IDWEEK 2016

2007

New Orleans, LA

October 26 - 30

Activity of Linezolid when Tested against Gram-Positive Isolates from the USA (Linezolid Experience and Accurate Determination of Resistance [LEADER]) from 2015

RK FLAMM[1], JM STREIT[1], RE MENDES[1], HS SADER[1], PA HOGAN[2]

[1]JMI Laboratories, North Liberty, IA, USA; [2]Pfizer, Inc, New York, NY, USA

Robert K. Flamm, PhD
JMI Laboratories
335 Beaver Kreek Centre
North Liberty, Iowa 52317
Phone: (319) 665-3370
robert-flamm@jmilabs.com

Abstract

Background: Linezolid (LZD) is active against Gram-positive (GP) organisms such as MRSA, drug-resistant (R) *S. pneumoniae* and vancomycin-R enterococci that are R to conventional drugs. The LEADER program has monitored the activity of LZD and comparator agents since 2004. Molecular characterization of isolates with elevated LZD MICs has been an integral part of this program.

Methods: A total of 3,031 *S. aureus* (SA), 924 coagulase-negative staphylococci (CoNS), 973 enterococci (ENT), 850 *S. pneumoniae* (SPN), 236 viridans group streptococci (VGS), and 727 β-hemolytic streptococci (BHS) from 60 medical centers were susceptibility (S) tested against LZD and comparator agents by reference broth microdilution methods. LZD-R isolates were confirmed by Etest (bioMérieux, Hazelwood, MO) and repeat reference S testing. PCR and sequencing was performed to detect mutations in the 23S rRNA, L3, L4, and L22 genes, and for the presence of acquired genes (*cfr*, *optrA*).

Results: LZD activity against 6,741 GP organisms was high (99.8% S). The $MIC_{50/90}$ for SA, MRSA, and MSSA was at 1/1 µg/ml. The MRSA rate, which has declined each year over the last eight years, was at 45.9%. For CoNS, MRCoNS, and MSCoNS, the $MIC_{50/90}$ for LZD was 0.5/1 µg/ml. LZD was active against all SPN and BHS with a $MIC_{50/90}$ of 1/1 µg/ml and VGS with an $MIC_{50/90}$ of 0.5/1 µg/ml. SPN penicillin non-susceptibility (NS; MIC, ≥0.12 µg/ml) occurred at a rate of 36.8% and ceftriaxone-NS at 1.7%. There was one LZD-R MRSA (MIC, 8 µg/ml), which harbored G2576T alterations. Among CoNS, seven *S. epidermidis* (0.76% of all 2015 CoNS isolates compared to 0.75% in 2014, 0.52% in 2013 and 0.92% in 2012) demonstrated LZD MIC results of ≥16 µg/ml. Four of these were from a single study site and three of these isolates were clonally-related (two contained *cfr* in addition to mutations in other drug target sites). The other resistant CoNS had combinations of 23S rRNA/L3/L4 alterations. One *E. faecalis* harbored *optrA*, while two *E. faecium* had G2576T mutations in 23S rRNA.

Conclusions: These *in vitro* results show continued potent activity of LZD. LZD R phenotypes remain uncommon (<1%), and most isolates were *S. epidermidis* carrying multiple R mechanisms. *cfr*-carrying isolates remain rare and associated with clonal dissemination, while detection of a newer mobile resistance mechanism (*optrA*) emphasizes the need for monitoring.

Introduction

The LEADER surveillance program has monitored linezolid activity, spectrum and resistance rates in the United States (USA) since 2004. This program has provided information on the emergence of resistance mechanisms to linezolid which have included ribosomal mutations and mobile mechanisms such as *cfr* and *optrA*. Overall in the LEADER Program, linezolid resistance has remained below 1%.

Linezolid was the first oxazolidinone class agent approved (2000) in the USA for clinical use. Linezolid is indicated for the treatment of complicated skin and skin structure infections (cSSSI) and nosocomial pneumonia caused by Gram-positive pathogens. This compound has emerged as a valuable parenteral/oral agent for the treatment of infections caused by Gram-positive organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant enterococci (VRE).

