of ESBL detection by CLSI screening methods.
ES-02	*S. aureus*	Methicillin-resistant (MRSA) community-acquired strain (USA-300-0114) typical of emerging clonal type. Monitored accuracy of MRSA detection by CLSI methods for key β-lactam, macrolide and fluoroquinolone resistances.
ES-03	*K. pneumoniae*	Carbapenem-resistant (KPC-3) and typical of emerging resistance type in northeast USA and worldwide. Monitored detection accuracy of this enzyme type via current CLSI methods.
2008		
ES-01	*S. mitis/oralis (S. peroris)*	High-level penicillin (β-lactam) resistance in a viridans streptococcus species associated with other antimicrobial resistances to clindamycin, macrolides, quinupristin-dalfopristin, fluoroquinolones, aminoglycosides, rifampin, tetracycline, and trimethoprim-sulfamethoxazole (TMP-SMX). Monitored accuracy of testing fastidious species.
ES-02	*K. oxytoca*	ESBL-like enzyme (OXY-series) having resistances to penicillins ± clavulanate, or sulbactam, or tazobactam, cephalosporins and aztreonam. Monitored for detection of enzyme more commonly found in this not-uncommon *Klebsiella* spp.
ES-03	*S. haemolyticus*	Multidrug-resistant (MDR) coagulase-negative staphylococcus (CoNS) with high monitored MIC results to methicillin, other β-lactams, macrolides, fluoroquinolones, and TMP-SMX.
2009		
ES-01	*P. aeruginosa*	MDR strain only susceptible to amikacin, gentamicin, tobramycin, and polymyxins (colistin, polymyxin B). Monitored ability to recognize the few remaining treatment antimicrobials.
ES-02	*S. pneumoniae*	An international quality control (QC) strain used to determine technical accuracy of CLSI and other test methods. Results were compared to published QC ranges of MICs and zone diameters. Only minor levels of β-lactam resistances were present in this well known strain.
ES-03	*A. baumannii*	*Acinetobacter* harbouring an OXA-23 carbapenemase producing a MDR organism emerging in the USA and worldwide. Only a few agents (aminoglycosides, ceftazidime, polymyxins, tigecycline and TMP/SMX) were clearly active with their identification accuracy assessed.
2010		
ES-01	*E. faecalis*	MDR strain with well defined oxazolidinone resistance (target site mutations) that have appeared in recent years. Monitored multiple methods to detect a rare but emerging resistance that could decrease utility of an important antimicrobial class.
ES-02	*S. marcescens*	MDR strain with a KPC-3 serine carbapenemase (see mechanism in ES-03, 2007) having susceptibility to few drugs (amikacin, gentamicin and tigecycline). Appraised the use of recently published CLSI breakpoints for carbapenems and confirmatory methods (Modified Hodge Test; MHT).
ES-03	*S. aureus*	Healthcare-associated strain (USA 100) with a characteristic MDR pattern but retaining susceptibility to at least 10 antimicrobials including parenteral and oral agents. Monitored testing accuracy for commonly tested agents and methods.

Table 2. Organism identification accuracy for API ES-series challenges for 2007-2010.

Organism and percentage acceptable performance	
Gram-negative species	
ES-01 (2007) *E. coli* at 99.2%	95.3%
ES-03 (2007) *K. pneumoniae* at 94.0%	
ES-02 (2008) *K. oxytoca* at 91.0%	
ES-01 (2009) *P. aeruginosa* at 96.6%	
ES-03 (2009) *A. baumannii* at 94.4%	
ES-02 (2010) *S. marcescens* at 96.7%	
Gram-positive species	
ES-02 (2007) *S. aureus* at 100.0%	97.0%
ES-01 (2008) *S. mitis/oralis or S. peroris* at 95.3%	
ES-03 (2008) *S. haemolyticus* at 95.7%	
ES-02 (2009) *S. pneumoniae* at 94.2%	
ES-01 (2010) *E. faecalis* at 97.8%	
ES-03 (2010) *S. aureus* at 99.1%	

Table 3. Categorical accuracy of disk diffusion (DD) and various MIC methods to determine susceptibility of 12 API Educational Sample challenge strains (2007-2010).

