A-117

Linezolid Experience and Accurate Determination of Resistance (LEADER) Program 2007: Assessing Oxazolidinone and Inducible Clindamycin Resistance

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ASM 2008

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AMENDED ABSTRACT

Background: The LEADER Program has completed an expanded fourth year of LZD surveillance in the USA, monitoring Gram-positive (GP) isolates in 60 medical centers. LZD has emerged as a viable therapeutic alternative for infections caused by R GP organisms such as methicillin-R *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE).

Methods: 6,305 GP isolates from 60 sites were sent to the monitor for identification confirmation and CLSI broth microdilution susceptibility (S) testing. Sites were distributed across the 9 USA census regions and each participant submitted 100 isolates in the following organism groups (no.): *S. aureus* (SA; 3,318), coagulase-negative staphylococci (CoNS; 1,020), enterococci (ENT, 705), *S. pneumoniae* (SPN; 622), viridans group (VGS; 249) and ß-haemolytic streptococci (BHS; 391). Molecular studies (PCR, PFGE) were performed to identify R mechanisms and possible clonality within or between hospitals.

Results: LZD-R rates remained very low (<1%) and consistent with previous results.

Table. LZD activity in the USA by year

	% non-susceptible or resistant			
Organisms (no., all years)	2004	2005	2006	2007
S. aureus (12,124)	0.00	0.03	0.03	0.06
CoNS (2,854)	0.20	1.13	1.61	1.76
Enterococci (2,659)	0.80	0.64	1.83	1.13
All streptococci[a] (3,241)	0.00	0.00	0.00	0.00
All organisms (20,878)	0.14	0.24	0.45	0.44

a. Includes: SPN (2,041), VGS (438) and BHS (762).

Two SA isolates (Texas and Ohio) were detected as non-S to LZD; One isolate was PCR-positive for the rare *cfr* gene. Inducible clindamycin (CC)-R was 38.1% among erythromycin-R, CC-S SA strains. In CoNS, 18 isolates were LZD-R (6 species; 8 states). Only 3 CoNS isolates were negative for the common G2576T mutation, with one (Arizona) PCR-positive for *cfr.* Eight LZD-R ENT strains were positive for the G2576T mutation and represented a slight decrease in R rate compared to 2006 results. VRE rates increased by 1.4% in 2007, against which LZD and daptomycin were most active. The LZD MIC_{50} for all organism groups was unchanged from 2006 except for SA and VGS which increased slightly (two-fold). The LZD MIC_{90} for all organism groups was stable.

Conclusion: LZD resistance rates were generally lower in 2007, even with the addition of 10 medical centers. The discovery of the multi-R *cfr* determinant in staphylococci has not been previously reported in the USA and proves the effectiveness of LEADER in detecting novel R. It is therefore critical to monitor for emerging LZD-R in the context of this new, mobile genetic threat.

INTRODUCTION

The LEADER Program has provided linezolid resistance surveillance data for four years in the United States (USA). Linezolid, the first oxazolidinone class agent to be licensed for use in clinical practice, has been used to treat Gram-positive pathogens in complicated skin and soft tissue infections (cSSTI) and nosocomial pneumonias, after its USA-Food and Drug Administration (FDA) approval in early 2000. Linezolid has emerged as a viable treatment alternative for infections caused by Gram-positive organisms that are resistant to conventional drugs, such as methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Streptococcus pneumoniae* (DRSP) and vancomycin-resistant enterococci (VRE). Therefore, it is prudent and required via regulatory agencies to monitor the potency and possible emerging resistances to linezolid as the use of this agent increases, both in volume and geographic distribution.

The linezolid 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. Among the documented uncommon occurrences of linezolid resistance reported to date for staphylococci and enterococci, G2576 or T2500 target site mutations have been the typical mechanism. For the first time (2007) in the LEADER Program, a *cfr*-mediated resistance mechanism to linezolid has emerged among staphylococci.

MATERIALS AND METHODS

The 60 medical centers were selected to represent all nine USA Bureau of Census geographic zones or 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 [3], Missouri [2], Nebraska [1]), West South Central (Arkansas [1], Texas [4]), East North Central (Indiana [1], Michigan [2], Ohio [3], Wisconsin [2]), East South Central (Kentucky [2], Mississippi [1], Tennessee [4]), New England (Connecticut [1], Maine [1], Massachusetts [3], Vermont [1]), Middle Atlantic (Pennsylvania [1], New York [3], New Jersey [3]), and South Atlantic (Florida [3], Maryland [1], North Carolina [1], Virginia [1], Washington DC [2]).

