Report of Linezolid Activity from the Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) Program 2014 (Europe, Latin America, Asia Pacific, Canada)

C-562

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ICAAC 2015
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ABSTRACT

Background: The ZAAPS program has monitored linezolid (LZD) resistance outside of the USA for 13 years. Herein, we report the results of this study which monitored 66 medical centers for 2014, a total of 7,541 Gram-positive (GP) organisms consecutively collected in 33 countries.

Methods: Susceptibility (S) tests were performed by reference CLSI broth microdilution methods, using validated panels. The number of strains tested were: *S. aureus* (SA; 3,560, 30.0% MRSA), coagulase-negative staphylococcal species (CoNS; 956, 68.8% methicillin-resistant [R]), *Enterococcus* spp. (ENT; 813 with 512 *E. faecalis* [EF] and 290 *E. faecium*), *S. pneumoniae* (SPN; 1,028), viridans gr. (495) and β-haemolytic streptococci (689). Strains with LZD MIC at ≥4 µg/mL were investigated by molecular methods (PCR/gene sequencing, PFGE) to determine LZD resistance mechanisms (23S rRNA, L3, L4 mutations and *cfr*).

Results: LZD potency remained stable (overall LZD R at 0.12%). Nearly all (97.1%) MIC values for LZD were 0.5, 1 or 2 µg/mL. All SA were S to LZD with $MIC_{50/90}$ at 1/1µg/mL. The overall MRSA rate decreased 1.2% from 2013 ZAAPS Program to 30.0%. Other agents with >90% S rates versus SA were: daptomycin (99.9%), teicoplanin (100.0%), trimethoprim/sulfamethoxazole (98.6%) and vancomycin (100.0%). The overall vancomycin-R ENT rate was 12.5%.

Nine isolates met the LZD–R screening criteria (6 CoNS; 5 *S. epidermidis* and 1 *S. haemolyticus* and 3 EF). The most prevalent R-mechanism was the 23S rRNA mutation G2576T found in 3 CoNS strains. Target site mutations or *cfr* were not detected among the 3 selected ENT.

Conclusion: Overall, LZD remained active against 99.88%, with only nine LZD non-S strains from the six GP pathogen groups tested for the 2014 ZAAPS Program and showed no escalation of R or MIC creep. The lack of R mechanisms identified in the ENT strains suggest the emergence of an additional R mechanism.

INTRODUCTION

Linezolid was the first oxazolidinone agent approved for clinical use by various national or regional regulatory agencies from 2000-2014, and it has become an important therapeutic agent for infections caused by more commonly occurring multidrug-resistant (MDR) 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 at ≤1%. The occurrence rates for *Staphylococcus aureus* and streptococci are even less frequent (<0.1%). These resistance occurrences have been associated with recognized risk factors such as prolonged therapeutic exposure; however, oxazolidinone-resistant strains have emerged in patients without prior linezolid exposure, many attributable to clonal dissemination from other patients in the same hospital environment. Also new mechanisms of resistance (*cfr, cfr*(B), *optr*A, L3 and/or L4 protein alteration) have been described in Gram-positive isolates, some of animal origin.

In this presentation, we report the results from the 2014 linezolid resistance surveillance for ex-USA nations.

MATERIALS AND METHODS

A total of 7,541 Gram-positive strains were collected from 66 medical centers on five continents (33 countries). Each continental region submitted the following number of Gram-positive organisms: Asia-West Pacific – 2,003; Europe – 4,260; Latin America – 1,082; and North America – 196. Each site submitted 250-500 isolates to reach a targeted 200 Gram-positive organisms per country except for Australia (600) and Japan (400). JMI Laboratories (North Liberty, Iowa USA) performed confirmatory organism identification tests and reference broth microdilution susceptibility testing by Clinical and Laboratory Standards Institute (CLSI) methods (M07-A10, 2015; M100-S25, 2015). Susceptibility testing was performed using validated, microdilution panels with cation-adjusted Mueller-Hinton broth (2.5-5% lysed horse blood added for testing streptococci).

