Report of Linezolid Resistance from the Zyvox® Annual Appraisal of Potency And Spectrum Program (Europe, Latin America, Asia Pacific)

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Abstract

Objectives: To monitor the in vitro activity and to detect resistance (R) to linezolid (LZD) in various geographic areas of the world, excluding the United States (USA), the Zyvox® Annual Appraisal of Potency and Spectrum Program (ZAAPS) surveillance program was established in 2002. LZD, the first oxazolidinone agent clinically applied, is an important therapeutic option for infections caused by antimicrobial-R Gram-positive (GP) pathogens. Although rare, LZD-R has been observed among coagulase-negative staphylococci (CoNS) in more frequency than enterococci (ENT). R rates remain extremely low for indicated *S. aureus* (SA) and streptococci.

Methods: 5,769 isolates were collected from 56 sites in 23 countries in 2011. Isolates were received from the following organism groups (n): SA (2,831), CoNS (656), ENT (747), *Streptococcus pneumoniae* (SPN; 878), viridans group streptococci (VGS; 244) and beta-haemolytic streptococci (BHS; 413). At least 200 isolates from each country (except China [n=800]; the United Kingdom and Japan [n=400]) were requested to be sent to a reference laboratory. CLSI broth microdilution susceptibility (S) testing was performed using TREK Diagnostics (Cleveland, Ohio, USA) panels. LZD-R isolates were confirmed with frozen broth microdilution, Etest (BioMerieux, Marcy l'Etoile, France) and disk diffusion methods. PCR and sequencing were performed to detect mutations in 23S rRNA, L3, L4, and L22 proteins, and acquired genes (*cfr*).

Results: Overall LZD-S in the ZAAPS study was >99.8% with only 9 isolates identified as non-susceptible (NS). LZD-S by organism group: SA 100.0%; CoNS 98.9%; ENT 99.7%; all streptococci had LZD MIC values of ≤2 mg/L (S). The *cfr* gene was identified in 3 CoNS strains with LZD MIC values of 4 mg/L (Mexico and France), also a *S. epidermidis* from Spain with a LZD MIC of 8 mg/L and a *S. epidermidis* from France had both a G2576T mutation and *cfr* gene with a LZD MIC of >128 mg/L (3 isolates with *cfr* in 2010 ZAAPS study). MRSA rate was 33.4% overall (27.7% Europe [EU]; 42.4% Latin America [LA]; 42.2% Asia Pacific [AP]). VRE rates were 9.2% in EU, 8.9% in LA and 1.1% in AP. SPN had overall penicillin and erythromycin R rates of 24.7% (MIC, ≥2 mg/L) and 45.0%, respectively.

Conclusions: LZD-R remains low at <1% among contemporary pathogens from surveyed organism groups. Continued worldwide monitoring of in vitro LZD activity allows detection of emerging resistance among targeted Gram-positive pathogens.

Introduction

The Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Program has completed its tenth year (2011) of resistance surveillance for linezolid, the first oxazolidinone class agent to be internationally developed and licensed for use in clinical practice. Since the United States Food and Drug Administration (USA-FDA) approval in 2000, linezolid has been used primarily to treat multidrug-resistant (MDR) Gram-positive pathogens causing complicated skin and skin structure infections (cSSSI) and nosocomial pneumonia.

Linezolid inhibits bacterial protein synthesis through a mechanism of action different from that of other antibacterial agents; therefore, cross resistance between linezolid and other classes of antibiotics is uncommon but may occur. Cases of linezolid resistance have been reported in staphylococci, enterococci, and streptococci with the G2576T or G2447T target site mutations as the dominant resistance mechanism. Recently, the *cfr* gene has been detected in coagulase-negative staphylococci (CoNS), *Staphylococcus aureus* and enterococci.

Linezolid has emerged as a viable alternative for infections caused by Gram-positive organisms that are resistant to conventional drugs, such as methicillin-resistant *S. aureus* (MRSA), drug-resistant *Streptococcus pneumoniae* (DRSP) and vancomycin-resistant enterococci (VRE). Therefore, it is important to continuously monitor the potency and potential for emerging resistance mechanisms to linezolid as the use of this agent increases, in volume and in geographic distribution.