Methods

Bacterial strain collection:

- A total of 6,741 Gram-positive pathogens cultured in 60 USA (35 states) medical centers (including medical centers specializing in children's healthcare) were selected to represent all nine USA Census Bureau regions (4-9 sample sites/region and 502-1,073 isolates/region).
- The isolates were distributed among the following organism groups (no.): *S. aureus* (3,031), coagulase-negative staphylococci (CoNS; 924), enterococci (973), *Streptococcus pneumoniae* (850), viridans group streptococci (VGS; 236), and β-hemolytic streptococci (BHS; 727).
- These pathogens were recovered from patients with bacteremia, respiratory tract infections, skin and soft tissue infections and urinary tract infections.

Antimicrobial susceptibility test methods:

- All susceptibility testing was performed utilizing broth microdilution methods (frozen-form 96-well plates; CLSI M07-A10, 2015) and published interpretive criteria (CLSI M100-S26, 2016).
- Linezolid-resistant isolates were confirmed by repeat broth microdilution testing.
- Molecular characterization was performed on isolates with elevated linezolid MICs (MIC, ≥4 µg/ml) to identify resistance mechanisms (*cfr, optrA*, and 23S rRNA, L3 and L4 mutations), and potential intra site clonality was evaluated using pulsed field gel electrophoresis (PFGE).

Results

- The activity of linezolid against the targeted six Gram-positive organism groups in the 2015 LEADER Program is presented in **Table 1**. Linezolid non-susceptibility occurred in *S. aureus*, CoNS, and enterococci. There were only 11 non-susceptible isolates (99.8% susceptible).
- Linezolid was highly potent against *S. aureus,* exhibiting a $MIC_{50/90}$ at 1 µg/ml. Its activity was similar for MRSA and MSSA ($MIC_{50/90}$ for MRSA and MSSA was 1 µg/ml). Resistance rates were high for MRSA for erythromycin (84.0%), levofloxacin (67.6%) and clindamycin (26.9%; **Table 2**).
- The MRSA rate was 45.9%. It has declined annually in the LEADER Program since 2007 when it was 58.2%
- The oxacillin (methicillin) resistance rate for CoNS was 58.8%. Only daptomycin and vancomycin at 100.0% susceptibility and linezolid (99.2% susceptible) exhibited high susceptibility rates (>90%; **Table 2**). The linezolid $MIC_{50/90}$ of 0.5/1 µg/ml was the same for CoNS, regardless of oxacillin susceptibility (**Table 1**).
- The VRE rate among the enterococci was 21.6% (3.6% for *E. faecalis* isolates and 68.9% among *E. faecium*; **Table 2**). Linezolid was highly active against enterococci, exhibiting a $MIC_{50/90}$ at 1 µg/ml and 99.7% susceptibility. Among the enterococci tested, the ampicillin-susceptible rate was 100.0% among *E. faecalis* (no. 676) and only 15.6% among *E. faecium* (no. 270, data not shown).
- Against *S. pneumoniae*, high rates of susceptibility were seen for amoxicillin-clavulanate (95.2%), ceftriaxone (98.4%), levofloxacin (99.3%), linezolid (100.0%), penicillin (96.7%, parenteral non-meningitis breakpoints), and vancomycin (100.0%; **Table 2**). Linezolid $MIC_{50/90}$ values were 1/1 µg/ml, respectively (**Table 2**). Erythromycin and clindamycin resistance was high among all *S. pneumoniae (*42.9 and 14.4%, respectively; **Table 2**).
- Linezolid was active against VGS ($MIC_{50/90}$, 0.5/1 µg/ml) and BHS ($MIC_{50/90}$, 1/1 µg/ml). Linezolid, daptomycin, tigecycline and vancomycin (all 100.0% susceptible), were highly active against those streptococci (data not shown).
- Among the 11 linezolid non-susceptible isolates (**Table 3**) there was one *S. aureus* (MIC, 8 µg/ml), seven CoNS (MIC, >8 µg/ml), and three enterococci (MIC, 4-8 µg/ml).
- The linezolid resistant *S. aureus* was a MRSA from Long Beach, California harboring a G2576T mutation (**Table 3**).

