Summary of 2009 Oxazolidinone Resistance Surveillance Studies Worldwide (LEADER and ZAAPS Programs)

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ABSTRACT

Background: Two comprehensive R surveillance programs (LEADER and ZAAPS) have monitored linezolid (LZD) activity for over 8 years in the USA and over 20 other nations; approximately 12,000 clinical Gram-positive (GP) samples/year. Overall results are analyzed for evolving R rates and mechanism types, where detected.

Methods: A total of 12,168 isolates (6,414/5,754 from USA/ex-USA) were susceptibility (S) tested by CLSI methods (M07-A8) collected from 123 hospitals in 23 countries in 2009. Six major GP pathogen groups were sampled: *S. aureus* (6,215; 37.9 to 51.4% [USA] MRSA), coagulase-negative staphylococci (CoNS; 1,643), enterococci (ENT; 1,761), *S. pneumoniae* (SPN; 1,297), viridans group (VGS; 478) and β-haemolytic (BHS; 776). GP strains with LZD-non-S (MIC, ≥4 μg/ml) were validated and R mechanisms determined by molecular methods.

Results: All SPN, VGS and BHS were LZD-S and had modal/MIC_{90} values at 1 μg/ml. Among *S. aureus,* 5 LZD-R strains (USA only; 0.15%) were noted in 5 states having the following R mechanisms: *cfr* (2), G2576T (2) and L3 S145 deletion (1). LZD-R CoNS were noted in USA (12; 1.47%) and ex-USA (4; Mexico and Italy, 0.48%) with the following R-mechanisms: *cfr* (6; 2 combined with L3 alterations), G2576T (6) and L3/L4 mutations (4). LZD MIC_{90} results were stable at 1 and 2 μg/ml for SA and CoNS, respectively. LZD-R among ENT were dominantly in *E. faecium* (11/12 strains; 0.54-0.79% by region), and produced by G2576T (11) or a single L4 (F101L) mutation (1); isolated in USA, Germany, Korea and China. R rates are declining in GP for the LEADER (0.45 [2006] to 0.34% [2009]) and ZAAPS (0.19 to 0.14%) Programs. Clonal outbreaks are frequent factors in LZD-R occurrences worldwide. Clindamycin inducible R in SA was 37.9%, encouraging use of D-testing.

Pathogen/Regional sample (no.)	LZD MIC distributions (cum. %) for all tested strains						
	≤0.25	0.5	1	2	4	8	%R[a]
S. aureus							
USA (3,257)	0.3	0.9	41.5	99.8	99.9	>99.9	0.15
ZAAPS (2,958)[b]	0.1	1.3	37.5	100.0	-	-	0.00
CoNS							
USA (816)	1.5	37.1	94.4	98.3	98.5	98.9	1.47
ZAAPS (827)	0.9	25.9	94.1	99.3	99.5	99.6	0.48
ENT							
USA (1,017)	0.1	4.0	53.4	98.9	99.2	99.6	0.79
ZAAPS (744)	0.0	4.3	51.5	99.5	99.5	99.7	0.54

a. CLSI and EUCAST criteria.
b. ZAAPS = ex-USA (32 nation sample)

Conclusion: Yearly LZD-R monitoring documents rare isolation of strains, usually CoNS and *E. faecium*. Numerous R-mechanisms have been detected, but the overall rates have remained stable in sampled nations since 2006 (<0.5% overall LZD-R).

INTRODUCTION

Over the past eight years, two surveillance programs have monitored emerging linezolid resistance, the Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Program for Asia-Pacific, Europe, Latin America, Canada and the LEADER Program (United States [USA]). Linezolid, the first oxazolidinone class agent to be licensed for clinical use, has been used primarily to treat multidrug-resistant (MDR) Gram-positive pathogens found in complicated skin and soft tissue infections (cSSTI) and nosocomial pneumonias, after its USA Food and Drug Administration (FDA) approval in early 2000. Those Gram-positive pathogens that linezolid has excellent activity against include organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), MDR *Streptococcus pneumoniae* and vancomycin-resistant enterococci (VRE). Linezolid use continues to increase worldwide and the need for potency and resistance surveillance becomes vital to the continued success of the oxazolidinone class.

