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LEADER Program Results for 2009: an Activity and Spectrum Analysis of Linezolid Using 6,414 Clinical Isolates from 56 Medical Centers in the United States▽†

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The LEADER Program monitors the *in vitro* activity of linezolid in sampled U.S. medical centers using reference broth microdilution methods with supporting molecular investigations in a central laboratory design. This report summarizes data obtained in 2009, the 6th consecutive year of this longitudinal study. A total of 6,414 isolates from 56 medical centers in all nine Census regions across the United States participated in 2009. For the six leading species/groups, the following linezolid MIC_{90} values were observed: *Staphylococcus aureus*, 2 μg/ml; coagulase-negative staphylococci (CoNS), 1 μg/ml; *Enterococcus* spp., 2 μg/ml; *Streptococcus pneumoniae*, 1 μg/ml; viridans group streptococci, 1 μg/ml; and beta-hemolytic streptococci, 1 μg/ml. Linezolid resistance was only 0.34% overall, with no evidence of significant increase in the LEADER Program since 2006. The predominant linezolid resistant mechanism found was a G2576T mutation in the 23S rRNA. L3/L4 riboprotein mutations were also found. The mobile multidrug-resistant *cfr* gene was found in four strains (two *S. aureus* strains and one strain each of *S. epidermidis* and *S. capitis*) from four different states, suggesting persistence but a lack of dissemination. Linezolid continues to exhibit excellent activity and spectrum, and this study documents the need for continued monitoring of emerging mechanisms of resistance over a wide geographic area.

Linezolid, which received U.S. Food and Drug Administration (FDA) approval in 2000 for adults and in 2005 for pediatric indications, has broad activity against many clinically important Gram-positive pathogens, including multidrug-resistant (MDR) subsets of *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), *Enterococcus faecalis* or *E. faecium*, *Streptococcus pneumoniae*, viridans group *Streptococcus* spp., various serogroups of beta-hemolytic streptococci, and other rarely isolated Gram-positive pathogens (3, 8, 34). Linezolid continues to demonstrate clinical success against a variety of infections, including serious cutaneous disease and nosocomial pneumonia, caused by prevalent Gram-positive organisms, including those resistant to conventional therapeutic agents (26, 27, 30, 32, 33).

Linezolid is an oxazolidinone agent which inhibits protein translation from mRNA by binding to the 50S ribosomal subunit (25). Although linezolid resistance remains very uncommon (<1.0%) among surveyed isolates (11, 16), individual cases have been widely reported and have been associated either with prolonged drug exposure in at-risk patient populations or with breaks in infection control practices leading to local outbreak or endemic occurrences (10, 12, 19, 20, 24, 29, 31). Most reports of oxazolidinone resistance describe mutations in the 23S rRNA peptidyl transfer center, usually at G2576T (13, 29, 31). More recently, a mobile element carrying a *cfr* rRNA methyltransferase that encodes resistances to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A ($phLOPS_A$) agents has been described previously (21) and has subsequently been identified in numerous human cases of staphylococcal infection (1, 22, 28).

The LEADER Program is an antibacterial resistance surveillance project designed to detect antimicrobial resistance development in the United States, against a range of clinically utilized antibacterial agents, with a specific focus on linezolid (9, 11). In this summary of the entire LEADER Program for 2009, we report linezolid and comparator resistance trends, details of the emerging resistance mechanisms, and geographic occurrences among a 6,414 isolate samples processed by reference broth microdilution tests with supporting molecular investigations.

MATERIALS AND METHODS

Sampling sites and organisms. A total of 56 medical centers from the United States participated in 2009, compared to 57 in 2008. These medical centers were selected to represent all nine U.S. Census Bureau regions (5 to 8 sites/region) as follows: Pacific (California [2 sites], Hawaii [1 site], Oregon [1 site], and Washington [3 sites]; 691 isolates), Mountain (Arizona [2 sites], Colorado [1 site], and Utah [1 site]; 525 isolates), West North Central (Iowa [1 site], Kansas [1 site], Missouri [2 sites], Minnesota [2 sites], and Nebraska [1 site]; 774 isolates); West South Central (Arkansas [1 site], Texas [3 sites], Oklahoma [1 site], and Louisiana [1 site]; 672 isolates), East North Central (Indiana [1 site], Illinois [1 site], Michigan [1 site], Ohio [3 sites], and Wisconsin [2 sites]; 776 isolates), East South Central (Kentucky [2 sites] and Tennessee [2 sites]; 692 isolates), New England (Connecticut [1 site], Maine [1 site], Massachusetts [3 sites], and Vermont [1 site]; 744 isolates), Middle Atlantic (Pennsylvania [1 site], New York [3 sites], and New Jersey [3 sites]; 823 isolates), and South Atlantic (Florida [4 sites], Maryland [1 site], North Carolina [1 site], and Virginia [1 site]; 717 isolates).

