A-084

Linezolid Experience and Accurate Determination of Resistance (LEADER) Program 2008: USA Surveillance Report

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ASM 2009
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AMENDED ABSTRACT

Background: Approved in 2000 by the USA-FDA, linezolid (LZD) is widely used to treat complicated skin and soft tissue infections and nosocomial pneumonia. LZD has emerged as a valuable treatment option for infections caused by Gram-positive organisms that are resistant (R) to conventional drugs, such as methicillin-R *Staphylococcus aureus* (MRSA), drug-R *Streptococcus pneumonia* and vancomycin-R enterococci. The LEADER Program has completed its fifth year of USA LZD activity surveillance (2008).

Methods: Over 6000 GP isolates were submitted from 57 medical centers distributed across 9 US Census Bureau Regions. Each center was instructed to send 100 GP isolates including the following organism groups (no. overall): *S. aureus* (SA; 3,156), coagulase-negative staphylococci (CoNS; 856), enterococci (ENT; 901), *S. pneumoniae* (619), viridans group (223), and ß-haemolytic streptococci (358). CLSI broth microdilution susceptibility was performed and identification was confirmed on all isolates. LZD-R isolates were confirmed using Etest and PCR performed to detect R mechanisms.

Results: Overall LZD-R decreased in 2008 to 0.36% (0.44%; 2007, 0.45%; 2006). Only three SA isolates were non-S to LZD, one containing the rare multidrug-R *cfr* gene. Among CoNS, 14 isolates (12 *S. epidermidis*, 1 *S. hominis*, 1 *S. haemolyticus*) were non-S to LZD with all but 3 isolates testing positive for the 23S G2576T mutation and one contained *cfr*. The first *cfr*-positive CoNS was reported in this program in 2007. The five LZD-R ENT isolates had the G2576T target mutation. Higher LZD-R rates in the Mid-Atlantic region represents clonality within medical centers. Vancomycin-R ENT rates varied by region from 24.5% (WN Central) to 41.3% (Mid-Atlantic). All streptococci were LZD-S (all MIC values, ≤2 µg/ml).

Table: Overall LZD-R and MRSA rates by USA Census regions.

USA Census region	LZD-R (% of all isolates)	MRSA rates (%)
1. Pacific	0.00	48.6
2. Mountain	0.02	51.0
3. West North Central	0.02	51.0
4. West South Central	0.02	68.2
5. East North Central	0.02	61.0
6. East South Central	0.08	58.3
7. New England	0.02	57.2
8. Mid-Atlantic	0.18	51.4
9. South Atlantic	0.02	55.0

Conclusions: LZD remains very active (99.64% susceptibility) with no evidence of MIC creep when compared to previous LEADER Program reports. The discovery of two *cfr* mediated multi-R isolates in staphylococci exhibits the need for continued monitoring as a potentially emerging resistance mechanism in the USA.

INTRODUCTION

The LEADER Program has been monitoring linezolid potency, spectrum and resistance in the United States (USA) since 2004, the last three years administered by JMI Laboratories (North Liberty, Iowa). Linezolid is the only commercially available oxazolidinone antibiotic and was approved by the FDA in 2000 for the treatment of complicated skin and soft tissue infections (cSSTI) and nosocomial pneumonias due to MRSA. In the past eight years, linezolid has emerged as a valuable treatment option for infections caused by Gram-positive organisms that are resistant (R) to conventional drugs, such as methicillin-R *Staphylococcus aureus* (MRSA), multi drug-R (MDR) *Streptococcus pneumoniae* and vancomycin resistant enterococci (VRE).

In the interest of antibiotic stewardship, it is prudent to monitor the in vitro activity of this agent. Since linezolid is reserved for serious infections, use is limited and resistance development is not expected to spread as rapidly as previously seen with other agents used to treat outpatient respiratory tract infections.

MATERIALS AND METHODS

Fifty-seven medical centers were selected to represent nine US Census Bureau Regions (5-8 sites/region) as follows: Pacific (California [2], Hawaii [1], Oregon [1], Washington [3]), Mountain (Arizona [2], Colorado [1], Montana [1], Utah [1]), West North Central (Iowa [1], Kansas [1], Missouri [1], Nebraska [1], Minnesota [1]), West South Central (Arkansas [1], Texas [3], Louisiana [1], Oklahoma [1]), East North Central (Indiana [1], Michigan [2], Ohio [3], Wisconsin [2]), East South Central (Kentucky [2], Mississippi [1], Tennessee [2]), New England (Connecticut [1], Maine [1], Massachusetts [3], Vermont [1]), Middle Atlantic (Pennsylvania [1], New York [4], New Jersey [3]), and South Atlantic (Florida [3], Maryland [1], North Carolina [1], Virginia [1], Washington DC [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 ß-haemolytic streptococci and viridans group streptococci (five strains each). The strains should be dominantly from bacteremias although isolates from documented pneumonia, cSSSI and urinary tract infections were acceptable. The forwarded clinical isolates (6,113 total strains) were distributed among the following organism groups as follows: *S. aureus* (3,156 strains), CoNS (856), enterococci (901), *S. pneumoniae* (619), viridans group streptococci (223) and ß-haemolytic streptococci (358).

