P1355

Activity of Linezolid when Tested Against Contemporary European Bacterial Clinical Isolates (2015)

RK FLAMM[1], RE MENDES[1], JM STREIT[1], HS SADER[1], PA HOGAN[2], RN JONES[1]
[1]JMI Laboratories, North Liberty, IA, USA; [2]Pfizer Inc., New York, NY, USA

ECCMID 2016
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
robert-flamm@jmilabs.com

ABSTRACT

Background: International bacterial surveillance has shown that although very uncommon, linezolid resistance has been observed among coagulase-negative staphylococci (CoNS) in more frequency than enterococci. Resistance remains even lower among *Staphylococcus aureus* and streptococci. In this study, an interim evaluation of in vitro activity of linezolid and comparators against isolates from the European and Israeli component of the 2015 Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) surveillance program are presented.

Methods: More than 4,000 isolates were collected from over 30 sites in Europe and Israel in 2015. Isolates were received from the following organism groups: *S. aureus,* CoNS, *Enterococcus* spp., *Streptococcus pneumoniae*, viridans group streptococci, and β-haemolytic streptococci. Isolates from each country were sent to a central monitoring laboratory for confirmatory identification and CLSI broth microdilution susceptibility testing. Susceptibility interpretations followed EUCAST breakpoint tables (Version 6.0, 2016). Isolates displaying elevated linezolid MIC results (≥4 mg/L) were retested using frozen broth microdilution, Etest and disk diffusion methods. PCR and sequencing were performed to detect mutations in 23S rRNA, L3, L4, and L22 genes, and acquired determinants (*cfr*; *optrA*).

Results: All *S. aureus* were susceptible to linezolid ($MIC_{50/90,}$ 1/1 mg/L), daptomycin, tigecycline, and vancomycin. MRSA represented 20.8% of *S. aureus*. Enterococci ($MIC_{50/90,}$ 1/2 mg/L), β-haemolytic streptococci ($MIC_{50/90,}$ 1/1 mg/L), viridans group streptococci ($MIC_{50/90,}$ 1/1 mg/L) and *S. pneumoniae* ($MIC_{50/90,}$ 1/1 mg/L) were all susceptible to linezolid. *S. pneumoniae* had overall penicillin and erythromycin resistant rates of 31.8% (MIC, ≥2mg/L) and 26.6%, respectively. Linezolid susceptibility for CoNS (64.7% methicillin-resistant CoNS) was 99.4%. Three linezolid-resistant CoNS were found. These were hospital-acquired isolates recovered from blood (two) and wound (one) specimens. These isolates originated from two sites in Italy and had linezolid MIC values of 16, 32 and 64 mg/L. Alterations in the 23S rRNA were observed in all three CoNS, but one isolate also carried *cfr* (linezolid MIC, 64 mg/L).

Conclusions: In this interim view of the 2015 European component of ZAAPS, linezolid-resistance remains uncommon (<1%). A linezolid resistance phenotype was only observed among CoNS, which were hospital-acquired isolates exhibiting a common linezolid resistance mechanism (target site alteration in 23S rRNA). Additionally, one isolate also had a plasmid-mediated resistance (*cfr*), emphasizing the importance of ongoing surveillance and molecular characterization of resistant isolates.

INTRODUCTION

The ZAAPS (Zyvox® Annual Appraisal of Potency and Spectrum) antibacterial surveillance program has monitored the activity of linezolid against Gram-positive pathogens for more than 15 years. During this time, linezolid resistance in the program has remained relatively low overall (<1%). When linezolid resistance occurs, it occurs most frequently in coagulase-negative staphylococci (CoNS) and enterococci. Linezolid resistance in streptococci and *Staphylococcus aureus* has occurred less often.

Mutations causing linezolid resistance are primarily target site alterations (changes in ribosomal RNA or ribosomal protein). However, acquired resistances such as *cfr* (found in several bacterial species) and *optr*A (currently only identified in Enterococcal strains) have been identified.

In this study, an interim evaluation of the in vitro activity of linezolid and comparators against isolates from the European and Israeli component of the 2015 Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) surveillance program is presented.

