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Linezolid Experience and Accurate Determination of Resistance (LEADER) Program for 2011: USA Surveillance Report

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ABSTRACT

Background: Approved in 2000, linezolid (LZD) is one of very few options available for the treatment of infections due to Gram-positive (GP) organisms that are resistant (R) to conventional drugs, such as MRSA, multidrug-R (MDR) *S. pneumoniae* and vancomycin-R enterococci (VRE). The LEADER Program has monitored LZD-R rates in the USA for eight years.

Methods: 60 medical centers from 9 USA Census regions were instructed to submit 100 GP isolates (7,303 total). The organism groups (no.overall) were: *S. aureus* (SA; 3,025), coagulase-negative staphylococci (CoNS; 761), enterococci (ENT; 1,160), *S. pneumoniae* (SPN; 943), viridans group (VGS; 519), and β-haemolytic streptococci (BHS; 895). CLSI broth microdilution susceptibility (S) testing was performed. LZD-R isolates were confirmed by 2 methods and PCR performed to detect R mechanisms.

Results: Overall LZD-R decreased in 2011 to 0.19%, lowest LZD-R rate since surveillance monitoring began in 2004. Only 3 SA isolates were non-susceptible (NS) to LZD, two containing the rare MDR *cfr* gene (Kentucky and California). Among CoNS, 9 *S. epidermidis* were LZD-R with the following mechanisms: G2576T mutation (8); L3 mutations (7); and L4 mutations (5). The four LZD-NS ENT isolates had the G2576T target mutation. LZD-R was not detected in four regions (highest LZD-R rate in W. S. Central region [0.87%]). A very rare LZD-R *S. sanguinis* isolate was found in Pennsylvania. MRSA rates varied by region from 39.4% (Mid Atlantic) to 59.2% (E. S. Central). VRE rates ranged from 19.4% (W. N. Central) to 38.5% (Mid Atlantic).

Table: Eight year trends in LZD-R rates observed in the LEADER Program (2004-2011; 47,482 isolates).

	% linezolid-NS or -R							
Organisms (no. tested)	2004	2005	2006	2007	2008	2009	2010	2011
SA (24,847)	0.00	0.03	0.03	0.06	0.10	0.15	0.06	0.10
CoNS (6,231)	0.20	1.13	1.61	1.76	1.64	1.47	1.48	1.18
ENT (6,671)	0.80	0.64	1.83	1.13	0.55	0.49	0.96	0.34
SPN (5,038)	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00
VGS (1,855)	NT	NT	0.00	0.00	0.00	0.00	0.00	**0.19**
BHS (3,020)	NT	NT	0.00	0.00	0.00	0.00	0.00	0.00
All organisms (47,482)	0.14	0.24	0.45	0.44	0.36	0.34	0.38	0.19

Conclusions: LZD remains very active (99.81% susceptibility) with no evidence of MIC creep when compared to previous LEADER Program reports. The continued evidence of *cfr* mediated MDR isolates in staphylococci emphasizes the need for continued linezolid surveillance.

INTRODUCTION

The LEADER surveillance program has monitored linezolid (an oxazolidinone) potency, spectrum and resistance rates in the United States (USA) since 2004, the most recent years (2006-2012) administered by JMI Laboratories (North Liberty, Iowa, USA). Linezolid was the first oxazolidinone class agent studied and approved (2000) in the USA for clinical use. Linezolid has been used to treat Gram-positive pathogens causing acute bacterial skin and skin structure infections (ABSSSI) and nosocomial pneumonias, after USA-Food and Drug Administration (FDA) review. This compound has emerged as a valuable treatment option for infections caused by multi-drug resistant Gram-positive organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Streptococcus pneumoniae* (DRSP) and vancomycin-resistant enterococci (VRE).Twelve years post approval, the LEADER program continues active surveillance of the in vitro activity of linezolid. The 2011 LEADER program is presented here representing data from sixty medical centers in the USA.