Each medical center was instructed to forward 100 organisms with the following species distribution: *S. aureus* (50 strains), coagulase-negative staphylococci (CoNS; 20 strains), enterococci (10 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, cutaneous wound infections and urinary tract infections were acceptable. The forwarded clinical isolates (6,305 total strains) were distributed among the following organism groups: *S. aureus* (3,318), CoNS (1,020), enterococci (705), *S. pneumoniae* (622), viridans group streptococci (249) and ß-haemolytic streptococci (391).

All susceptibility tests were performed by a GLP-compliant reference laboratory using Clinical and Laboratory Standards Institute (CLSI; formerly the NCCLS) broth microdilution methods and published interpretive criteria (CLSI M100-S18, 2008). 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. 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-S18 recommended methods.

RESULTS

- A total of 3,318 *S. aureus* strains were tested by the reference broth microdilution method (CLSI, M7-A7, 2006) with collected samples varying from 263 (WS Central) to 469 (EN Central) isolates per region. MRSA rates were determined via a prevalence mode of testing, overall rate at 58.2%. Participant sites complied with the request and the MRSA rate varied from 52.6% (Mountain) to 67.1% (ES Central).
- The CLSI D-test detected an overall resistance induction rate of 38.1% among erythromycin-resistant, clindamycin-susceptible (ERCS) phenotypic *S. aureus*; 39.4% in 2006. The distribution of rates by Census region and year varied widely between 17 and 62% (Table 1).
- Linezolid demonstrated excellent comparative activity across all *S. aureus* tested (Tables 2 and 3). The linezolid MIC_{90} for *S. aureus* was 2 µg/ml and when the MRSA and MSSA were examined separately, the MIC_{50} and modal MIC for both groups was 1 µg/ml. This observation differed slightly from 2006 results (for LEADER and ZAAPS), where MSSA strains had a two-fold higher linezolid MIC mode.
- A total of 1,020 CoNS isolates were processed by CLSI reference tests for linezolid and 15 comparison agents. The all region linezolid MIC_{90} was 1 µg/ml (Table 3) 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 (58.7 to 78.9%) with the highest rates detected in the WN Central, ES Central and Mid-Atlantic regions. The overall OR CoNS rate was 72.7%, decreased from 76.9% in 2006.
- Linezolid potency tested against CoNS isolates demonstrated a $MIC_{50/90}$ of only 1 µg/ml (Table 1) without any adverse influence by oxacillin susceptibility or resistance. However, 18 (1.76%) isolates were observed to have linezolid MIC results at ≥8 µg/ml, e.g. non-susceptible (Tables 2 and 3). These strains were isolated from 13 hospitals in eight states (New York [3], Texas [5], New Jersey [4], Arizona [2] and one strain each from Kentucky, Massachusetts, Tennessee and Wisconsin). The most frequent linezolid-resistant CoNS species were: *S. epidermidis* (10 strains), *S. haemolyticus* (two), *S. simulans* (two), *S. xylosis* (two), *S. capitis* (one) and *S. hominis* (one).
- The tested enterococcal species strains (705) were most likely to be identified as *E. faecalis* (436; 61.8%) or *E. faecium* (249; 35.3%%). Among these strains, the ampicillin-susceptible rate was only 67.0% and VRE rates varied by census region ranging from 24.5% (WN Central) to 41.3% (Mid-Atlantic). The VRE rate for the entire enterococcal sample was 29.8% (28.0% in 2006) and the VanA resistance phenotype represented 93.0% of the VRE isolates. High-level aminoglycoside resistance (gentamicin and streptomycin) was only 25.2 to 29.2%.
- Linezolid and daptomycin were the most active agents tested against enterococci with susceptibility rates at 98.9% and 100.0%, respectively (Tables 2 and 3). A total of eight enterococci (all *E. faecium*) had a linezolid MIC at ≥8 µg/ml (Table 4). These strains were found in Kansas (2), Kentucky (1), Maryland (1), Tennessee (1), Texas (1), Utah (1) and Virginia (1).

Table 1. Inducible clindamycin resistance among *S. aureus* listed by census region.

Region (%2007/2006)	
1.	Pacific (20.5/21.5)
2.	Mountain (29.2/30.8)
3.	West North Central (39.0/39.7)
4.	West South Central (16.9/25.5)
5.	East North Central (37.8/44.6)
6.	East South Central (39.3/33.1)
7.	New England (61.7/65.2)
8.	Mid-Atlantic (52.9/53.6)
9.	South Atlantic (39.6/48.4)

Table 2. Linezolid activity compared to 14 other agents when tested in the LEADER Program (USA, 2007), 6,305 strains.