MATERIALS AND METHODS

Bacterial isolate collection. Medical centres (34 in total) from 18 countries (**Table 1**) were instructed to forward ≥100 organisms with the following species or genus distribution targets: *S. aureus* (50 isolates), CoNS (15 isolates), enterococci (15 isolates), *Streptococcus pneumoniae* (10 isolates), β-haemolytic streptococci (5 isolates) and viridans group streptococci (5 isolates). The isolates were mostly from bloodstream infections, respiratory tract, and skin and soft tissue infections. Isolates (one per infection episode) were identified by the local laboratory and sent to the central monitoring laboratory (JMI Laboratories, North Liberty, Iowa USA) for confirmatory identification and reference antimicrobial susceptibility testing. A total 4,338 Gram-positive pathogens were submitted to the monitor and included *S. aureus* (2,434 strains), CoNS (465), enterococci (426), *S. pneumoniae* (632), viridans group streptococci (93), and β-haemolytic streptococci (288; **Table 2**).

Antimicrobial susceptibility test methods. Susceptibility tests were performed using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods (frozen panels; CLSI M07-A10, 2015). Interpretive criteria used were those of EUCAST (Version 6.0, 2016) and (CLSI M100-S26, 2016). Isolates exhibiting a linezolid MIC value of ≥4 mg/L were confirmed by repeat broth microdilution testing.

Screening for linezolid resistance mechanisms. Isolates displaying confirmed linezolid MIC results of ≥4 mg/L were then screened for the presence of *cfr*, *optrA* and mutations in the 23S rRNA and ribosomal proteins (L3 and L4) by PCR and sequencing.

RESULTS

- The activity of linezolid when tested against the six groups of Gram-positive organisms collected in Europe and Israel (2015) is presented in **Table 2**. The "all organism" MIC_{50} and MIC_{90} were 1 and 1 mg/L, respectively with only 3 (0.07%) non-susceptible isolates (all CoNS).
- A total of 20.8% of *S. aureus* were MRSA (**Table 2**, **3**). The linezolid MIC distributions for MRSA and MSSA were similar with identical MIC_{50} and MIC_{90} values at 1 and 1 mg/L, respectively (**Table 2**). *S. aureus* linezolid MIC values ranged from 0.25 to 2 mg/L. The modal MIC was at 1 mg/L with 97.9% of MIC values at either 0.5 or 1 mg/L (**Table 2**).
- All *S. aureus* were susceptible to linezolid, daptomycin (MIC_{50} and $MIC_{90,}$ 0.25 and 0.5 mg/L), tigecycline (MIC_{50} and $MIC_{90,}$ 0.06 and 0.12 mg/L), and vancomycin (MIC_{50} and $MIC_{90,}$ 0.5 and 1 mg/L; **Table 3**). Susceptibility was lower for MRSA compared to MSSA for a number of agents including erythromycin (MRSA, 33.3/34.1% [CLSI/EUCAST]; MSSA, 82.5/82.6% [CLSI/EUCAST]); clindamycin (MRSA, 73.5% [CLSI/EUCAST]; MSSA, 98.8/98.7% [CLSI/EUCAST]); levofloxacin (MRSA, 18.2% [CLSI/EUCAST]; MSSA, 95.3% [CLSI/EUCAST]); and tetracycline (MRSA, 85.4/85.2% [CLSI/EUCAST]; MSSA, 94.2/93.8% [CLSI/EUCAST]).
- For CoNS, the linezolid modal MIC was at 0.5 mg/L with 90.3% of CoNS displaying MIC values in the range of 0.5-1 mg/L (**Table 3**). All isolates were susceptible to daptomycin (MIC_{50} and MIC_{90}, 0.5 and 0.5 mg/L), and vancomycin (MIC_{50} and MIC_{90}, 1 and 2 mg/L; **Table 3**). A total of 64.7% of isolates were methicillin-resistant (**Table 3**).
- The modal linezolid MIC for the enterococci was 1 mg/L (MIC_{50} and $MIC_{90,}$ 1 and 2 mg/L; **Table 2**). All enterococcal isolates were susceptible to linezolid (**Table 3**). All isolates of *E. faecalis* but only 6.8% of *E. faecium* isolates were susceptible to ampicillin. Vancomycin resistance among *E. faecium* was at 16.5% and only 0.3% in *E. faecalis*.
- Linezolid (100.0% susceptible) was highly potent (MIC_{50} and MIC_{90}, 1 mg/L) against β-haemolytic streptococci and *S. pneumoniae*. Penicillin resistance in *S. pneumoniae* was 13.8% (CLSI oral breakpoint). Erythromycin susceptibility was 73.1% for *S. pneumoniae* and 55.9% (CLSI interpretive criteria) for viridans group streptococci (**Table 3**)
- There were three CoNS displaying linezolid-resistant MIC values of 16 - 64 mg/L (**Table 4**). These isolates had combinations of alterations at the 23S rRNA and/or L3 and L4 ribosomal proteins, and/or *cfr* (**Table 4**). These three CoNS were collected in hospitals in Italy, and two isolates originated from the same medical centre.

