Linezolid Experience and Accurate Determination of Resistance (LEADER) Program: United States (USA) Results for 2009

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AMENDED ABSTRACT

Background: The USA's largest oxazolidinone surveillance program (LEADER) specifically designed to monitor linezolid (LZD) activity has completed six years of testing. Since approval (FDA, 2000) for treatment of complicated skin and soft tissue infections, nosocomial and community- acquired pneumonias, LZD has become a valuable treatment option for Gram-positive (GP) infections due to methicillin-resistant *S. aureus* (MRSA), multidrug-resistant (MDR) *S. pneumoniae* and vancomycin-resistant enterococci.

Methods: Over 6,200 GP isolates were submitted to a reference laboratory from 56 hospitals in all 9 USA Census Regions. Each center was instructed to send 100 isolates from six organism groups (no.): *S. aureus* (SA; 3,257), coagulase-negative staphylococci (CoNS; 816), enterococci (ENT; 1,017), *S. pneumoniae* (659), viridans group (264) and β-haemolytic streptococci (401). A central laboratory confirmed identification and performed CLSI broth microdilution susceptibility testing. Molecular studies (PCR, PFGE) were used to identify resistance (R) mechanisms and possible relatedness of strains within or between hospitals.

Results: LZD remained very active with an overall R rate of only 0.34% (0.36%; 2008) and no evidence of MIC creep. Five SA isolates were non-S with 2 having a *cfr* gene (Kentucky and Ohio), 2 containing ribosomal target G2576T mutations (California and Kansas) and 1 containing L3 deletion (S145; Connecticut) was noted. One isolate (Maryland) had a LZD MIC of 4 µg/ml (S) and also contained the *cfr* gene. Among CoNS, 12 isolates (11 *S. epidermidis*, 1 *S. capitis*) had a LZD MIC of ≥8 µg/ml with 2 positive for *cfr* (Arizona and Michigan). Only 8 ENT isolates were LZD-R (0.49%; 0.55% in 2008), all with a G2576T mutation. Four LZD-R ENT were from one site in Kentucky, highly probable outbreak. Overall MRSA rates decreased to 51.4% (55.5%, 2008).

Table. Number of isolates detected in LEADER Program with LZD resistance mechanism

Year (no.)	G2576T	T2504A	G2447T	*cfr*	L3 or L4 deletion	Unknown	R rate (%)
2009 (25)	16	0	0	4	5	0	0.34
2008 (27)	22	1	0	2	0	2	0.36
2007 (30)	26	0	0	2	0	2	0.44
2006 (24)	21	0	3	0	0	0	0.45
Total (106)	85	1	3	8	5	4	0.44

Conclusions: With a stable overall R rate of 0.34%, LZD remains highly active against indicated pathogens after nine years of clinical use. The emergence of the mobile *cfr* gene and detection of undetermined R mechanisms underscore the need for continued surveillance of LZD potencies versus MDR GP pathogens.

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 (2006-2009) 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 complicated skin and skin-structure infections (cSSSI) and nosocomial pneumonias, after USA-Food and Drug Administration (FDA) review. This compound has emerged as a valuable treatment option for infections caused by multidrug-resistant Gram-positive organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Streptococcus pneumoniae* (DRSP) and vancomycin-resistant enterococci (VRE).

Ten years post approval, the LEADER program continues active surveillance of the in vitro activity of linezolid.

MATERIALS AND METHODS

Fifty-six medical centers were selected to represent nine USA Census Bureau Regions (4-8 sites/region) as follows: Pacific (California [2], Hawaii [1], Oregon [1], Washington [3]); Mountain (Arizona [2], Colorado [1], Utah [1]); West North Central (Iowa [1], Kansas [1], Missouri [2], Nebraska [1], Minnesota [2]); West South Central (Arkansas [1], Texas [3], Louisiana [1], Oklahoma [1]); East North Central (Illinois [1], Indiana [1], Michigan [1], Ohio [3], Wisconsin [2]); East South Central (Kentucky [2], Tennessee [2]), New England (Connecticut [1], Maine [1], Massachusetts [3], Vermont [1]); Middle Atlantic (Pennsylvania [1], New York [3], New Jersey [3]); and South Atlantic (Florida [4], Maryland [1], North Carolina [1], Virginia [1]).

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 pneumonia, cSSSI and urinary tract infections were acceptable. The forwarded clinical isolates (6,414 total strains) were distributed among the following organism groups as follows: *S. aureus* (3,257 strains); CoNS (816); enterococci (1,017); *S. pneumoniae* (659); viridans group streptococci (264); and β-haemolytic streptococci (401).