	MIC (µg/ml)			% by category:
Pathogen (no. tested/antimicrobial agent)	50%	90%	Range	Susceptible/Resistant
S. aureus (3,318)				
Linezolid	1	2	0.25->8	99.9 / -
Ciprofloxacin	1	>4	≤0.03->4	51.2 / 47.3
Clindamycin	≤0.25	>2	≤0.25->2	75.8 / 24.2
Erythromycin	>2	>2	≤0.25->2	33.3 / 66.4
Gentamicin	≤2	≤2	≤2->8	97.5 / 2.4
Oxacillin[a]	>2	>2	≤0.25->2	41.8 / 58.2
Quinupristin/dalfopristin	0.5	0.5	≤0.25-2	99.9 / 0.1
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->2	98.0 / 2.0
Vancomycin	1	1	≤0.12-4	>99.9 / 0.0
Coagulase-negative staphylococci (1,020)				
Linezolid	1	1	≤0.06->8	98.2 / -
Ciprofloxacin	4	>4	≤0.03->4	43.0 / 55.6
Clindamycin	≤0.25	>2	≤0.25->2	62.5 / 35.1
Erythromycin	>2	>2	≤0.25->2	29.3 / 70.3
Gentamicin	≤2	>8	≤2->8	71.9 / 17.7
Oxacillin[a]	>2	>2	≤0.25->2	27.3 / 72.7
Quinupristin/dalfopristin	≤0.25	0.5	≤0.25-2	99.8 / 0.0
Trimethoprim/sulfamethoxazole	≤0.5	>2	≤0.5->2	65.2 / 34.8
Vancomycin	1	2	≤0.12-8	99.9 / 0.0
Enterococci (705)				
Linezolid	1	2	0.25->8	98.9 / 1.1
Ampicillin[a]	≤1	>16	≤1->16	67.0 / 33.0
Ciprofloxacin	>4	>4	0.25->4	43.3 / 53.6
Gentamicin (high-level)	≤500	>1000	≤500->1000	74.8 / 25.2
Quinupristin/dalfopristin	>2	>2	≤0.25->2	32.2 / 62.7
Streptomycin (high level)	≤1000	>2000	≤1000->2000	70.8 / 29.2
Teicoplanin	≤2	>16	≤2->16	72.3 / 25.0
Vancomycin	1	>16	0.25->16	70.2 / 29.4
S. pneumoniae (622)				
Linezolid	1	1	≤0.12-2	100.0 / -
Amoxicillin/clavulanic acid	≤1	8	≤1-16	84.7 / 12.2
Ceftriaxone	≤0.25	1	≤0.25-8	92.3 / 2.3
Ciprofloxacin	1	2	≤0.03->4	(2.3)[b]
Clindamycin	≤0.25	>2	≤0.25->2	80.5 / 19.1
Erythromycin	≤0.25	>2	≤0.25->2	65.9 / 33.4
Levofloxacin	1	1	≤0.5->4	99.8 / 0.0
Penicillin	≤0.03	4	≤0.03-8	61.6 / 19.0
Vancomycin	≤1	≤1	≤1	100.0 / -
Viridans group streptococci (249)				
Linezolid	1	1	0.12-1	100.0 / -
Ceftriaxone	≤0.25	1	≤0.25-8	93.2 / 3.2
Ciprofloxacin	1	4	0.12->4	(14.1)[b]
Clindamycin	≤0.25	0.5	≤0.25->2	88.4 / 9.6
Erythromycin	1	>2	≤0.25->2	43.4 / 55.8
Levofloxacin	1	2	≤0.5->4	94.8 / 4.0
Penicillin[a]	0.06	1	≤0.015-32	71.5 / 4.4
Vancomycin	0.5	0.5	≤0.12-1	100.0 / -
ß-haemolytic streptococci (391)				
Linezolid	1	1	0.25-2	100.0 / -
Ceftriaxone	≤0.25	≤0.25	≤0.25-0.5	100.0 / -
Ciprofloxacin	0.5	1	≤0.03->4	(0.8)[b]
Clindamycin	≤0.25	>2	≤0.25->2	88.2 / 11.3
Erythromycin	≤0.25	>2	≤0.25->2	72.1 / 27.4
Levofloxacin	≤0.5	1	≤0.5->4	99.2 / 0.5
Penicillin[a]	≤0.015	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.

- Linezolid was active against all streptococci (MIC_{50} and MIC_{90}, 1 µg/ml, Tables 2 and 3) and only 16 strains had MIC values of 2 µg/ml.
- 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). These isolates were the first noted in humans in the USA.

Table 3. 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, 2007)[a]; 6,305 strains.

