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Antimicrobial Susceptibility Studies

LEADER Surveillance program results for 2010: an activity and spectrum analysis of linezolid using 6801 clinical isolates from the United States (61 medical centers)☆

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ABSTRACT

The LEADER program monitors the in vitro activity of linezolid and comparator agents across the United States using reference broth microdilution and supportive molecular susceptibility-based investigations. This report summarizes the data from the 2010 program, the seventh consecutive year. A total of 61 medical centers from the USA including 7 medical centers specializing in children's healthcare provided a total of 6801 Gram-positive pathogens. The medical centers represented all 9 US Bureau of Census geographic regions. The organisms tested by reference broth microdilution were 3105 *Staphylococcus aureus,* 944 coagulase-negative staphylococci (CoNS), 934 Enterococci, 803 *Streptococcus pneumoniae,* 604 β-haemolytic streptococci, and 411 viridans group and other streptococci. The MIC_{90} value for each of the above 6 targeted groups of organisms was 1 μg/mL. The "all organism" linezolid-resistant and nonsusceptible rate was 0.38%, which has been constant at 0.34% (2009) to 0.45% (2006) for the last 4 years. For *Staphylococcus aureus*, only 0.06% of the isolates were linezolid-resistant (MIC, ≥8 μg/mL); however, 2 additional methicillin-resistant *Staphylococcus aureus* had a *cfr* and a MIC of only 4 μg/mL. Resistance to linezolid was detected in 7 enterococci (0.75%) and 14 CoNS isolates (1.48%). This also represents a stable rate of resistance noted since the 2006 LEADER program report. Of note, for the first time in the 7 years of the Leader Program a linezolid-resistant *Streptococcus pneumoniae* was encountered. Overall, the results of the LEADER program demonstrate that linezolid maintains excellent in vitro activity against target Gram-positive pathogens across the USA. The LEADER program continues to provide valuable reference and molecular-level monitoring of linezolid activity.

1. Introduction

The LEADER surveillance program has monitored linezolid (an oxazolidinone) activity, spectrum, and resistance rates in the USA since 2004 (Draghi et al., 2005, 2006; Farrell et al., 2009, 2011; Jones et al., 2007a, 2008). Oxazolidinone resistance surveillance for the USA was originally provided by the 2002 and 2003 ZAAPS program that sampled countries around the world including the USA (Anderegg et al., 2005). However, the 2004–2010 ZAAPS program only sampled countries other than the USA (Anderegg et. al., 2005; Jones et. al., 2006; Jones et al., 2007b; Jones et. al., 2009a; Jones et. al., 2009b; Ross et. al., 2005; Ross et. al., 2007), while the USA component was separated into the LEADER program and expanded to 60 or more laboratory sites in an effort to concentrate on emerging resistance development in various regions in the USA (Draghi et al., 2005, 2006; Farrell et al., 2009, 2011; Jones et al., 2007a, 2008; Pillar et al., 2008).

Linezolid is the first oxazolidinone class agent studied and approved (in 2000) in the USA for clinical use (Diekema and Jones, 2001; Fung et al., 2001; Stevens et al., 2004). It has a broad spectrum of activity against many clinically important Gram-positive pathogens including methicillin-resistant *Staphylococcus aureus*, CoNS, *Enterococcus faecalis* or *Enterococcus faecium*, *Streptococcus pneumoniae*, viridans group and other streptococci, β-hemolytic streptococci, and other rarely isolated Gram-positive pathogens (Brickner, 1996; Ford et al., 2001; Jones et al., 2007c). It has been used for therapy for a variety of infections including complicated skin and skin structure infection and nosocomial pneumonia caused by Gram-positive pathogens (Shorr et al., 2005; Stevens et al., 2002; Weigelt et al., 2005; Wunderink et al., 2003a,b). In addition, this compound has emerged as a valuable parenteral/oral agent for infections caused by multidrug-resistant pathogens—methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Streptococcus pneumoniae*, and vancomycin-resistant enterococci (VRE)—that are refractory to many commonly used agents (Jones et al., 1996, 2009a; Stevens et al., 2002; Wunderink et al., 2003a, 2012; Zurenko et al., 1996). Therefore, it is prudent to routinely monitor the in vitro

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Table 1
Number of sites and organisms listed by US census region (LEADER Program, 2010).

Region	States (number of sites)	No. of organisms tested
1. Pacific	AK (1), CA (1), HI (1), OR (1), WA (3)	744
2. Mountain	AZ (2), CO (1), NM (1), UT (1)	543
3. West North Central	IA (1), MO (2), ND (1), NE (1), MN (2)	820
4. West South Central	AR (1), TX (3), LA (2), OK (1)	782
5. East North Central	IL (1), IN (1), MI (1), OH (3), WI (2)	929
6. East South Central	AL (2), KY (2), TN (2)	625
7. New England	CT (1), MA (3), ME (1), VT (1)	720
8. Middle Atlantic	NY (3), NJ (3), PA (2)	907
9. South Atlantic	FL (4), MD (1), NC (1), VA (1)	741

activity of linezolid and possible evolving resistances as its use expands worldwide.

