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In vitro activity of linezolid as assessed through the 2013 LEADER surveillance program

Robert K. Flamm [a,*], Rodrigo E. Mendes [a], Patricia A. Hogan [b], James E. Ross [a], David J. Farrell [a,c], Ronald N. Jones [a,d]

[a] JMI Laboratories, North Liberty, IA 52317, USA
[b] Pfizer Inc, Collegeville, PA 19426, USA
[c] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada
[d] Tufts University School of Medicine, Boston, MA 02111, USA

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ABSTRACT

The 2013 LEADER surveillance program monitored the in vitro activity of linezolid and comparator agents against Gram-positive bacteria at 60 medical centers in the United States. A total of 7183 pathogens were contributed from 6 predetermined pathogen groups. The groups were *Staphylococcus aureus*, coagulase-negative staphylococci, enterococci, *Streptococcus pneumoniae*, β-hemolytic streptococci, and viridans group streptococci. The MIC_{90} value for each of the 6 pathogen groups was 1 μg/mL. Susceptibility of "all organisms" to linezolid was 99.83%. Only 12 isolates (2 *S. aureus*, 3 *Staphylococcus epidermidis*, 1 *Streptococcus sanguinis*, 5 *Enterococcus faecium*, and 1 *Enterococcus faecalis*) were nonsusceptible to linezolid (0.17%). Three of these (2 *S. aureus* and 1 *E. faecium*) harbored the *cfr* resistance mechanism. The findings indicate that linezolid activity remains stable, although there are examples of clonal dissemination within several monitored institutions.

1. Introduction

In vitro resistance development studies indicated that the protein synthesis inhibitors, the oxazolidinones, exhibited a low propensity to select for resistance mutations (Zurenko et al., 1996). However, when linezolid, the first approved oxazolidinone, was in widespread use, target site (ribosomal) mutations in domain V of the 23S rRNA leading to linezolid resistance were found to occur (Gonzales et al., 2001; Tsiodras et al., 2001). These resistant isolates tended to occur primarily in patients undergoing long-term therapy (Burleson et al., 2004; Gonzales et al., 2001; Tsiodras et al., 2001; Wilson et al., 2003). With the continued use of linezolid, an additional resistance mechanism, *cfr*, was found to lead to linezolid resistance (Arias et al., 2008; Long et al., 2006; Mendes et al., 2008; Toh et al., 2007). As *cfr* has been shown to reside on plasmids and is flanked by insertion sequences, there was concern about its potential to spread widely. Fortunately, although ribosomal mutations and *cfr* have occurred in a number of the target Gram-positive species, their overall prevalence has remained relatively low (Draghi et al., 2005; Farrell et al., 2009, 2011; Flamm et al., 2012, 2013a; Jones et al., 2007, 2008; Mendes et al., 2014b; Zhanel et al., 2013).

The LEADER Program established in 2004 was designed as a national initiative to monitor the activity of linezolid and comparator agents against target Gram-positive pathogens (Draghi et al., 2005). This program has provided information not only on the activity of linezolid and comparator agents but has also provided insight into the epidemiology and resistance mechanisms of the oxazolidinones (Draghi et al., 2005, 2006; Farrell et al., 2009, 2011; Flamm et al., 2012; Jones et al., 2007, 2008; Mendes et al., 2014b). More than 60,000 Gram-positive bacteria have been collected over the course of the program, and although new resistance mechanisms have emerged and the number of genera harboring these mechanisms has changed, isolates nonsusceptible to linezolid remain relatively uncommon (approximately 0.3%).

In this report of the 2013 LEADER Program, we present the results of testing 7183 clinically significant Gram-positive isolates from 60 medical centers from the 9 US Bureau of Census geographic zones.

2. Materials and methods

2.1. Organisism collection

A total of 7183 Gram-positive pathogens were submitted from 60 US (36 states) medical centers. The medical centers were selected to represent all 9 US Census Bureau regions. There were 4–10 participating sites/region and 549–1281 strains/region. Each recruited medical center was instructed to forward ≥100 organisms collected during 2013 with the following species or genus distribution: *Staphylococcus aureus* (50 strains), coagulase-negative staphylococci (CoNS; 15 strains), enterococci (15 strains), *Streptococcus pneumoniae* (10 strains), β-hemolytic streptococci (BHS; 5 isolates), and viridans group streptococci (VGS; 5 isolates). The strains were predominantly from bloodstream infections, pneumonia (respiratory tract), and skin and soft tissue infections. Species identification that was performed at the participant medical center was confirmed at the monitoring laboratory (JMI Laboratories, North Liberty, IA, USA) using the Vitek 2 System (bioMerieux, Hazelwood,

* Corresponding author. Tel.: +1-319-665-3370; fax: +1-319-655-3371.
E-mail address: robert-flamm@jmilabs.com (R.K. Flamm).

