Tracking Linezolid Antimicrobial Activity and Resistance in North America: Results from the LEADER Program for 2013

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REVISED ABSTRACT

Background: The LEADER Program has monitored the activity of linezolid (LZD) and comparator agents in USA medical centers since 2004. The percent of non-susceptible (NS) Gram-positive (GP) monitored isolates has remained below 1% (range, 0.14-0.45%; 0.17% in 2012).

Methods: In 2013, a total of 7,183 GP pathogens were sampled from 60 medical centers across the USA. Isolates were susceptibility (S) tested by CLSI reference broth microdilution methods. LZD NS isolates were confirmed by repeated reference S testing, with the LZD Etest (bioMerieux, Hazelwood, Missouri, USA) and CLSI disk diffusion methods.

Results: A total of 3,035 *S. aureus* strains were submitted. Methicillin-resistant *S. aureus* (MRSA; 47.9%) varied from 35.1% (Middle Atlantic) to 58.7% (East South Central). Resistance rates among MRSA for many antimicrobial agents were much higher than in MSSA. Examples included the β-lactam agents, levofloxacin (MRSA, 64.2%, MSSA, 11.2%), clindamycin (26.7%, 5.3%), and erythromycin (87.8%, 31.9%). The LZD $MIC_{50/90}$ for *S. aureus* was 1/1 µg/ml (S, 99.9%). There were two LZD NS isolates (MRSA from California and Michigan); both contained *cfr*. The MIC_{50} and modal MIC for MRSA and MSSA were the same. A total of 580 CoNS isolates exhibited a LZD $MIC_{50/90}$ at 0.5/1 µg/ml (S, 99.5%). The three LZD NS CoNS isolates contained mutations at 23S rRNA and L3 and/or L4. LZD was active against Enterococci with a $MIC_{50/90}$ at 1/1 µg/ml and 99.4% S. All LZD NS enterococci had a G2476T mutation and one also contained a *cfr*. S to LZD for 399 viridans group streptococci (VGS) and 964 β-hemolytic streptococci was 99.7 and 100.0%, respectively. There was one *S. sanguinis* isolate (LZD NS [MIC, 4 µg/ml]), which demonstrated a mutation at the G2576 nucleotide of the 23S rRNA. LZD S for all organisms tested (7,183) was 99.83% with only 12 isolates (6 enterococci, 2 *S. aureus,* 3 *S. epidermidis,* 1 VGS) testing NS.

Conclusions: LZD among USA medical centers demonstrated excellent activity and a sustained S rate of 99.83%. LZD MIC population distributions remain stable without evidence of "MIC creep" among monitored species. These data show no evidence of widespread dissemination of the *cfr* resistance determinant in LEADER Program monitored USA medical centers.

INTRODUCTION

Linezolid, approved by the Food and Drug Administration (FDA) in 2000, is an important therapeutic agent used to treat uncomplicated and complicated skin and skin structure infections (cSSSI) and nosocomial pneumonia caused by commonly occurring Gram-positive pathogens. 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 treatment option against Gram-positive, such as methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Streptococcus pneumoniae* (DRSP) and VRE isolates that are resistant to conventional drugs.

Oxazolidinone resistance has been detected, mainly among *Enterococcus* species and coagulase-negative staphylococci (CoNS), but the occurrence rates remain rare for *S. aureus* and streptococci. The oxazolidinone mechanism of action has been described as selective binding to the 50S ribosomal subunit of the 23S rRNA molecule with resultant inhibition of protein synthesis. Target site mutations and a mobile *cfr*-mediated resistance mechanism to linezolid have been detected among *Staphylococcus* spp. isolates.

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 benchmark data to which local susceptibility patterns may be compared. In addition, molecular testing of isolates with decreased linezolid susceptibility allows detection of emerging resistance that would not be possible in routine clinical laboratory practice.

MATERIALS AND METHODS

Bacterial strain collection. A total of 7,183 Gram-positive pathogens were submitted to JMI Laboratories and distributed among the following organism groups: *S. aureus* (3,035 strains), *S. pneumoniae* (1281), β-hemolytic streptococci (964), enterococci (924), CoNS (580), and viridans group streptococci (399).
The nine USA Census Bureau Regions were represented by sixty medical centers. Each recruited medical center was instructed to forward ≥100 organisms with the following species or genus distribution: *S. aureus* (50 strains), coagulase-negative staphylococci (CoNS; 15 strains), enterococci (15 strains), *S. pneumoniae* (10 strains), β-hemolytic streptococci (5 strains) and viridans group streptococci (5 strains). The strains were predominantly from bacteremias, although isolates from pneumonia (respiratory tract), cutaneous wound infections or cSSSI, and urinary tract infections were acceptable.