	Cum. % inhibited at linezolid MIC (µg/ml):							
Organism group (no. tested)	≤0.12	0.25	0.5	1	2	4	8	>8
Viridans group streptococci (249)	0.8	4.4	42.6	98.4	100.0	-	-	-
S. pneumoniae (622)	0.3	1.9	36.7	98.6	100.0	-	-	-
ß-haemolytic streptococci (391)	0.0	0.3	21.2	99.2	100.0	-	-	-
CoNS (1,020)	0.4	1.3	45.0	96.9	98.0	98.2	98.3[b]	100.0[b]
Enterococci (705)	0.0	0.4	4.3	68.1	98.9	98.9	99.4[c]	100.0[c]
S. aureus (3,318)	0.0	0.1	0.9	58.8	99.9	>99.9	>99.9[d]	100.0[d]

a. Organism groups were ranked in decreasing order of susceptibility to the oxazolidinone.
b. 18 strains from eight states, includes six species.
c. Eight *E. faecium* strains from seven states.
d. One strain at each MIC (8 and >8 µg/ml) from Texas and Ohio.

Table 4. Staphylococcus and Enterococcus species resistant to linezolid (MIC, ≥8 µg/ml).

Isolate ID number	Organism	State	Linezolid MIC	Mechanism	PFGE	LEADER sample[a]
25-6328A	*S. epidermidis*	Texas	>8	G2576T		Yes
15-5690A	*S. epidermidis*	New York	>8	G2576T		Yes
129-1200A	*S. epidermidis*	New Jersey	>8	G2576T	129B	Yes
426-3147L	*S. epidermidis*	Arizona	>8	cfr +	426A	Yes
426-3159L	*S. epidermidis*	Arizona	>8	G2576T	426B	Yes
442-3207L	*S. epidermidis*	New Jersey	>8	Negative[b]	442B	Yes
442-3204L	*S. epidermidis*	New Jersey	8	Negative[b]	442A	Yes
417-801L	*S. epidermidis*	Wisconsin	>8	G2576T		Yes
82-3650A	*S. epidermidis*	New York	8	G2576T	82C	No
82-7521A	*S. epidermidis*	New York	>8	G2576T	82D	Yes
82-7522A	*S. epidermidis*	New York	>8	G2576T	82C1	No
52-7675A	*S. epidermidis*	Massachusetts	>8	G2576T		Yes
107-3386A	*S. haemolyticus*	Kentucky	>8	G2576T		Yes
432-1923L	*S. haemolyticus*	Tennessee	>8	G2576T		Yes
409-659L	*S. simulans*	Texas	>8	G2576T	409C	Yes
409-660L	*S. simulans*	Texas	>8	G2576T	409C	Yes
407-2847L	*S. hominis*	New Jersey	>8	G2576T		Yes
409-664L	*S. capitis*	Texas	>8	G2576T	409C	Yes
409-663L	*S. xylosus*	Texas	>8	G2576T	409D	Yes
17-998C	*S. xylosus*	New York	>8	G2576T		Yes
409-641L	*S. aureus*	Texas	>8	G2576T		Yes
4-737X	*S. aureus*	Ohio	8	cfr +		Yes
30-142A	*E. faecium*	Virginia	>8	G2576T	30F1	No
30-3053A	*E. faecium*	Virginia	8	G2576T	30F1	No
30-1896X	*E. faecium*	Virginia	>8	G2576T	30F	No
30-6832A	*E. faecium*	Virginia	8	G2576T	30F	No
30-901C	*E. faecium*	Virginia	>8	Negative[b]	30F1	No
30-8040A	*E. faecium*	Virginia	8	G2576T	30F1	Yes
425-394L	*E. faecium*	Kansas	>8	G2576T	425A	Yes
425-381L	*E. faecium*	Kansas	>8	G2576T	425A	Yes
401-559L	*E. faecium*	Maryland	8	G2576T		Yes
51-1562A	*E. faecium*	Utah	8	G2576T		Yes
24-5739A	*E. faecium*	Texas	>8	G2576T		Yes
107-4184X	*E. faecium*	Kentucky	>8	G2576T		Yes
436-1710L	*E. faecium*	Tennessee	>8	G2576T		Yes

a. Yes = used in surveillance report and No = follow up studies of local clonality.
b. Mechanism of resistance remains unknown.

CONCLUSIONS

- Linezolid susceptibility testing of Gram-positive isolates (6,305) from 60 USA medical centers showed excellent retained activity and a sustained susceptibility rate of 99.56% overall (99.55% in 2006).
- Linezolid MIC population distributions remained unchanged without evidence of "MIC creep" among indicated species (Table 3).
- Molecular epidemiology (PFGE) and mechanisms of resistance (target mutations or *cfr*) testing for the linezolid-resistant strains implicate epidemic clonal dissemination in monitored medical centers. This type of spread along with the novel emerging mobile resistance (plasmidic *cfr*) appears to be most responsible for the modest resistance rates observed in the LEADER Program (2007). Continued monitoring of the oxazolidinone resistance rates and mechanisms remain a prudent practice.

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