MATERIALS AND METHODS

Study sites: Sixty medical centers were selected to represent nine USA Census Bureau Regions (4-10 sites/region) as follows: Pacific Region (California [2], Hawaii [1], Oregon [1], Washington [3]); Mountain Region (Arizona [1], Colorado [1], New Mexico [1], Utah [1]); West North Central Region (Iowa [1], Missouri [2], North Dakota [1], Nebraska [1], Minnesota [2]); West South Central Region (Arkansas [1], Texas [3], Louisiana [1], Oklahoma [1]); East North Central Region (Illinois [1], Indiana [1], Michigan [2], Ohio [4], Wisconsin [2]); East South Central Region (Alabama [2], Kentucky [2], Tennessee [2]); New England (Maine [1], Massachusetts [3], Vermont [1]); Middle Atlantic Region (Pennsylvania [2], New York [3], New Jersey [2]); and South Atlantic Region (Florida [4], Georgia [1], North Carolina [1], Virginia [1]).

Organisms tested: Each medical center was instructed to forward 100 organisms with the following species distributions: *S. aureus* (50 strains); coagulase-negative staphylococci (CoNS; 15 strains); enterococci (15 strains); *S. pneumoniae* (10 strains); and β-haemolytic streptococci and viridans group streptococci (five strains each). The strains were predominantly from bacteremias although isolates from documented pneumonias, cSSSI and urinary tract infections were acceptable. The forwarded clinical isolates (7,303 total strains) were distributed among the following organism groups: *S. aureus* (3,025 strains); CoNS (761); enterococci (1,160); *S. pneumoniae* (943); viridans group streptococci (519); and β-haemolytic streptococci (895).

Susceptibility methods: All susceptibility tests were performed by a GLP-compliant reference laboratory (JMI Laboratories) using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods and published interpretive criteria (CLSI M100-S22, 2012). Isolates displaying linezolid MICs ≥4 μg/ml were confirmed by repeated reference frozen-form broth microdilution testing and with the linezolid Etest (AB bioMeriuex, Marcy l'Etoile, France) and disk diffusion susceptibility testing (CLSI M02-A11, 2012). Furthermore, *S. aureus* strains found to be resistant to erythromycin and susceptible to clindamycin were screened by the CLSI D-test to detect inducible clindamycin resistance per M100-S22 recommended methods.

Molecular testing was performed on selected isolates to detect recognized target site mutations (23S rRNA and L3 and L4 ribosomal proteins), *cfr* and potential clonality using pulsed field gel electrophoresis (PFGE).

RESULTS

- A total of 3,025 *S. aureus* strains were tested by the reference broth microdilution method with Census Region organism sample sizes ranging from 200 (Mountain) to 512 (East North Central) isolates (**Table 1**). An overall MRSA rate of 49.8% (50.8% in 2010; declining since 2007 [58.2%]) was observed (data not shown).
- The CLSI D-test detected an overall clindamycin resistance induction rate of 29.6% among erythromycin-resistant clindamycin-susceptible (ERCS) *S. aureus*; range 30.6-39.4% in 2006-2010. The distribution of ERCS rates showed 52 of 60 (86.7%) sites had inducible resistance with rates ranging between 11 and 60%.
- Linezolid demonstrated excellent comparative activity in all regions, as well as across all *S. aureus* tested (**Tables 2-3**). Activity of linezolid was not affected by methicillin resistance in *S. aureus* ($MIC_{50/90}$ was 1/1 μg/ml for MRSA and 1/2 μg/ml for MSSA, respectively). The isolates inhibited by linezolid at ≤1 μg/ml for MRSA was 85.6% compared to 92.2% of MSSA at ≤1 μg/ml.
- A total of 761 CoNS isolates were tested against linezolid and 8 comparator agents (**Table 3**). The overall linezolid MIC_{90} was 1 μg/ml and no significant differences were noted in linezolid MIC distributions when comparing methicillin (oxacillin)-resistant and -susceptible isolates. The oxacillin-resistant (OR) rates varied by census region (56.6 to 76.7%; overall rate at 63.5%) with the highest rates detected in the Mountain region.
- The tested enterococcal isolates (1,160) were predominantly identified as *E. faecalis* (766; 66.0%) and *E. faecium* (355; 30.6%). Among these strains, the ampicillin-susceptible rate was 100.0% for *E. faecalis* and 10.7% for *E. faecium*. VRE rates varied by census region ranging from 19.4% (West North Central) to 38.5% (Middle Atlantic). The VRE rate for the entire enterococcal sample was 26.6% (**Table 3**) and the VanA resistance phenotype consisted of 93.1% of the VRE isolates.
- Linezolid was active against all *S. pneumoniae* (MIC_{50} and MIC_{90}, 1 μg/ml; **Tables 2** and **3**).
- Three *S. aureus* isolates from three different states were linezolid-resistant (**Table 4**). One strain had a G2576T point mutation (Texas), one strain was positive for *cfr* (Kentucky) and one strain had both resistance mechanisms (California).
- Among the CoNS, nine (1.18%) isolates were observed to have linezolid MIC values at ≥8 μg/ml, e.g. resistant (**Table 4**) and demonstrated amino acid alteration in the 23S rRNA and/or ribosomal proteins (L3/L4).
- A total of four enterococci (three *E. faecium* and one *E. faecalis*) had linezolid MIC values at ≥4 μg/ml (**Table 4**). All were positive for the G2576T mutation.
- A linezolid non-susceptible viridans group streptococcus was encountered for the first time in this program (**Tables 2, 3** and **4**). This *Streptococcus sanguinis* exhibited a MIC value of 32 μg/ml and contained multiple mutations at 23S rRNA (G2576T, C2610T), and L22 (I59V, V110A; see poster C1-1343).