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, 2015); 6,741 isolates.

Organisms / Organism Groups	No. of isolates at MIC (µg/ml; cumulative %)						
	≤0.25	0.5	1	2	4	8	>8
Staphylococcus aureus (3,031)	26 (0.9%)	1186 (40.0%)	1773 (98.5%)	45 (>99.9%)	0 (>99.9%)	1 (100.0%)	
MSSA (1,640)	11 (0.7%)	571 (35.5%)	1023 (97.9%)	35 (100.0%)			
MRSA (1,391)	15 (1.1%)	615 (45.3%)	750 (99.2%)	10 (99.9%)	0 (99.9%)	1 (100.0%)	
Coagulase-negative staphylococci (924)	107 (11.6%)	562 (72.4%)	240 (98.4%)	8 (99.2%)	0 (99.2%)	0 (99.2%)	7 (100.0%)
MSCoNS (381)	51 (13.4%)	232 (74.3%)	97 (99.7%)	1 (100.0%)			
MRCoNS (543)	56 (10.3%)	330 (71.1%)	143 (97.4%)	7 (98.7%)	0 (98.7%)	0 (98.7%)	7(100.0%)
Enterococcus spp. (973)	26 (2.7%)	267 (30.1%)	605 (92.3%)	72 (99.7%)	1 (99.8%)	2 (100.0%)	
Enterococcus faecalis (676)	11 (1.6%)	192 (30.0%)	429 (93.5%)	43 (99.9%)	1 (100.0%)		
Enterococcus faecium (270)	14 (5.2%)	65 (29.3%)	164 (90.0%)	25 (99.3%)	0 (99.3%)	2 (100.0%)	
Streptococcus pneumoniae (850)	3 (0.4%)	193 (23.1%)	620 (96.0%)	34 (100.0%)			
Viridans group streptococci (236)	14 (5.9%)	134 (62.7%)	88 (100.0%)				
β-haemolytic streptococci (727)		180 (24.8%)	547 (100.0%)				

- Among the linezolid resistant CoNS, all seven isolates were *S. epidermidis*. There were two *cfr* containing isolates which also contained rDNA and ribosomal protein mutations. One isolate contained L3 and L4 mutations only. The remaining four contained one or more mutations in rDNA gene(s) and either L3 or L3 and L4 mutations.
- Three enterococcus (0.3%) were linezolid-non-susceptible (4-8 µg/ml), and one of these contained *optrA*.

Table 2. Linezolid activity compared to other agents when tested in the 2015 LEADER Program; 6,741 isolates.