For 2014, all results were interpreted to susceptibility category by both CLSI (M100-S25, 2015) and EUCAST (2015) criteria/breakpoints, where available. Tigecycline breakpoints were those found in the United States-Food and Drug Administration (USA-FDA) approved product package insert.

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. Molecular testing was then performed to identify the target site mutation (23S rRNA, L3 and/or L4 proteins) or gene (*cfr, optrA*)-based mechanisms, as well as possible epidemic clonality using pulsed field gel electrophoresis (PFGE).

RESULTS

- The activity of linezolid in the 2014 ZAAPS Program sampling of 66 medical centers (7,541 Gram-positive strains) is presented in **Table 1**. The "all organism" MIC_{50} and MIC_{90} results were 1 and 1 µg/mL, respectively, with only nine (0.12%) linezolid-non-susceptible isolates.
- The linezolid *S. aureus* MIC values were distributed across a very narrow range (99.9% of linezolid MIC values were at 0.25-2 µg/mL). The global (ex-USA) MRSA rate was only 30.0%. When the linezolid MRSA MIC distribution was compared to the MSSA strains (**Tables 1** and **2**), the $MIC_{50/90}$ results for both MRSA and MSSA isolates were identical (1/1 µg/mL).
- The linezolid modal MIC and MIC_{90} results for CoNS were at 0.5 µg/mL with 99.1% of isolates displaying MIC values in the range of 0.25-2 µg/mL. There were 0.6% (six) strains with elevated linezolid MICs, an increase from 2013 (0.4% [4]) and a decrease from 2012 (0.9% [8]). Linezolid MIC values were generally two-fold lower for CoNS when compared to *S. aureus* results (**Table 2**).
- All *S. aureus* isolates were susceptible to vancomycin and teicoplanin (**Table 2**). Erythromycin and clindamycin resistances against all *S. aureus* were at 27.4 and 12.7% (CLSI), respectively (**Table 2**), and for MRSA were 64.2 and 37.5% (data not shown).
- Linezolid MIC distributions for enterococci were generally between 0.5 and 2 µg/mL (98.9% of values), consistent with previous ZAAPS surveillance reports with only three linezolid-non-susceptible isolates (0.37%; **Table 1**).
- Linezolid activity remained highly potent (MIC_{50} and MIC_{90}, 1 µg/mL) for β-haemolytic streptococci and *Streptococcus pneumoniae*. Viridans group streptococci had slightly lower MIC results for linezolid, with a $MIC_{50/90}$ at 0.5/1 µg/mL (**Table 2**).
- There were six CoNS with elevated linezolid MIC values (five *S. epidermidis* and one *S. haemolyticus)*. Three isolates (two *S. epidermidis* and the *S. haemolyticus* isolate) contained the 23S rRNA (G2576T) mutation only. The isolates with the highest MIC results (>128 µg/mL) contained multiple mutations at 23S rRNA (T2504A +/- C2534T) and other ribosomal proteins (either at L3 or L3 and L4).
- Three linezolid-non-susceptible enterococci (MIC, 4-8 µg/mL) from Ireland (Galway and Dublin) and Malaysia had the newly identified ABC transporter (*optrA*).

Table 3. Linezolid (LZD)-non-susceptible strains found in the 2014 ZAAPS surveillance program.

Organism (no. of strains)	Country (LZD MIC; µg/mL)	Resistance mechanism
CoNS (6)[a]	Mexico (4)	L3 mutation
	Brazil (8)	G2576T
	Brazil (8)	G2576T
	Greece (>128)	T2504A, C2534T, L3 and L4 mutation
	Korea (16)	G2576T
	Portugal (>128)	T2504A, L3 mutation
E. faecalis (3)	Ireland (8)	*optrA*
	Ireland (4)	*optrA*
	Malaysia (4)	*optrA*

a. CoNS = coagulase-negative staphylococci, five *S. epidermidis* and one *S. haemolyticus*.

Table 1. Cumulative % inhibited results at each linezolid MIC when testing against six different groups of Gram-positive cocci isolated on four continents (ZAAPS, 2014)[a].