The linezolid mechanism of action has been described as binding to the 50S ribosomal subunit of the 23S rRNA molecule with inhibition of protein synthesis. Among the rare cases of linezolid resistance reported to date among staphylococci and enterococci, G2576U or G2447T target site mutations have been the detected mechanism, however, a mobile resistance element (*cfr*) has been described in staphylococci.

MATERIALS AND METHODS

Organism collection. A total of 12,168 Gram-positive isolates were forwarded to the central monitoring site (JMI Laboratories, North Liberty, Iowa, USA) from 23 nations and 123 medical centers. Each participating site in the USA fowarded a target total of 100 clinically significant Gram-positive isolates in a prevalence style sampling design. Outside of the USA, multiple centers contributed designated numbers of strains for a total of 200 Gram-positive isolates per country.

Isolates were grouped for analysis as follows: *S. aureus* (6,215 strains), coagulase-negative staphylococci (CoNS; 1,643 strains), β-haemolytic streptococci (776 strains), viridans group streptococci (478 strains), *S. pneumoniae* (1,295 strains) and enterococci (1,761 strains). All processed organisms were identified by the submitting laboratory and confirmed by the central facility using the Vitek standard system (bioMerieux, Hazelwood, Missouri, USA) or molecular methods, when needed.

Susceptibility testing. Antimicrobial susceptibility testing was performed using validated microdilution panels with cation-adjusted Mueller-Hinton broth (2-5% lysed horse blood added for testing streptococci) prepared by TREK Diagnostics (Cleveland, Ohio, USA). The categorical interpretations of MIC results followed Clinical and Laboratory Standards Institute (CLSI) document M100-S20-U. Quality control (QC) organism (*S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619) results were within the acceptable MIC QC ranges as published by CLSI (2010).

All isolates were tested against antimicrobial agents active against Gram-positive organisms including: linezolid, ciprofloxacin, erythromycin, levofloxacin, penicillin, quinupristin/dalfopristin, rifampin, teicoplanin, and vancomycin. Other drugs tested against selected pathogen subgroups were: ampicillin, ceftriaxone, clindamycin, piperacillin/tazobactam, tetracycline, and trimethoprim/sulfamethoxazole (TMP/SMX).

All linezolid-resistant isolates (MIC, ≥8 μg/mL), if detected, were confirmed by Etest (bioMerieux) and disk diffusion methods. The determination of the domain V 23S ribosomal target mutation(s) was performed by polymerase chain reaction (PCR) amplification and sequence analysis. Furthermore, molecular tests to identify the *cfr* gene encoding resistances to oxazolidinones in staphylococci were tested as described by Mendes et al, [2008]. Other potential target site modifications (L3 and L4) associated with increased linezolid MIC results were also examined.

