Report of Linezolid Activity from the Linezolid Experience and Accurate Determination of Resistance (LEADER) Program for 2014: Monitoring Resistance Trends and Mechanisms

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ABSTRACT

Background: Linezolid (LZD) was the first oxazolidinone approved in the USA (2000) and is indicated for the treatment of complicated skin and skin structure infection and nosocomial pneumonia. It is active against Gram-positive (GP) organisms such as MRSA, multidrug-resistant (R) *S. pneumoniae* and vancomycin-R enterococci that are resistant to conventional drugs. The LEADER Program has monitored LZD activity, spectrum and R rates since 2004.

Methods: A total of 6,865 GP pathogens from 60 medical centers from 36 states were submitted. The organism groups (no. overall) were: *S. aureus* (SA; 3,106), coagulase-negative staphylococci (CoNS; 797), enterococci (ENT; 855), *S. pneumoniae* (SPN; 874), viridans group (VGS; 359), and β-hemolytic streptococci (BHS; 874). All susceptibility (S) testing was performed by reference broth microdilution methods. LZD-R isolates were confirmed by Etest (bioMerieux, Hazelwood, MO) and by repeated reference S testing. PCR and sequencing was performed to detect mutations in 23S rRNA, L3, L4, and L22 proteins, and an acquired gene (*cfr*).

Results: LZD activity against GP organisms remains high (99.78% S). The $MIC_{50/90}$ for SA was at 1/1 μg/mL. MRSA rates were at 47.2% (47.9% in 2013; declining since 2007 [58.2%]). Among the LZD-non-S (NS) MRSA, one strain harbored *cfr* only (MIC, 4 μg/mL), one harbored G2576T (MIC, 8 μg/mL) and one contained *cfr* and G2576T with L3 changes (MIC, ≥8 μg/mL). Among CoNS, six isolates, 0.75% of all strains (0.52% in 2013, 0.92% in 2012) demonstrated LZD MIC results of ≥4 μg/mL. Five of these were identified as *S. epidermidis*; 4 of which contained *cfr* in addition to the presence of mutations in the ribosomal proteins L3 and L4, alone or in combination with 23S rRNA (G2576T) mutations. Six ENT (0.7%) were LZD-NS (≥4 μg/mL; G2576T mutations and one with an additional *cfr*). LZD was active against all SPN and BHS with a $MIC_{50/90}$ of 1/1 μg/mL and VGS with a $MIC_{50/90}$ of 0.5/1 μg/mL. SPN penicillin-NS (MIC, ≥0.12 μg/mL) occurred at a rate of 41.5% and NS ceftriaxone at only 7.2%.

Conclusion: LZD demonstrates excellent activity and a sustained susceptibility rate of 99.78% overall (99.62-99.83% during 2008-2013 LEADER Program). LZD MIC population distributions remained unchanged without evidence of "MIC creep" among monitored, indicated species.

INTRODUCTION

Linezolid, the initial oxazolidinone, was approved by the Food and Drug Administration (FDA) in 2000. Linezolid is indicated for the treatment of complicated skin and skin structure infection (cSSSI) and nosocomial pneumonia. Linezolid is also indicated for the treatment of vancomycin-resistant *Enterococcus faecium* (VRE) infections (including cases with concurrent bacteremia). This compound has emerged as a valuable parenteral/oral agent for the treatment of infections caused by Gram-positive organisms such as MRSA, drug-resistant *Streptococcus pneumoniae* and VRE that are also resistant to commonly used agents.

The Linezolid Experience and Accurate Determination of Resistance (LEADER) surveillance program has monitored linezolid activity, spectrum and resistance rates in the United States (USA) since 2004. This program serves to generate national *in vitro* data for linezolid and comparator agents to provide longitudinal data to which local susceptibility patterns may be compared. In addition, molecular testing of isolates with decreased linezolid susceptibility allows detection of emerging resistance mechanisms that would not be possible in routine clinical laboratory practice.