Each medical center forwarded ≥100 organisms, with the following minimal species distribution: *S. aureus*, 50 strains (in a prevalence design); CoNS, 20 strains; enterococci, 10 strains; *S. pneumoniae*, 10 strains; and beta-hemolytic streptococci and viridans group streptococci, 5 strains each. The isolates were

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TABLE 1. Cumulative percentages of inhibition results at each linezolid MIC with testing of six different groups of Gram-positive cocci isolated from all U.S. Census regions (LEADER Program, 2009; 6,414 strains)

Organism group (no. tested)	Cumulative % inhibited at linezolid MIC (μg/ml) of:							
	≤0.12	0.25	0.5	1	2	4	8	>8
Viridans group streptococci (264)	1.1	2.7	34.5	97.7	100.0			
S. pneumoniae (659)	0.6	3.8	43.4	96.5	100.0			
Beta-hemolytic streptococci (401)	0.5	0.5	11.2	99.5	100.0			
CoNS (816)	0.1	1.4	37.1	94.4	98.3	98.5	98.9	100.0[a]
Enterococci (1,017)	0.0	0.1	4.0	53.4	98.9	99.2	99.6[b]	100.0[b]
S. aureus (3,257)	0.1	0.2	0.9	41.5	99.8	99.9	>99.9[c]	100.0[c]

[a] Nine strains from eight states (all *S. epidermidis*, and eight strains were methicillin resistant).
[b] Eight strains from 5 states, all *E. faecium* (four strains with MICs of 8 μg/ml and four strains with MICs of >8 μg/ml).
[c] Five strains from 5 states (three strains with MICs of 8 μg/ml and two strains with MICs of >8 μg/ml); all were MRSA.

dominantly from bacteremias, pneumonias, wound infections, and urinary tract infections. The collection of 6,414 clinical isolates (compliance target of 6,000 isolates; 106.9% compliance rate) was distributed among the following organism groups: *S. aureus* (3,257 isolates), CoNS (816 isolates), enterococci (1,017 isolates), *S. pneumoniae* (659 isolates), viridans group streptococci (264 isolates), and beta-hemolytic streptococci (401 isolates).

Antimicrobial susceptibility testing. All tests were performed in a GLP reference laboratory (JMI Laboratories, North Liberty, IA) using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods and published interpretive criteria (6, 7). Linezolid-resistant isolates were confirmed with alternative methods such as Etest (AB Biodisk, Solna, Sweden), disk diffusion susceptibility testing (5), and an extended MIC (range up to 128 μg/ml) reference frozen-form panel. *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 (7). Molecular testing was performed on all isolates nonsusceptible to linezolid to identify the 23S rRNA target site mutations, *cfr*- or L3/L4 protein-mediated resistances, and potential clonality using pulsed-field gel electrophoresis (PFGE), as previously described (22, 23). Per convention, 23S ribosomal mutations refer to *Escherichia coli* 23S rRNA positions.

RESULTS

Linezolid activity against staphylococci. A total of 3,257 *S. aureus* strains were tested with Census region sample sizes ranging from 291 (Mountain) to 390 (East South Central) isolates. MRSA rates were determined via a prevalence mode of sample testing, with the overall rate at 51.4% (declining since 2007 [58.2%]). Participant sites complied with the consecutive sampling pattern request, and the MRSA rate varied only modestly by region, from 43.3% (Mountain) to 60.0% (West South Central), the latter region also having the highest rate in 2008. The other antimicrobial resistance rates decreasing in 2009 were as follows: levofloxacin, 45.2 to 42.4%; clindamycin, 23.9 to 19.1%; erythromycin, 67.6 to 62.6%; gentamicin, 1.7 to 1.4%; trimethoprim-sulfamethoxazole (TMP-SMX), 1.8 to 1.5%; and tetracycline, 4.6 to 3.5%. The CLSI D test detected an overall inducible-clindamycin-resistance induction rate of 37.9% among erythromycin-resistant, clindamycin-susceptible *S. aureus* isolates, compared to 39.4, 38.1, and 39.3% observed in 2006, 2007, and 2008, respectively. The highest rates of clindamycin-induced resistance were located in New England (67.5%; site range, 45.5 to 80.0%).