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MO, USA) or MALDI-TOF (Bruker Daltonics, Bremen, Germany), when necessary.

2.2. Susceptibility testing

Susceptibility tests were performed according to Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution methods and published interpretive criteria (CLSI, 2012a, 2014). Linezolid-resistant isolates were confirmed by repeated reference broth microdilution testing and by performing a linezolid Etest (bioMerieux) and CLSI disk diffusion susceptibility test (CLSI, 2012a, 2012b).

2.3. Molecular testing

Molecular testing was performed on isolates with elevated linezolid MICs (MIC, 4 or ≥8 μg/mL) to identify target site mutations and potential clonality using pulsed-field gel electrophoresis (PFGE) and various PCR procedures, as previously described (Diaz et al., 2012; Mendes et al., 2010, 2012, 2013a). All strains were also screened for the chloramphenicol, florphenicol-class resistance mediating gene (*cfr*) as well as 23S rRNA, L3, L4, and L22 ribosomal protein mutations. Furthermore, *S. aureus* and CoNS strains found to be resistant to erythromycin but susceptible to clindamycin (ERCS) were screened by the CLSI broth dilution inducible clindamycin screening test, as outlined in M100-S24 (CLSI, 2014).

3. Results

A total of 3035 *S. aureus* strains were tested by the reference broth microdilution method (CLSI, 2012a). The linezolid MIC_{50} and MIC_{90} for *S. aureus* was 1 μg/mL (Tables 1 and 2). The MIC_{50} and MIC_{90} values were the same for methicillin (oxacillin)–resistant *S. aureus* (MRSA) and methicillin (oxacillin)–susceptible *S. aureus* (MSSA; MIC_{50} and MIC_{90}, 1 μg/mL). MRSA represented 47.9% of total *S. aureus* (Tables 1 and 2). The percentage of MRSA has decreased yearly, a 10.3% decline since 2007 (Jones et al., 2008). MRSA rates varied by region from 35.1% (Middle Atlantic) to 58.7% (East South Central; data not shown). Levofloxacin, clindamycin, and erythromycin susceptibility for *S. aureus* was 36.6%, 15.6%, and 58.5%, respectively (data not shown). Resistance occurrence to these 3 antimicrobials was higher in MRSA than MSSA (levofloxacin [MRSA, 64.2%; MSSA, 11.2%], clindamycin [26.7%, 5.3%], and erythromycin [87.8%, 31.9%]) (Table 2). Linezolid, daptomycin, teicoplanin, vancomycin, and tigecycline retained 99.9–100.0% activity against both MRSA and MSSA. Susceptibility to trimethoprim/sulfamethoxazole, gentamicin, and tetracycline was 97.9%, 97.3%, and 94.9% for MRSA and 99.4%, 99.2%, and 95.9% for MSSA (Table 2).

When CLSI interpretive criteria for broth microdilution MIC determination were applied, 15.6% of *S. aureus* were clindamycin resistant. Further, 26.7% of MRSA isolates were clindamycin resistant compared to 5.3% of MSSA. As clindamycin resistance was determined by broth microdilution susceptibility testing, the true rate of clindamycin resistance was underestimated, as there is a population of inducibly resistant strains that may test as susceptible. Broth microdilution screening for inducible resistance was performed on *S. aureus* strains that tested as ERCS. The results indicated the true clindamycin resistance rate for all *S. aureus* strains was 27.2% and for MRSA was 37.1% (data not shown). A total of 17.1% ERCS MRSA were inducible in 2013 (Table 3). This represented 10.4% of all MRSA. A total of 91.7% (55/60) of sites had *S. aureus* exhibiting inducible resistance (data not shown). Among ERCS MRSA, the highest rate of clindamycin inducible resistance occurred in New England (Table 3). Inducible ERCS MRSA regional rates varied from 2.7% (Mountain) to 40.8% (New England; Table 3). Among the MSSA, 48.7% of ERCS were inducibly resistant (data not shown). Rates ranged from 39.0% in the Mountain region to 59.0% in the West North Central region (data not shown).

