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Report of Linezolid Activity from the Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) Program 2012 (Europe, Latin America, Asia Pacific, Canada)

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ABSTRACT

Background: To report the most recent full-year of linezolid (LZD) resistance surveillance (ZAAPS Program) monitoring 73 medical centers for 2012, a total of 7,972 Gram-positive (GP) organisms were consecutively collected (prevalence design) in 33 countries. Results represent the eleventh yearly sample for this central laboratory-based study.

Methods: All susceptibility (S) tests were performed applying reference CLSI broth microdilution methods, using validated panels. The number of strains tested were: *S. aureus* (SA; 4,077, 32.2% MRSA), coagulase-negative staphylococcal species (CoNS; 905, 76.5% methicillin-resistant [R]), *Enterococcus* spp. (ENT; 797 with 434 *E. faecalis* [EF] and 363 *E. faecium* [EFM]), *S. pneumoniae* (SPN; 1,210), viridans gr. (VGS, 400) and β-hemolytic streptococci (BHS; 583). Strains with LZD MIC at ≥4 μg/ml were tested by molecular methods (PCR/gene sequencing, PFGE) to determine LZD resistance (R) mechanisms (23S target, L3, L4 mutations and *cfr*).

Results: LZD potency for indicated species in the ZAAPS Program remained stable (overall LZD R at 0.16% since 2008). Nearly all (97.8%) MIC values for LZD among all monitored species were 0.5, 1 or 2 μg/ml. LZD-non-S isolates were detected among SA (3), CoNS (1 *S. cohnii*, 1 *S. haemolyticus*, and 6 *S. epidermidis*) and ENT (4 EF, 1 EFM and 1 *E. avium*), see **Table**. LZD R mechanisms included *cfr* (3 strains), L4 mutation (6), L3 mutation (4), G2576T (6) and C2319T (1). Nine countries had LZD-R or –intermediate (I) strains with LZD MIC values at 4-32 μg/ml. Other agents with >90% S rates versus SA were: daptomycin (99.9%), teicoplanin (>99.9%), trimethoprim/sulfamethoxazole (97.9%) and vancomycin (100.0%).

Conclusion: Overall, LZD remained active against 99.79%, with only 17 LZD non-S strains from the six GP pathogen groups tested for the 2012 worldwide ZAAPS Program and showed no escalation of R or MIC creep.

Table. LZD non-susceptible strains in the 2012 ZAAPS surveillance program

Organism (number)	Country (LZD MIC; μg/ml)	Resistance Mechanism
S. aureus (3)[a]	Italy (8)	G2576T
	Italy (8), Hong Kong (8)	None detected
CoNS (8)	Mexico (>8), Italy (>8)	L3 mutation, L4 mutation
	Brazil (>8), Brazil (>8), Italy (>8)	G2576T
	Italy (>8)	C2319T, L3 mutation, L4 mutation
	Italy (>8)	*cfr*, L3 mutation, L4 mutation
	Mexico (>8)	*cfr*
Enterococci (6)	Poland (4), Ireland (4)	L4 mutation
	Poland (8), Germany (8)	G2576T
	China (4), Taiwan (4)	None detected

a. There was a SA strain from Brazil with a LZD MIC of 4 μg/ml (susceptible) with *cfr*.

INTRODUCTION

Linezolid continues to be the only oxazolidinone agent approved for clinical use by various national or regional regulatory agencies, and is an important therapeutic agent for infections caused by commonly occurring antimicrobial-resistant Gram-positive pathogens. Oxazolidinone resistance has been detected, mainly among *Enterococcus* species and the coagulase-negative staphylococci (CoNS; *Staphylococcus epidermidis* and some other species), but remains relatively rare ≤1%. The occurrence rates for *S. aureus* and streptococci are even less frequent (<0.1%). These resistance occurrences have been associated with recognized risk factors such as prolonged therapeutic exposure, but oxazolidinone-resistant strains have emerged in patients without prior linezolid exposure, each attributable to clonal dissemination from other patients in the same hospital environment. Also, new mechanisms of resistance (*cfr,* L3 and/or L4 protein alteration) have emerged in CoNS, some of animal origin; but in the more recent ZAAPS and LEADER surveillance reports, the mobile *cfr* gene has been observed in *S. aureus* and CoNS human infections on several continents.