Materials and Methods

Organism collection. Gram-positive isolates (5,769) from 56 medical centers in 23 countries were sent to a central laboratory for processing (Table 1). Each participating country with the exception of the United Kingdom, Japan and China which submitted more isolates, forwarded a target total of 200 consecutively sampled, non-duplicate patient isolates from infections of the bloodstream, respiratory tract, urinary tract, or wound/SSSI.

All isolates were identified by the submitting laboratory and confirmed by the central facility using standardized and commercial methods (VITEK 2 system; bioMerieux, Hazelwood, Missouri, USA). Isolates were grouped for analysis as follows: *S. aureus* (2,831 strains), CoNS (656 strains), β-haemolytic streptococci (413 strains), viridans group streptococci (244 strains), *S. pneumoniae* (878 strains), and enterococci (747 strains), see Table 1.

Susceptibility testing. Antimicrobial susceptibility testing (linezolid and comparators) was performed using validated microdilution panels with cation-adjusted Mueller-Hinton broth (2.5-5% lysed horse blood supplement for testing fastidious streptococci) produced by ThermoFisher Scientific (formerly TREK Diagnostics Cleveland, Ohio, USA). The categorical interpretations of MIC results were those published by the Clinical and Laboratory Standards Institute (CLSI, formerly the NCCLS) in M100-S22 [2012] and by EUCAST [2012]. Quality control (QC) organism (*S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *S. pneumoniae* ATCC 49619) results were within the acceptable published ranges.

Isolates having linezolid MIC values in the non-susceptible or resistant range were repeated by the CLSI M07-A9 method and further subjected to alternative tests using disk diffusion and Etest (bioMérieux, Durham, North Carolina, USA) methods.

Methods-Continued

Molecular testing was performed to identify the 23S rRNA target site mutation and possible clonal relatedness using pulsed-field gel electrophoresis (PFGE). Furthermore, molecular tests to identify the *cfr* gene encoding resistances to oxazolidinones were performed as described by Mendes et al. [2008]. Other potential target site modifications associated with increased linezolid MIC results were also examined (L3, L4 and L22 proteins, etc).

Results

- The MRSA rates among 2,831 *S. aureus* isolates tested included: Latin America (average at 42.2%; range 33.2% [Brazil] to 67.9% [Chile]); Europe (average at 27.7%; range 1.0% [Sweden] to 61.5% [Portugal]); Canada (32.6%) and the APAC region (average at 42.1%; range 36.8% [China] to 51.7% [Singapore]).
- The $MIC_{50/90}$ results for linezolid when tested against *S. aureus* were at 1 and 2 mg/L respectively with 93.1% of all *S. aureus* inhibited at 1 or 2 mg/L. For MRSA, the MIC_{90} decreased to 1 mg/L. None of the *S. aureus* isolates had a linezolid MIC greater than 2 mg/L.
- Among 626 CoNS isolates, oxacillin resistance increased to 77.6% (73.8% in ZAAPS Program 2010) with rates ranging from 63.1% (Germany) to 93.3% (China).
- Linezolid MIC values were generally two-fold lower for CoNS when compared to *S. aureus*. The modal and MIC_{90} results for CoNS continue to be at 1 mg/L (same as 2008; Table 2).
- Seven CoNS (six *S. epidermidis* and one *S. capitis*) were detected with linezolid MIC results at ≥8 mg/L. Two *S. epidermidis* (Spain and France) contained the *cfr* gene, with one isolate (France) also possessing the G2576T mutation. Three *S. epidermidis* from Sao Paulo, Brazil were clonal by PFGE (Table 3). In addition, three CoNS with linezolid MIC values at 4 mg/L were positive for *cfr*.
- The overall VRE rate was 8.0% (Table 2) with the vast majority of these exhibiting the VanA resistance type (88.3%). Two enterococci had linezolid MIC values of ≥8 mg/L (Germany and Ireland) both containing the G2576T target mutation.
- The overall penicillin and erythromycin non-susceptible rates for 878 *S. pneumoniae* isolates were 24.7 and 45.0%, respectively. No linezolid MIC was greater than 2 mg/L for this species (Table 2).
- β-haemolytic streptococci and viridans group streptococci both had similar linezolid $MIC_{50/90}$ results of 1 mg/L with no MIC values observed above 2 mg/L (Table 2). One β-haemolytic streptococcus Group B isolate from Japan was penicillin and ceftriaxone non-susceptible.

