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Multicenter Assessment of Linezolid Spectrum and Potency in the United States Using the Standardized Disk Diffusion Method: Report From the Zyvox® Antimicrobial Potency Study (KB-ZAPS)

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ABSTRACT

BACKGROUND: New and novel resistances in gram-positive (G+) species have escalated, necessitating more reliable therapies, such as the oxazolidinones. However, their continued use may compromise spectrum, and this possibility requires monitoring.

METHODS: The activity of linezolid (LZD) against common G+ pathogens was compared to that of penicillins, vancomycin (VANCO), quinupristin/dalfopristin (Q/D), and 5 other drugs by NCCLS disk diffusion (DD) method. 106 USA centers (31 states) tested recent clinical isolates of *S. aureus*, CoNS, *E. faecium*, *E. faecalis*, *S. pneumoniae*, and other streptococci (3,100 strains; 97% compliance). Testing used the standardized method and concurrent QC-qualified sites. Strains with LZD zones ≤ 20 mm were to be referred to the national monitor.

RESULTS: LZD susceptibility (zone, ≥ 21 mm) was reported for 100% and 99.4% of staphylococci and streptococci, respectively. Susceptibility (zone, ≥ 23 mm) of enterococci to LZD was 96.0% with 3 isolates (0.4%) reported as resistant (zones, ≤ 20 mm; unconfirmed). Among a total of 9 isolates (0.3%) overall reported to have zone diameters at 20 mm, 6 were not available for further testing, 2 were contaminated with G- bacilli, and one was determined to be LZD-susceptible. There were no differences in LZD susceptibility in the VANCO- or oxacillin- or penicillin-resistant subsets of strains, and LZD spectrum was routinely greater than that of VANCO and Q/D.

CONCLUSIONS: The observed susceptibility pattern of G+ species for United States medical centers indicates an excellent and nearly complete LZD activity against the key pathogens. Essentially all strains observed locally as LZD-resistant were not confirmed, and such strains as they appear should always be confirmed by reference laboratories.

BACTERIAL STRAINS

A total of 3,100 strains were tested with acceptable accompanying quality control results and distributed as follows:

- *Staphylococcus aureus* – 1,290 strains (623 oxacillin-resistant)
- Coagulase-negative staphylococci (CoNS) – 488 strains (351 oxacillin-resistant)
- *Enterococcus faecalis* – 332 strains (33 vancomycin-resistant)
- *Enterococcus faecium* – 169 strains (130 vancomycin-resistant)
- Other enterococci not identified to species level – 371 strains
- *Streptococcus pneumoniae* – 225 strains
- Other streptococci – 240 strains

All organisms were isolated from positive blood cultures and wound, abdominal cavity, respiratory tract, and urinary tract infections. Strains with linezolid zones ≤ 20 mm (possible resistance) were to be repeated by each participant. Isolates with reproducibly small zones were forwarded to the microbiology monitor for confirmation. Three strains were referred, all determined to be susceptible to linezolid (one organism) or to be contaminated by a gram-negative bacillus (two organisms). Six strains with linezolid zone diameters of ≤ 20 mm were not available for retesting (Table 1). Only one laboratory's results (30 strains) were omitted from the tabulations because of unacceptable quality assurance results.

RESULTS AND DISCUSSION

- All staphylococci tested exhibited linezolid zone diameters ≥ 21 mm (susceptible). Vancomycin was also active with only 9 *S. aureus* strains (0.7% overall) having zone diameter measurements in the non-susceptible range (zones 11-14 mm) (Table 1).
- Rates of oxacillin resistance for the surveillance organism collection were 48.3% and 71.9% for *S. aureus* and CoNS, respectively. Levofloxacin was markedly less active compared with oxacillin-resistant strains (12.0% and 47.5% susceptible, respectively). Both quinupristin/dalfopristin and nitrofurantoin had very wide spectrums of activity, each covering $\geq 98.1\%$ of isolates.
- Linezolid was active against all pneumococci; however, 3 other *Streptococcus* strains had zone diameters of 19 mm or 20 mm (breakpoint zone for susceptible at ≥ 21 mm). These strains unavailable for retesting had penicillin zones of 30 to 35 mm (highly susceptible) and were additionally susceptible to macrolides, clindamycin, nitrofurantoin, and older cephalosporins.
- Macrolide resistance among the *S. pneumoniae* isolates was 28.8% compared with 17.6% for clindamycin.
- Levofloxacin resistance in *S. pneumoniae* increased to 1.4%, a modest increase compared with the 1999 results. Fluoroquinolone resistance (3.4%) was greater among the "other streptococci."
- Generally, quinupristin/dalfopristin was active against nearly all streptococci.
- Vancomycin non-susceptible strains (1.7%), as defined by currently published NCCLS criteria, were not uncommon when testing non-pneumococcal streptococci. Three of the 4 strains in this latter category had vancomycin zones at 16 mm, which is 1 mm below the breakpoint.
- Using the interpretive criteria found in the linezolid product package insert, 93.1% to 98.1% of various enterococcal groups were susceptible (Table 1). Only 3 strains (0.3% overall) were discovered with zone diameters at 20 mm. A total of 163 vancomycin-resistant *E. faecalis* or *E. faecium* were tested against linezolid, with only one strain determined to be resistant by the disk diffusion method. All other non-susceptible isolates (1.9% to 5.7%) had zone diameters of 21 or 22 mm, which would have been categorized as susceptible if the strain was *Staphylococcus* or *Streptococcus* species.
- Figure 1 illustrates the population distribution of zone diameters around the 30- μ g linezolid disk (3,100 results) in 2 mm groupings.
- Table 2 presents the detailed occurrence rates of linezolid zone diameters found adjacent to the recommended susceptible breakpoints for the four organism groups. Organisms with linezolid zones ≤ 20 mm were quite rare and included 0.4% of enterococci, 0.6% of streptococci, and no staphylococci.
- The recommendation for clinical laboratories using linezolid disk diffusion tests against gram-positive cocci is as follows:
 - 1) If a zone of ≤ 20 mm is noted, repeat the test to confirm the result and purity of isolate.
 - 2) Once confirmed, forward the strain to a reference or public health laboratory for further investigation.
 - 3) Notify the physician/hospital of a possible linezolid non-susceptible isolate.

