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Surveillance of Linezolid Activity Against Staphylococci in the United States (USA; 2010)

RN JONES, JE ROSS, M CASTANHEIRA, RE MENDES, DJ FARRELL

JMI Laboratories, North Liberty, Iowa, USA

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JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

ABSTRACT

Background: Linezolid (LZD) remains the only oxazolidinone antimicrobial available in the USA after more than 10 years of clinical use. Structured surveillance of potency and for signs of emerging LZD resistance (R) have been functional worldwide since 1999. We report the 2010 USA LZD results for staphylococci (5,216 strains).

Methods: A total of 4,540 *S. aureus* (SA; 2,259 MRSA), and 676 coagulase-negative staphylococci (CoNS; 468 methicillin-R [MR]) were collected from 55 medical centers in 36 states (representing all 9 Census Regions). Most strains (55.0%) were from bacteremias; others from documented infections (pneumonias, 15.8%; wounds 29.2%). A central monitoring site used reference CLSI (M07-A8) susceptibility (S) methods and LZD-R (MIC, ≥8 µg/ml) staphylococci were tested by PCR/sequencing for R mechanisms in 23S rRNA targets or presence of cfr gene.

Results: LZD activity against staphylococci remains high with MIC_{90} and modal MIC results at 1 µg/ml (98.7% of MIC values at 0.5-2 µg/ml). LZD MICs were rare at 4 µg/ml (S breakpoint concentration; 0.06%) and at ≥8 µg/ml (R; 0.17%). Only 1 SA (OH state; G2576T mutant) and 8 CoNS (KY [2], LA, MA, MO, OH, TN states; G2576T [3], T2504A, L3 [5], L4 [3], and cfr [3] mechanisms) strains were LZD-R, most with multiple mechanisms. No clonal occurrences were detected. Vancomycin-S rates for SA were the same as LZD (Table).

Conclusions: LZD maintained high potency against SA and CoNS without MIC creep or increasing R rates since 2005 (LEADER Program). Various R mechanisms were identified including as many as six types in each of 3 CoNS strains. 2010 surveillance continues to provide evidence of low LZD-R at only 0.17% of staphylococci.

	LZD MIC (µg/ml)			
Pathogen group (no.)	50%	90%	Range	%S
SA, all (4,540)	1	1	≤0.12-8	99.98
MSSA (2,281)	1	1	≤0.12-4	100.0
MRSA (2,259)	1	1	≤0.12-8	99.96
CoNS, all (676)	0.5	1	≤0.12->8	98.82
MS-CoNS (208)	0.5	1	≤0.12-1	100.0
MR-CoNS (468)	0.5	1	0.25->8	98.29
All staphylococci (5,216)	1	1	≤0.12->8	99.83

INTRODUCTION

Linezolid was the first oxazolidinone class agent studied and approved (2000) in the United States (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 multi-drug resistant Gram-positive organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Streptococcus pneumoniae* (DRSP) and vancomycin-resistant enterococci (VRE).

The LEADER surveillance program has monitored linezolid potency, spectrum and resistance rates in the USA since 2004, the most recent years (2006-2010) administered by JMI Laboratories (North Liberty, Iowa, USA). Ten years post approval, the LEADER Program continues active monitoring of the in vitro activity of linezolid.

MATERIALS AND METHODS

Fifty-five medical centers were selected to represent nine USA Census Bureau Regions (5-8 sample sites/region) as follows: Pacific (Alaska [1], California [1], Hawaii [1], Oregon [1], Washington [2]); Mountain (Arizona [1], Colorado [1], New Mexico [1], Utah [1]); West North Central (Iowa [1], Missouri [2], Nebraska [1], North Dakota [1], Minnesota [1]); West South Central (Arkansas [1], Texas [3], Louisiana [1], Oklahoma [1]); East North Central (Illinois [1], Indiana [1], Michigan [2], 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 ß-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. A total of 4,540 *S. aureus* and 676 CoNS strains were submitted to the central monitoring laboratory.

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 and published interpretive criteria (CLSI M100-S21, 2011). Linezolid-resistant isolates were confirmed by repeated reference broth microdilution testing and with the linezolid Etest (AB bioMeriuex, Marcy l'Etoile, France) 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), 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-S21 recommended methods.