Organism/antimicrobial agent (no. tested)	MIC (µg/ml)			CLSI[a]	
	MIC_{50}	MIC_{90}	Range	%S	%I	%R
S. aureus (3,031)						
Linezolid	1	1	≤0.12 — 8	>99.9	-	<0.1
Clindamycin	≤0.25	>2	≤0.25 — >2	84.4	0.3	15.3
Daptomycin	0.25	0.5	≤0.12 — 1	100.0	-	-
Erythromycin	>8	>8	≤0.06 — >8	40.7	5.5	53.7
Gentamicin	≤1	≤1	≤1 — >8	97.5	0.1	2.5
Levofloxacin	0.25	>4	≤0.03 — >4	62.0	1.0	37.1
Tetracycline	≤0.5	≤0.5	≤0.5 — >8	95.1	1.0	3.9
Tigecycline	0.06	0.12	≤0.015 — 0.5	100.0	-	[b]
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5 — >4	98.4	-	1.6
Vancomycin	0.5	1	≤0.12 — 2	100.0	0.0	0.0
S. aureus, methicillin-resistant (1,391)						
Linezolid	1	1	≤0.12 — 8	99.9	-	0.1
Clindamycin	≤0.25	>2	≤0.25 — >2	72.6	0.5	26.9
Daptomycin	0.25	0.5	≤0.12 — 1	100.0	-	-
Erythromycin	>8	>8	≤0.06 — >8	12.5	3.5	84.0
Gentamicin	≤1	≤1	≤1 — >8	96.1	0.1	3.8
Levofloxacin	4	>4	0.12 — >4	30.7	1.7	67.6
Tetracycline	≤0.5	≤0.5	≤0.5 — >8	94.2	1.0	4.8
Tigecycline	0.06	0.12	≤0.015 — 0.5	100.0	-	[b]
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5 — >4	97.2	-	2.8
Vancomycin	0.5	1	≤0.12 — 2	100.0	0.0	0.0
Coagulase-negative staphylococci (924)						
Linezolid	0.5	1	≤0.12 — >8	99.2	-	0.8
Clindamycin	≤0.25	>2	≤0.25 — >2	71.5	2.3	26.2
Daptomycin	0.5	0.5	≤0.12 — 1	100.0	-	-
Erythromycin	>8	>8	≤0.06 — >8	40.5	2.6	56.9
Gentamicin	≤1	>8	≤1 — >8	78.9	2.1	19.0
Levofloxacin	0.25	>4	≤0.03 — >4	58.3	1.9	39.7
Tetracycline	≤0.5	>8	≤0.5 — >8	86.7	1.4	11.9
Tigecycline	0.06	0.12	≤0.015 — 0.5	-	-	-
Trimethoprim-sulfamethoxazole	≤0.5	4	≤0.5 — >4	74.6	-	25.4
Vancomycin	1	2	≤0.12 — 2	100.0	0.0	0.0
Enterococci (973)[c]						
Linezolid	1	1	≤0.25 — 8	99.7	0.1	0.2
Ampicillin	1	>8	≤0.5 — >8	76.6	-	23.4
Levofloxacin	1	>4	≤0.5 — >4	59.3	1.7	39.0
Daptomycin	1	2	≤0.25 — >8	99.8	-	-
Teicoplanin	≤2	>16	≤2 — >16	79.1	2.5	18.4
Vancomycin	1	>16	≤0.5 — >16	78.3	0.1	21.6
S. pneumoniae (850)						
Linezolid	1	1	0.25 — 2	100.0	-	-
Penicillin	≤0.06	1	≤0.06 — 4	96.7	3.3	0.0 [d]
Amoxicillin/clavulanic acid	≤0.03	2	≤0.03 — >4	95.2	2.9	1.9
Ceftriaxone	0.03	1	≤0.015 — >2	98.4	1.2	0.5 [e]
Clindamycin	≤0.12	>1	≤0.12 — >1	85.0	0.6	14.4
Erythromycin	0.03	>2	≤0.015 — >2	56.5	0.6	42.9
Levofloxacin	1	1	0.25 — >4	99.3	0.0	0.7
Tetracycline	0.25	>4	≤0.12 — >4	80.1	0.2	19.7
Vancomycin	0.25	0.25	≤0.03 — 0.5	100.0	-	-
Viridans group streptococci (236)						
Linezolid	0.5	1	≤0.06 — 1	100.0	-	-
Ceftriaxone	0.12	0.5	≤0.03 — >4	97.5	0.8	1.7
Clindamycin	0.03	>2	≤0.015 — >2	83.5	0.4	16.1
Erythromycin	0.5	>4	≤0.03 — >4	47.5	4.7	47.9
Levofloxacin	1	2	≤0.03 — >4	91.9	0.8	7.2
Penicillin	≤0.03	0.5	≤0.03 — >4	80.1	17.4	2.5
Vancomycin	0.5	0.5	≤0.06 — 1	100.0	-	-
β-haemolytic streptococci (727)						
Linezolid	1	1	0.5 — 1	100.0	-	-
Ceftriaxone	≤0.03	0.06	≤0.03 — 0.25	100.0	-	-
Clindamycin	0.06	>2	≤0.015 — >2	78.7	0.4	20.9
Erythromycin	0.06	>4	≤0.03 — >4	60.8	1.0	38.2
Levofloxacin	0.5	1	0.06 — >4	99.3	0.4	0.3
Penicillin	≤0.03	0.06	≤0.03 — 0.12	100.0	-	-
Vancomycin	0.25	0.5	≤0.06 — 1	100.0	-	-

a. Criteria as published by the CLSI [2016]
b. Breakpoints from FDA Package Insert revised 12/2014
c. Includes 676 *E. faecalis* and 270 *E. faecium*
d. Parenteral nonmeningitis breakpoints (≤2/4/8 µg/ml)
e. Nonmeningitis breakpoints (≤1/2/4 µg/ml)

Table 3. Isolates with elevated or resistant-level linezolid MIC values (≥4 µg/ml) in the 2015 LEADER Program.