TABLE 1. Spectrums of activity for linezolid and selected comparison agents tested by the Kirby-Bauer disk diffusion method against 3,100 gram-positive isolates from 31 states (106 medical centers)

Organism (no. tested)	Antimicrobial Agent	% By Susceptibility Category*		
		Susceptible	Intermediate	Resistant
<i>S. aureus</i> oxacillin-susceptible (667)				
	Linezolid	100.0	— ^b	— ^b
	Erythromycin	62.6	5.4	32.0
	Clindamycin	91.4	2.3	6.3
	Quinupristin/dalfopristin ^c	99.7	0.2	0.2
	Levofloxacin	87.2	0.8	12.0
	Nitrofurantoin	99.3	0.7	0.0
	Vancomycin	99.6	— ^b	— ^b
oxacillin-resistant (623)				
	Linezolid	100.0	— ^b	— ^b
	Erythromycin	4.5	0.8	94.7
	Clindamycin	26.4	1.8	71.8
	Quinupristin/dalfopristin ^c	98.8	0.2	1.0
	Levofloxacin	12.0	2.3	85.7
	Nitrofurantoin	98.1	1.1	0.8
	Vancomycin	99.0	— ^b	— ^b
Coagulase-negative staphylococci oxacillin-susceptible (137)				
	Linezolid	100.0	— ^b	— ^b
	Erythromycin	52.6	1.5	46.0
	Clindamycin	86.8	2.2	11.0
	Quinupristin/dalfopristin ^c	99.3	0.0	0.7
	Levofloxacin	82.5	3.6	13.9
	Nitrofurantoin	100.0	0.0	0.0
	Vancomycin	100.0	— ^b	— ^b
oxacillin-resistant (351)				
	Linezolid	100.0	— ^b	— ^b
	Erythromycin	20.0	1.4	78.6
	Clindamycin	54.0	2.0	44.0
	Quinupristin/dalfopristin ^c	99.1	0.0	0.9
	Levofloxacin	47.5	9.0	43.5
	Nitrofurantoin	99.7	0.0	0.3
	Vancomycin	99.4	— ^b	— ^b
Streptococci				
<i>S. pneumoniae</i> (225)				
	Linezolid	100.0	— ^b	— ^b
	Penicillin	78.9 ^d	— ^b	— ^b
	Erythromycin	70.2	5.8	24.0
	Clindamycin	82.4	11.8	5.9
	Quinupristin/dalfopristin ^c	83.1	16.9	0.0
	Levofloxacin	98.6	0.5	0.9
	Vancomycin	100.0	— ^b	— ^b
Other species (240)				
	Linezolid	98.8	— ^b	— ^b
	Penicillin	92.8 ^e	— ^b	— ^b
	Erythromycin	66.1	20.1	13.8
	Clindamycin	75.4	16.7	7.9
	Quinupristin/dalfopristin ^c	92.5	4.6	2.9
	Levofloxacin	96.6	2.1	1.3
	Vancomycin	98.3	— ^b	— ^b
<i>E. faecalis</i> vancomycin-susceptible (299)				
	Linezolid	94.0	5.7	0.3 ^f
	Ampicillin	99.0	— ^b	1.0
	Erythromycin	11.5	29.6	58.9
	Quinupristin/dalfopristin ^c	3.7	3.7	92.6
	Levofloxacin	55.0	3.1	41.9
	Nitrofurantoin	98.7	1.0	0.3
vancomycin-resistant (33)				
	Linezolid	97.0	3.0	0.0
	Ampicillin	87.9	— ^b	12.1
	Erythromycin	0.0	15.2	84.8
	Quinupristin/dalfopristin ^c	15.2	0.0	84.8
	Levofloxacin	6.1	3.0	90.9
	Nitrofurantoin	97.0	3.0	0.0
<i>E. faecium</i> vancomycin-susceptible (39)				
	Linezolid	94.9	5.1	0.0
	Ampicillin	33.3	— ^b	66.7
	Erythromycin	13.2	18.4	68.4
	Quinupristin/dalfopristin ^c	69.2	17.9	12.9
	Levofloxacin	31.6	7.9	60.5
	Nitrofurantoin	35.9	15.4	48.7