Antimicrobial susceptibility test methods. All susceptibility tests were performed in a CLIA-certified and GLP-compliant reference laboratory (JMI Laboratories) using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods (frozen- and dry-form 96-well plates; CLSI M07-A9, 2012) and published interpretive criteria (CLSI M100-S24, 2014). Frozen-form reference broth microdilution testing, with the linezolid Etest (bioMerieux, Hazelwood, Missouri, USA) and CLSI disk diffusion susceptibility testing methods (CLSI M02-A11, 2012) were used to confirm isolates exhibiting a linezolid MIC value of ≥4 µg/ml.

Staphylococcal isolates (*S. aureus*, CoNS) found to be resistant to erythromycin, but susceptible to clindamycin (ERCS) were screened by the CLSI broth dilution inducible clindamycin screening test as outlined in the M100-S24 (2014) document.

Molecular screening was performed on isolates displaying confirmed linezolid MIC results of ≥4 µg/ml to identify the presence of *cfr*, target site mutations (23S rRNA and ribosomal proteins L3 and/or L4) and possible epidemic clonality using pulsed field gel electrophoresis (PFGE).

RESULTS

- A total of 3,035 *S. aureus* strains were submitted for testing by the reference broth microdilution method. The MRSA rates, determined via a prevalence mode of sample testing, varied by region from 35.1% (Mid-Atlantic) to 58.7% (East South Central) with the overall rate at 47.9% (data not shown). The overall LEADER MRSA rate has decreased 10.3% since 2007 (**Figure 1**).
- Resistance rates, in particular, for MRSA in 2013 were much higher than in MSSA including levofloxacin (MRSA, 64.2%, MSSA, 11.2%), clindamycin (26.7%, 5.3%), and erythromycin (87.8%, 31.9%; **Table 1**). However, linezolid, daptomycin, and vancomycin retained 99.9-100.0% activity against both MRSA and MSSA with linezolid exhibiting a $MIC_{50/90}$ at 1/1 µg/ml (**Tables 1** and **2**).
- CLSI interpretive criteria indicated that clindamycin resistance in *S. aureus* was 15.6%, MRSA (26.7%), and MSSA (5.3%). Screening for inducible clindamycin resistance indicated an overall resistance induction rate of 27.1% among ERCS *S. aureus* and 17.1% among MRSA. The results indicated the true clindamycin resistance rate for all *S. aureus* strains was 27.2% and for MRSA was 37.1% (data not shown).
- A total of 580 CoNS isolates exhibited a linezolid $MIC_{50/90}$ at 0.5/1 µg/ml (99.5% susceptible) and was unaffected by oxacillin resistance (**Table 1**). Resistance rates for other comparator agents ranged from 16.0% for gentamicin to 68.6% for oxacillin. Linezolid, daptomycin and vancomycin exhibited very high susceptibility rates (99.5-100%; **Table 1**).
- Linezolid was very active against all enterococci with a $MIC_{50/90}$ at 1/1 µg/ml and 99.4% susceptible and highly active against VRE (98.0% susceptible) exhibiting $MIC_{50/90}$ at 1/1 µg/ml also.
- Linezolid was active against all *S. pneumoniae* (MIC_{50}, and MIC_{90} at 1 µg/ml). A total of 43.6% of *S. pneumoniae* isolates were non-susceptible to penicillin (MIC, ≥0.12 µg/ml), a rate that has increased 5.6% since 2010 (38.0%). Erythromycin and clindamycin resistance were high among all *S. pneumoniae (*46.5 and 18.3%, respectively; **Table 1**).
- Susceptibility to linezolid for 399 viridans group streptococci and 964 β-hemolytic streptococci was 99.7 and 100.0%, respectively. Linezolid, daptomycin, and vancomycin (>99.0% susceptible), were highly active against all viridans group streptococci and β-hemolytic streptococci tested (**Table 1**).
- Two linezolid-resistant MRSA were detected. They both contained *cfr* (one strain each from California [linezolid MIC, 32 µg/ml] and Michigan [linezolid MIC, 8 µg/ml]). The three non-susceptible CoNS isolates all identified as *S. epidermidis* and originated from Michigan, North Carolina and Texas. The North Carolina isolate matched the PFGE profile noted from two isolates surveyed last year (SEPI454E). All linezolid non-susceptible enterococci had a G2476T mutation and one also contained a *cfr*. There was one non-susceptible *S. sanguinis* isolate (MIC, 4 µg/ml), which demonstrated a mutation at the G2576 nucleotide of the 23S rRNA (**Table 3**).

