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Sustained *In Vitro* Activity of Linezolid and Evolving Resistance Mechanisms: Summary of the Zyvox® Annual Appraisal of Potency and Spectrum Program for 2016

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Introduction

- Oxazolidinones are a class of synthetic antimicrobial agents that bind to the A-site of the peptidyl transferase center (PTC) in domain V of the 23S rRNA component of the 50S subunit
  - Binding at the PTC prevents the formation of the *N*-formylmethionyl-tRNA-ribosome-mRNA ternary complex and inhibits initiation of bacterial protein synthesis
- This unique mechanism of action of linezolid makes cross-resistance with other antimicrobial agents and the development of resistance due to multiple numbers of 23S rRNA copies unlikely
- Linezolid has intravenous and oral formulations
  - The oral formulation is 100% bioavailable, allows step-down therapy, and may help to expedite hospital discharge
- The *in vitro* activity of linezolid has been monitored many years through the Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) and Linezolid Experience and Accurate Determination of Resistance (LEADER) programs
  - In addition, the epidemiology of gram-positive isolates demonstrating elevated MIC results to linezolid and respective resistance mechanisms have been monitored
- This study investigated and reported the linezolid activity against gram-positive isolates and resistance mechanisms associated with elevated MIC results

Materials and Methods

Bacterial strain

- A total of 8,325 isolates were collected from 76 centers (42 countries in 5 continents) and submitted to the coordinating monitoring laboratory (JMI Laboratories, North Liberty, Iowa, USA)
  - Isolates were recovered from hospitalized patients and selected by participating centers following specific protocols, as part of the SENTRY Antimicrobial Surveillance Program for 2016
- Bacterial identifications were confirmed by standard algorithms and matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany)
- Isolates that displayed linezolid MIC results at ≥4 µg/mL were selected for further molecular characterization

Antimicrobial susceptibility testing

- Susceptibility testing was performed by broth microdilution methods, according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (M07-A10, 2015)
- Inoculum density was monitored by colony counts to ensure an adequate number of cells for each testing event
- MIC values were validated by concurrently testing CLSI-recommended quality control reference strains (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Streptococcus pneumoniae* ATCC 49619)
- MIC interpretations were based on the CLSI M100-S27 and European Committee on Antimicrobial Susceptibility Testing (EUCAST) documents, when available

Screening for linezolid resistance mechanisms and epidemiology typing

- Isolates were screened for the presence of *cfr*, *cfr*(B), *cfr*(C), and *optrA* and mutations in the 23S rRNA-, L3-, and L4-encoding genes
- Complete genomes were sequenced on a MiSeq Sequencer (JMI Laboratories) following the manufacturer's instructions (Illumina, San Diego, California, USA)
- Assembled genomes were subjected to a proprietary software (JMI Laboratories) to screen genes of interest or to extract DNA sequences (23S rRNA, L3, and L4 genes) for mutation analysis
- Pulsed-field gel electrophoresis (PFGE) was performed on isolates of the same species that were recovered from the same medical site and exhibited decreased susceptibility to linezolid
- Multilocus sequence typing was performed using standard protocols

Results

In vitro antimicrobial activity

- Overall, linezolid showed MIC_{50} results of 0.5 µg/mL when tested against coagulase-negative staphylococci (CoNS) and 1 µg/mL against *S. aureus*, enterococci, and streptococci (Table 1)
- Linezolid ($MIC_{50/90}$, 1/1 µg/mL; 100.0% susceptible) was active against *S. aureus* and methicillin-resistant *S aureus* (MRSA; Tables 1 and 2)
  - Daptomycin (100.0% susceptible), vancomycin (100.0% susceptible), teicoplanin (96.7%–100.0% susceptible), and trimethoprim-sulfamethoxazole (96.4% susceptible) were also active against MRSA (Table 2)
- Linezolid ($MIC_{50/90}$, 0.5/1 µg/mL; 99.6% susceptible) and daptomycin ($MIC_{50/90}$, 0.5/1 µg/mL; 99.8% susceptible) displayed equivalent MIC values against CoNS, which had MIC values 2-fold lower than vancomycin ($MIC_{50/90}$, 1/2 µg/mL; 100.0% susceptible) and 4-fold lower than teicoplanin ($MIC_{50/90}$, 2/4 µg/mL; 92.0%–99.5% susceptible); tigecycline was very active against CoNS, while other agents had limited coverage (Tables 1 and 2)
- Linezolid ($MIC_{50/90}$, 1/2 µg/mL; 98.5% susceptible), ampicillin ($MIC_{50/90}$, 1/2 µg/mL; 99.8%–100.0% susceptible), daptomycin ($MIC_{50/90}$, 1/1 µg/mL; 100.0% susceptible), and vancomycin ($MIC_{50/90}$, 1/2 µg/mL; 99.6% susceptible) showed similar potencies against *E. faecalis* (Table 2)
- Tigecycline ($MIC_{50/90}$, 0.03/0.06 µg/mL; 99.7% susceptible), linezolid ($MIC_{50/90}$, 1/1 µg/mL; 99.3% susceptible), and daptomycin ($MIC_{50/90}$, 2/2 µg/mL; 99.7% susceptible) were active against *Enterococcus faecium* (Table 2)
- Streptococci were very susceptible to linezolid ($MIC_{50/90}$, 1/2 µg/mL; >99.9% susceptible), except for 1 *Streptococcus gallolyticus* (linezolid MIC, 4 µg/mL) and 1 *Streptococcus mitis* (MIC, 16 µg/mL; Table 3)