The oxazolidinone mechanism of action has been described as selective binding to the 50S ribosomal subunit of the 23S rRNA molecule resulting in inhibition of protein synthesis. Oxazolidinone resistance has been detected, mainly among *Enterococcus* species and the coagulase-negative staphylococci (CoNS), but the occurrence rates of resistance have remained rare for *Staphylococcus aureus* and streptococci. Among the detected cases of linezolid resistance, target site mutations in 23S rRNA, L3 and L4 ribosomal proteins have been the most prevalent mechanisms. In addition, plasmid-encoded efflux-pump (*optrA*) and methylase (*cfr* and *cfr*[B]) genes have been detected among several species of Gram-positive isolates of human and animal origin.

MATERIALS AND METHODS

Bacterial strain collection: A total of 6,865 Gram-positive pathogens from 60 medical centers in 36 states were submitted to JMI Laboratories (North Liberty, Iowa, USA). The medical centers represented all nine USA Bureau of Census geographic regions. The strains were distributed among the following organism groups (no.): *S. aureus* (3,106), CoNS (797), enterococci (855), *S. pneumoniae* (874), viridans group (VGS; 359), and β-hemolytic streptococci (BHS; 874). The predominant sources for these pathogens were bacteremias, respiratory tract, SSSI, and urinary tract infections.

Antimicrobial susceptibility test methods: All susceptibility testing was performed utilizing Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods (frozen- and dry-form 96-well plates; CLSI M07-A10, 2015) and published interpretive criteria (CLSI M100-S25, 2015). Linezolid-resistant isolates were confirmed by frozen-form reference broth microdilution testing. Molecular testing was performed on isolates with elevated linezolid MICs (MIC, ≥4 μg/mL) to identify resistance mechanisms (*cfr,* and 23S rRNA, L3 and L4 mutations) and potential clonality was evaluated using pulsed field gel electrophoresis (PFGE).

RESULTS

- The activity of linezolid in the 2014 LEADER Program sampling of 60 medical centers (6,865 Gram-positive strains) is presented in **Table 1**. The linezolid activity against monitored organisms remains high (99.78% susceptible), with only 15 (0.22%) isolates displaying elevated MIC values (i.e. ≥4 μg/mL).
- Linezolid was highly potent against *S. aureus* exhibiting a $MIC_{50/90}$ at 1 μg/mL. Erythromycin, ciprofloxacin and clindamycin resistance rates were at 85.2, 69.0 and 26.8% when tested against MRSA, respectively. In MSSA, resistance rates to the above tested drugs were lower (27.4, 11.5 and 5.2%), while linezolid, daptomycin, vancomycin, gentamicin and trimethoprim-sulfamethoxazole were very active (≥96.0% susceptible) against MSSA and MRSA (**Table 2**).
- MRSA rates were at 47.2% (47.9% in 2013; declining since 2007 [58.2%]), see **Figure 1**.
- Linezolid demonstrated a $MIC_{50/90}$ of 0.5 μg/mL when tested against all CoNS isolates, regardless of oxacillin susceptibility (**Table 2**). Only linezolid, daptomycin and vancomycin exhibited high susceptibility rates at >90% (**Table 2**).
- Linezolid was highly active against VRE (99.3% susceptible) exhibiting a $MIC_{50/90}$ at 1 μg/mL. Among the enterococci tested (855), the ampicillin-susceptible rate was only 74.9% (**Table 2**), and VRE rates varied by Census Region ranging from 8.4% (West North Central) to 30.3% (South-Atlantic) (data not shown).
- Linezolid was active against all *S. pneumoniae* with MIC_{50}, MIC_{90} and MIC_{100} of 1, 1 and 2 μg/mL, respectively (**Table 2**). Penicillin non-susceptibility (MIC, ≥0.12 μg/mL) occurred at a rate of 41.5% and ceftriaxone–non-susceptibility was only 7.2%. Erythromycin and clindamycin resistance was high among all *S. pneumoniae* (45.9 and 17.2%, respectively; **Table 2**).
- Linezolid was active against all VGS and BHS ($MIC_{50/90}$, 0.5/1 μg/mL for VGS and $MIC_{50/90}$, 1/1 μg/mL for BHS). Linezolid, daptomycin, tigecycline and vancomycin (all 100.0% susceptible) were highly active those streptococci (data not shown).
- **Table 3** displays the list of isolates with elevated linezolid MIC values (≥4 μg/mL) in the 2014 LEADER Program. Among the MRSA, one strain from Louisiana harbored only *cfr* (MIC, 4 μg/mL), two strains from California harbored G2576T and one also contained *cfr* (MIC, 8-16 μg/mL).
- Among CoNS, six isolates, 0.75% of all strains (0.52% in 2013, 0.92% in 2012) demonstrated linezolid MIC results of 8->128 μg/mL. Five of these organisms were identified as *S. epidermidis*; four of which contained *cfr* in addition to the presence of mutations in the ribosomal proteins L3 and L4, alone or in combination with 23S rRNA (G2576T) mutations.
- Six enterococcus (0.70%) were linezolid-non-susceptible (4-16 μg/mL; G2576T mutations and one with an additional *cfr*).