Organism	City	State	Linezolid MIC (µg/ml)	Resistance mechanisms	PFGE[a]
S. aureus	Long Beach	California	8	G2576T	
S. epidermidis	Long Beach	California	16	G2576T, L3 (V154L, M156T)	
S. epidermidis	Memphis	Kentucky	128	G2576T, L3 (Q136L, H146R, M156T), L4 (71G72 insertion)	SEPI412F
S. epidermidis	Houston	Texas	16	L3 (V96D, H146Q, V154L, A157R), L4 (71G72 insertion)	SEPI116G
S. epidermidis	Houston	Texas	16	C2534T, L3 (H146Q, V154L, A157R), L4 (71G72 insertion)	SEPI116E1
S. epidermidis	Houston	Texas	128	C2534T, *cfr*, L3 (V154L, A157R), L4 (71G72 insertion)	SEPI116E
S. epidermidis	Houston	Texas	>128	C2534T, *cfr*, L3 (H146Q, V154L, A157R)	SEPI116E
S. epidermidis	Winston Salem	North Carolina	16	G2576T, L3 (H146P, M156T)	
E. faecalis	Milwaukee	Wisconsin	4	*optrA*	
E. faecium	Houston	Texas	8	G2576T	
E. faecium	Seattle	Washington	8	G2576T, L3 (K95T)	

a. Pulsed-field gel electrophoresis. PFGE profile SEPI412F was also observed in a linezolid-resistant isolate collected during the 2014 surveillance program. PFGE SEPI116E has been noted in linezolid-resistant isolates collected during 2011 and 2014.

Conclusions

- In the 2015 LEADER Program, linezolid activity was shown to remain high with a MIC_{90} value for all Gram-positive organism groups tested at 1 µg/ml. Of the 6,741 isolates tested from 60 USA medical centers in 35 states, there were only 11 linezolid non-susceptible isolates (99.8% susceptible).
- In the 2014 LEADER program, 8/15 (53.3%) of the linezolid non-susceptible isolates harbored *cfr*. In this 2015 LEADER Program, only 2/11 (18.2%) linezolid non-susceptible isolates harbored *cfr* (both of which were *S. epidermidis*), indicating that although *cfr* is potentially highly mobile, the *cfr* rate did not show an increase in the most current surveillance year.
- Other resistant mechanisms included (no. isolates with at least one mutation/class): C2534T (3), G2576T (6), L3 mutations (8), L4 mutations (4), and *optrA* (1; *E. faecalis*). Overall, the "all organism" linezolid-resistant and non-susceptible rate (0.16%) is not higher than what was identified in the 2005 (0.24%) LEADER Program.
- Longitudinal monitoring for novel emerging resistances to new agents provides a valuable tool for tracking antimicrobial activity for that agent and other antimicrobial classes with a similar spectrum of activity.

Acknowledgements

The authors would like to thank all participating centers for contributing isolates to this surveillance protocol. This study was sponsored by Pfizer Inc.

References

1. Clinical and Laboratory Standards Institute (2015). *M07-A10. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard- tenth edition.* Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute (2016). *M100-S26. Performance standards for antimicrobial susceptibility testing: 26th informational supplement.* Wayne, PA: CLSI.
3. 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.
4. 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.
5. Flamm RK, Mendes RE, Hogan PA, Ross JE, Farrell DJ, Jones RN (2015). In vitro activity of linezolid as assessed through the 2013 LEADER surveillance program. *Diagn Microbiol Infect Dis* 81: 283-289.
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. 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.
8. Mendes RE, Flamm RK, Hogan PA, Ross JE, Jones RN (2014). Summary of linezolid activity and resistance mechanisms detected during the 2012 LEADER surveillance program for the United States. *Antimicrob Agents Chemother* 58: 1243-1247.