			Categorical accuracy by method
Year	Sample	Target susceptibility testing topic[a]	MIC / Disk Diffusion
2007	ES-01	ESBL (SHV-5) detection in *E. coli*	96.9 / 92.7
	ES-02	MRSA (USA300-0114)	93.1 / 92.3
	ES-03	Carbapenemases (KPC-3) in Enterobacteriaceae	92.9 / 89.5
2008	ES-01	High-level PEN-R in streptococci (*S. peroris*)	96.6 / 95.0
	ES-02	ESBL-like enzyme (OXY-2a) in *K. oxytoca*	87.7 / 86.9
	ES-03	MDR *S. haemolyticus*	93.2 / 98.0
2009	ES-01	MDR *P. aeruginosa*	81.8[b] / 96.9
	ES-02	Control *S. pneumoniae* to assess QC	99.4 / 98.9
	ES-03	MDR *A. baumannii* (OXA-23)	94.6 / 97.1
2010	ES-01	MDR *E. faecalis*, linezolid-resistant	97.3 / 98.8
	ES-02	MDR *S. marcescens* (KPC-3)	85.5[c] / 83.2[c]
	ES-03	MRSA (USA100 hospital-strain)	97.1 / 99.3

a. ESBL = extended spectrum β-lactamase; MDR = multidrug-resistant.
b. Driven to low rate by poor ceftazidime performance (only 23.3% accuracy).
c. Slow adoption of current CLSI breakpoint criteria for ESBL-producing strains.

Table 4. List of susceptibility testing interpretation and methods concerns documented in the API ES Program for Microbiology EQA.

ES-01 (2007) had significant numbers of laboratories not applying ESBL screening concentrations of ≥2 µg/ml for aztreonam or ceftriaxone or ceftaroline for the SHV-5 producing *E. coli* strain.
ES-02 (2007) provided educational content to update methods to recognize MRSA by oxacillin and cefoxitin disks and false-susceptible rates were 2.0 - 6.7% for this strain and other β-lactams were often not reported as resistant. Inappropriate agents active only versus UTI isolates were reported (nitrofurantoin as an example).
ES-03 (2007) challenged laboratories with a KPC-3 producing serine carbapenemase in a *K. pneumoniae* that was called susceptible to imipenem (33.1-40.0%) and other carbapenem. This was due to yet to be published, modified (lower) breakpoints for marketed carbapenem; see ES-02 (2010). False-susceptible results were common during this period.
ES-01 (2008) a multidrug-resistant (MDR) *S. peroris* had susceptibilities accurately assessed but some laboratories reported disk diffusion susceptibility categories where no criteria were published in CLSI tables for viridans group streptococci.
ES-02 (2008) was a *K. oxytoca* strain having an ESBL-like β-lactamase (OXY-2a) and MIC results at ≥2 µg/ml for aztreonam and ceftriaxone e.g. ESBL phenotype. All methods and automated systems (even those with "Expert Software Programs") failed to recognize significant resistance per CLSI criteria.
ES-01 (2010), an *E. faecalis* with oxazolidinone target site mutational resistance, was noted to be accurately found as resistant (MIC, >8 µg/ml), but less than 50% of participants tested linezolid. Moreover, this enterococcus was wrongly called ampicillin-non-susceptible by 1.8-5.9% of participants.
ES-02 (2010) was the second Enterobacteriaceae (*S. marcescens*) among ES challenges having a KPC-3 carbapenemase. Only three drugs were active (amikacin, gentamicin, tigecycline) and poor performance was documented with false-susceptible rates for carbapenems due to non-application of CLSI screening criteria for serine carbapenemases (CLSI M100-S20 U). Unacceptable numbers of laboratories continue to report false-susceptible results (20.4-90.9%) for carbapenems and other broad-spectrum β-lactam agents.

CONCLUSIONS

- The API ES Microbiology Program provided 12 highly successful EQA samples from 2007-2010; documenting acceptable levels of organism identification and categorical performance of antimicrobial susceptibility testing (disk diffusion and commercial MIC methods).

- Delays in the implementation of methods to identify emerging resistances in some species have resulted in the potential reporting of false-susceptible results to guide infection chemotherapy. Thus, this ongoing practice could increase morbidity and mortality of infected patients.

- The API ES Program will be extended to routinely provide ongoing education to participating laboratories as an EQA process directed at susceptibility testing accuracy.

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