Linezolid demonstrated excellent comparative activity in all Census regions, as well as across all *S. aureus* isolates tested. The linezolid $MIC_{50/90}$ for *S. aureus* was 2 μg/ml (Table 1), and for methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA), the MIC_{50} and modal MIC for both groups were also 2 μg/ml. MRSA and MSSA linezolid MIC_{50}, MIC_{90}, and modal MIC values, as in 2008, remained at 2 μg/ml (Table 2). Five linezolid-resistant MRSA isolates were detected in California, Connecticut, Kansas, Kentucky, and Ohio medical centers (five different Census regions; MIC, ≥8 μg/ml) (see Table 4). Molecular studies for establishing the mechanisms of resistance showed a G2576T mutation in the 23S rRNA (California and Kansas strains). Isolate 27-1687A from Louisville, KY, had a linezolid MIC of 16 μg/ml and PCR evidence for the presence of the *cfr* gene (see Table 4). In 2008, a medical center in Lexington, KY, also had a *cfr*$^+$ strain. Another MRSA isolate from Ohio had a *cfr* gene, isolated from a medical center in Akron where prior epidemics have been documented by the LEADER Program, the SENTRY Antimicrobial Resistance Program, and separate investigator reports (2, 11, 22). One strain from Connecticut (424-2665L) had a linezolid MIC at 8 μg/ml and a L3 mutation (S415 deletion). In the 2008 LEADER Program report, two organisms (one each *S. aureus* and *S. epidermidis*) had no detectable resistance mechanism and a linezolid MIC at 8 μg/ml; these strains will be further examined for L3, L4, and L22 mutations (11).

For 816 coagulase-negative staphylococcus (CoNS) isolates, the overall linezolid MIC_{50} and MIC_{90} values were both 1 μg/ml (Table 1), with no differences noted in linezolid $MIC_{50/90}$ when methicillin (oxacillin)-resistant and -susceptible isolates were compared (Table 2). The rates of oxacillin-resistant (OR) isolates differed by Census region (67.0 to 91.0%), with the highest rates detected in the East South Central Region, as noted in 2007 and 2008. The overall OR-CoNS rate was 73.9%, increased from 72.7% in 2007 and 70.8% in 2008. Twelve isolates (1.47%; 1.64% in 2008) (Table 3) were observed to have linezolid MIC results at ≥8 μg/ml, i.e., resistant (Table 1). These isolates came from 11 hospitals in 10 states (Michigan [2 isolates], Arizona [2 isolates], and Ohio, Tennessee, Utah, Kentucky, Minnesota, Texas, New Jersey, and Massachusetts [1 isolate each]). The most frequently identified linezolid-resistant species were *S. epidermidis* (11 isolates) and *S. capitis* (1 isolate). The resistance mechanisms detected in these isolates are summarized in Table 4 and were as follows: G2576T (6 CoNS isolates [50.0%]), L3 and L4 mutations (4 isolates [33.3%]), and *cfr* (2 isolates [16.7%]). Further studies are in progress to determine the resistance mechanism in *S. epidermidis* strains from prior years where 23S rRNA alterations or *cfr* was not detected, e.g., screening of L3, L4, and L22 proteins.

TABLE 2. Comparative activity of linezolid tested against 6,414 Gram-positive pathogens isolated during the 2009 LEADER Program