RESULTS

- The total number of Gram-positive cocci submitted to the ZAAPS and LEADER Programs was over 12,000 during 2009. All tests were performed in a reference laboratory by standardized CLSI methods (Tables 1-2 and Figure 1).
- Overall MRSA rates were 37.9% in the ZAAPS study and 51.4% in the LEADER study. Resistance to methicillin did not adversely affect the linezolid activity (data not shown).
- Linezolid activity among CoNS was the same for the two programs with the $MIC_{50/90}$ results at 1 μg/ml. However, twelve isolates in LEADER Program and four isolates in ZAAPS Program were found to have linezolid MIC results of ≥8 μg/ml (14 *S. epidermidis*, 1 *S. cohnii* and 1 *S. capitis*; Table 2). Overall linezolid resistance rates were only 0.48% (ZAAPS) and 1.47% (LEADER).
- The VRE rate was 11.7% in ZAAPS (highest rate in Taiwan, 41.5%) and 29.0% in LEADER (highest rate in Mid-Atlantic Census Region, 41.3%). Overall linezolid resistance was 0.5% (4 strains; ZAAPS) and 1.1% (8 strains; LEADER).
- Among the tested *S. pneumoniae*, non-susceptible penicillin rates (MIC, ≥2 μg/ml) were greatly increased in 2009 for the ZAAPS Program (30.7%; 11.5% in 2008), but remained stable in the LEADER Program (21.5%; 22.0% in 2008).
- Linezolid $MIC_{50/90}$ values among all streptococci in both programs was consistent at 1 μg/ml. Similar to 2008, all streptococci isolates were susceptible to linezolid.
- The most common linezolid resistance mechanisms were: G2576T mutations (20) and *cfr* gene (8), see Table 2.
- The overall linezolid resistance rates for each study were only 0.14% (ZAAPS) and 0.34% (LEADER), with nearly all MIC values occurring at 0.5, 1 or 2 μg/ml (Figure 1).

Table 1. Linezolid activity as measured by the ZAAPS/LEADER Programs (2009) for 12,168 Gram-positive pathogens, and compared to selected other antimicrobials (7-9) by reference (CLSI) methods.