Table 1. Frequency of organisms tested, listed by Census Region (LEADER Surveillance Program, 2011)

	Organism group (no. tested):						Region Total
Region (no. of sites)	*S. aureus*	CoNS	ENT	SPN	VGS	BHS	
1. New England (5)	250	50	101	80	31	73	585
2. Mid Atlantic (7)	363	138	174	77	55	98	905
3. E N Central (10)	512	113	190	185	104	162	1,266
4. W N Central (7)	349	103	103	123	59	89	826
5. S Atlantic (7)	367	66	150	104	39	78	804
6. E S Central (6)	304	70	106	95	62	111	748
7. W S Central (6)	278	110	126	68	39	69	690
8. Mountain (4)	200	43	69	68	50	88	518
9. Pacific (8)	402	68	141	143	80	127	961
Organism Group Total	3,205	761	1,160	943	519	895	7,303

Abbreviations: CoNS = Coagulase-negative staphylococci; ENT = Enterococci; SPN = *Streptococcus pneumoniae*; VGS = Viridans group streptococci; BHS = Beta-haemolytic streptococci.

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, 2011); 7,303 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-haemolytic streptococci (895)	0	4	231	657	3	-	-	-
S. pneumoniae (943)	3	26	334	567	13	-	-	-
Enterococci (1,160)	1	3	111	933	108	3[a]	1[a]	-
S. aureus (3,025)	0	2	195	2,492	331	2	3[a]	-
MRSA (1,505)	0	0	123	1,265	112	2	3[a]	-
MSSA (1,520)	0	2	72	1,227	219	-	-	-
Viridans group streptococci (519)	1	20	189	296	12	0	0	1[a]
CoNS (761)	2	53	536	158	3	0	0	9[a]
MRCoNS (483)	1	41	335	95	3	0	0	8[a]
MSCoNS (278)	1	12	201	63	0	0	0	1[a]

a. Linezolid-non-susceptible strains

Table 3. Linezolid activity compared to 8 other agents when tested in the LEADER Program (USA, 2011), 7,303 strains.