All susceptibility tests were performed by a GLP-compliant reference laboratory (JMI Laboratories, North Liberty, Iowa) using Clinical and Laboratory Standards Institute (CLSI; formerly the NCCLS) broth microdilution methods and published interpretive criteria (CLSI M100-S19, 2009). Linezolid-resistant isolates were confirmed by repeated reference broth microdilution testing and with the linezolid Etest (AB BIODISK, 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 or U 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-S19 recommended methods.

RESULTS

- A total of 3,156 *S. aureus* strains were tested by the reference broth microdilution method with sample sizes varying from 299 (New England) to 401 (mid-Atlantic) isolates per region. MRSA rates were determined via a prevalence mode of testing, overall rate at 55.5% (58.2% in 2007).
- The CLSI D-test detected an overall clindamycin resistance induction rate of 39.3% among erythromycin-R clindamycin-S (ERCS) *S. aureus*; 39.4/38.1% in 2006/2007. The distribution of ERCS rates showed 50 of 57 (87.7%) sites had inducible resistance with rates between 11 and 70%.
- 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).
- A total of 856 CoNS isolates was tested vs 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 (60.9 to 76.5%; average at 70.8%) with the highest rates detected in the ES Central and Mid-Atlantic regions.
- Linezolid potency against CoNS isolates demonstrated a $MIC_{50/90}$ of only 1 µg/ml (Table 2) without any adverse influence by oxacillin susceptibility or resistance. However, 18 (1.76%) isolates were observed to have linezolid MIC values at ≥8 µg/ml, e.g. non-susceptible.
- The tested enterococcal species strains (901) were identified as *E. faecalis* (586; 65.0%) and *E. faecium* (270; 30.0%). Among these strains, the ampicillin-susceptible rate was only 71.3% and VRE rates varied by census region ranging from 24.5% (WN Central) to 41.3% (Mid-Atlantic). The VRE rate for the enterococcal sample was 27.3% (Table 1) and the VanA resistance phenotype was 91.9% of the VRE isolates. A total of five enterococci (all *E. faecium*) were resistant to linezolid with MIC values ≥8 µg/ml. All were positive for G2576T mutation.
- Linezolid was active against all streptococci (MIC_{50} and MIC_{90}, 1 µg/ml; Table 1).
- Two staphylococcus isolates (*S. aureus* and *S. epidermidis*) displayed linezolid-non-susceptible phenotypes and had a positive PCR result using *cfr*-specific primers (confirmed by sequencing).

Table 1. Linezolid activity compared to 9 other agents when tested in the LEADER Program (USA, 2008), 6,113 strains.