Pathogen (No. of isolates ZAAPS/LEADER)	Antimicrobial agent	MIC (μg/ml): ZAAPS/LEADER 50%	MIC (μg/ml): ZAAPS/LEADER 90%	CLSI % Susceptible[a] ZAAPS/LEADER	EUCAST % Susceptible[a] ZAAPS/LEADER
S. aureus (2,958/3,257)	Linezolid	2/2	2/2	100.0 / 99.8	100.0 / 99.8
	Oxacillin	1/>2	>2/>2	62.1 / 48.6	62.1 / 48.6
	Erythromycin	0.5/>2	>2/>2	55.6 / 36.9	56.4 / 37.2
	Clindamycin	≤0.25/≤0.25	>2/>2	72.4 / 80.4	72.0 / 79.7
	Daptomycin	0.5/0.5	>2/0.5	100.0 / 100.0	100.0 / 100.0
	Gentamicin	≤2/≤2	>8/≤2	75.7 / 98.4	75.3 / 98.0
	Levofloxacin	≤0.5/≤0.5	>4/>4	64.8 / 57.4	64.8 / 57.4
	Tetracycline	≤2/≤2	>8/≤2	77.9 / 95.9	77.3 / 95.0
	TMP/SMX[b]	≤0.5/≤0.5	≤0.5/≤0.5	94.8 / 98.5	94.8 / 98.5
	Vancomycin	1/1	1/1	100.0 / 100.0	100.0 / 100.0
CoNS[c] (827/816)	Linezolid	1/1	1/1	99.5 / 98.5	99.5 / 98.5
	Oxacillin	>2/>2	>2/>2	19.8 / 26.1	19.8 / 26.1
	Erythromycin	>2/>2	>2/>2	36.4 / 33.1	36.4 / 33.6
	Clindamycin	≤0.25/≤0.25	>2/>2	65.1 / 67.3	63.4 / 66.1
	Daptomycin	0.25/0.25	0.5/0.5	100.0 / 100.0	100.0 / 100.0
	Gentamicin	4/≤2	>8/>8	51.0 / 73.4	46.4 / 69.4
	Levofloxacin	4/4	>4/>4	44.0 / 43.6	44.0 / 43.6
	Tetracycline	≤2/≤2	>8/>8	86.8 / 85.2	81.5 / 81.7
	TMP/SMX[b]	≤0.5/≤0.5	>2/>2	62.8 / 60.1	62.8 / 60.1
	Vancomycin	2/1	2/2	100.0 / 100.0	98.5 / 99.1
Enterococci (744/1,017)	Linezolid	1/1	2/2	99.5 / 98.9	99.5 / 99.2
	Ampicillin	2/2	>16/>16	64.7 / 67.8	62.8 / 67.6
	Ciprofloxacin	>4/>4	>4/>4	33.9 / 37.9	- / -[d]
	Daptomycin	1/1	2/2	100.0 / 99.9	- / -
	Levofloxacin	>4/>4	>4/>4	44.8 / 45.4	- / -
	Tetracycline	>8/>8	>8/>8	39.1 / 30.4	- / -
	Teicoplanin	≤2/≤2	8/>16	90.3 / 71.8	89.5 / 71.2
	Vancomycin	1/2	>16/>16	87.4 / 70.3	87.4 / 70.3
S. pneumoniae (636/659)	Linezolid	1/1	1/1	100.0 / 100.0	100.0 / 100.0
	Penicillin[d]	≤0.03/≤0.03	4/4	56.8 / 57.7	56.8 / 57.7
	Amox/clav	≤1/≤1	8/8	81.1 / 81.8	- / -
	Ceftriaxone	≤0.25/≤0.25	2/2	80.5 / 87.1	67.8 / 77.2
	Erythromycin	≤0.25/≤0.25	>2/>2	50.8 / 58.1	50.8 / 58.1
	Clindamycin	≤0.25/≤0.25	>2/>2	60.4 / 77.8	61.3 / 78.4
	Levofloxacin	1/1	2/1	98.3 / 99.1	98.3 / 99.1
	Tetracycline	≤2/>8	≤2/>8	53.0 / 75.9	53.0 / 75.9
	TMP/SMX[b]	≤0.5/≤0.5	>2/>2	56.2 / 63.7	62.4 / 67.2
	Vancomycin	≤1/≤1	≤1/≤1	100.0 / 100.0	100.0 / 100.0
β-haemolytic streptococci (375/401)	Linezolid	1/1	1/1	100.0 / 100.0	100.0 / 100.0
	Penicillin	≤0.015/0.03	0.06/0.06	100.0 / 100.0	100.0 / 100.0
	Ceftriaxone	≤0.25/≤0.25	≤0.25/≤0.25	100.0 / 100.0	80.8 / 100.0
	Erythromycin	≤0.25/≤0.25	>2/>2	81.1 / 62.8	81.1 / 62.8
	Clindamycin	≤0.25/≤0.25	0.5/>2	89.8 / 81.8	90.4 / 82.8
	Levofloxacin	1/≤0.5	1/1	98.1 / 99.5	90.9 /96.8
	Daptomycin	0.12/0.12	0.25/0.25	100.0 / 100.0	100.0 / 100.0
	Vancomycin	0.5/0.5	0.5/0.5	100.0 / 100.0	100.0 / 100.0
Viridans group streptococci (214/264)	Linezolid	1/1	1/1	100.0 / 100.0	- / -
	Penicillin	0.06/0.06	1/1	71.0 / 77.3	80.8 / 86.4
	Ceftriaxone	≤0.25/≤0.25	2/1	88.8 / 91.7	82.7 / 89.4
	Erythromycin	≤0.25/1	>2/>2	60.3 / 46.2	- / -
	Clindamycin	≤0.25/≤0.25	≤0.25/0.5	90.2 / 88.6	91.6 / 90.9
	Levofloxacin	1/1	2/2	95.8 / 90.2	- / -
	Daptomycin	0.25/0.25	0.5/1	99.5 / 99.6	- / -
	Vancomycin	0.5/0.5	1/1	100.0 / 99.6	100.0 / 100.0

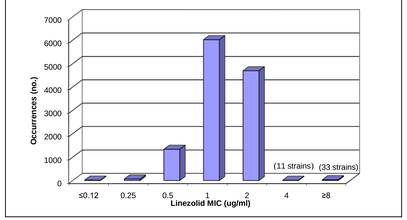
a. Interpretive breakpoint criteria of the CLSI and EUCAST (2010).
b. Amox/clav = amoxacillin/clavulanic acid; TMP/SMX = trimethoprim/sulfamethoxazole; CoNS = coagulase-negative staphylococci
c. - = no criteria published
d. Criteria at ≤0.06 μg/ml for both organizations.