Table 1. Distribution of organism identifications for the 2011 ZAAPS sample indexed by nation of origin (5,769 strains).

Nation (no. medical centers)	No. of strains						
	SA	CoNS	ENT	SPN	VGS	βHS	Total
Canada (2)	89	41	10	20	7	7	174
Argentina (2)	85	1	18	12	7	15	138
Brazil (4)	187	57	44	23	2	13	326
Chile (2)	81	18	33	5	12	13	162
Mexico (2)	194	74	107	22	15	34	446
Belgium (1)	49	1	25	11	3	18	107
France (5)	406	77	107	228	58	93	969
Germany (3)	167	65	56	30	9	25	352
Greece (1)	24	7	11	69	0	36	147
Ireland (2)	148	3	45	94	19	31	340
Israel (1)	27	0	3	8	1	2	41
Italy (2)	136	48	34	36	0	1	255
Portugal (1)	104	41	36	0	0	4	185
Slovakia (1)	41	0	0	9	6	0	56
Spain (3)	140	36	47	33	16	18	290
Sweden (2)	101	21	39	93	22	29	305
Turkey (2)	93	37	28	41	5	0	204
United Kingdom (2)	237	23	14	18	7	35	334
China (10)	185	45	45	58	28	0	361
India (3)	69	3	16	1	5	5	99
Japan (3)	186	53	19	49	11	10	328
Singapore (1)	58	0	0	8	10	20	96
Thailand (1)	24	5	10	10	1	4	54
TOTAL (56)	2831	656	747	878	244	413	5769

SA=*S. aureus*; ENT=Enterococci; SPN=*S. pneumoniae*; VGS=viridans group streptococci; βHS=β-haemolytic streptococci.

Table 3. Linezolid-resistant (R) isolates found in the 2011 ZAAPS Program.

Species	City/Country	Linezolid MIC (mg/L)	R- mechanism
E. faecium	Leipzig/Germany	8	G2576T
E. faecium	Dublin/Ireland	8	G2576T
S. capitis	Athens/Greece	16	G2576T
S. epidermidis	Sao Paulo/Brazil	8	G2576T
S. epidermidis	Sao Paulo/Brazil	8	G2576T
S. epidermidis	Sao Paulo/Brazil	16	G2576T
S. epidermidis	Madrid/Spain	8	*cfr*
S. epidermidis	Genova/Italy	16	G2576T
S. epidermidis	Lille Cedex/France	>128	G2576T and *cfr*

a. Note that 3 CoNS with linezolid MIC at 4 mg/L were positive for *cfr*.

Conclusions

- Overall, linezolid remained active against 5,760 of 5,769 (99.84% susceptible) strains that were processed in the 2011 ZAAPS Program. This resistance rate (0.16%) was less than that of any glycopeptides or lipopeptide due to escalating rates of VRE and other MDR strains.
- Linezolid resistance rates remained stable in CoNS (1.2%) and enterococci (0.27%) with the dominant mechanism of resistance being a G2576T mutation (eight isolates) and two CoNS isolates were observed with the *cfr* gene.
- Linezolid continues to demonstrate potent in vitro activity against Gram-positive pathogens on four monitored continents. Linezolid refractory strains still appear to be unusual, without escalating occurrence (<1%) overall and when present can be clonal.

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Table 2. Comparative activity of linezolid tested against 5,769 Gram-positive cocci from 23 nations in the ZAAPS Program (2011).