In this study, we report the results from the 2012 linezolid resistance surveillance for ex-USA nations.

METHODS

A total of 7,972 Gram-positive strains were collected from 73 medical centers on five continents (33 countries, **Table 1**). Each site submitted 250-500 isolates to reach a targeted 200 Gram-positive organisms per country except for China (600) and Japan (400). JMI Laboratories (North Liberty, Iowa, USA) or Women's and Children's Hospital (Adelaide, Australia; Australia and New Zealand isolates only) performed confirmatory organism identification tests and reference broth microdilution susceptibility testing by Clinical and Laboratory Standards Institute (CLSI) methods (M07-A9, 2012; M100-S23, 2013). Susceptibility testing was performed using validated microdilution panels with cation-adjusted Mueller-Hinton broth (2-5% lysed horse blood added for testing streptococci).

Isolates with elevated MIC values to linezolid (MIC, ≥4 μg/ml) upon initial testing were confirmed by repeat dry- and frozen-form broth microdilution testing, Etest and disk diffusion methods (CLSI M02-A10, 2012). Molecular testing was performed to identify the target site mutation (23S rRNA, L3 and/or L4 proteins) or gene (*cfr*)-based mechanism as well as possible epidemic clonality using pulsed field gel electrophoresis (PFGE) and/or various other PCR-based tests.

For 2012, all results were interpreted to susceptibility category by both CLSI (M100-S23, 2013) and EUCAST (2013) criteria/breakpoints, where available. Tigecycline breakpoints were those found in the USA-FDA approved product package insert.

RESULTS

- The activity of linezolid in the 2012 ZAAPS Program sampling of 73 medical centers (7,972 Gram-positive strains) is presented in **Table 1**. The "all organism" MIC_{50} and MIC_{90} were 1 and 2 μg/mL, respectively with only 17 (0.21%) non-susceptible isolates.
- *S. aureus* linezolid MIC values were distributed across a very narrow range (99.9% of linezolid MIC values were at 0.25-2 μg/ml). The global methicillin-resistant *S. aureus* (MRSA) rate was 32.2%. When the MRSA MIC distribution for linezolid was compared to the methicillin-susceptible *S. aureus* (MSSA) strains (**Table 1**), the $MIC_{50/90}$ results for both were 1/2 μg/ml, respectively with a mode at 1 μg/ml.
- Modal linezolid MIC values varied from 0.5 μg/ml (CoNS) to 1 μg/ml (all other monitored species; **Tables 1** and **3**), results consistent with all prior ZAAPS surveillance years (2002-2011). Linezolid MIC values were generally lower by two-fold for CoNS when compared to *S. aureus*.
- All *S. aureus* isolates were susceptible to vancomycin and 99.9% to teicoplanin (**Table 2**). Erythromycin and clindamycin resistance were at 35.6 and 19.4%, respectively and for MRSA were 73.7 and 48.6%, (**Table 2**).
- Linezolid MIC distributions for enterococci were consistent with previous ZAAPS surveillance with $MIC_{50/90}$ of 1/2 μg/ml.
- Linezolid activity remained highly potent (MIC_{50} and MIC_{90}, 1 μg/ml) for all monitored streptococci groups (β-hemolytic, viridans group and pneumococci).
- The distribution of sampled Gram-positive organisms and location/type of resistance are presented in **Table 2**.
  - There were 3 *S. aureus* strains with linezolid MIC values at ≥8 μg/mL, one from Catania, Italy, one from Genova, Italy, and one from Hong Kong. These three isolates represented <0.1% of all *S. aureus* collected. In addition, there was one MSSA (linezolid susceptible, MIC = 4 μg/ml) containing a *cfr* and an L4 mutation from Sao Paulo, Brazil (**Table 2**).
  - There were 0.9% (8) linezolid-resistant CoNS strains, a decrease from 1.2% (11) in 2011. Six *S. epidermidis*, one *S. haemolyticus*, and one *S. cohnii* had linezolid MIC values at ≥8 μg/ml. A *S. cohnii* from Mexico and a *S. epidermidis* from Italy both were found to contain *cfr.*
  - Six linezolid-non-susceptible (≥4 μg/ml) enterococci were documented in five countries with no evidence of clonal spread. The vancomycin-resistance rate among enterococci was 17.7% (19.1% including intermediate category).