Table 1. Number of isolates inhibited at each linezolid MIC when testing six different groups of Gram-positive cocci isolated from all USA census regions (LEADER Program, 2014); 6,865 strains.

	Number of isolates inhibited at linezolid MIC (μg/mL):							
Organism group (no. tested)	≤0.12	0.25	0.5	1	2	4	8	>8
β-hemolytic streptococci (874)	0	16	415	443	-	-	-	-
S. pneumoniae (874)	3	28	289	551	3	-	-	-
Enterococci (855)	0	7	167	640	35	5[a]	1	-
S. aureus (3,106)	0	23	921	2131	28	1	1	1
MRSA (1,465)	0	14	499	941	8	1	1	1
MSSA (1,641)	0	9	422	1190	20	-	-	-
Viridans group streptococci (359)	3	22	209	125	-	-	-	-
CoNS (797)	5	220	524	42	0	1[b]	0	5
MRCoNS (457)	2	119	305	26	0	1[b]	0	4
MSCoNS (340)	3	101	219	16	0	0	0	1

a. Four of these strains had MICs of ≥8 μg/mL when tested using reference frozen-form MIC panels.
b. This strain had a MIC of 8 μg/mL when tested using reference frozen-form MIC panels.

Table 3. Isolates with elevated linezolid MIC values (≥4 μg/mL) in the 2014 LEADER Program.

Isolate ID number	Organism	City	State	Age/Sex	Linezolid MIC (μg/mL)	Resistance mechanisms	PFGE
468-15900	*S. aureus*	Long Beach	California	21/F	8	G2576T	SA468A
468-24497	*S. aureus*	Long Beach	California	23/M	16	*cfr*, G2576T, L3 (H146 deletion, P151L)	SA468B
448-24858	*S. aureus*	New Orleans	Louisiana	54/F	4	*cfr*	-
116-6817	*S. epidermidis*	Houston	Texas	39/M	64	G2576T, L3 (G137S, H146P, M156T), L4 (71G72insertion)	SEPI116D[a]
116-16061	*S. epidermidis*	Houston	Texas	68/M	128	*cfr*, L3 (H146Q, V154L, A157R), L4 (71G72insertion)	SEPI116E[a]
468-169	*S. epidermidis*	Long Beach	California	80/F	128	*cfr*, L3 (G137S, H146Q, V154L, A157R), L4 (71G72 insertion)	-
470-39182	*S. epidermidis*	San Francisco	California	52/M	>128	*cfr*, L3 (H146Q, V154L, A157R), L4 (71G72 insertion)	SEPI470A
412-12722	*S. epidermidis*	Memphis	Tennessee	NA	>128	*cfr*, G2576T, L4 (G137S, H146P, F147Y, M156T), L4 (71G72 insertion)	-
413-48138	*S. hominis*	Seattle	Washington	38/F	8	*cfr*, L3 (M169L)	-
30-36528	*E. faecium*	Charlottesville	Virginia	42/M	4	G2576T (+)	-
461-4485	*E. faecium*	Fairbanks	Alaska	62/M	8	G2576T (+)	EFM461A
461-16441	*E. faecium*	Fairbanks	Alaska	62/M	8	G2576T (+)	EFM461A1
467-20417	*E. faecium*	Los Angeles	California	34/M	16	G2576T (+)	EFM467B[b]
149-8289	*E. faecium*	Atlanta	Georgia	6/M	8	*cfr*, G2576T (+)	-
122-4664	*E. faecalis*	Burlington	Vermont	58/F	8	G2576T (-)	-