Organism/antimicrobial agent (no. tested)	MIC (μg/ml)			CLSI	EUCAST
	MIC_{50}	MIC_{90}	Range	% S / %R	% S / %R
MR-*S. aureus* (1,505)					
Linezolid	1	1	0.5-8	99.8 / 0.2	99.8 / 0.2
Ciprofloxacin	>4	>4	≤0.03->4	29.5 / 68.8	29.5 / 70.5
Clindamycin	≤0.25	>2	≤0.25->2	72.3 / 27.6[b]	72.1 / 27.7
Daptomycin	0.25	0.5	0.12-1	100.0 / -	100.0 / 0.0
Erythromycin	>16	>16	≤0.12->16	10.1 / 88.5	10.2 / 89.6
Gentamicin	≤1	≤1	≤1->8	97.3 / 2.4	97.1 / 2.9
Oxacillin[a]	>2	>2	>2	0.0 / 100.0	0.0 / 100.0
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->4	97.7 / 2.3	97.7 / 2.1
Vancomycin	1	1	0.25-2	100.0 / 0.0	100.0 / 0.0
MS-*S. aureus* (1,520)					
Linezolid	1	2	0.25-2	100.0 / 0.0	100.0 / 0.0
Ciprofloxacin	0.25	>4	≤0.03->4	87.5 / 11.3	87.5 / 12.5
Clindamycin	≤0.25	≤0.25	≤0.25->2	95.4 / 4.5[b]	95.2 / 4.6
Daptomycin	0.25	0.5	≤0.06-1	100.0 / -	100.0 / 0.0
Erythromycin	0.25	>16	≤0.12->16	65.5 / 30.9	65.8 / 33.0
Gentamicin	≤1	≤1	≤1->8	98.9 / 1.0	98.6 / 1.4
Oxacillin[a]	0.5	0.5	≤0.25-2	100.0 / 0.0	100.0 / 0.0
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->4	99.0 / 1.0	99.0 / 0.8
Vancomycin	1	1	0.25-2	100.0 / 0.0	100.0 / 0.0
Coagulase-negative staphylococci (761)					
Linezolid	0.5	1	≤0.12->8	98.8 / 1.2	98.8 / 1.2
Ciprofloxacin	0.5	>4	≤0.03->4	52.3 / 47.2	52.3 / 47.7
Clindamycin	≤0.25	>2	≤0.25->2	70.6 / 26.7	69.8 / 29.4
Daptomycin	0.25	0.5	≤0.06-2	99.9 / -	99.9 / 0.1
Erythromycin	>16	>16	≤0.12->16	39.4 / 58.0	40.5 / 59.1
Gentamicin	≤1	>8	≤1->8	78.2 / 17.0	76.1 / 23.9
Oxacillin[a]	1	>2	≤0.25->2	36.5 / 63.5	36.5 / 63.5
Trimethoprim/sulfamethoxazole	≤0.5	>4	≤0.5->4	64.3 / 35.7	64.3 / 20.8
Vancomycin	1	2	≤0.12-4	100.0 / 0.0	99.6 / 0.4

a. Criteria as published by the CLSI, β-lactam susceptibility should be directed by the oxacillin test results with staphylococci. Enterococcal susceptibility was predicted by ampicillin results and penicillin was the agent used for streptococcal activity for selected β-lactams.
b. The clindamycin rate, as determined by susceptibility testing, underestimates the true rate of clindamycin resistance as the population of strains that test susceptible by reference MIC testing may include inducible strains.
c. Percentages in parentheses are the strains having a ciprofloxacin MIC at ≥4μg/ml, possible QRDR mutations.

Organism/antimicrobial agent (no. tested)	MIC (μg/ml)			CLSI	EUCAST
	MIC_{50}	MIC_{90}	Range	% S / %R	% S / %R
Enterococci (1,160)					
Linezolid	1	1	≤0.12-8	99.7 / 0.1	99.9 / 0.1
Ampicillin[a]	1	>8	≤0.25->8	71.9 / 28.1	71.7 / 28.1
Ciprofloxacin	2	>4	≤0.03->4	42.8 / 49.9	- / -
Piperacillin/tazobactam	4	>64	≤0.5->64	71.9 / -	71.9 / -
Teicoplanin	≤2	>16	≤2->16	74.6 / 24.5	74.1 / 25.9
Vancomycin	1	>16	0.25->16	72.7 / 26.6	72.7 / 27.3
S. pneumoniae (943)					
Linezolid	1	1	≤0.12-2	100.0 / -	100.0 / 0.0
Amoxicillin/clavulanic acid	≤1	8	≤1->8	91.7 / 14.7	- / -
Ceftriaxone	≤0.06	2	≤0.06-8	87.6 / 1.2	76.5 / 1.2
Ciprofloxacin	1	2	≤0.03->4	(4.0)[c]	0.2 / 4.0
Clindamycin	≤0.25	>2	≤0.25->2	77.5 / 22.0	78.0 / 22.0
Erythromycin	≤0.12	>16	≤0.12->16	55.1 / 44.5	55.1 / 44.5
Levofloxacin	1	1	≤0.12->4	98.9 / 1.1	98.9 / 1.1
Penicillin	≤0.06	4	≤0.06-8	57.7 / 22.8	57.7 / 15.6
Vancomycin	0.25	0.5	≤0.12-1	100.0 / -	100.0 / 0.0
Viridans group streptococci (519)					
Linezolid	1	1	≤0.12->8	99.8 / -	- / -
Ceftriaxone	0.12	1	≤0.06-8	91.7 / 3.9	87.9 / 12.1
Ciprofloxacin	1	4	≤0.03->4	(21.6)[c]	- / -
Clindamycin	≤0.25	>2	≤0.25->2	84.0 / 15.0	85.0 / 15.0
Erythromycin	1	>16	≤0.12->16	46.2 / 51.6	- / -
Levofloxacin	1	2	≤0.12->4	91.3 / 7.7	- / -
Penicillin[a]	≤0.06	1	≤0.06->8	74.0 / 3.3	82.9 / 3.3
Vancomycin	0.5	1	≤0.12-1	100.0 / -	100.0 / 0.0
β-haemolytic streptococci (895)					
Linezolid	1	1	0.25-2	100.0 / -	100.0 / 0.0
Ceftriaxone	≤0.06	0.12	≤0.06-1	99.9 / -	100.0 / 0.0
Ciprofloxacin	0.5	1	≤0.03->4	(1.0)[c]	- / -
Clindamycin	≤0.25	>2	≤0.25->2	82.3 / 17.4	82.6 / 17.4
Erythromycin	≤0.12	>16	≤0.12->16	65.4 / 33.4	65.4 / 33.4
Levofloxacin	≤0.5	1	≤0.12->4	99.2 / 0.7	95.6 / 0.8
Penicillin[a]	≤0.06	≤0.06	≤0.06-0.12	100.0 / -	100.0 / 0.0
Vancomycin	0.5	0.5	≤0.12-1	100.0 / -	100.0 / 0.0