Table 1. Cumulative % inhibited at each linezolid MIC when testing six different groups of Gram-positive cocci isolated on four continents (ZAAPS, 2012)[a].

	Cum. % inhibited at linezolid MIC (μg/ml)						
Organism group (no. tested)	≤0.12	0.25	0.5	1	2	4	≥8
β-hemolytic streptococci (583)	0.0	0.0	22.3	99.8	100.0	-	-
Viridans group streptococci (400)	0.5	5.5	47.5	98.3	100.0	-	-
S. pneumoniae (1,210)	0.2	2.9	38.2	97.2	100.0	-	-
S. aureus (4,077)	0.0	0.5	11.6	81.5	99.90	99.9	100.0
Enterococci (797)	0.0	0.6	17.2	88.3	99.2	99.7	100.0
CoNS (905)	0.0	8.1	73.7	98.1	99.1	99.1	100.0
All Organisms (7,972)	0.1	1.9	25.8	88.6	99.8	99.8	100.0

a. Organism groups were ranked in decreasing order of susceptibility to the oxazolidinone.

Table 2. Distribution of non-susceptible organisms by country and location/type of oxazolidinone resistance.

Organism	Country[a]	Linezolid MIC (μg/ml)	Resistance mechanism
S. aureus	Italy	8	L3 (Q136H and H146Δ), L4 (G69A, T70P, G71S)
S. aureus	Italy	8	G2576T
S. aureus	Hong Kong	8	None detected
S. aureus	Brazil	8[b]	Cfr, L4 (V142)
S. cohnii	Mexico	>8	Cfr , L4 (V155I, A133T), L3 (D159Y)
S. haemolyticus	Brazil	>8	G2576T
S. epidermidis	Italy	>8	C2319T, L4 (71G72 ins), L3 (V154L, H146Q)
S. epidermidis	Italy	>8	L4 (71G72 ins), L3 (V154L, H146Q, A157R)
S. epidermidis	Italy	>8	G2576T
S. epidermidis	Italy	>8	Cfr, L3 (F147L)
S. epidermidis	Brazil	>8	G2576T
S. epidermidis	Mexico	>8	Cfr, L3 (D159Y)
E. avium	Poland	4	L4 (P171S)
E. faecalis	Poland	8	G2576T
E. faecalis	Ireland	4	L4 (F101L)
E. faecalis	China	4	None detected
E. faecalis	Taiwan	4	None detected
E. faecium	Germany	8	G2576T

a. Percentage of non-susceptible isolates by country (number of isolates); Italy 6/352, 1.7%; Taiwan 1/68, 1.5%; Poland 2/181, 1.1%; Hong Kong 1/93, 1.1%; Mexico 2/206, 1.0%; Brazil 3/435, 0.7%; Ireland 1/212, 0.5%; Germany 1/359, 0.3%; China 1/523, 0.2%. Non-susceptible isolates were not found in the following countries (number of isolates): Canada (199), Argentina (203), Chile (222), Belgium (198), Czech Republic (121), France (330), Greece (203), Hungary (89), Israel (54), Portugal (187), Russia (263), Slovenia (107), Spain (300), Sweden (298), Turkey (297), Ukraine (101), United Kingdom (426), Australia (813), Japan (389), Korea (240), Malaysia (100), New Zealand (256), Singapore (97), Thailand (50).

b. Isolate considered susceptible by CLSI breakpoint (≥8 μg/ml) but contains *cfr* gene.

Table 3. Comparative activity of linezolid tested against 7,972 Gram-positive cocci from 33 nations in the ZAAPS Program (2012).