Organism, resistance group (no. tested), and antimicrobial agent	MIC (μg/ml)			% of isolates susceptible/ % resistant[a]
	50%	90%	Range	
S. aureus				
Oxacillin susceptible (1,584)				
Linezolid	2	2	≤0.06–2	100.0/0.0
Oxacillin	0.5	1	≤0.25–2	100.0/0.0
Ceftriaxone	4	4	≤0.25–32	99.4/0.0
Ciprofloxacin	≤0.5	2	≤0.5–>4	88.3/9.9
Clindamycin	≤0.25	≤0.25	≤0.25–>2	95.1/4.5
Daptomycin	0.25	0.5	≤0.06–1	100.0/−
Erythromycin	0.5	>2	≤0.25–>2	67.2/32.1
Gentamicin	≤2	≤2	≤2–>8	99.2/0.5
Levofloxacin	≤0.5	2	≤0.5–>4	89.7/9.7
Penicillin	2	16	≤0.015–>32	22.9/77.1
Quinupristin-dalfopristin	0.5	0.5	≤0.25–2	99.9/0.0
Teicoplanin	≤2	≤2	≤2–4	100.0/0.0
Tetracycline	≤2	≤2	≤2–>8	96.8/2.3
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5–>2	99.0/1.0
Vancomycin	1	1	≤0.12–2	100.0/0.0
Oxacillin resistant (1,673)				
Linezolid	2	2	0.25–>8	99.7/0.3
Ciprofloxacin	>4	>4	≤0.5–>4	25.3/73.2
Clindamycin	≤0.25	>2	≤0.25–>2	66.5/33.0
Daptomycin	0.5	0.5	≤0.06–1	100.0/−
Erythromycin	>2	>2	≤0.25–>2	8.3/91.5
Gentamicin	≤2	≤2	≤2–>8	97.6/2.3
Levofloxacin	>4	>4	≤0.5–>4	26.9/72.2
Quinupristin-dalfopristin	0.5	1	≤0.25–2	99.6/0.0
Teicoplanin	≤2	≤2	≤2–8	100.0/0.0
Tetracycline	≤2	≤2	≤2–>8	95.0/4.7
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5–>2	98.1/1.9
Vancomycin	1	1	0.25–2	100.0/0.0
CoNS				
Oxacillin susceptible (213)[b]				
Linezolid	1	1	≤0.06–8	99.5/0.5
Ceftriaxone	2	4	≤0.25–16	98.1/0.0
Ciprofloxacin	≤0.5	>4	≤0.5–>4	80.3/19.7
Clindamycin	≤0.25	2	≤0.25–>2	88.7/9.9
Daptomycin	0.25	0.5	≤0.06–1	100.0/−
Erythromycin	≤0.25	>2	≤0.25–>2	56.6/42.5
Gentamicin	≤2	≤2	≤2–>8	98.1/0.9
Levofloxacin	≤0.5	>4	≤0.5–>4	79.8/19.7
Quinupristin-dalfopristin	≤0.25	≤0.25	≤0.25–1	100.0/0.0
Teicoplanin	≤2	4	≤2–8	100.0/0.0
Tetracycline	≤2	8	≤2–>8	89.2/9.4
Trimethoprim-sulfamethoxazole	≤0.5	>2	≤0.5–>2	87.3/12.7
Vancomycin	1	2	≤0.12–2	100.0/0.0
Oxacillin resistant (603)[c]				
Linezolid	1	1	0.25–>8	98.2/1.8
Ciprofloxacin	>4	>4	≤0.5–>4	31.2/67.7
Clindamycin	≤0.25	>2	≤0.25–>2	59.7/38.8
Daptomycin	0.25	0.5	≤0.06–1	100.0/−
Erythromycin	>2	>2	≤0.25–>2	24.9/73.8
Gentamicin	≤2	>8	≤2–>8	64.7/27.4
Levofloxacin	>4	>4	≤0.5–>4	30.8/67.2
Quinupristin-dalfopristin	≤0.25	0.5	≤0.25–2	99.8/0.0
Teicoplanin	≤2	8	≤2–16	96.2/0.0
Tetracycline	≤2	>8	≤2–>8	83.7/15.8
Trimethoprim-sulfamethoxazole	2	>2	≤0.5–>2	50.5/49.5
Vancomycin	2	2	≤0.12–4	100.0/0.0
Enterococcus spp. (1,017)[d]				
Linezolid	1	2	0.25–>8	98.9/0.8