Two linezolid-resistant MRSA (linezolid MIC, 8 and >8 μg/mL, respectively) were detected (Table 4). Both MRSA were shown to harbor *cfr*. One strain was from Long Beach (linezolid MIC, 32 μg/mL) and the other from Detroit (linezolid MIC, 8 μg/mL). The strain with the higher MIC value (32 μg/mL) contained a ribosomal mutation at G2576 in addition to cfr.

The linezolid MIC_{50} and MIC_{90} for all 580 CoNS was 0.5 and 1 μg/mL (Tables 1 and 2), and no major differences were noted in linezolid MIC distributions when comparing methicillin (oxacillin)–resistant and methicillin (oxacillin)–susceptible isolates (Table 1). Methicillin-resistant rates across the Census regions varied from 60.7% to 78.1% with the highest rate detected in the Mountain region (data not shown). Overall, the methicillin-resistant CoNS rate was 68.6% (Tables 1 and 2). Other resistances of note for all CoNS were the high rate of fluoroquinolone resistance (levofloxacin [39.8%], ciprofloxacin [40.9%], and tetracycline [15.0%]) (data not shown). Daptomycin (100.0% susceptible), vancomycin (100.0% susceptible), and teicoplanin (97.7% susceptible) were highly active against methicillin-resistant CoNS (Table 2).

Erythromycin and clindamycin resistance were elevated at 59.7% and 30.3% for CoNS, respectively (data not shown). Testing for inducible resistance to clindamycin in CoNS strains, which were ERCS, indicated that 29.5% of the ERCS strains were inducible. Regional percentages of inducibly resistant CoNS ranged from 14.3% in the Pacific region to 40.0% in West North Central region (data not shown).

Three CoNS isolates (0.52%) demonstrated linezolid MIC results of ≥8 μg/mL, which were confirmed by Etest, disk diffusion, and broth microdilution frozen-form panels (MIC, 32–128 μg/mL; Table 4). All were identified as *S. epidermidis* isolates, which originated from 3 states: Michigan, North Carolina, and Texas (Table 4). The resistance mechanisms detected in the 3 CoNS from the 2013 Program are described in Table 4. Overall, these linezolid-resistant *S. epidermidis* isolates demonstrated the presence of mutations in the ribosomal proteins L3 and L4, alone or in combination with 23S rRNA (G2576T) mutations. *cfr*-positive *S. epidermidis* strains were not detected in 2013.

Table 1
Cumulative frequency (in percentages) of isolates inhibited at each linezolid MIC when testing 6 different groups of Gram-positive cocci isolated from all US Census regions (LEADER Program, 2013); 7183 strains.

Organism (number)	MIC in μg/mL									
	≤0.12	0.25	0.5	1	2	4	8	>8	MIC_{50}	MIC_{90}
Staphylococcus aureus (3035)	0.1	0.5	19.8	96.7	99.9	99.9	100.0	100.0	1	1
MSSA (1581)	0.1	0.5	16.5	96.1	100.0				1	1
MRSA (1454)	0.2	0.6	23.4	97.4	99.9	99.9	99.9	100.0	1	1
CoNS (580)	0.0	14.5	87.6	99.3	99.5	99.5	99.5	100.0	0.5	1
MSCoNS (182)	0.0	13.7	89.0	100.0	–	–	–	–	0.5	1
MRCoNS (398)	0.0	14.8	86.9	99.0	99.2	99.2	99.2	100.0	0.5	1
Enterococcus spp. (924)	0.0	0.8	17.1	94.9	99.4	99.4	99.7	100.0	1	1
Vancomycin nonsusceptible (MIC, ≥8 mg/L) (199)	0.0	1.0	21.1	96.0	98.0	98.0	99.5	100.0	1	1
Vancomycin susceptible (MIC, ≤4 mg/L) (725)	0.0	0.7	16.0	94.6	99.7	99.7	99.7	100.0	1	1
Streptococcus pneumoniae (1281)	0.2	2.0	37.8	99.0	100.0				1	1
VGS (399)	1.3	5.5	61.2	99.7	99.7	100.0			0.5	1
BHS (964)	0.1	0.3	49.4	100.0					1	1