RESULTS

- A total of 4,540 *S. aureus* strains were tested by the reference broth microdilution method. MRSA rates were determined via a prevalence mode of testing, overall rate at 49.8% (51.4% in 2009).
- The CLSI D-test detected an overall clindamycin resistance induction rate of 30.6% among erythromycin-R clindamycin-S (ERCS) *S. aureus*; range 37.8-39.4% in 2006-2009. The distribution of ERCS rates showed 83.6% of sites had inducible resistance with rates ranging between 11 and 50%.
- 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 1/1 µg/ml for both MRSA and MSSA subsets; Figure 1).
- One *S. aureus* isolate was linezolid-resistant from Ohio and was found to have a rRNA G2576T mutation. Two more *S. aureus* strains had linezolid MIC values at 4 µg/ml (Utah and Michigan) and both had a positive PCR result using *cfr*-specific primers (confirmed by sequencing).
- A total of 676 CoNS isolates were tested against linezolid and 8 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-resistant and -susceptible isolates. The methicillin-resistant (MR) rates varied by census region (65.4 to 75.2%; average at 70.6%) with the highest rates detected in the Mid-Atlantic Census region.

Table 1. Linezolid activity compared to eight other agents when tested in the LEADER Program (USA, 2010), 5,216 strains.

	MIC (µg/ml)			% by category:
Pathogen (no. tested/antimicrobial agent)	50%	90%	Range	Susceptible/Resistant
S. aureus (4,540)				
Linezolid	1	1	≤0.12-8	>99.9 / <0.1
Ciprofloxacin	0.5	>4	≤0.03->4	58.1 / 40.4
Clindamycin	≤0.25	>2	≤0.25->2	82.3 / 17.4
Erythromycin	>4	>4	≤0.25->4	38.2 / 60.0
Gentamicin	≤1	≤1	≤1->8	97.6 / 2.1
Oxacillin[a]	2	>2	≤0.25->2	50.2 / 49.8
Quinupristin/dalfopristin	≤0.5	≤0.5	≤0.5-2	99.9 / 0.0
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->4	98.5 / 1.5
Vancomycin	1	1	0.25-4	99.9 / 0.0
Coagulase-negative staphylococci (676)				
Linezolid	0.5	1	≤0.12->8	98.8 / 1.2
Ciprofloxacin	4	>4	≤0.03->4	45.7 / 53.0
Clindamycin	≤0.25	>2	≤0.25->2	71.3 / 27.0
Erythromycin	>4	>4	≤0.25->4	35.6 / 62.4
Gentamicin	≤1	>8	≤1->8	77.2 / 15.4
Oxacillin[a]	2	>2	≤0.25->2	30.8 / 69.2
Quinupristin/dalfopristin	≤0.5	≤0.5	≤0.5-1	100.0 / 0.0
Trimethoprim/sulfamethoxazole	≤0.5	>4	≤0.5->4	65.0 / 35.0
Vancomycin	2	2	0.25-4	100.0 / 0.0

a. Criteria as published by the CLSI, ß-lactam susceptibility should be directed by the oxacillin test results with staphylococci.

- Linezolid potency against CoNS isolates demonstrated a MIC_{90} of only 1 µg/ml without any adverse influence by MR. However, 8 (1.18%) isolates were observed to have linezolid MIC values at ≥8 µg/ml. Seven *S. epidermidis* and one *S. haemolyticus* were identified as resistant to linezolid (≥8 µg/ml). Each isolate contained a known resistant mechanism (four G2576T, three *cfr* mediated resistances, and various mutations and insertions in the L3 and L4 region; see Table 2).
- Three linezolid-resistant *S. epidermidis* isolates from Lexington, Kentucky (2) and Akron, Ohio (1) were found to be identical to clones that were previously reported in the LEADER Program.

Table 3. Seven year trends in linezolid resistance rates observed in the LEADER Program (2004-2010; 28,279 isolates).