All susceptibility tests were performed by a GLP-compliant reference laboratory (JMI Laboratories) using Clinical and Laboratory Standards Institute (CLSI; formerly the NCCLS) broth microdilution methods (CLSI M07-08, 2009) and published interpretive criteria (CLSI M100-S20, 2010). Linezolid-resistant isolates were confirmed by repeated reference broth microdilution testing and with the linezolid Etest (AB bioMeriuex, Solna, Sweden) and disk diffusion susceptibility testing (CLSI M02-A10, 2009). Molecular (PCR) testing was performed on linezolid-resistant isolates to identify recognized target site mutations and potential clonality using pulsed-field gel electrophoresis (PFGE), automated ribotyping and various PCR procedures. Staphylococci that were negative for the common G2576T mutation were also screened for the phenicol resistance mediating gene, *cfr*. 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-S20 recommended methods.

RESULTS

- A total of 3,257 *S. aureus* strains were tested by the reference broth microdilution method with sample sizes varying from 291 (Mountain) to 390 (East South Central) isolates per region. MRSA rates were determined via a prevalence mode of testing, overall rate at 51.4% (55.5% in 2008).
- The CLSI D-test detected an overall clindamycin resistance induction rate of 37.9% among erythromycin-R clindamycin-S (ERCS) *S. aureus*; 39.4/38.1/39.3% in 2006/2007/2008. The distribution of ERCS rates showed 41 of 56 (73.2%) sites had inducible resistance with rates ranging between 21 and 60%.
- Linezolid demonstrated excellent comparative activity in all regions, as well as across all *S. aureus* tested (Table 1). Activity of linezolid was not affected by methicillin resistance in *S. aureus* ($MIC_{50/90}$ was 2 µg/ml for both MRSA and MSSA subsets).
- Five *S. aureus* isolates were linezolid-resistant from five different states. Two isolates (Ohio and Kentucky) had a positive PCR result using *cfr*-specific primers (confirmed by sequencing). One strain (Connecticut) had a L3 mutation (S415). The other strains (California and Kansas) contained the G2576T 23S rRNA mutation.
- A total of 816 CoNS isolates were tested against linezolid and 9 comparator agents (Table 1). 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 (67.0 to 91.0%; average at 73.9%) with the highest rates detected in the East South Central region (also in 2007 and 2008).
- Linezolid potency against CoNS isolates demonstrated a $MIC_{50/90}$ of only 1 µg/ml (Table 2) without any adverse influence by OR. However, 12 (1.47%) isolates were observed to have linezolid MIC values at ≥8 µg/ml, e.g. resistant.
- The tested enterococcal species isolates (1,017) were predominantly identified as *E. faecalis* (635; 62.4%) and *E. faecium* (339; 33.3%). Among these strains, the ampicillin-susceptible rate was only 67.8% and VRE rates varied by census region ranging from 28.9% (West North Central) to 41.3% (Middle Atlantic). The VRE rate for the enterococcal sample was 29.0% (Table 1) and the VanA resistance phenotype was 92.4% of the VRE isolates. A total of eight enterococci (all *E. faecium;* four isolates were clonal) were resistant to linezolid with MIC values at ≥8 µg/ml. All were positive for the G2576T mutation.
- Linezolid was active against all streptococci (MIC_{50} and MIC_{90}, 1 µg/ml; Tables 1 and 2).

Table 1. Linezolid activity compared to 9 other agents when tested in the LEADER Program (USA, 2009), 6,414 strains.

Pathogen (no. tested/antimicrobial agent)	MIC (µg/ml) 50%	90%	Range	% by category: Susceptible/Resistant
S. aureus (3,257)				
Linezolid	2	2	≤0.06->8	99.9 / 0.1
Ciprofloxacin	≤0.5	>4	≤0.5->4	55.9 / 42.4
Clindamycin	≤0.25	>2	≤0.25->2	80.4 / 19.1
Erythromycin	>2	>2	≤0.25->2	36.9 / 62.6
Gentamicin	≤2	≤2	≤2->8	98.4 / 1.4
Oxacillin[a]	>2	>2	≤0.25->2	48.6 / 51.4
Quinupristin/dalfopristin	0.5	0.5	≤0.25-2	99.8 / 0.0
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->2	98.5 / 1.5
Vancomycin	1	1	≤0.12-2	100.0 / 0.0
Coagulase-negative staphylococci (816)				
Linezolid	1	1	≤0.06->8	98.5 / 1.5
Ciprofloxacin	>4	>4	≤0.5->4	44.0 / 55.1
Clindamycin	≤0.25	>2	≤0.25->2	67.3 / 31.3
Erythromycin	>2	>2	≤0.25->2	33.1 / 65.6
Gentamicin	≤2	>8	≤2->8	73.4 / 20.5
Oxacillin[a]	>2	>2	≤0.25->2	26.1 / 73.9
Quinupristin/dalfopristin	≤0.25	0.5	≤0.25-2	99.9 / 0.0
Trimethoprim/sulfamethoxazole	≤0.5	>2	≤0.5->2	60.1 / 39.9
Vancomycin	1	2	≤0.12-4	100.0 / 0.0
Enterococci (1,017)				
Linezolid	1	2	0.25->8	98.9 / 0.8
Ampicillin[a]	2	>16	≤1->16	67.8 / 32.2
Ciprofloxacin	>4	>4	≤0.5->4	37.9 / 53.9
Quinupristin/dalfopristin	>2	>2	≤0.25->2	31.7 / 63.9
Teicoplanin	≤2	>16	≤2->16	71.8 / 26.8
Vancomycin	2	>16	0.25->16	70.3 / 29.0