a. One isolate each (from 2011) displayed the same SEPI116D and SEPI116E patterns as the isolates from 2014.
b. PFGE profile distinct from the one noted for non-S isolates detected from this site during the 2013 (EFM467A) Program.

Figure 1. MRSA and MSSA rate by year for the LEADER Program.

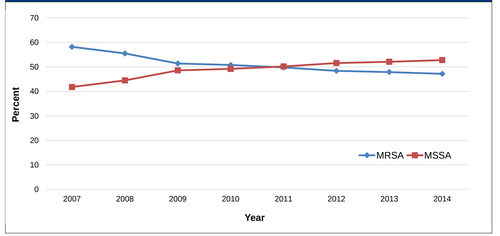

Table 2. Linezolid activity compared to other agents when tested in the LEADER Program (USA, 2014), 6,835 strains.

Organism/antimicrobial agent (no. tested)	MIC (μg/mL)			CLSI[a]
	MIC_{50}	MIC_{90}	Range	%S / %I / %R
S. aureus, methicillin-resistant (1,465)				
Linezolid	1	1	0.25->8	99.9 / - / 0.1
Ciprofloxacin	>4	>4	0.12->4	28.6 / 2.5 / 69.0
Clindamycin	≤0.25	>2	≤0.25->2	72.8 / 0.4 / 26.8
Daptomycin	0.25	0.5	0.12-2	99.9 / - / -
Erythromycin	>16	>16	≤0.12->16	10.7 / 4.2 / 85.2
Gentamicin	≤1	≤1	≤1->8	96.3 / 0.4 / 3.3
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->4	97.7 / - / 2.3
Vancomycin	1	1	0.25-2	100.0 / 0.0 / 0.0
S. aureus, methicillin-susceptible (1,641)				
Linezolid	1	1	0.25-2	100.0 / - / 0.0
Ciprofloxacin	0.5	>4	0.03->4	86.1 / 2.4 / 11.5
Clindamycin	≤0.25	≤0.25	≤0.25->2	94.6 / 0.2 / 5.2
Daptomycin	0.25	0.5	≤0.06-1	100.0 / - / -
Erythromycin	0.25	>16	≤0.12->16	65.9 / 6.7 / 27.4
Gentamicin	≤1	≤1	≤1->8	99.0 / 0.0 / 1.0
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->4	99.3 / - / 0.7
Vancomycin	1	1	0.25-2	100.0 / 0.0 / 0.0
Coagulase-negative staphylococci (797)[b]				
Linezolid	0.5	0.5	≤0.12->8	99.4 /- / 0.6
Ciprofloxacin	0.25	>4	≤0.03->4	60.2 / 0.8 / 39.0
Clindamycin	≤0.25	>2	≤0.25->2	71.6 / 1.5 / 26.9
Daptomycin	0.25	0.5	≤0.06-1	99.9 / - / -
Erythromycin	16	>16	≤0.12->16	39.1 / 2.8 / 58.2
Gentamicin	≤1	>8	≤1->8	82.1 / 3.6 / 14.3
Oxacillin	1	>2	≤0.25->2	42.7 / 0.0 / 57.3
Trimethoprim/sulfamethoxazole	≤0.5	>4	≤0.5->4	75.8 / - / 24.2
Vancomycin	1	2	0.25-4	100.0 / 0.0 / 0.0
Enterococci (855)[c]				
Linezolid	1	1	0.25->8	99.3 / 0.6 / 0.1
Ampicillin	1	>8	≤0.25->8	74.9 / - / 25.1
Ciprofloxacin	2	>4	≤0.03->4	46.3 / 8 / 45.8
Piperacillin/tazobactam	4	>64	≤0.5->64	- / - / -
Teicoplanin	≤2	>16	≤2->16	79.9 / 1.6 / 18.5
Vancomycin	1	>16	0.25->16	78.4 / 0.4 / 21.3
S. pneumoniae (874)				
Linezolid	1	1	≤0.12-2	100.0 / - / -
Penicillin[d]	≤0.06	2	≤0.06-8	58.5 / 27.8 / 13.7
Amoxicillin/clavulanic acid	≤1	4	≤1->8	88.8 / 4.5 / 6.8
Ceftriaxone	≤0.06	1	≤0.06-8	92.8 / 5.8 / 1.4
Clindamycin	≤0.25	>2	≤0.25->2	82.0 / 0.8 / 17.2
Erythromycin	≤0.12	>16	≤0.12->16	53.5 / 0.6 / 45.9
Levofloxacin	1	1	0.25->4	98.2 / 0.3 / 1.5
Vancomycin	0.25	0.5	≤0.12-0.5	100.0 / - / -
Viridans group streptococci (359)[e]				
Linezolid	0.5	1	≤0.12-1	100.0 / - / -
Ceftriaxone	0.25	0.5	≤0.06-8	96.7 / 1.9 / 1.4
Clindamycin	≤0.25	>2	≤0.25->2	88.3 / 0.8 / 10.9
Erythromycin	≤0.12	8	≤0.12->16	53.6 / 3.9 / 42.5
Levofloxacin	1	2	≤0.12->4	92.8 / 0.6 / 6.7
Penicillin	≤0.06	0.5	≤0.06->8	81.3 / 17.3 / 1.4
Vancomycin	0.5	0.5	≤0.12-1	100.0 / - / -
β-hemolytic streptococci (874)[f]				
Linezolid	1	1	0.25–1	100.0 / - / -
Ceftriaxone	≤0.06	0.12	≤0.06-0.5	100.0 / - / -
Clindamycin	≤0.25	>2	≤0.25->2	78.4 / 1.1 / 20.5
Erythromycin	≤0.12	>16	≤0.12->16	62.4 / 1.4 / 36.2
Levofloxacin	0.5	1	≤0.12->4	99.4 / 0.0 / 0.6
Penicillin	≤0.06	≤0.06	≤0.06-0.12	100.0 / - / -
Vancomycin	0.25	0.5	≤0.12-0.5	100.0 / - / -