	No. of isolates (cum. %) inhibited at linezolid MIC (µg/mL):[b]						
Organism group (no. tested)[a]	0.12	0.25	0.5	1	2	4	≥8
β-haemolytic streptococci (689)	--	1 (0.1)	273 (39.8)	415 (100.0)	--	--	--
Viridans group streptococci (495)	4 (0.8)	39 (8.7)	276 (64.4)	175 (99.8)	1 (100.0)	--	--
S. pneumoniae (1,028)	5 (0.5)	37 (4.1)	412 (44.2)	569 (99.5)	5 (100.0)	--	--
S. aureus (3,560)	4 (0.1)	28 (0.9)	1056 (30.6)	2428 (98.8)	44 (100.0)	--	--
MRSA (1,067)	4 (0.4)	15 (1.8)	379 (37.3)	659 (99.1)	10 (100.0)	--	--
MSSA (2,493)	--	13 (0.5)	677 (27.7)	1769 (98.6)	34 (100.0)	--	--
Enterococci (813)	--	6 (0.7)	151 (19.3)	592 (92.1)	61 (99.6)	2[c] (99.9)	1[c] (100.0)
CoNS (956)	3 (0.3)	246 (26.0)	616 (90.5)	84 (99.3)	1 (99.4)	2[d] (99.6)	4[c] (100.0)
MR-CoNS (658)	3 (0.5)	183 (28.3)	405 (89.8)	60 (98.9)	1 (99.1)	2[d] (99.4)	4[c] (100.0)
MS-CoNS (298)	--	63 (21.1)	211 (91.9)	24 (100.0)	--	--	--
All Organisms (7,541)	(0.2)	(2.8)	(32.2)	(94.7)	(99.9)	(99.9)	(100.0)

a. Organism groups were ranked in decreasing order of susceptibility to the oxazolidinone. MRSA = Methicillin-resistant *S. aureus*; MSSA = Methicillin-susceptible *S. aureus*; MR-CoNS = Methicillin-resistant coagulase-negative staphylococci; MS-CoNS = Methicillin-susceptible coagulase-negative staphylococci.
b. MIC values were confirmed using frozen-form broth microdilution panels containing an extended dilution range for linezolid (1-128 µg/mL).

Table 2. Comparative activity of linezolid tested against 7,541 Gram-positive cocci from 33 nations in the ZAAPS Program (2014).