Table 1. Linezolid activity compared to other agents when tested in the LEADER Program (USA, 2013), 7,183 strains.

Organism/antimicrobial agent (no. tested)	MIC (µg/ml) MIC_{50}	MIC_{90}	Range	CLSI[a] %S / %I / %R
S. aureus, methicillin-resistant (1,454)				
Linezolid	1	1	≤0.12->8	99.9 / 0.0 / 0.1
Ciprofloxacin	>4	>4	0.12->4	31.8 / 1.4 / 66.8
Clindamycin	≤0.25	>2	≤0.25->2	73.0 / 0.3 / 26.7
Daptomycin	0.25	0.5	0.06-2	99.9 / - / -
Erythromycin	>16	>16	≤0.12->16	9.9 / 2.3 / 87.8
Gentamicin	≤1	≤1	≤1->8	97.3 / 0.0 / 2.7
TMP/SMX[b]	≤0.5	≤0.5	≤0.5->4	97.9 / 0.0 / 2.1
Vancomycin	1	1	0.25-2	100.0 / 0.0 / 0.0
S. aureus, methicillin-susceptible (1,581)				
Linezolid	1	1	≤0.12-2	100.0 / 0.0 / 0.0
Ciprofloxacin	0.25	>4	0.06->4	86.6 / 1.5 / 11.9
Clindamycin	≤0.25	≤0.25	≤0.25->2	94.4 / 0.3 / 5.3
Daptomycin	0.25	0.25	≤0.06-1	100.0 / - / -
Erythromycin	0.25	>16	≤0.12->16	64.9 / 3.2 / 31.9
Gentamicin	≤1	≤1	≤1->8	99.2 / 0.2 / 0.6
TMP/SMX	≤0.5	≤0.5	≤0.5->4	99.4 / 0.0 / 0.6
Vancomycin	1	1	0.25-2	100.0 / 0.0 / 0.0
Coagulase-negative staphylococci (580)[c]				
Linezolid	0.5	1	0.25->8	99.5 / 0.0 / 0.5
Ciprofloxacin	0. 5	>4	≤0.03->4	58.8 / 0.3 / 40.9
Clindamycin	≤0.25	>2	≤0.25->2	67.9 / 1.8 / 30.3
Daptomycin	0.25	0.5	≤0.06-1	100.0 / - / -
Erythromycin	>16	>16	≤0.12->16	38.4 / 1.9 / 59.7
Gentamicin	≤1	>8	≤1->8	78.1 / 5.9 / 16.0
Oxacillin	1	>2	≤0.25->2	31.4 / 0.0 / 68.6
TMP/SMX	≤0.5	>4	≤0.5->4	67.1 / 0.0 / 32.9
Vancomycin	1	2	0.25-2	100.0 / 0.0 / 0.0
Enterococci (924)[d]				
Linezolid	1	1	0.25->8	99.4 / 0.0 / 0.6
Ampicillin	1	>8	≤0.25->8	75.2 / 0.0 / 24.8
Ciprofloxacin	1	>4	0.25->4	51.4 / 7.5 / 41.1
Piperacillin/tazobactam	4	>64	≤0.5->64	75.2 / - / -
Teicoplanin	≤2	>16	≤2->16	79.8 / 0.9 / 19.3
Vancomycin	1	>16	0.25->16	78.5 / 0.6 / 20.9
S. pneumoniae (1,281)				
Linezolid	1	1	≤0.12-2	100.0 / - / -
Amoxicillin/clavulanic acid	≤1	4	≤1->8	86.0 / 4.2 / 9.8
Ceftriaxone	≤0.06	1	≤0.06-8	91.6 / 8.1 / 0.3
Ciprofloxacin	1	2	0.12->4	- / - / -
Clindamycin	≤0.25	>2	≤0.25->2	81.1 / 0.6 / 18.3
Erythromycin	≤0.12	>16	≤0.12->16	52.5 / 1.0 / 46.5
Levofloxacin	1	1	0.25->4	98.6 / 0.3 / 1.1
Penicillin[e]	≤0.06	2	≤0.06-8	56.4 / 26.0 / 17.6
Vancomycin	0.25	0.5	≤0.12-0.5	100.0 / - / -
Viridans group streptococci (399)[f]				
Linezolid	0.5	1	≤0.12-4	99.7 / - / -
Ceftriaxone	0.25	0.5	≤0.06-8	95.2 / 2.5 / 2.3
Ciprofloxacin	1	4	0.06->4	- / - / -
Clindamycin	≤0.25	>2	≤0.25->2	87.6 / 0.5 / 11.9
Erythromycin	0.25	16	≤0.12->16	50.6 / 3.3 / 46.1
Levofloxacin	1	2	≤0.12->4	95.0 / 0.5 / 4.5
Penicillin	≤0.06	0.5	≤0.06->8	76.7 / 20.8 / 2.5
Vancomycin	0.5	0.5	≤0.12-1	100.0 / - / -
β-hemolytic streptococci (964)[g]				
Linezolid	1	1	≤0.12–1	100.0 / - / -
Ceftriaxone	≤0.06	0.12	≤0.06-0.12	100.0 / - / -
Ciprofloxacin	0.5	1	≤0.03->4	- / - / -
Clindamycin	≤0.25	>2	≤0.25->2	79.3 / 0.2 / 20.5
Erythromycin	≤0.12	>16	≤0.12->16	61.0 / 1.1 / 37.9
Levofloxacin	0.5	1	≤0.12->4	99.2 / 0.3 / 0.5
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 [2014].
b. Trimethoprim/sulfamethoxazole.
c. Includes 15 species.
d. Includes seven species.
e. Criteria as published by the CLSI [2014] for 'Penicillin oral penicillin V' (S ≤0.06, I=0.121, R≥2 µg/ml).
f. Includes 27 species.
g. Includes: *Streptococcus agalactiae* (523 strains), *Streptococcus dysgalactiae* (78 strains), and *Streptococcus pyogenes* (363 strains).

