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Critical Evaluation of Linezolid Susceptibility Testing Using Two Automated Systems (Vitek, Vitek 2)

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ABSTRACT

Background:
Linezolid (LZD), a valuable Gram-positive-active oxazolidinone, has generally suffered from false-resistant (FR) susceptibility (S) testing results due to trailing MIC endpoints or fuzzy borders on agar diffusion methods (disk diffusion [DD], Etest). In contrast, user experience and proficiency sample programs (CAP) have cited potential false-S (FS) errors produced by some commercial MIC devices. We investigate Vitek (V) and Vitek 2 (V2) accuracy using genetically defined LZD-R strains.

Methods:
70 organisms were tested, 35 LZD-R (18 enterococci; 5 *S. aureus* [SA]; 12 CoNS) with confirmed mechanisms (G2576T mutations) and phenotypes by reference-quality tests (DD, Etest, CLSI MIC). Equal numbers of LZD-S strains were tested with identical species distributions. V and V2 tests were performed by manufacturer instructions with discordant results repeated in triplicate.

Results:
All errors tabulated were reproducible and included unacceptable FS (very major [VM]) and false-intermediate (minor) types by V (29 and 14%, respectively). The manufacturer noted poor V accuracy for LZD-enterococci in Dec '06, but our results also detected suboptimal performance with staphylococci; V2 had no discords with LZD-R strains. For LZD-S and -I challenges, FR was recorded for each system (3-11%), both with staphylococci and enterococci and FS only with V (9%). LZD-FS has been noted (19%) but FR rarely (<1%) in CAP Surveys (2003-2006) for V or V2.

	No. (%) by Vitek System error type:		
LZD-R species	FS (very major)	False-intermediate (minor)	Total % Errors
E. faecalis (5)	0 (0)	1 (20)	20
E. faecium (13)	8 (62)	4 (31)	92
S. aureus (5)	2 (40)	-	40
CoNS (12)[a]	0 (0)	-	0
All (35)	10 (29)	5 (14)	43

a. Includes: *S. epidermidis* (9), *S. haemolyticus* (2) and *S. capitis* (1).

Conclusions:
Unacceptable error rates were observed (FS 43%; FR 3%) for V as well as V2 (FR 11%). Warnings to users of V should be expanded to cover all Gram-positive cocci, and V2 users for potential FR. Therapy with LZD is best guided by manual reference-quality tests (DD, Etest, M7-A7).

INTRODUCTION

Linezolid, the first oxazolidinone class agent to be clinically applied, has become a welcome therapeutic addition for infections caused by commonly occurring antimicrobial-resistant Gram-positive pathogens. Resistance to linezolid initially was very infrequent; however, documented occurrences have been noted, usually among enterococci (*Enterococcus faecium* > *Enterococcus faecalis*) and, more recently, coagulase-negative staphylococci (CoNS). The occurrence of linezolid resistance must be classified as very rare among *Staphylococcus aureus* and streptococci, and is usually associated with prolonged therapeutic exposure or infected indwelling devices. Some oxazolidinone-resistant strains have emerged in patients without prior drug exposure, each being attributed to clonal epidemic spread from other patients in the hospital environment.

As linezolid use continues to increase, the need for resistance monitoring also becomes vital to the continued success of this novel antimicrobial class. In vitro studies (ZAPS protocols) of linezolid activity prior to the release by the US-FDA indicated that an excellent spectrum of activity could be expected against essentially all Gram-positive pathogens isolated in North America, Latin America, Europe and the Asia-Western Pacific region (33,642 strains worldwide). The occurrence of linezolid-resistant isolates in the ZAPS international studies was extremely rare (1 case, 0.003%). When local laboratories detected resistance by either MIC or disk diffusion methods, the susceptibility test result was consistently determined to be erroneous by reference laboratories. This problem of false-resistant testing results reported from commonly used manual methods (broth microdilution, disk diffusion and Etest [AB BIODISK, Solna, Sweden]) has persisted and has been clearly attributed to the so-called "trailing endpoints" or "fuzzy zone borders" producing misleading reports of resistance. More recently, false-susceptible results were detected for automated systems by the College of American Pathologists (CAP; 2005).

This report follows the recommendation by the CAP (2005) to determine the origin and extent of false-susceptible and other linezolid testing errors. Preliminary data from the CAP indicated automated susceptibility testing error rates at unacceptable levels that both confirm and contradict published system (Vitek 2) evaluations by such investigators as Abele-Horn et al. (2006) and Qi et al. (2006). In one of these cited studies, no linezolid-resistant strains were tested. Like Qi and colleagues, we challenged two of these commercial products (Vitek and Vitek 2) with well-characterized linezolid-resistant Gram-positive cocci representing recent isolates from six species.

MATERIALS AND METHODS

Tested organisms were obtained from laboratories in the United States participating in various antimicrobial resistance surveys (2005-2007).