The oxazolidinone 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 (Shinabarger, 1999). Among the detected cases of linezolid resistance reported among staphylococci and enterococci, G2576T, G2447T, or T2504A 23S rRNA target site mutations have been most prevalent. However, in this report and other LEADER summaries, a mobile *cfr*-mediated resistance mechanism to linezolid has emerged among *Staphylococcus* spp. isolates (*Staphylococcus aureus* and CoNS) (Arias et al., 2008; Long et al., 2006; Mendes et al., 2008; Toh et al., 2007). So far, it has persisted, as evidenced by its continued finding in the LEADER program, but has not widely disseminated as only a few isolates carrying this mechanism continue to be identified; some in localized epidemic settings (Bonilla et al., 2010).

In this report of the 2010 LEADER Program, we present the findings from a 61-laboratory sample distributed among the 9 US census regions: a total of 6801 strains. Linezolid and comparator agent resistance trends as well as molecular characterization of oxazolidinone resistance mechanism are presented.

2. Materials and methods

2.1. Organism collection and susceptibility testing

A total of 61 medical centers from the USA including 7 medical centers specializing in children's healthcare (Table 1) provided a total of 6801 Gram-positive pathogens in 2010. The medical centers were selected to represent all 9 US Bureau of Census geographic zones or regions (5–8 sampling sites/region and 543–929 strains/region).

Each medical center forwarded ≥100 organisms with the following target species or genus distribution: *Staphylococcus aureus* (50 isolates), CoNS (15 isolates), Enterococci (15 isolates), *Streptococcus pneumoniae* (10 isolates), β-haemolytic streptococci and viridans group and other streptococci (5 isolates each; total 10). The strains were mostly from invasive bloodstream infections, although isolates from pneumonia (respiratory tract), acute bacterial skin and skin structure infections (ABSSSI), and urinary tract infections were acceptable.

All susceptibility tests were performed using dry form panels produced by TREK Diagnostics (Cleveland, OH, USA) in a GLP-compliant reference laboratory (JMI Laboratories, North Liberty, IA, USA) using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods and published interpretive criteria (CLSI, 2009b, 2011). Linezolid-resistant isolates were confirmed by repeated reference broth microdilution testing (CLSI, 2009b) using frozen-form broth microdilution panels (JMI Laboratories) and with the linezolid Etest (AB BIODISK; bioMérieux, Hazelwood, MO, USA) and CLSI disk diffusion susceptibility testing methods (CLSI, 2009a).

2.2. Molecular characterization

Molecular testing was performed on isolates exhibiting elevated linezolid MIC results (MIC ≥4 μg/mL) to identify recognized target site mutations or genes and potential clonality using pulsed-field gel electrophoresis (PFGE). Staphylococci and enterococci were screened for *cfr* and mutations in the central loop of domain V region of 23S rRNA, L3, and L4 ribosomal proteins (Locke et al., 2009a, 2009b; Mendes et al., 2008, 2010). Targeted genes were polymerase chain reaction–amplified and amplicons sequenced on both strands. Nucleotide and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI, USA) and respective proteins compared with those from wild-type linezolid-susceptible ATCC strains. Furthermore, *Staphylococcus aureus* strains found to be resistant to erythromycin, but susceptible to clindamycin, were screened by the CLSI D-test (disk approximation) to detect inducible clindamycin resistance (CLSI, 2011).

3. Results

3.1. Activity of linezolid against staphylococci

A total of 3105 *Staphylococcus aureus* strains were tested by the reference broth microdilution method with census region organism sample sizes ranging from 256 (Mountain) to 420 (Mid-Atlantic) isolates (Table 2). MRSA rates were determined via a prevalence mode of sample testing, with overall rate at 50.8% (51.4% in 2009; declining since 2007 [58.2%]). MRSA rates varied by region ranging from 43.1% (South Atlantic) to 64.6% (West South Central), the latter region also having the highest rate in 2008 and 2009. Other antimicrobial resistance rates decreasing since 2008 were levofloxacin (45.0 to 41.4%), clindamycin (23.9 to 18.9%), and erythromycin (67.6 to 62.1%). However, gentamicin (1.7 to 3.0%) increased and trimethoprim/sulfamethoxazole (TMP/SMX; 1.8 to 1.9%) and tetracycline (4.6 to 4.1%) were somewhat stable for resistance rates.

Table 2
Frequency of organisms tested, listed by census region (LEADER Program, 2010).

Region (no. of sites)	Organism group (no. tested)						Region total
	Staphylococcus aureus	Coagulase-negative staphylococci	Enterococci	*Streptococcus pneumoniae*	Viridans group and other streptococci	Beta-haemolytic streptococci	
1. Pacific (7)	350	68	107	98	50	71	744
2. Mountain (5)	256	74	83	54	28	48	543
3. West North Central (7)	359	109	105	105	45	87	810
4. West South Central (7)	350	133	108	85	33	73	782
5. East North Central (8)	405	143	124	112	59	86	929
6. East South Central (6)	315	68	94	59	30	59	625
7. New England (6)	300	130	89	88	44	69	720
8. Mid Atlantic (8)	420	145	117	107	63	55	907
9. South Atlantic (7)	350	74	107	95	59	56	741
Total (61)	3105	944	934	803	411	604	6801