Pathogen (no. tested/antimicrobial agent)	MIC (µg/ml) 50%	90%	Range	% by category: Susceptible/Resistant
S. pneumoniae (659)				
Linezolid	1	1	≤0.12-2	100.0 / -
Amoxicillin/clavulanic acid	≤1	8	≤1-16	81.8 / 15.5
Ceftriaxone	≤0.25	2	≤0.25-8	87.1 / 2.1
Ciprofloxacin	1	2	≤0.5->4	(3.9)[b]
Clindamycin	≤0.25	>2	≤0.25->2	77.8 / 21.6
Erythromycin	≤0.25	>2	≤0.25->2	58.1 / 41.1
Levofloxacin	1	1	≤0.5->4	99.1 / 0.9
Penicillin	≤0.03	4	≤0.03->4	57.7 / 21.5
Vancomycin	≤1	≤1	≤1	100.0 / -
Viridans group streptococci (264)				
Linezolid	1	1	0.12-2	100.0 / -
Ceftriaxone	≤0.25	1	≤0.25->32	91.7 / 3.8
Ciprofloxacin	2	>4	≤0.5->4	(19.7)[b]
Clindamycin	≤0.25	0.5	≤0.25->2	88.6 / 9.1
Erythromycin	1	>2	≤0.25->2	46.2 / 50.8
Levofloxacin	1	2	≤0.5->4	90.2 / 8.0
Penicillin[a]	0.06	1	≤0.015-32	77.3 / 3.0
Vancomycin	0.5	1	≤0.12-2	99.6 / -
β-haemolytic streptococci (401)				
Linezolid	1	1	≤0.06-2	100.0 / -
Ceftriaxone	≤0.25	≤0.25	≤0.25-0.5	100.0 / -
Ciprofloxacin	≤0.5	1	≤0.5->4	(0.7)[b]
Clindamycin	≤0.25	>2	≤0.25->2	81.8 / 17.2
Erythromycin	≤0.25	>2	≤0.25->2	62.8 / 36.4
Levofloxacin	≤0.5	1	≤0.5->4	99.5 / 0.5
Penicillin[a]	0.03	0.06	≤0.015-0.12	100.0 / -
Vancomycin	0.5	0.5	≤0.12-1	100.0 / -

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. Percentages in parentheses are the strains having a ciprofloxacin MIC at ≥4 µg/ml, possible QRDR mutations

Table 2. Cumulative percentage inhibited results at each linezolid MIC when testing six different groups of Gram-positive cocci isolated from all USA census regions (LEADER Program, 2009); 6,414 strains.

Organism group (no. tested)	Cum. % inhibited at linezolid MIC (µg/ml): ≤0.12	0.25	0.5	1	2	4	8	>8
Vir. grp streptococci (264)	1.1	2.7	34.5	97.7	100.0	-	-	-
S. pneumoniae (659)	0.6	3.8	43.4	96.5	100.0	-	-	-
β-haem. streptococci (401)	0.5	0.5	11.2	99.5	100.0	-	-	-
CoNS (816)	0.1	1.4	37.1	94.4	98.3	98.5	98.9	100.0
Enterococci (1,017)	0.0	0.1	4.0	53.4	98.9	99.2	99.6	100.0
S. aureus (3,257)	0.1	0.2	0.9	41.5	99.8	99.9	>99.9	100.0

Table 3. Six year trends in linezolid resistance rates observed in the LEADER Program (2004-2009; 33,378 isolates).

Organisms (no. tested)	% linezolid-non-susceptible or resistant 2004	2005	2006	2007	2008	2009
S. aureus (18,537)	0.00	0.03	0.03	0.06	0.10	0.15
CoNS (4,526)	0.20	1.13	1.61	1.76	1.64	1.47
Enterococci (4,577)	0.80	0.64	1.83	1.13	0.55	0.49
S. pneumoniae (3,292)	0.00	0.00	0.00	0.00	0.00	0.00
Vir. grp. streptococci (925)	NT	NT	0.00	0.00	0.00	0.00
β-haem. streptococci (1,521)	NT	NT	0.00	0.00	0.00	0.00
All organisms (33,378)	0.14	0.24	0.45	0.44	0.36	0.34

CONCLUSIONS

- Linezolid surveillance susceptibility testing of Gram-positive isolates (6,414) from 56 USA medical centers in 2009 showed excellent sustained activity and a high susceptibility rate of 99.66% overall (99.64% in 2008; see Table 3).
- Linezolid MIC population distributions remained unchanged without evidence of "MIC creep" among indicated species (Table 2).
- 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 33,378 strains over six surveillance years using reference CLSI methods (Table 3).

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