Table 2. 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, 2013); 7,183 total strains.

Organism group (no. tested)	Number of isolates inhibited at linezolid MIC (µg/ml): ≤0.12	0.25	0.5	1	2	4	8	>8
β-hemolytic streptococci (964)	1	2	473	488[a]	-	-	-	-
S. pneumoniae (1,281)	3	23	458	484	13	-	-	-
Enterococci (924)	0	7	151	719	41	0	3	3
S. aureus (3,035)	4	12	585	2335	97	0	1	1
MRSA (1,454)	3	5	332	1076	36	0	1	1
MSSA (1,581)	1	7	253	1259	61	0	0	0
Viridans group streptococci (399)	5	17	222	154	0	1	-	-
CoNS (580)	0	84	424	68	1	0	0	3
MRCoNS (396)	0	59	287	48	1	0	0	3
MSCoNS (182)	0	25	137	20	0	0	0	0

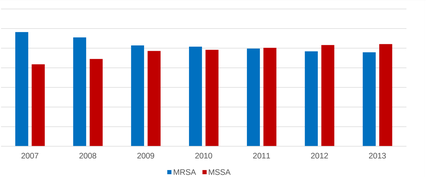
a. Underlined value represents MIC_{50}.

Table 3. Isolates with elevated or resistant-level linezolid MIC values (≥4 µg/ml) in the 2013 LEADER Program.

Isolate ID number	Organism	City	State	Age/ Sex	Linezolid MIC (µg/ml) Frozen -form	Dry-form	Resistance mechanisms	PFGE
146-371	*S. aureus*	Long Beach	California	21/M	32	>8	*cfr*, G2576T, L3 (D159E, G152D)	SA146A[a]
003-21572	*S. aureus*	Detroit	Michigan	59/M	8	8	*cfr*	
454-10665	*S. epidermidis*	Winston Salem	North Carolina	20/M	32	>8	G2576T, L3 (H146P, M156T)	SEPI454E[b]
116-11612	*S. epidermidis*	Houston	Texas	21/M	64	>8	G2576T, L3 (H146R, M156T), L4 (71GT2 ins)	SEPI116F[c]
003-29089	*S. epidermidis*	Detroit	Michigan	53/F	128	>8	G2576T, L3 (G137S, H146P, M156T), L4 (71GT2 ins)	SEPI3K[d]
422-26617	*S. sanguinis*	Aurora	Colorado	21/M	4	4	G2576T	NA
116-11589	*E. faecium*	Houston	Texas	54/F	16	>8	G2576T (+)	EFM116C[e]
004-14445	*E. faecium*	Akron	Ohio	64/F	32	>8	G2576T (+)	NA
467-18556	*E. faecium*	Los Angeles	California	36/F	32	>8	G2576T (+)	NA
448-18961	*E. faecium*	New Orleans	Louisiana	63/M	8	8	*cfr*, G2576T (+)	EFM448B[f]
464-24785	*E. faecium*	Maywood	Illinois	55/F	8	8	G2576T (+)	
453-23688	*E. faecalis*	Hershey	Pennsylvania	44/F	8	8	G2576T (+)	