Organism (no. tested)/ antimicrobial agent	MIC (µg/mL) 50%	90%	Range	% by category[a] Susceptible/Resistant CLSI	EUCAST
S. aureus					
All strains (3,560)					
Linezolid	1	1	≤0.12-2	100.0 / 0.0	100.0 / 0.0
Ceftriaxone[a]	4	>8	0.5->8	70.0 / 30.0	70.0 / 30.0
Clindamycin	≤0.25	>2	≤0.25->2	87.2 / 12.7	86.9 / 12.8
Erythromycin	0.25	>16	≤0.12->16	68.8 / 27.4	69.1 / 29.9
Gentamicin	≤1	>8	≤1->8	89.0 / 10.5	88.6 / 11.4
Levofloxacin	0.25	>4	≤0.12->4	75.8 / 24.0	75.8 / 24.0
Oxacillin[a]	0.5	>2	≤0.25->2	70.0 / 30.0	70.0 / 30.0
Tetracycline	≤0.5	>8	≤0.5->8	88.6 / 10.5	88.2 / 11.7
TMP/SMX[b]	≤0.5	≤0.5	≤0.5->4	98.6 / 1.4	98.6 / 1.4
Teicoplanin	≤2	≤2	≤2-16	100.0 / 0.0	98.8 / 1.2
Vancomycin	1	1	0.25-2	100.0 / 0.0	100.0 / 0.0
MRSA (1,067)[b]					
Linezolid	1	1	≤0.12-2	100.0 / 0.0	100.0 / 0.0
MSSA (2,493)[b]					
Linezolid	1	1	0.25-2	100.0 / 0.0	100.0 / 0.0
CoNS (956)[c]					
Linezolid	0.5	0.5	≤0.12->8	99.6 / 0.4	99.6 / 0.4
Ceftriaxone[a]	>8	>8	0.12->8	31.2 / 68.8	31.2 / 68.8
Clindamycin	≤0.25	>2	≤0.25->2	73.2 / 26.3	71.9 / 26.8
Erythromycin	16	>16	≤0.12->16	41.4 / 57.1	37.7 / 62.1
Gentamicin	≤1	>8	≤1->8	59.3 / 35.8	56.2 / 43.8
Levofloxacin	2	>4	≤0.12->4	49.9 / 44.7	49.9 / 44.7
Oxacillin[a]	>2	>2	≤0.25->2	31.2 / 68.8	31.2 / 68.8
Tetracycline	≤0.5	>8	≤0.5->8	86.9 / 11.6	82.7 / 14.2
TMP/SMX[b]	≤0.5	>4	≤0.5->4	72.4 / 27.6	72.4 / 13.1
Teicoplanin	≤2	8	≤2->16	97.5 / 0.4	83.8 / 16.2
Vancomycin	1	2	≤0.12-4	100.0 / 0.0	100.0 / 0.0
Enterococci					
All strains (813)[d]					
Linezolid	1	1	0.25-8	99.6 / 0.1	99.9 / 0.1
Ampicillin	1	>8	≤0.25->8	67.7 / 32.3	66.9 / 32.3
Erythromycin	>16	>16	≤0.12->16	5.7 / 62.2	- / -
Levofloxacin[e]	4	>4	≤0.12->4	49.6 / 48.6	51.4 / 48.6
Piperacillin/tazobactam[a]	4	>64	≤0.5->64	67.7 / 32.3	66.9 / 32.3
Teicoplanin	≤2	16	≤2->16	89.1 / 9.5	88.9 / 11.1
Vancomycin	1	>16	0.25->16	87.1 / 12.5	87.1 / 12.9
VRE (105)[f]					
Linezolid	1	1	0.5-2	100.0 / 0.0	100.0 / 0.0
VSE (708)[f]					
Linezolid	1	1	0.25-8	99.6 / 0.1	99.1 / 0.1
S. pneumoniae (1,028)					
Linezolid	1	1	≤0.12-2	100.0 / -	100.0 / 0.0
Amoxacillin/clavulanic acid	≤1	4	≤1->8	87.8 / 9.1	63.0 / 8.6
Ceftriaxone	≤0.06	2	≤0.06->8	88.5 / 2.0	77.7 / 2.0
Ciprofloxacin	1	2	≤0.12->4	(8.7)[f]	0.3 / 8.7
Clindamycin	≤0.25	>2	≤0.25->2	75.3 / 24.0	76.0 / 24.0
Erythromycin	≤0.12	>16	≤0.12->16	65.9 / 33.5	65.9 / 33.5
Levofloxacin	1	1	0.25->4	97.8 / 1.8	97.8 / 2.2
Penicillin[g]	≤0.06	2	≤0.06->8	63.0 (91.4)[f] 18.0 (1.2)	63.0 / 8.6
Tetracycline	≤0.5	>8	≤0.5->8	66.5 / 33.1	66.5 / 33.1
TMP/SMX[b]	≤0.5	>4	≤0.5->4	63.4 / 26.8	69.7 / 26.8
Vancomycin	0.25	0.25	≤0.12-0.5	100.0 / -	100.0 / 0.0
Viridans group streptococci (495)[h]					
Linezolid	0.5	1	≤0.12-2	100.0 / -	- / -
Ceftriaxone	0.25	2	≤0.06->8	89.3 / 7.5	85.1 / 14.9
Clindamycin	≤0.25	0.5	≤0.25->2	89.3 / 9.9	90.1 / 9.9
Erythromycin	≤0.12	8	≤0.12->16	59.8 / 37.1	- / -
Levofloxacin	1	2	≤0.12->4	96.2 / 3.4	- / -
Penicillin[a]	≤0.06	1	≤0.06->8	72.1 / 6.7	78.6 / 6.7
Tetracycline	≤0.5	>8	≤0.5->8	66.7 / 30.1	- / -
Vancomycin	0.5	0.5	0.25-1	100.0 / -	100.0 / 0.0
β-haemolytic streptococci (689)[i]					
Linezolid	1	1	0.25-1	100.0 / -	100.0 / 0.0
Amoxacillin/clavulanic acid	≤1	≤1	≤1	100.0 / -	100.0 / 0.0
Ceftriaxone	≤0.06	0.12	≤0.06-0.25	100.0 / -	100.0 / 0.0
Clindamycin	≤0.25	2	≤0.25->2	88.4 / 10.9	89.1 / 10.9
Erythromycin	≤0.12	>16	≤0.12->16	79.4 / 19.5	79.4 / 19.5
Levofloxacin	0.5	1	≤0.12->4	97.2 / 2.8	94.6 / 2.8
Penicillin[a]	≤0.06	≤0.06	≤0.06-0.12	100.0 / -	100.0 / 0.0
Tetracycline	≤0.5	>8	≤0.5->8	54.4 / 43.3	53.1 / 45.5
Vancomycin	0.25	0.5	≤0.12-1	100.0 / -	100.0 / 0.0