Organism (no. tested)/ antimicrobial agent	MIC (mg/L)			% by category[a] Susceptible/Resistant	
	50%	90%	Range	CLSI	EUCAST
S. aureus - All strains (2,831)					
Linezolid	1	2	0.25-2	100.0 / 0.0	100.0 / 0.0
Ceftriaxone[a]	4	>8	0.12->8	65.2 / 33.4	66.6 / 33.4
Clindamycin	≤0.25	>2	≤0.25->2	81.7 / 18.3	81.3 / 18.3
Erythromycin	0.25	>16	≤0.12->16	59.9 / 37.4	60.0 / 39.2
Gentamicin	≤1	>8	≤1->8	85.3 / 13.6	84.2 / 15.8
Levofloxacin	0.25	>4	≤0.12->4	67.0 / 31.8	67.0 / 31.8
Oxacillin[a]	0.5	>2	≤0.25->2	66.6 / 33.4	66.6 / 33.4
Tetracycline	≤0.25	8	≤0.25->8	89.6 / 9.9	88.9 / 10.7
TMP/SMX[b]	≤0.5	≤0.5	≤0.5->4	97.9 / 2.1	97.9 / 1.8
Teicoplanin	≤2	≤2	≤2-4	100.0 / 0.0	99.9 / 0.1
Vancomycin	1	1	≤0.12-2	100.0 / 0.0	100.0 / 0.0
MRSA (945) - Linezolid	1	1	0.25-2	100.0 / 0.0	100.0 / 0.0
MSSA (1,886) - Linezolid	1	2	0.25-2	100.0 / 0.0	100.0 / 0.0
Coagulase-negative staphylococci (656)[c]					
Linezolid	0.5	1	≤0.12->128	99.1 / 0.9	99.1 / 0.9
Ceftriaxone[a]	>8	>8	0.25->8	22.4 / 77.6	22.4 / 77.6
Clindamycin	≤0.25	>2	≤0.25->2	66.5 / 32.9	64.8 / 33.5
Erythromycin	>16	>16	≤0.12->16	33.5 / 66.0	33.5 / 66.2
Gentamicin	4	>8	≤1->8	50.9 / 40.7	45.7 / 54.3
Levofloxacin	4	>4	≤0.12->4	41.6 / 54.1	41.6 / 54.1
Oxacillin[a]	>2	>2	≤0.25->2	22.4 / 77.6	22.4 / 77.6
Tetracycline	1	>8	≤0.25->8	81.7 / 16.8	67.7 / 20.6
TMP/SMX[b]	≤0.5	>4	≤0.5->4	60.5 / 39.5	60.5 / 22.9
Teicoplanin	≤2	8	≤2->16	97.3 / 0.3	87.5 / 12.5
Vancomycin	1	2	0.25-4	100.0 / 0.0	99.1 / 0.9
Enterococci - All strains (747)[d]					
Linezolid	1	2	≤0.12-8	99.6 / 0.4	99.6 / 0.4
Ampicillin	1	>8	≤0.25->16	66.9 / 33.1	66.7 / 33.1
Erythromycin	>16	>16	≤0.12->2	6.6 / 64.5	- / -
Levofloxacin	2	>4	≤0.12->4	50.6 / 46.7	- / -
Piperacillin/tazobactam	8	>64	≤0.5->64	66.7 / -	66.7 / -
Tetracycline	>8	>8	≤0.25->8	38.0 / 61.2	- / -
Teicoplanin	≤2	≤2	≤2->16	92.5 / 7.1	92.4 / 7.6
Vancomycin	1	4	0.25->16	91.4 / 8.0	91.4 / 8.6
VRE (64) - Linezolid	1	2	0.5-8	96.9 / 3.1	96.9 / 3.1
VSE (683) - Linezolid	1	2	≤0.12-8	99.9 / 0.1	99.9 / 0.1