Continued on following page

TABLE 2—*Continued*

Organism, resistance group (no. tested), and antimicrobial agent	MIC (μg/ml)			% of isolates susceptible/ % resistant[a]
	50%	90%	Range	
Ampicillin	2	>16	≤1–>16	67.8/32.2
Ciprofloxacin	>4	>4	≤0.5–>4	37.9/53.9
Daptomycin	1	2	≤0.06–8	99.9/−
Erythromycin	>2	>2	≤0.25–>2	9.0/70.1
Levofloxacin	>4	>4	≤0.5–>4	45.4/52.6
Quinupristin-dalfopristin	>2	>2	≤0.25–>2	31.7/63.9
Teicoplanin	≤2	>16	≤2–>16	71.8/26.8
Vancomycin	2	>16	0.25–>16	70.3/29.0
S. pneumoniae (659)				
Linezolid	1	1	≤0.12–2	100.0/−
Amoxicillin-clavulanate	≤1	8	≤1–16	81.8/15.5
Ceftriaxone	≤0.25	2	≤0.25–8	87.1/2.1
Clindamycin	≤0.25	>2	≤0.25–>2	77.8/21.6
Erythromycin	≤0.25	>2	≤0.25–>2	58.1/41.1
Levofloxacin	1	1	≤0.5–>4	99.1/0.9
Penicillin[e]	≤0.03	4	≤0.03–>4	83.6/2.4
Penicillin[f]	≤0.03	4	≤0.03–>4	57.7/21.5
Quinupristin-dalfopristin	0.5	0.5	≤0.25–2	99.5/0.0
Tetracycline	≤2	>8	≤2–>8	75.9/23.8
Trimethoprim-sulfamethoxazole	≤0.5	>2	≤0.5–>2	63.7/29.6
Vancomycin	≤1	1	≤1–1	100.0/−
Viridans group streptococci (264)[g]				
Linezolid	1	1	0.12–2	100.0/−
Ceftriaxone	≤0.25	1	≤0.25–>32	91.7/3.8
Clindamycin	≤0.25	0.5	≤0.25–>2	88.6/9.1
Daptomycin	0.25	1	≤0.06–2	99.6/−
Erythromycin	1	>2	≤0.25–>2	46.2/50.8
Levofloxacin	1	2	≤0.5–>4	90.2/8.0
Penicillin	0.06	1	≤0.015–32	77.3/3.0
Quinupristin-dalfopristin	0.5	1	≤0.25–>2	97.7/0.4
Tetracycline	≤2	>8	≤2–>8	60.6/36.4
Vancomycin	0.5	1	≤0.12–2	99.6/−
Beta-hemolytic streptococci (401)[h]				
Linezolid	1	1	≤0.06–2	100.0/−
Ceftriaxone	≤0.25	≤0.25	≤0.25–0.5	100.0/−
Clindamycin	≤0.25	>2	≤0.25–>2	81.8/17.2
Daptomycin	0.12	0.25	≤0.06–0.5	100.0/−
Erythromycin	≤0.25	>2	≤0.25–>2	62.8/36.4
Levofloxacin	≤0.5	1	≤0.5–>4	99.5/0.5
Penicillin	0.03	0.06	≤0.015–0.12	100.0/−
Quinupristin-dalfopristin	≤0.25	0.5	≤0.25–1	100.0/0.0
Tetracycline	>8	>8	≤2–>8	39.2/58.9
Vancomycin	0.5	0.5	≤0.12–1	100.0/−

[a] Criteria as published by the CLSI (2010); β-lactam susceptibility should be directed by the oxacillin test results. −, no interpretive criteria for this category.

[b] Includes *Staphylococcus capitis* (9 isolates), *Staphylococcus epidermidis* (47 isolates), *Staphylococcus haemolyticus* (1 isolate), *Staphylococcus hominis* (7 isolates), *Staphylococcus intermedius* (2 isolates), *Staphylococcus lugdunensis* (1 isolate), *Staphylococcus saccharolyticus* (1 isolate), *Staphylococcus saprophyticus* (2 isolates), *Staphylococcus schleiferi* (1 isolate), *Staphylococcus warnerii* (4 isolates), and coagulase-negative staphylococci not determined to the species level (138 isolates).

[c] Includes *Staphylococcus auricularis* (3 isolates), *Staphylococcus capitis* (3 isolates), *Staphylococcus epidermidis* (187 isolates), *Staphylococcus haemolyticus* (14 isolates), *Staphylococcus hominis* (22 isolates), *Staphylococcus lugdunensis* (14 isolates), *Staphylococcus saprophyticus* (8 isolates), *Staphylococcus simulans* (3 isolates), *Staphylococcus warnerii* (7 isolates), and coagulase-negative staphylococci not determined to the species level (342 isolates).

[d] Includes *Enterococcus avium* (9 isolates), *Enterococcus casseliflavus* (9 isolates), *Enterococcus durans* (5 isolates), *Enterococcus faecalis* (635 isolates), *Enterococcus faecium* (339 isolates), *Enterococcus gallinarum* (9 isolates), *Enterococcus hirae* (2 isolates), *Enterococcus raffinosus* (3 isolates), and enterococci not determined to the species level (6 isolates).

[e] CLSI (2010) criteria for "penicillin parenteral (nonmeningitis)" (susceptible at ≤2 μg/ml and resistant at ≥8 μg/ml).

[f] CLSI (2010) criteria for "penicillin parenteral (oral penicillin V)" (susceptible at ≤0.06 μg/ml and resistant at ≥2 μg/ml).