	% linezolid-non-susceptible or resistant						
Organisms (no. tested)	2004	2005	2006	2007	2008	2009	2010
S. aureus (23,077)	0.00	0.03	0.03	0.06	0.10	0.15	0.02
CoNS (5,202)	0.20	1.13	1.61	1.76	1.64	1.47	1.18

Figure 1. Linezolid MIC distribution for *S. aureus* tabulated by MSSA (2,281) and MRSA (2,259) groups.

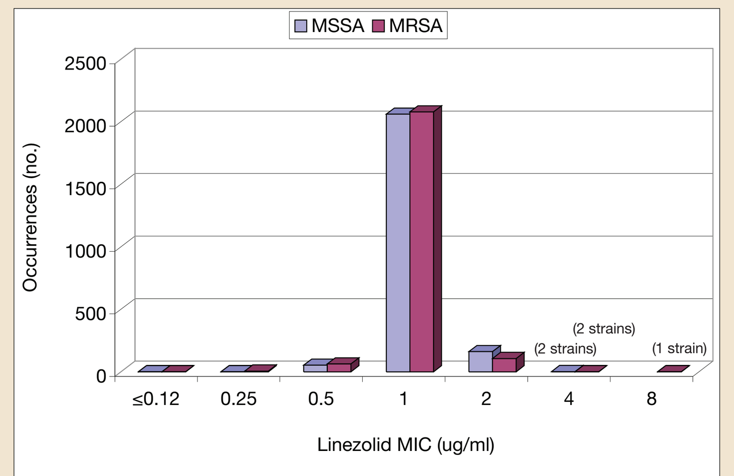

Table 2. *Staphylococcus* species with elevated or resistant-level linezolid MIC values (≥4 µg/ml) in the 2010 LEADER Program.

Isolate ID number	Organism	City	State	Age/Sex	Linezolid MIC	Resistance Mechanism	PFGE[a]
433-32739	*S. aureus*	Cincinnati	Ohio	20/M	8	G2576T	
051-22689	*S. aureus*	Salt Lake City	Utah	55/M	4	cfr	
460-22631	*S. aureus*	Lansing	Michigan	34/M	4	cfr	
107-2907	*S. epidermidis*	Lexington	Kentucky	55/F	128	*cfr,* L3 (V154L, A157R), L4 (N158S)	SEPI107A[b]
449-4042	*S. epidermidis*	Kansas City	Missouri	49/F	16	*cfr,* L3 (A157R)	
004-12676	*S. epidermidis*	Akron	Ohio	61/F	>128	*cfr,* L3 (V154L, H146Q, A157R), L4 (N158S, 71G72 insertion)	SEPI4A[c]
448-18006	*S. epidermidis*	New Orleans	Louisiana	50/M	32	G2576T, L3 (M156T, H146P)	
412-22291	*S. epidermidis*	Memphis	Tennessee	53/M	16	L3 (V154L, H146Q, A157R), L4 (N158S, 71G72 insertion)	
441-25437	*S. epidermidis*	Boston	Massachusetts	26/M	128	G2576T, L3 (V154L, M156T, H146R, G137D), L4 (71G72 insertion)	
441-25438	*S. epidermidis*	Boston	Massachusetts	61/M	128	G2576T, L3 (M156R, H146P, G137S), L4 G69R, 71G72 insertion)	
107-29423	*S. haemolyticus*	Lexington	Kentucky	68/M	32	G2576T, L3 (M156T)	SH107A[c]

a. PFGE performed only when multiple isolates were recovered from the same medical site.
b. PFGE profile indistinguishable from isolate recovered in 2009 from the same medical institution.
c. Epidemic clone detected in 2005 – 2008 and 2010.

CONCLUSIONS

- Linezolid surveillance susceptibility testing of staphylococci isolates (5,216) from 55 USA medical centers in 2010 showed excellent sustained activity and a high susceptibility rate of 99.83% overall.
- Linezolid MIC population distributions remained unchanged without evidence of "MIC creep" among tested staphylococci. Table 3 shows resistance rates, not MICs, over time.
- Monitoring of linezolid for changing patterns of resistance should be continued, although no increasing recent trends have been observed since 2006. The LEADER Program has now sampled 28,279 staphylococci strains over seven surveillance years using reference CLSI methods (Table 3).

ACKNOWLEDGEMENT

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