Table 2. Listing of various linezolid non-susceptible Gram-positive cocci detected in ZAAPS and LEADER medical centers during the 2009 ZAAPS resistance surveillance program.

Program	Organism	City	Country	Linezolid MIC (MIC)[a]	Mechanism
ZAAPS	*S. cohnii*	Guadalajara	Mexico	>8 (32)	*cfr*
	S. epidermidis	Guadalajara	Mexico	>8 (32)	*cfr*
	S. epidermidis	Guadalajara	Mexico	>8 (32)	*cfr*
	S. epidermidis	Roma	Italy	8	*cfr*
	E. faecium	Seoul	Korea	>8 (32)	G2576T
	E. faecium	Frankfurt	Germany	>8 (16)	G2576T
	E. faecalis	Frankfurt	Germany	8	G2576T
	E. faecalis	Shenzhen	China	8	G2576T
LEADER	*S. aureus*	Akron	Ohio	8 (16)	*cfr*
	S. aureus	Palo Alto	California	>8 (16)	G2576T
	S. aureus	Hartford	Connecticut	8 (8)	L3 (S145 deletion)
	S. aureus	Louisville	Kentucky	>8 (16)	*cfr*
	S. aureus	Wichita	Kansas	>8 (16)	G2576T
	S. epidermidis	Akron	Ohio	>8 (128)	G2576T
	S. epidermidis	Memphis	Tennessee	8 (8)	L3 (V154L, L101V, A157R), L4 (P171S)
	S. epidermidis	Tempe	Arizona	>8 (>128)	cfr
	S. epidermidis	Tempe	Arizona	>8 (32)	G2576T
	S. epidermidis	Lexington	Kentucky	8 (16)	L3 (A157R, L101V, V154L)
	S. epidermidis	St. Paul	Minnesota	>8 (32)	G2576T
	S. epidermidis	Detroit	Michigan	>8 (16)	L3 (H146Q)
	S. capitis	Detroit	Michigan	8 (8)	*cfr*
	S. epidermidis	Boston	Massachusetts	>8 (16)	L3 (H146Q)
	S. epidermidis	New Brunswick	New Jersey	>8 (128)	G2576T
	S. epidermidis	Salt Lake City	Utah	>8 (128)	G2576T
	S. epidermidis	Houston	Texas	>8 (128)	G2576T
	E. faecium	Louisville	Kentucky	>8 (32)	G2576T
	E. faecium	Louisville	Kentucky	8 (16)	G2576T
	E. faecium	Louisville	Kentucky	8 (8)	G2576T
	E. faecium	Louisville	Kentucky	8 (8)	G2576T
	E. faecium	Wichita	Kansas	>8 (16)	G2576T
	E. faecium	Charlottesville	Virginia	8 (8)	G2576T
	E. faecium	Salt Lake City	Utah	8 (16)	G2576T
	E. faecium	Seattle	Washington	>8 (8)	G2576T

a. MIC from a reference frozen-form panel with a MIC linezolid range to 128 μg/ml.

Figure 1. Linezolid MIC distribution for all isolates in the 2009 ZAAPS and LEADER Programs (12,168 strains).



CONCLUSIONS

- Worldwide linezolid resistance remains unusual (<0.3%) and focused among staphylococci (CoNS > *S. aureus*) and two species of enterococci (Table 2).
- Linezolid-resistant strains were observed in five countries (Germany, Italy, Mexico, Korea and China) and 15 different states in the USA with clonal occurrences documented in several medical centers.
- A wide variety of resistance mechanisms were identified in 2009, including rRNA target site mutation G2576T, *cfr*, and L3 and L4 mutations/deletions (Table 2).
- As other commonly used antimicrobials become compromised by evolving resistances, linezolid refractory strains continue to be relatively rare and without escalating occurrence.

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