[g] Includes *Streptococcus anginosus* (15 isolates), *Streptococcus bovis* (9 isolates), *Streptococcus constellatus* (7 isolates), *Streptococcus equinus* (1 isolate), *Streptococcus gallolyticus* (2 isolates), *Streptococcus gordonii* (1 isolate), *Streptococcus intermedius* (4 isolates), *Streptococcus milleri* (2 isolates), *Streptococcus mitis* (42 isolates), *Streptococcus oralis* (7 isolates), *Streptococcus parasanguinis* (7 isolates), *Streptococcus porcinus* (1 isolate), *Streptococcus salivarius* (14 isolates), *Streptococcus sanguinis* (8 isolates), *Streptococcus vestibularis* (1 isolate), alpha-hemolytic streptococci not determined to the species level (3 isolates), and viridans group streptococci not determined to the species level (140 isolates).

[h] Includes *Streptococcus dysgalactiae* (8 isolates), group A streptococci (121 isolates), group B streptococci (217 isolates), group C streptococci (15 isolates), and group G streptococci (40 isolates).

TABLE 3. Six-year trends in linezolid resistance rates observed in the LEADER Program (2004 to 2009; 33,378 isolates)

Organism (no. tested)	% of isolates nonsusceptible or resistant to linezolid[a]					
	2004	2005	2006	2007	2008	2009
S. aureus (18,537)	0.00	0.03	0.03	0.06	0.10	0.15
CoNS (4,526)	0.20	1.13	1.61	1.76	1.64	1.47
Enterococci (4,577)	0.80	0.64	1.83	1.13	0.55	0.49
S. pneumoniae (3,292)	0.00	0.00	0.00	0.00	0.00	0.00
viridans group streptococci (925)	NT	NT	0.00	0.00	0.00	0.00
Beta-hemolytic streptococci (1,521)	NT	NT	0.00	0.00	0.00	0.00
All organisms (33,378)	0.14	0.24	0.45	0.44	0.36	0.34

[a] CLSI interpretation criteria from M100-S20-U (2010). NT, not tested.

TABLE 5. *E. faecium* isolates resistant to linezolid (MIC, ≥8 μg/ml) in the 2009 LEADER Program[a]

Isolate	City and state	Age (yr)/sex[b]	Linezolid MIC (μg/ml)[c]
027-6948A	Louisville, KY	29/M	32
027-5561A	Louisville, KY	31/M	16
027-870A	Louisville, KY	39/M	8
027-1686A	Louisville, KY	39/M	8
427-414L	Wichita, KS	56/M	16
030-2539A	Charlottesville, VA	6/M	8
051-6128A	Salt Lake City, UT	19/M	16
021-939D	Seattle, WA	53/F	8

[a] For all isolates listed, the resistance mechanism was G2576T and the PFGE pattern was EFM27B1.
[b] M, male; F, female.
[c] MIC from a reference frozen-form panel with a linezolid MIC range to 128 μg/ml.

Linezolid activity against enterococci. The tested enterococcal species strains (1,017 strains) were most likely to be identified as *E. faecalis* (635 strains [62.4%]) or *E. faecium* (339 strains [33.3%]). Among these strains, the ampicillin susceptibility rate was only 67.8% (decreasing from 71.3% in 2008) and vancomycin-resistant-enterococcus (VRE) rates differed by Census region, ranging from 24.5% (West North Central) to 41.3% (Mid-Atlantic). The VRE rate for the entire enterococcal sample was 29.0% (27.2 to 29.8% in 2006 to 2008), and the VanA resistance phenotype represented 92.4% of the VRE identified.

Linezolid and daptomycin were the most active agents tested against enterococci, with susceptibility rates at 98.9 and 99.9%, respectively (Table 2). A total of eight enterococci (all *E. faecium*) had linezolid MICs at ≥8 μg/ml, and all eight strains harbored the G2576T mutation in the 23S rRNA (Table 5). These strains were found in Kentucky (4 strains), Utah (1 strain), Virginia (1 strain), Kansas (1 strain), and Washington (1 strain). Only three enterococci had linezolid MIC values at 4 μg/ml (intermediate), one each from three different Census regions (Pacific, West North Central, and Mid-Atlantic). As noted with the CoNS, clonal analyses by molecular methods indicated that where more than one enterococcal strain was documented in the LEADER Program site sampling (for example, Louisville, KY), a clonal outbreak was continuing (Table 5), as described in the LEADER 2008 study (11). Therefore, only five (0.49%) *E. faecium* strains were classified as linezolid resistant in this 2009 surveillance, the lowest percentage found in the past six study years (2004 to 2009) (Table 3).