Table 1. Distribution of isolates from Europe and Israel.

Country (Number of sites)	Number of isolates
Belarus (1)	53
Belgium (1)	123
Czech Republic (1)	117
France (4)	642
Germany (3)	498
Greece (1)	59
Hungary (1)	121
Ireland (2)	292
Israel (1)	95
Italy (4)	571
Poland (1)	26
Portugal (1)	132
Romania (1)	107
Russia (3)	240
Slovenia (1)	138
Spain (3)	410
Turkey (2)	177
United Kingdom (3)	537

Table 2. Cumulative percent inhibited at each linezolid MIC when testing against six different groups of Gram-positive cocci isolated from Europe (2015).

	Number of isolates (cumulative %) inhibited at linezolid MIC in mg/L:								
Organism group (no. tested)	≤0.25	0.5	1	2	4	8	>8	MIC_{50}	MIC_{90}
S. aureus (2,434)	21 (0.9)	700 (29.6)	1683 (98.8)	30 (100.0)				1	1
MRSA (506)	4 (0.8)	198 (39.9)	302 (99.6)	2 (100.0)				1	1
MSSA (1,928)	17 (0.9)	502 (26.9)	1381 (98.5)	28 (100.0)				1	1
CoNS (465)	39 (8.4)	282 (69.0)	138 (98.7)	3 (99.4)	0 (99.4)	0 (99.4)	3 (100.0)	0.5	1
MRCoNS (301)	21 (7.0)	194 (71.4)	81 (98.3)	2 (99.0)	0 (99.0)	0 (99.0)	3 (100.0)	0.5	1
MSCoNS (164)	18 (11.0)	88 (64.6)	57 (99.4)	1 (100.0)				0.5	1
BHS (288)	1 (0.3)	85 (29.9)	202 (100.0)					1	1
Enterococcus (426)	6 (1.4)	79 (20.0)	289 (87.8)	52 (100.0)				1	2
Viridans group streptococci (93)	2 (2.2)	38 (43.0)	53 (100.0)					1	1
S. pneumoniae (632)	11 (1.7)	155 (26.3)	446 (96.8)	20 (100.0)				1	1
"All organisms" (4,338)	80 (1.8)	1339 (32.7)	2811 (97.5)	105 (>99.9)	0 (>99.9)	0 (>99.9)	3 (100.0)	1	1

MRSA= methicillin-resistant *Staphylococcus aureus*, MSSA= methicillin-susceptible *Staphylococcus aureus*, MRCoNS=methicillin-resistant coagulase negative staphylococci, MSCoNS= methicillin-susceptible coagulase negative staphylococci.

Table 3. Comparative activity of linezolid tested against 4,338 Gram-positive cocci from European component of the ZAAPS program (2015).