a. One *cfr*-positive SA detected in 2011 from this site displayed the same PFGE profile.
b. Two linezolid-resistant SEPI displaying the same PFGE profile were detected during the 2012 Program.
c. Linezolid-resistant SEPI isolates originating from this site during previous years (2006 – 2011) had distinct PFGE profiles.
d. PFGE profile identical to that from isolate (LZD MIC 128 µg/ml) detected in 2012.
e. PFGE profile distinct from those noted for non-S isolates detected from this site during the 2011 (EFM 116A) and 2012 (EFM116B) Program.
f. Two non-S EFM showing distinct PFGE profiles were detected from site number 448 during the 2012 Program. One isolate (from 2012) displayed the same EFM448B pattern as the isolate from 2013.

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



CONCLUSIONS

- Susceptibility to linezolid was 99.83% for 7,183 organisms tested from 60 USA medical centers with only 12 isolates (six Enterococci, two *S. aureus*, three *S. epidermidis,* and one *S. sanguinis*) testing non-susceptible.
- Linezolid resistant isolates included mutations in rRNA, L3, L4, L22 and *cfr* (**Table 3**). The majority of resistant isolates harbored ribosomal mutations and not *cfr*.
- Linezolid continues to demonstrate excellent in vitro activity against targeted Gram-positive pathogens with no evidence of "MIC creep" among monitored species. Although no increasing resistance trends have been observed, continued monitoring of linezolid activity through the LEADER Program should be continued to detect future trends in resistance and geographic variance.

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REFERENCES

1. Clinical and Laboratory Standards Institute (2012). *M02-A11. Performance standards for antimicrobial disk susceptibility tests; approved standard: eleventh edition*. Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute (2012). *M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: ninth edition*. Wayne, PA: CLSI.
3. Clinical and Laboratory Standards Institute (2014). *M100-S24. Performance standards for antimicrobial susceptibility testing: 24th informational supplement.* Wayne, PA: CLSI.
4. Flamm RK, Farrell DJ, Mendes RE, Ross JE, Sader HS, Jones RN (2012). LEADER Surveillance program results for 2010: an activity and spectrum analysis of linezolid using 6801 clinical isolates from the United States (61 medical centers). *Diagn Microbiol Infect Dis* 74: 54-61.
5. Flamm RK, Mendes RE, Ross JE, Sader HS, Jones RN (2013). Linezolid surveillance results for the United States: LEADER Surveillance Program 2011. *Antimicrob Agents Chemother* 57: 1077-1081.
6. Jones RN, Farrell DJ, Sader HS (2011). Comparative activity of linezolid against respiratory tract infection isolates of *Staphylococcus aureus*: An 11-year report from the SENTRY Antimicrobial Surveillance Program. *Int J Antimicrob Agents* 37: 584-585.
7. Jones RN, Fritsche TR, Sader HS, Ross JE (2007). LEADER surveillance program results for 2006: an activity and spectrum analysis of linezolid using clinical isolates from the United States (50 medical centers). *Diagn Microbiol Infect Dis* 59: 309-317.
8. Jones RN, Ross JE, Castanheira M, Mendes RE (2008). United States resistance surveillance results for linezolid (LEADER Program for 2007). *Diagn Microbiol Infect Dis* 62: 416-426.
9. Mendes RE, Deshpande LM, Castanheira M, DiPersio J, Saubolle MA, Jones RN (2008). First report of *cfr*-mediated resistance to linezolid in human staphylococcal clinical isolates recovered in the United States. *Antimicrob Agents Chemother* 52: 2244-2246.