Linezolid activity against streptococcal species. The comparative linezolid activity tested against 659 *S. pneumoniae* is found in Table 2. Significant sample size was achieved across all Census regions (51 to 84 strains; average, 69 strains/region, or 10.9 strains/site). Resistance to oral penicillin V (MIC, ≥2 μg/ml; CLSI M100-S20-U) was noted in 21.5% of strains (range, 16.2% [Pacific] to 26.8% [Mountain]) and was increased from 13.2% in 2006 (15) and 19.0% in 2007 (17) but appears stable compared to the 22.0% reported in 2008 (11). Erythromycin resistance (MIC, ≥1 μg/ml) was 41.1%, markedly increased from 2008 (34.2%). Macrolide resistance in pneumococci continues to escalate more rapidly than β-lactam or other class resistances, mainly due to the expansion of the multidrug-resistant serotype 19A clone (14, 18). Other drugs whose resistance rates have increased since 2008 include clin-

TABLE 4. *Staphylococcus* species resistant to linezolid (MIC, ≥8 μg/ml) in the 2009 LEADER Program

Isolate	Organism	City and state	Age (yr)/sex[a]	Linezolid MIC (μg/ml)[b]	Resistance mechanism(s)
004-272C	*S. aureus*	Akron, OH	52/M	16	*cfr*
444-2031L	*S. aureus*	Palo Alto, CA	14/F	16	G2576T
424-2265L	*S. aureus*	Hartford, CT	68/M	8	L3 (S145 deletion)
027-1687A	*S. aureus*	Louisville, KY	53/M	16	*cfr*
427-99L	*S. aureus*	Wichita, KS	17/M	16	G2576T
004-3417A	*S. epidermidis*	Akron, OH	89/M	128	G2576T
412-2466L	*S. epidermidis*	Memphis, TN	71/M	8	L3 (V154L, L101V, A157R), L4 (P171S)
426-2174L	*S. epidermidis*	Tempe, AZ	55/M	128	*cfr*
426-2179L	*S. epidermidis*	Tempe, AZ	73/F	32	G2576T
107-7715A	*S. epidermidis*	Lexington, KY	40/M	16	L3 (A157R, L101V, V154L), L4 (N158S)
443-2409L	*S. epidermidis*	St. Paul, MN	84/M	32	G2576T
003-4596A	*S. epidermidis*	Detroit, MI	64/F	16	L3 (H146Q), L4 (N158S)
003-4593A	*S. capitis*	Detroit, MI	74/F	8	*cfr*
441-1590L	*S. epidermidis*	Boston, MA	59/M	16	L3 (H146Q), L4 (N158S)
129-25A	*S. epidermidis*	New Brunswick, NJ	71/M	128	G2576T
051-2286A	*S. epidermidis*	Salt Lake City, UT	55/F	128	G2576T
116-13800A	*S. epidermidis*	Houston, TX	24/M	128	G2576T

[a] M, male; F, female.
[b] MIC from a reference frozen-form panel with a linezolid MIC range to 128 μg/ml.

damycin (19.5 to 21.6%), levofloxacin (0.5 to 0.9%), ceftriaxone (1.6 to 2.1%), tetracycline (21.6 to 23.8%), TMP-SMX (24.1 to 29.6%), and, importantly, amoxicillin-clavulanate (12.9 to 15.5%). Ciprofloxacin MIC values at ≥4 μg/ml accounted for 3.9% (4.4% in 2008) of the *S. pneumoniae* isolates, a statistic indicating possible single-step target mutations (usually in *parC*) (4). However, the levofloxacin resistance remained low at only 0.9% (MIC, ≥8 μg/ml).

Linezolid was active against all *S. pneumoniae* strains (MIC_{50} and MIC_{90}, 1 μg/ml), and only 3.9% of strains had MICs at 2 μg/ml (susceptibility breakpoint) (Table 1). Other agents with high activity (Table 2) against these pneumococci were amoxicillin-clavulanic acid (MIC_{50}, ≤1 μg/ml; 81.8% susceptible), ceftriaxone (MIC_{50}, ≤0.25; 87.1% susceptible), levofloxacin (MIC_{50}, 1; 99.1% susceptible), quinupristin-dalfopristin (Q-D) (MIC_{50}, 0.5; 99.5% susceptible), and vancomycin (MIC_{50}, ≤1; 100.0% susceptible), all with rates clearly lower than those observed in 2008 (for example, ceftriaxone susceptibility decreasing from 91.4 to 87.1%).