	MIC (mg/L)			% by category[a] Susceptible/Resistant	
Organism (no. tested) / antimicrobial agent	50%	90%	Range	CLSI [2016]	EUCAST [2016]
S. aureus					
All strains (2,434)					
Linezolid	1	1	0.25 to 2	100.0 / 0.0	100.0 / 0.0
Clindamycin	≤0.25	≤0.25	≤0.25 to >2	93.5 / 6.3	93.4 / 6.5
Daptomycin	0.25	0.5	≤0.12 to 1	100.0 / -	100.0 / 0.0
Erythromycin	0.25	>8	≤0.06 to >8	72.3 / 25.0	72.5 / 26.7
Gentamicin	≤1	≤1	≤1 to >8	94.4 / 5.4	94.1 / 5.9
Levofloxacin	0.12	>4	0.06 to >4	79.3 / 20.3	79.3 / 20.3
Oxacillin[a]	0.5	>2	≤0.25 to >2	79.2 / 20.8	79.2 / 20.8
Teicoplanin	≤0.5	≤0.5	≤0.5 to 8	100.0 / 0.0	99.9 / 0.1
Tetracycline	≤0.5	≤0.5	≤0.5 to >8	92.4 / 6.5	92.0 / 7.8
Tigecycline	0.06	0.12	≤0.015 to 0.5	100.0 / -	100.0 / -
TMP/SMX[b]	≤0.5	≤0.5	≤0.5 to >4	99.8 / 0.2	99.8 / <0.1
Vancomycin	0.5	1	≤0.12 to 2	100.0 / 0.0	100.0 / 0.0
MRSA (506)[c]					
Linezolid	1	1	0.25 to 2	100.0 / 0.0	100.0 / 0.0
MSSA (1,928)[c]					
Linezolid	1	1	0.25-2	100.0 / 0.0	100.0 / 0.0
Coagulase-negative staphylococci (465)[d]					
Linezolid	0.5	1	0.25 to >8	99.4 / 0.6	99.4 / 0.6
Clindamycin	≤0.25	>2	≤0.25 to >2	77.8 / 21.5	76.3 / 22.2
Daptomycin	0.5	0.5	≤0.12 to 1	100.0 / -	100.0 /0.0
Erythromycin	>8	>8	≤0.06 to >8	38.5 / 60.4	38.5 / 61.1
Gentamicin	≤1	>8	≤1 to >8	55.1 / 40.2	52.0 / 48.0
Levofloxacin	2	>4	0.06 to >4	49.2 / 46.5	49.2 / 46.5
Oxacillin[a]	>2	>2	≤0.25 to >2	35.3 / 64.7	35.3 / 64.7
Teicoplanin	2	4	≤0.5 to 8	100.0 / 0.0	93.1 / 6.9
Tetracycline	≤0.5	>8	≤0.5 to >8	86.7 / 12.0	83.0 / 15.5
Tigecycline	0.12	0.25	≤0.015 to 0.5	- / -	100.0 / 0.0
TMP/SMX[b]	≤0.5	>4	≤0.5 to >4	67.5 / 32.5	67.5 /15.5
Vancomycin	1	2	≤0.12 to 2	100.0 / 0.0	100.0 / 0.0
Enterococci					
All strains (426)[e]					
Linezolid	1	2	≤0.25 to 2	100.0 /0.0	100.0 /0.0
Ampicillin	1	>8	≤0.5 to >8	70.9 / 29.1	70.9 / 29.1
Daptomycin	1	2	≤0.25 to 4	100.0 /-	- / -
Levofloxacin	2	>4	≤0.5 to >4	55.2 / 43.2	56.8 / 43.2
Pip/tazobactam[b]	4	>16	≤2 to >16	- / -	70.9 / 29.1
Teicoplanin	≤2	≤2	≤2 to >16	95.3 / 4.2	95.3 / 4.7
Vancomycin	1	2	≤0.5 to >16	94.6 / 5.4	94.6 / 5.4
VRE (23)[c]					
Linezolid	1	2	≤0.5 to 2	100.0 /0.0	100.0 /0.0
VSE (403)[c]					
Linezolid	1	2	≤0.5 to 2	100.0 /0.0	100.0 /0.0