Pathogen (no. tested/antimicrobial agent)	MIC (µg/ml) 50%	MIC (µg/ml) 90%	MIC (µg/ml) Range	% by category: Susceptible/Resistant
S. aureus (3,156)				
Linezolid	2	2	0.25->8	99.9 / -
Ciprofloxacin	0.5	>4	≤0.03->4	52.4 / 45.2
Clindamycin	≤0.25	>2	≤0.25->2	75.8 / 23.9
Erythromycin	>2	>2	≤0.25->2	31.7 / 67.6
Gentamicin	≤2	≤2	≤2->8	98.1 / 1.7
Oxacillin[a]	>2	>2	≤0.25->2	44.5 / 55.5
Quinupristin/dalfopristin	0.5	0.5	≤0.25-2	99.8 / 0.0
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->2	98.2 / 1.8
Vancomycin	1	1	≤0.12-2	100.0 / 0.0
Coagulase-negative staphylococci (856)				
Linezolid	1	1	≤0.06->8	98.4 / -
Ciprofloxacin	4	>4	0.06->4	46.0 / 53.2
Clindamycin	≤0.25	>2	≤0.25->2	65.9 / 32.1
Erythromycin	>2	>2	≤0.25->2	34.6 / 65.0
Gentamicin	≤2	>8	≤2->8	70.1 / 22.7
Oxacillin[a]	>2	>2	≤0.25->2	29.2 / 70.8
Quinupristin/dalfopristin	≤0.25	0.5	≤0.25-2	99.9 / 0.0
Trimethoprim/sulfamethoxazole	≤0.5	>2	≤0.5->2	60.5 / 39.5
Vancomycin	1	2	0.25-4	100.0 / 0.0
Enterococci (901)				
Linezolid	1	2	0.25->8	99.3 / 0.6
Ampicillin[a]	≤1	>16	≤1->16	71.3 / 28.7
Ciprofloxacin	>4	>4	0.06->4	41.3 / 54.1
Gentamicin (high-level)	≤500	>1000	≤500->1000	75.4 / 24.6
Quinupristin/dalfopristin	>2	>2	≤0.25->2	28.6 / 66.3
Streptomycin (high level)	≤1000	>2000	≤1000->2000	69.6 / 30.4
Teicoplanin	≤2	>16	≤2->16	74.0 / 25.1
Vancomycin	1	>16	0.25->16	72.0 / 27.3
S. pneumoniae (619)				
Linezolid	1	1	≤0.12-2	100.0 / -
Amoxicillin/clavulanic acid	≤1	8	≤1-16	83.4 / 12.9
Ceftriaxone	≤0.25	1	≤0.25-8	91.4 / 1.6
Ciprofloxacin	2	2	0.12->4	(4.4)[b]
Clindamycin	≤0.25	>2	≤0.25->2	80.1 / 19.5
Erythromycin	≤0.25	>2	≤0.25->2	65.3 / 34.2
Levofloxacin	1	1	≤0.5->4	99.4 / 0.5
Penicillin	≤0.03	4	≤0.03-8	60.1 / 22.0
Vancomycin	≤1	≤1	≤1	100.0 / -
Viridans group streptococci (223)				
Linezolid	1	1	0.12-2	100.0 / -
Ceftriaxone	≤0.25	1	≤0.25-16	91.9 / 3.6
Ciprofloxacin	2	>4	0.12->4	(23.3)[b]
Clindamycin	≤0.25	0.5	≤0.25->2	89.7 / 9.9
Erythromycin	1	>2	≤0.25->2	44.4 / 51.6
Levofloxacin	1	>4	≤0.5->4	87.4 / 10.8
Penicillin[a]	0.06	1	≤0.015-16	74.4 / 5.4
Vancomycin	0.5	0.5	≤0.12-1	100.0 / -
ß-haemolytic streptococci (358)				
Linezolid	1	1	0.25-2	100.0 / -
Ceftriaxone	≤0.25	≤0.25	≤0.25-0.5	100.0 / -
Ciprofloxacin	0.5	1	0.12->4	(1.4)[b]
Clindamycin	≤0.25	>2	≤0.25->2	86.9 / 13.1
Erythromycin	≤0.25	>2	≤0.25->2	72.1 / 27.4
Levofloxacin	≤0.5	1	≤0.5->4	98.6 / 1.1
Penicillin[a]	0.03	0.06	≤0.015-0.12	100.0 / -
Vancomycin	0.5	0.5	0.25-1	100.0 / -

a. Criteria as published by the CLSI, ß-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 ß-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, 2008; 6,113 strains).

Organism group (no. tested)	Cumulative % inhibited at MIC (µg/ml): ≤0.12	0.25	0.5	1	2	4	8	>8
Viridans group streptococci (223)	0.0	3.6	45.7	98.2	100.0	-	-	-
S. pneumoniae (619)	1.1	4.8	37.5	98.9	100.0	-	-	-
ß-haemolytic streptococci (358)	0.0	0.3	24.0	99.7	100.0	-	-	-
CoNS (856)	0.2	1.6	38.3	96.7	98.2	98.4	98.4	100.0
Enterococci (901)	0.0	0.7	6.2	60.4	99.4	99.6	99.9	100.0
S. aureus (3,156)	0.0	0.1	0.5	42.0	99.8	99.9	>99.9	100.0

Table 3. Five year trends in linezolid non-susceptible results in the LEADER Program (2004-2008; 26,964 isolates).

Organisms (no. tested)	% non-susceptible or resistant 2004	2005	2006	2007	2008
S. aureus (15,280)	0.00	0.03	0.03	0.06	0.10
CoNS (3,710)	0.20	1.13	1.61	1.76	1.64
Enterococci (3,560)	0.80	0.64	1.83	1.13	0.55
S. pneumoniae (2,633)	0.00	0.00	0.00	0.00	0.00
Viridans group streptococci (661)	NT	NT	0.00	0.00	0.00
ß-haemolytic streptococci (1,120)	NT	NT	0.00	0.00	0.00
All organisms (26,964)	0.14	0.24	0.45	0.44	0.36

CONCLUSIONS

- Linezolid susceptibility testing of Gram-positive isolates (6,113) from 57 USA medical centers in 2008 showed excellent retained activity and a sustained susceptibility rate of 99.64% overall (99.56% in 2007; see Table 3).
- Linezolid MIC population distributions remained unchanged without evidence of "MIC creep" among indicated species (Table 2).
- Increasing VRE rates as well as the escalating MRSA rates (nearly 60%) were noted in the LEADER Program samples, indicate the continuing need for the oxazolidinone class.
- Monitoring of linezolid for changing patterns of emerging resistance should be continued, although no recent trends have been noted since 2006.

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