Organism (no. tested)/ antimicrobial agent	MIC (μg/ml)			% by category[a] (Susceptible/Resistant)	
	50%	90%	Range	CLSI [2013]	EUCAST [2013]
S. aureus					
All strains (4,077)					
Linezolid	1	2	≤0.12-8	99.9 / 0.1	99.9 / 0.1
Ceftriaxone	4	>8	≤0.06->8	67.8 / 32.2	67.8 / 32.2
Clindamycin	≤0.25	>2	≤0.25->2	80.4 / 19.4	80.0 / 19.6
Erythromycin	0.25	>16	≤0.12->16	62.6 / 35.6	62.9 / 36.9
Gentamicin	≤1	>8	≤1->8	83.9 / 15.2	83.3 / 16.7
Levofloxacin	0.25	>4	≤0.12->4	70.3 / 29.2	70.3 / 29.2
Oxacillin	0.5	>2	≤0.25->2	67.8 / 32.2	67.8 / 32.2
Tetracycline	≤0.25	>8	≤0.25->8	85.5 / 13.7	85.0 / 14.7
TMP/SMX[b]	≤0.5	≤0.5	≤0.5->4	97.9 / 2.1	97.9 / 1.7
Teicoplanin	≤2	≤2	≤2-16	>99.9 / <0.1	99.0 / 1.0
Vancomycin	1	1	≤0.12-2	100.0 / 0.0	100.0 / 0.0
MRSA (1,312)					
Linezolid	1	2	0.25-8	99.8 / 0.2	99.8 / 0.2
MSSA (2,765)					
Linezolid	1	2	≤0.12-4	100.0 / 0.0	100.0 / 0.0
Coagulase-negative staphylococci (905)[c]					
Linezolid	0.5	1	0.25->8	99.1 / 0.9	99.1 / 0.9
Ceftriaxone	>8	>8	0.25->8	23.5 / 76.5	23.5 / 76.5
Clindamycin	≤0.25	>2	≤0.25->2	73.2 / 25.7	71.0 / 26.8
Erythromycin	>16	>16	≤0.12->16	37.4 / 61.7	37.7 / 62.1
Gentamicin	≤1	>8	≤1->8	56.1 / 36.5	50.8 / 49.2
Levofloxacin	1	>4	≤0.12->4	51.3 / 46.7	51.3 / 46.7
Oxacillin[a]	>2	>2	≤0.25->2	23.5 / 76.5	23.5 / 76.5
Tetracycline	0.5	>8	≤0.25->8	84.3 / 14.0	73.8 / 16.9
TMP/SMX[b]	≤0.5	>4	≤0.5->4	65.6 / 34.4	65.6 / 20.6
Teicoplanin	≤2	8	≤2->16	94.5 / 0.7	80.2 / 19.8
Vancomycin	1	2	0.25-4	100.0 / 0.0	100.0 / 0.0
Enterococci					
All strains (797)[d]					
Linezolid	1	2	0.25-8	99.2 / 0.3	99.7 / 0.3
Ampicillin	2	>8	≤0.25->8	61.0 / 39.0	59.1 / 39.0
Erythromycin	>16	>16	≤0.12->16	6.9 / 69.6	- / -
Levofloxacin	>4	>4	0.25->4	43.8 / 54.1	- / -
Piperacillin/tazobactam	16	>64	1->64	61.0 / -	61.0 / -
Teicoplanin	≤2	>16	≤2->16	84.7 / 14.1	84.2 / 15.8
Vancomycin	1	>16	0.25->16	80.9 / 17.7	80.9 / 19.1
VRE (152)					
Linezolid	1	2	0.5-8	99.3 / 0.7	99.3 / 0.7
VSE (645)					
Linezolid	1	2	0.25-8	99.2 / 0.2	99.8 / 0.2
S. pneumoniae					
All strains (1,210)					
Linezolid	1	1	≤0.12-2	100.0 / -	100.0 / 0.0
Amoxacillin/clavulanic acid	≤1	4	≤1->8	87.5 / 9.8	- / -
Ceftriaxone	≤0.06	2	≤0.06->8	87.5 / 2.5	78.4 / 2.5
Ciprofloxacin	1	2	0.06->4	(5.8)[e]	0.2 / 5.8
Clindamycin	≤0.25	>2	≤0.25->2	75.7 / 23.9	76.1 / 23.9
Erythromycin	≤0.12	>16	≤0.12->16	63.1 / 36.6	63.1 / 36.6
Levofloxacin	1	1	≤0.12->4	98.3 / 1.3	98.3 / 1.7
Penicillin[f]	≤0.06	4	≤0.06-8	63.7 (89.7) / 19.6 (1.9)	63.7 / 10.3
Tetracycline	0.5	>8	≤0.25->8	67.2 / 31.3	66.8 / 32.8
TMP/SMX[b]	≤0.5	>4	≤0.5->4	62.8 / 26.1	70.0 / 26.1
Vancomycin	0.25	0.5	≤0.12-0.5	100.0 / -	100.0 / 0.0
Viridans group streptococci (400)[g]					
Linezolid	1	1	≤0.12-2	100.0 / -	- / -
Ceftriaxone	0.25	1	≤0.06->8	93.0 / 4.8	87.8 / 12.3
Clindamycin	≤0.25	>2	≤0.25->2	85.0 / 14.3	85.7 / 14.3
Erythromycin	≤0.12	>16	≤0.12->16	57.8 / 40.3	- / -
Levofloxacin	1	2	≤0.12->4	95.7 / 3.3	- / -
Penicillin[f]	≤0.06	1	≤0.06->4	77.0 / 3.8	84.0 / 3.8
Tetracycline	0.5	>8	≤0.25->8	61.9 / 36.6	- / -
Vancomycin	0.5	1	≤0.12-1	100.0 / -	100.0 / 0.0
β-hemolytic streptococci (583)[h]					
Linezolid	1	1	0.5-1	100.0 / -	100.0 / 0.0
Amoxacillin/clavulanic acid	≤1	≤1	≤1	- / -	100.0 / 0.0
Ceftriaxone	≤0.06	0.12	≤0.06-0.5	100.0 / -	100.0 / 0.0
Clindamycin	≤0.25	≤0.25	≤0.25->2	91.9 / 8.1	91.9 / 8.1
Erythromycin	≤0.12	4	≤0.12->16	82.3 / 16.7	82.3 / 16.7
Levofloxacin	0.5	1	0.25->4	96.6 / 3.4	93.7 / 3.4
Penicillin[f]	≤0.06	≤0.06	≤0.06-0.12	100.0 / -	100.0 / 0.0
Tetracycline	0.5	>8	≤0.25->8	56.8 / 40.4	55.8 / 43.2
Vancomycin	0.25	0.5	≤0.12-1	100.0 / -	100.0 / 0.0