	MIC (mg/L)			% by category[a] Susceptible/Resistant	
Organism (no. tested) / antimicrobial agent	50%	90%	Range	CLSI [2016]	EUCAST [2016]
S. pneumoniae					
All strains (632)					
Linezolid	1	1	0.25 to 2	100.0 / -	100.0 / 0.0
Amoxicillin/clav acid[b]	≤0.03	2	≤0.03 to >4	92.2 / 4.7	- / -
Ceftriaxone[e]	0.03	1	≤0.015 to >2	96.0 / 0.8	85.1 / 0.8
Clindamycin	≤0.12	>1	≤0.12 to >1	81.0 / 17.9	82.1 / 17.9
Erythromycin	0.03	>2	≤0.015 to >2	73.1 / 26.6	73.1 / 26.6
Levofloxacin	1	1	≤0.12 to >4	98.6 / 1.4	98.6 / 1.4
Penicillin	≤0.06	2	≤0.06 to 8	68.2 / 13.8[g]	68.2 / 5.4[f]
				94.6 / 0.2[h]	- / -
Tetracycline	0.25	>4	≤0.12 to >4	75.2 / 24.8	75.2 / 24.8
TMP/SMX[b]	≤0.5	>4	≤0.5 to >4	69.3 / 20.1	76.9 / 20.1
Vancomycin	0.25	0.25	0.06 to 0.5	100.0 / -	100.0 / 0.0
Viridans group streptococci (93)					
Linezolid	1	1	0.12 to 1	100.0 / -	- / -
Ceftriaxone	0.12	0.5	≤0.03 to 4	96.8 / 1.1	91.4 8.6
Clindamycin	0.03	0.25	≤0.015 to >2	90.3 /9.7	90.3 /9.7
Erythromycin	≤0.03	>4	≤0.03 to >4	55.9 / 39.8	- / -
Levofloxacin	1	1	≤0.03 to 4	98.9 / 0.0	- / -
Penicillin	0.06	0.5	≤0.03 to >4	76.3 / 4.3	80.6 / 4.3
Tetracycline	0.5	>8	≤0.25 to >8	71.0 / 25.8	- / -
Vancomycin	0.5	0.5	0.12 to 1	100.0 / -	100.0 / 0.0
β-hemolytic streptococci (288)[i]					
Linezolid	1	1	0.25 to 1	100.0 / -	100.0 / 0.0
Amoxicillin/clav acid[b]	≤0.03	0.06	≤0.03 to 0.06	100.0 / -	100.0 / 0.0
Ceftriaxone	≤0.03	0.06	≤0.03 to 0.12	100.0 / -	100.0 / 0.0
Clindamycin	0.06	>2	0.03 to >2	88.9 / 11.1	88.9 / 11.1
Erythromycin	≤0.03	4	≤0.03 to >4	84.0 / 15.3	84.0 / 15.3
Levofloxacin	0.5	1	0.25 to >4	97.9 / 1.7	96.2 / 2.1
Tetracycline	≤0.25	>8	≤0.25 to >8	52.4 / 47.2	52.4 / 47.6
Vancomycin	0.25	0.5	0.12 to 0.5	100.0 / -	100.0 / 0.0

a. Criteria as published by the CLSI [2016] and EUCAST [2016].
b. TMP/SMX=trimethoprim/sulfamethoxazole; Pip/tazobactam = Piperacillin/tazobactam; Amoxicillin/clav acid = Amoxicillin/clavulanic acid.
c. MRSA = methicillin-resistant *staphylococcus aureus*, MSSA = methicillin-resistant *staphylococcus aureus*, VRE = vancomycin-resistant enterococci, VSE = vancomycin-susceptible enterococci.
d. Includes: *Staphylococcus epidermidis* (263), *S. hemolyticus* (84) and 12 other species.
e. Includes: *Enterococcus faecalis* (288 strains), *E. faecium* (133 strains), and 5 other enterococci.
f. Using non-meningitis breakpoints.
g. Using Oral breakpoints.
h. Using parenteral, non-meningitis breakpoints.
i. Includes: *S. agalactiae* (131 strains), *S. dysgalactiae* (47 strains), *S. pyogenes* (110 strains).