a. Criteria as published by the CLSI [2015] and EUCAST [2015], beta-lactam susceptibility should be directed by the oxacillin test results for staphylococci and by ampicillin or penicillin for Enterococcus or specified streptococci.
b. TMP/SMX=trimethoprim/sulfamethoxazole; VRE=vancomycin-resistant enterococci; VSE=vancomycin-susceptible enterococci; MRSA = Methicillin-resistant *S. aureus*; MSSA = Methicillin-susceptible *S. aureus*.
c. Includes: 18 species (955 strains) and unidentified coagulase-negative staphylococci (one strain).
d. Includes: *Enterococcus faecalis* (512 strains), *Enterococcus faecium* (290 strains), and 11 other enterococci.
e. Breakpoints for uncomplicated UTI, only.
f. Percentage of pneumococci or other streptococci with ciprofloxacin MICs at ≥4 µg/mL, indicating possible QRDR mutations.
g. CLSI 2015 susceptibility breakpoints for penicillin oral penicillin V (nonmeningitis in parenthesis).
h. Includes: 23 species (493 strains), *Streptococcus bovis* group (two strains).
i. Includes: Group A (318 strains), Group B (254 strains), *S. dysgalatiae* (116 strains), and *S. equi* (one strain).

CONCLUSIONS

- Across all six Gram-positive pathogen groups that were monitored in the 33 nations, linezolid activity was stable and without escalating occurrence of isolates having linezolid MIC values of ≥4 µg/mL (nine strains, 0.12%; nine strains [0.09%] in 2013 and 13 strains or 0.16% in 2012).
- As rates of MDR MRSA and enterococci continue to be elevated in many countries, linezolid-refractory strains still appear to be unusual and without escalating occurrence across the last 5-8 years. These resistant strains most often occurred among the enterococci and CoNS species. In this survey, only one linezolid-resistant isolate (*S. haemolyticus* from Brazil) was shown to be related to a clone that was previously documented in that participating survey site.
- The number of Gram-positive clinical isolates showing elevated MIC results for linezolid (i.e. ≥4 µg/mL) during the 2014 surveillance program was very low, but an array of resistance mechanisms were observed, including a newly described ABC transporter (*optrA*).
- Although there have been concerns about the potential for the rapid spread of the mobile *cfr*, our results from the 2014 ZAAPS Program did not detect any *cfr*-mediated resistance. However, this study demonstrates that isolates carrying the newly identified linezolid resistance mechanism, the plasmid-encoded *optrA* gene, are not restricted to China.

ACKNOWLEDGEMENT

The authors would like to thank all participating centers worldwide for contributing isolates to this surveillance protocol. This study was sponsored by Pfizer (New York, NY).

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