a. Criteria as published by the CLSI [2013], beta-lactam susceptibility should be directed by the oxacillin test results for staphylococci and by ampicillin or penicillin for Enterococcus or streptococci.
b. TMP/SMX=trimethoprim/sulfamethoxazole.
c. Includes: 20 species (898 strains) and unidentified coagulase-negative staphylococci (7 strains).
d. Includes: *Enterococcus faecalis* (434 strains), *E. faecium* (333 strains), and 30 other enterococci.
e. Percentage of pneumococci or other streptococci with ciprofloxacin MICs at ≥4 μg/ml, indicating possible QRDR mutations.
f. CLSI 2013 susceptibility breakpoints for penicillin oral penicillin/*V* (nonmeningitis in parenthesis).
g. Includes: 17 species (388 strains), *Streptococcus bovis* group (8 strains), unidentified viridans group streptococci (4 strains).
h. Includes; Group A (292 strains), Group B (169 strains), Group C (25 strains), Group F (2 strains), Group G (67 strains), and two other species (28 strains).

CONCLUSIONS

- Linezolid continues to be highly active against contemporary (2012) Gram-positive pathogens from indicated organism groups including *S. aureus* (MIC_{90}, 2 μg/ml), CoNS (MIC_{90}, 1 μg/ml), various enterococcal species (MIC_{90}, 2 μg/ml) and three groups of streptococci (MIC_{90}, 1 μg/ml). These results are consistent with all previous ZAAPS studies.
- Overall, 99.79% (17 non-susceptible) or 99.77% (if one includes the *cfr*-containing *S. aureus* with MIC value at 4 μg/ml) of isolates were susceptible to linezolid in the 2012 ZAAPS Program.
- As rates of multidrug-resistant MRSA and enterococci continue to be elevated in most countries, linezolid-refractory strains appear to be unusual and without escalating occurrence.
- Linezolid-resistance mechanisms continue to be diverse, five different resistance mechanisms were found in the 2012 surveillance (L4 mutation [7], L3 mutation [6], G2576T [6], *cfr* [4], and C2319T [1]), consistent with previous years. Although there have been concerns about the potential for the rapid spread of *cfr*, in the ZAAPS Program *cfr* continues to occur but remains uncommon.

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