Table 4. Linezolid non-susceptible strains in the European component of the ZAAPS (2015) program.

					Linezolid MIC (mg/L)		
Isolate No.	Organism[a]	Site	City	Country	Surveillance	Custom[b]	Resistance mechanism
2498	*S. epidermidis*	075	Genova	Italy	>8	16	L3 (H146Q, V154L, A157R), L4 (71G72 ins)
30831	*S. epidermidis*	075	Genova	Italy	>8	64	*cfr*, 23S rRNA (G2576T), L3 (H146Q)
10136	*S. epidermidis*	085	Milan	Italy	>8	32	23S rRNA (G2576T), L3 (M156T)

a. Isolates from Genova were collected from patients hospitalized in the same medical centre.
b. Custom frozen-form 96-well plates with an extended linezolid MIC range (i.e. 1 - 128 mg/L).

CONCLUSIONS

- Linezolid was highly potent against a collection of 4,338 Gram-positive isolates collected from medical centres in Europe and Israel during 2015. The linezolid MIC_{90} value for all organisms tested was 1 mg/L (1 mg/L for five of organism groups and 2 mg/L for enterococci). Linezolid overall susceptibility was 99.93% (three linezolid-resistant isolates).
- Linezolid resistance was detected in three CoNS isolates from Italy and demonstrated multiple commonly observed oxazolidinone resistance mechanisms (**Table 4**).
- Linezolid continues to demonstrate excellent activity against targeted Gram-positive pathogens with linezolid resistance rates remaining below 1%.

ACKNOWLEDGEMENTS

The authors would like to thank all participating centres for contributing isolates to this surveillance protocol. This study was sponsored by Pfizer Inc.

REFERENCES

1. Biedenbach DJ, Farrell DJ, Mendes RE, Ross JE, Jones RN (2010). Stability of linezolid activity in an era of mobile oxazolidinone resistance determinants: Results from the 2009 Zyvox(R) Annual Appraisal of Potency and Spectrum program. *Diagn Microbiol Infect Dis* 68: 459-467.
2. Clinical and Laboratory Standards Institute (2016). *M100-S26. Performance standards for antimicrobial susceptibility testing: 26th informational supplement.* Wayne, PA: CLSI.
3. EUCAST (2016). Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0, January 2016. Available at: http://www.eucast.org/clinical_breakpoints/. Accessed January 2016 Mendes RE, Hogan PA, Streit JM, Jones RN, Flamm RK (2014). Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) Program: Report of linezolid activity over nine years (2004 - 2012). *J Antimicrob Chemother* 69: 1582-1588.
4. Mendes RE, Hogan PA, Streit JM, Jones RN, Flamm RK (2015). Update on the linezolid *in vitro* activity through the Zyvox(R) Annual Appraisal of Potency and Spectrum Program (2013). *Antimicrob Agents Chemother* 59: 2454-2457.
5. Morales G, Picazo JJ, Baos E, Candel FJ, Arribi A, Pelaez B, Andrade R, de la Torre MA, Fereres J, Sanchez-Garcia M (2010). Resistance to linezolid is mediated by the *cfr* gene in the first report of an outbreak of linezolid-resistant *Staphylococcus aureus*. *Clin Infect Dis* 50: 821-825.
6. Tygacil® Package Insert (2015). Available at http://labeling.pfizer.com/ShowLabeling.aspx?id=491. Accessed August 2015.
7. Wang Y, Lv Y, Cai J, Schwarz S, Cui L, Hu Z, Zhang R, Li J, Zhao Q, He T, Wang D, Wang Z, Shen Y, Li Y, Fessler AT, Wu C, Yu H, Deng X, Xia X, Shen J (2015). A novel gene, optrA, that confers transferable resistance to oxazolidinones and phenicols and its presence in Enterococcus faecalis and *Enterococcus faecium* of human and animal origin. *J Antimicrob Chemother* 70: 2182-2190.