a. Criteria as published by the CLSI [2015].
b. Includes 22 species.
c. Includes eight species.
d. Criteria as published by the CLSI [2015] for Oral Penicillin breakpoints (≤0.06/>2 μg/mL).
e. Includes 22 species.
f. Includes: *Streptococcus pyogenes* (342 strains), *S. agalactiae* (417 strains), *S. dysgalactiae* (113 strains), *S. equi* (1), and *S. pseudoporcinus* (1).

CONCLUSIONS

- Linezolid resistance surveillance testing of Gram-positive isolates (6,865) from 60 USA medical centers in 2014 showed excellent sustained activity in all geographic regions and a high linezolid-susceptibility rate of 99.78% (99.62 - 99.83% during 2008-2013).
- There were 15 non-susceptible isolates, eight of which (8/15 [53.3%]) harbored *cfr*. This increase in the presence of *cfr* in the linezolid non-susceptible isolates is concerning and next year's LEADER surveillance results will show if this is an outlier or repeats as a trend. Other resistant mechanisms included (no. strains): G2576T (10), L3 mutation (6), and L4 mutations (6). Overall, the "all organism" linezolid-resistant and non-susceptible rate (0.22%) is essentially the same as in 2005 (0.24%) with variation from 0.14-0.45% over the monitored 11 year period.
- MRSA rates remained high, but overall has consistently declined. Treatment options for infections due to MDR pathogens are limited, however, linezolid continues to demonstrate excellent *in vitro* activity against these organisms.

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