A total of 70 isolates (35 linezolid-susceptible or -intermediate and 35 linezolid-resistant) were tested and the species distribution was as follows: *S. aureus* (10 strains), *S. capitis* (two strains), *S. epidermidis* (18 strains), *S. haemolyticus* (four strains), *E. faecalis* (10 strains) and *E. faecium* (26 strains).

All reference susceptibility tests were performed in a GLP laboratory (JMI Laboratories, North Liberty, IA) using Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) broth microdilution methods and published interpretive criteria. Vitek and Vitek 2 tests were performed according to manufacturers (bioMerieux, Hazelwood, MO) instructions.

Linezolid-resistant isolates were confirmed with the linezolid Etest (AB BIODISK) and disk diffusion susceptibility testing. Molecular testing was performed on linezolid-resistant isolates to identify the 23S rRNA target site mutations (typically G2576T) and potential clonality using pulsed-field gel electrophoresis (PFGE), automated ribotyping and various PCR procedures.

CAP Survey critiques were reviewed for relevant linezolid testing results from 2004 to date. Table 1 was derived from CAP sample D-05 (2005).

RESULTS

- CAP Survey D-A (2005) for a linezolid-resistant *S. aureus* (Table 1).
  - Disk diffusion false-susceptible rate at 18.9%.
  - MIC systems false-susceptible rate at 18.7%.
  - Participant modal MIC at ≥8 µg/ml (resistant).
  - Error rates varied widely by system used, highest errors for Vitek 2 (38.2%) and MicroScan (22.3%).
  - False-resistant error for vancomycin was only 0.3%.

Table 1. Distributions of false-susceptible or -intermediate results reported to the CAP Survey (sample DA, 2005) by various commercial susceptibility testing systems for a linezolid-resistant strain of *S. aureus*.

Linezolid error (no. occurrences)	Automated system (no./%)
False-susceptible (44)	MicroScan (30/21.6) Vitek (1/1.4) Vitek 2 (13/38.2)
False-intermediate (2)	MicroScan (1/0.7) Vitek (1/1.4)
Correct response (199)	MicroScan (108/77.7) Vitek (70/97.2) Vitek 2 (21/61.8)

- CAP Surveys for Gram-positive cocci (2004-2006) using linezolid-susceptible strains; data not shown.
  - Disk diffusion false-resistant error rates were at 0.0 to 1.0% (highest for *E. faecalis*, 2006).
  - MIC system false-resistant error rates were at 0.0 to 0.6% (highest for *E. faecalis*, 2006).

- Among well-characterized linezolid-susceptible or -intermediate (35 strains) or -resistant strains (35 total), testing showed:
  - All but four resistant strains had a target mutation at G2576T.
  - False-susceptible and -intermediate results were most prevalent among Vitek results (Abstract table) with 27% errors overall.
  - Trends toward greater false resistance were noted with Vitek 2 (Table 2) and 7% errors overall (range 0-10%; 8-10% for enterococci) were documented.
  - Vitek 2 major and minor errors were at unacceptable levels for CoNS and *E. faecalis*, respectively.

- Manufacturer letter dated December 22, 2006 recommended an alternative method for enterococcal linezolid tests by Vitek users due to inaccurate results; however, no warning was issued for Vitek 2. Vitek system testing errors for linezolid are also observed at unacceptable levels with staphylococci (Table 2).

Table 2. Distribution of linezolid interpretation errors for Vitek and Vitek 2 systems when testing 70 Gram-positive organisms (includes 35 linezolid-resistant strains).

		Error types (no.)[a]			
Organism (no. tested)	System	Very Major	Major	Minor	% all errors
E. faecalis (10)	Vitek	-	-	2	20%
	Vitek 2	-	-	1	10%
E. faecium (26)	Vitek	8	-	6	54%
	Vitek 2	-	-	2	8%
S. aureus (10)	Vitek	2	-	NA	20%
	Vitek 2	-	-	NA	0%
CoNS (24)b	Vitek	-	1	NA	4%
	Vitek 2	-	2	NA	8%
All (70)	Vitek	10	1	8	27%
	Vitek 2	-	2	3	7%

a. Very major = false-susceptible, major = false-resistant and minor = intermediate versus susceptible or resistant.
b. Includes: *S. capitis* (2; 1 resistant), *S. epidermidis* (18 ; 9 resistant) and *S. haemolyticus* (4; 2 resistant).

CONCLUSIONS

- Linezolid susceptibility testing can be compromised by fuzzy zone diameter edges for disk diffusion and Etest methods requiring attention to technical details about endpoint determinations published in CLSI (M100-S18, 2008 [in press]) and manufacturer (AB BIODISK) documents.
- False-susceptible (Vitek) and false-resistant (Vitek 2) results can be generated by contemporary automated commercial susceptibility test systems at a high level of occurrence.
- These results confirm the CAP Survey findings of linezolid MIC testing errors, particularly among some automated systems.
- Clinically, linezolid resistance remains rare (USA and international surveillance trial results at <1%; see references) and, when detected, should be evaluated further, including submission to a reference laboratory for confirmation.

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