Table 4. Isolates with elevated linezolid MIC values in the 2011 LEADER Program.

Organism	City	State	Age/Sex	Linezolid MIC (μg/ml)	Resistance Mechanism
S. aureus	Akron	Ohio	85/M	4	*cfr*
S. aureus	Louisville	Kentucky	67/M	8	*cfr*
S. aureus	Long Beach	California	20/M	8	*cfr*, G2576T
S. aureus	Houston	Texas	67/M	8	G2576T
S. aureus	Milwaukee	Wisconsin	28/M	4	L3 (S145 del)
S. epidermidis	Cleveland	Ohio	69/F	32	G2576T, L3 (M156T, H146P, G137S, F147Y), L4 (71G72 ins)
S. epidermidis	New Orleans	Louisiana	61/M	64	G2576T
S. epidermidis	Memphis	Tennessee	55/F	128	G2576T, L3 (V154L, M156T, H146R), L4 (71G72 ins)
S. epidermidis	Memphis	Tennessee	82/M	128	G2576T, L3 (V154L, M156T, H146R), L4 (71G72 ins)
S. epidermidis	Hershey	Pennsylvania	32/F	64	G2576T, L3 (V154L, M156T, H146R)
S. epidermidis	Hackensack	New Jersey	84/F	64	G2576T, L3 (V154L, M156T, H146R, G137D), L4 (71G72 ins)
S. epidermidis	Houston	Texas	62/F	64	G2576T, L3 (M156T, H146P, G137S)
S. epidermidis	Houston	Texas	70/M	16	L3 (V154L, H146Q, A157R), L4 (71G72 ins)
S. epidermidis	New Brunswick	New Jersey	37/M	64	G2576T
E. faecalis	New York	New York	54/M	8	G2576T (+)
E. faecium	Houston	Texas	68/M	8	G2576T (+)
E. faecium	Houston	Texas	24/M	4	G2576T (+)
E. faecium	Birmingham	Alabama	58/M	4	G2576T (+)
S. sanguinis	Hershey	Pennsylvania	71/M	32	G2576T, C2610T, L22 (I59V, V110A)

CONCLUSIONS

- Linezolid surveillance susceptibility testing of Gram-positive isolates (7,303) from 60 USA medical centers in 2011 showed excellent sustained activity and a high susceptibility rate of 99.81% overall (99.62% in 2010).
- Linezolid MIC population distributions remained unchanged without evidence of "MIC creep" among indicated species (**Table 2**).
- For the first time in the LEADER Program, a linezolid resistant viridans group streptococcus (*S. sanguinis*) was discovered. Linezolid non-susceptible strains contained the following resistant mechanisms (**Table 4**): G2576T (79%), L3 mutation (42%), L4 mutation (26%), *cfr* (14%), L22 mutation (5%).
- Monitoring of linezolid for changing patterns of emerging resistance should be continued, although no increasing recent trends have been observed since 2006. The LEADER Program has now sampled 47,482 strains over eight surveillance years using reference CLSI methods.

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