A total of 264 viridans group streptococcus isolates that included 15 or more different species were tested, although most laboratories only reported these organisms as alpha-hemolytic or viridans group streptococci not determined to the species level (Table 2). Several older antimicrobial agents are compromised in their coverage of these streptococcal species, including erythromycin (46.2% susceptible), tetracycline (60.6% susceptible), and penicillin (77.3% susceptible). Penicillin resistance (MIC, ≥4 μg/ml) was noted in only 3.0% of these species, however. This resistance pattern minimally differed across Census regions but showed increased susceptibility for some agents in 2009. Clindamycin was active against 88.6% of isolates, and Q-D inhibited 97.7% of strains at ≤1 μg/ml (0.4% resistance). A remarkable finding was the level of fluoroquinolone resistance as represented by levofloxacin. This has escalated from 5.9 to 10.8% over the 2006-to-2008 interval, but in 2009, the rate stabilized downward to 8.0%. Also, the ciprofloxacin MIC value at ≥4 μg/ml was 19.7%, a slight decrease from 23.3% found in 2008, indicating high potential for target mutational events.

The linezolid MIC values among these streptococci were dominantly 0.5 or 1 μg/ml (MIC_{90}, 1 μg/ml) (Tables 1 and 2). Only 2.3% of viridans group strains were found with a linezolid MIC at the susceptible breakpoint of 2 μg/ml (Table 1). Like linezolid, two other agents, daptomycin (99.6% susceptible) and vancomycin (99.6% susceptible), were highly active against all or nearly all viridans group streptococcal species tested (Table 2).

Among 401 isolates of beta-hemolytic streptococci, the most common serogroups were *S. agalactiae* group B (217 isolates [54.1%]) and *S. pyogenes* group A (121 isolates [30.2%]), with groups C and G accounting for the vast majority of the remaining isolates. Significant resistances (Table 2) were identified for erythromycin (36.4%, increasing from 27.4% in 2008), clindamycin (17.2%), and tetracycline (58.9%). The clindamycin resistances continue to differ yearly and, when paired with the erythromycin MIC results, provide information about the occurrence of constitutive (47.3% overall) versus efflux resistance phenotypes. Linezolid, ceftriaxone, daptomycin, penicillin, Q-D, and vancomycin inhibited all beta-hemolytic streptococci tested at their CLSI susceptibility breakpoints (Table 2). Like the viridans group streptococci, levofloxacin resistance (0.5%) was observed (0.5% also in 2007) in these beta-hemolytic species. The linezolid MIC range was ≤0.06 to 2 μg/ml, with clear modal MIC, MIC_{50}, and MIC_{90} values at 1 μg/ml (Table 1). Only 0.5% of strains had linezolid MICs at the breakpoint of 2 μg/ml (Table 1).

DISCUSSION

The overall activities of linezolid versus these tested isolates from the six organism groups are shown in Table 1. Generally, linezolid MIC distributions are narrow, with nearly 100.0% of results within 3 or 4 doubling dilutions. This characteristic of potent central tendencies has been consistent within ZAPS, ZAAPS, and LEADER Program publications for over 10 years. Six-year trends in linezolid resistance rates from 33,378 isolates tested in the LEADER Program (2004 to 2009) (Table 3) show that linezolid resistance rates have not increased significantly since 2006, but new occurrences of resistance mechanisms have been discovered, particularly in staphylococci (*cfr*, T2504A, and L protein mutations) (11, 17, 22). Some recent increases in nonsusceptibility have been driven by clonal dissemination of mutant strains within several LEADER Program-monitored hospitals (2). Continued surveillance seems to be an important tool for assisting these sites in addressing evolving resistance problems.

In conclusion, the LEADER Program (2009) monitoring for linezolid resistance in significant sample sizes (testing 6,414 Gram-positive pathogens from 56 U.S. medical centers) continues to show excellent activity and a sustained susceptibility rate of 99.66% overall (99.64% in 2008). Also, linezolid MIC population distributions remained unchanged, without evidence of "MIC creep," among all indicated species. Over the past four LEADER study years, several genetically identical strains nonsusceptible to linezolid (including mobile *cfr*-encoded strains) have been found in the same medical centers, which demonstrates persistence but also suggests a lack of significant dissemination. These important findings from the LEADER Program (2009) document the need for continued monitoring and illustrate the sensitivity of this surveillance network for recognition of new and emerging mechanisms of oxazolidinone resistance within the United States.

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