Organism (no. tested)/ antimicrobial agent	MIC (mg/L)			% by category[a] Susceptible/Resistant	
	50%	90%	Range	CLSI	EUCAST
S. pneumoniae - All strains (878)					
Linezolid	1	1	≤0.12-2	100.0 / -	100.0 / 0.0
Amoxicillin/clavulanic acid	≤1	4	≤1->8	88.5 / 6.3	- / -
Ceftriaxone	≤0.06	1	≤0.06-4	90.3 / 1.1	71.5 / 1.1
Ciprofloxacin	1	2	≤0.03->4	(4.4)[e]	0.5 / 4.4
Clindamycin	≤0.25	>2	≤0.25->2	67.8 / 31.8	68.2 / 31.8
Erythromycin	≤0.12	>16	≤0.12->16	54.9 / 45.0	54.9 / 45.0
Levofloxacin	1	1	≤0.12->4	98.2 / 1.6	98.2 / 1.8
Penicillin[f]	≤0.06	4	≤0.06-8	57.7 (89.7)/ 24.7 (1.4)	57.7 / 10.3
Tetracycline	≤0.5	>8	≤0.25->8	61.6 / 37.9	61.3 / 38.4
TMP/SMX[b]	≤0.5	>4	≤0.5->4	66.5 / 21.0	76.3 / 21.0
Vancomycin	0.25	0.5	≤0.12-1	100.0 / -	100.0 / 0.0
MDR-3[g] (150) - Linezolid	0.5	1	0.25-1	100.0 / -	100.0 / 0.0
MDR-4[h] (135) - Linezolid	0.5	1	0.25-1	100.0 / -	100.0 / 0.0
MDR-5[i] (79) - Linezolid	0.5	1	0.25-1	100.0 / -	100.0 / 0.0
Viridans group streptococci (244)[j]					
Linezolid	1	1	≤0.12-2	100.0 / -	- / -
Ceftriaxone	0.25	2	≤0.06->8	87.3 / 8.6	82.0 / 18.0
Clindamycin	≤0.25	>2	≤0.25->2	77.5 / 22.1	77.9 / 22.1
Erythromycin	1	>16	≤0.12->16	43.9 / 56.1	- / -
Levofloxacin	1	2	0.25->4	91.0 / 9.0	- / -
Penicillin[a]	≤0.06	2	≤0.06->8	63.5 / 8.2	75.4 / 8.2
Tetracycline	≤1	>8	≤0.25->8	56.6 / 40.6	- / -
Vancomycin	0.5	1	≤0.25-1	100.0 / -	100.0 / 0.0
β-haemolytic streptococci (413)[k]					
Linezolid	1	1	0.5-1	100.0 / -	100.0 / 0.0
Amoxicillin/clavulanic acid[a]	≤1	≤1	≤1	- / -	100.0 / 0.0
Ceftriaxone	≤0.06	0.12	≤0.06-1	99.8 / -	100.0 / 0.0
Clindamycin	≤0.25	≤0.25	≤0.25->2	91.3 / 8.7	91.3 / 8.7
Erythromycin	≤0.12	8	≤0.12->16	78.0 / 21.5	78.0 / 21.5
Levofloxacin	0.5	1	≤0.12->4	97.6 / 1.7	92.5 / 2.4
Penicillin[a]	≤0.06	≤0.06	≤0.06-0.25	99.8 / -	100.0 / 0.0
Tetracycline	2	>8	≤0.25->8	51.3 / 46.5	49.9 / 48.7
Vancomycin	0.5	0.5	0.25-1	100.0 / -	100.0 / 0.0

a. Criteria as published by the CLSI [2012] and EUCAST [2012], beta-lactam susceptibility should be directed by the oxacillin test results for staphylococci and by ampicillin or penicillin for Enterococcus or streptococci.
b. TMP/SMX=trimethoprim/sulfamethoxazole.
c. Includes: 15 species (491 strains) and unidentified coagulase-negative staphylococci (165 strains).
d. Includes: *Enterococcus faecalis* (446 strains), *Enterococcus faecium* (206 strains), and 31 other enterococci.
e. Percentage of pneumococci or other streptococci with ciprofloxacin MICs at ≥4 mg/L, indicating possible QRDR mutations.
f. CLSI 2012 susceptibility breakpoints for high-dose parenteral penicillin (nonmeningitis in parenthesis).
g. MDR-3 = resistant to three agents eg. penicillin ≥2 mg/L, erythromycin ≥1 mg/L, and clindamycin ≥1 mg/L.
h. MDR-4 = resistant to four agents eg. penicillin ≥2 mg/L, erythromycin ≥1 mg/L, clindamycin ≥1 mg/L, and tetracycline ≥8 mg/L.
i. MDR-5 = resistant to five agents eg. penicillin ≥2 mg/L, erythromycin ≥1 mg/L, clindamycin ≥1 mg/L, tetracycline ≥8 mg/L, and trimethoprim-sulfamethoxazole ≥4 mg/L.
j. Includes: 15 species (133 strains), *Streptococcus bovis* group (8 strains), unidentified viridans group streptococci (103 strains).
k. Includes: Group A (160 strains), Group B (156 strains), Group C (20 strains), Group F (3 strains), Group G (63 strains), and two other species (11 strains).