Organism (no. tested)	Antimicrobial Agent	% By Susceptibility Category*		
		Susceptible	Intermediate	Resistant
<i>E. faecium</i> vancomycin-resistant (130)				
	Linezolid	93.1	5.4	1.5 ^f
	Ampicillin	2.4	— ^b	97.6
	Erythromycin	0.0	4.7	95.3
	Quinupristin/dalfopristin ^c	90.3	4.0	5.7
	Levofloxacin	0.8	0.0	99.2
	Nitrofurantoin	40.8	17.7	41.5
Other enterococci (356)				
	Linezolid	98.1	1.9	0.0
	Ampicillin	81.7	— ^b	18.3
	Erythromycin	13.0	26.6	60.4
	Quinupristin/dalfopristin ^c	19.6	5.7	74.7
	Levofloxacin	50.6	1.6	47.8
	Vancomycin	82.2	3.5	14.3
	Nitrofurantoin	88.7	4.8	6.5

*Susceptibility criteria were those provided by the NCCLS or the linezolid product package insert (2000); for staphylococci and streptococci, susceptible only at ≥ 21 mm (MIC ≤ 4 and ≤ 2 μ g/mL, respectively); and for enterococci, susceptible at ≥ 23 mm and resistant at ≤ 20 mm.

^b—No criteria for this category were found in the NCCLS recommendations or product package insert.
^c—Quinupristin/dalfopristin tested in a 30/70 ratio.
^d—Penicillin susceptibility defined by an inhibitory zone diameter of ≥ 24 mm as used for non-pneumococcal streptococci.
^e—Resistance rates among *S. pneumoniae* could be underestimated by these centers.
^f—Three unconfirmed strains of *Streptococcus* spp. were reported as having zone diameters of 19 mm (1 strain) or 20 mm (2 strains).

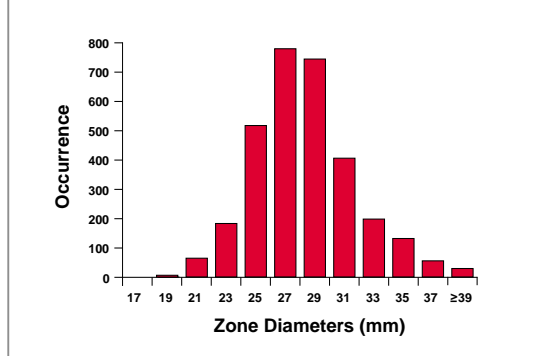
^g—Linezolid-resistant strains by disk diffusion test were not referred to or confirmed by the monitor and included *E. faecalis* (1 strain) and *Enterococcus* spp., NOS (2 strains) with zone diameters of 20 mm.

TABLE 2. Resistant or intermediate breakpoint details of linezolid zone diameters listed by genus or species groups

Organism group (no. tested)	% At Each Zone Diameter (mm)				
	19	20	21	22	≥ 23
Enterococci (857)	—	0.4	0.5	3.1	96.0 ^a
<i>S. aureus</i> (1,290)	—	—	0.2 ^a	0.8	99.0
Coagulase-negative staphylococci (488)	—	—	— ^a	0.4	99.6
Streptococci (465)	0.2	0.4	1.7 ^a	2.2	95.5

^aSusceptible zone diameter selected by the US Food and Drug Administration (Linezolid product package insert, 2000). No resistant or intermediate category has been recommended for staphylococci or streptococci. The resistant interpretive zone for enterococci was ≤ 20 mm.

FIGURE 1. Distribution of zone diameters around a 30- μ g linezolid disk for 3,100 gram-positive isolates



CONCLUSIONS

- Clearly, linezolid possesses the most complete antimicrobial spectrum (by disk diffusion tests) against all species of staphylococci, streptococci, and enterococci when directly compared with the clinically available streptogramin or vancomycin (Table 1).
- The zone diameters surrounding the 30- μ g linezolid disks in this surveillance trial illustrated the extremely rare occurrence of isolates (0.0% to 0.6% by genus group) with potentially elevated linezolid MIC values (≥ 8 μ g/mL). **Such strains should be studied further in reference laboratories.**
- Linezolid was proven in this comprehensive sample of more than 3,000 strains (~ 100 US medical centers) to have near complete coverage of contemporary gram-positive cocci. All strains with participant-measured zone diameters ≤ 20 mm that were available for retesting failed to be confirmed. The remaining linezolid non-susceptible strains had zones clustered near the breakpoint zone (19 or 20 mm), were not monitor confirmed, and were unlikely to have a reproducible linezolid MIC ≥ 8 μ g/mL.

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