Table 1 Antimicrobial activity of linezolid tested against the main organisms and organism groups of isolates as part of the 2016 ZAAPS Program

Organism / organism group[a] (no. of isolates)	No. of isolates at MIC (µg/mL; cumulative %)									MIC_{50}	MIC_{90}
	≤0.06	0.12	0.25	0.5	1	2	4	8	>8		
S. aureus (3,990)		1 <0.1	23 0.6	649 16.9	2,959 91.0	357 >99.9	1 100.0			1	1
MRSA (1,139)		1 0.1	6 0.6	232 21.0	816 92.6	84 100.0				1	1
MSSA (2,851)			17 0.6	417 15.2	2,143 90.4	273 >99.9	1 100.0			1	1
CoNS (1,140)		2 0.2	81 7.3	681 67.0	354 98.1	18 99.6	0 99.6	0 99.6	4 100.0	0.5	1
Oxacillin-resistant (767)		1 0.1	43 5.7	460 65.7	242 97.3	17 99.5	0 99.5	0 99.5	4 100.0	0.5	1
Oxacillin-susceptible (373)		1 0.3	38 10.5	221 69.7	112 99.7	1 100.0				0.5	1
Enterococcus spp. (854)			5 0.6	155 18.7	539 81.9	145 98.8	8 99.8	2 100.0		1	2
E. faecalis (531)			4 0.8	98 19.2	309 77.4	112 98.5	8 100.0			1	2
E. faecium (292)				49 16.8	214 90.1	27 99.3	0 99.3	2 100.0		1	1
S. pneumoniae (1,136)			5 0.4	153 13.9	796 84.0	182 100.0				1	2
VGS (469)		3 0.6	16 4.1	121 29.9	307 95.3	20 99.6	1 99.8		1 100.0	1	1
BHS (736)				33 4.5	638 91.2	65 100.0				1	1

[a] MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; CoNS, coagulase-negative staphylococci; VGS, viridans group streptococci; BHS, β-hemolytic streptococci
CoNS includes *Staphylococcus arlettae* (1), *S. auricularis* (1), *S. capitis* (77), *S. caprae* (19), *S. carnosus* (1), *S. cohnii* (4), *S. epidermidis* (588), *S. gallinarum* (1), *S. haemolyticus* (159), *S. hominis* (106), *S. lentus* (2), *S. lugdunensis* (102), *S. pasteuri* (3), *S. pettenkoferi* (3), *S. pseudintermedius* (4), *S. saprophyticus* (27), *S. schleiferi* (1), *S. sciuri* (5), *S. simulans* (10), *S. warneri* (25), *S. xylosus* (1)
VGS includes *Streptococcus anginosus* (107), *S. anginosus* group (18), *S. australis* (2), *S. bovis* group (1), *S. constellatus* (17), *S. cristatus* (3), *S. equinus* (2), *S. gallolyticus* (44), *S. gordonii* (8), *S. infantis* (1), *S. intermedius* (4), *S. lutetiensis* (4), *S. mitis* (1), *S. mitis* group (159), S. mitis/oralis (6), *S. mutans* (3), *S. oralis* (2), *S. parasanguinis* (27), *S. salivarius* (9), *S. salivarius* group (12), *S. salivarius/vestibularis* (11), *S. sanguinis* (21), *S. vestibularis* (7)
BHS includes *Streptococcus agalactiae* (265), *S. canis* (2), *S. dysgalactiae* (137), *S. equi* (1), *S. pyogenes* (331)

Table 2 Activity of linezolid and comparator antimicrobial agents when tested against gram-positive clinical isolates as part of the 2016 ZAAPS Program

Organism/group[a] (no.) antimicrobial agent	MIC (µg/mL): MIC_{50}	MIC_{90}	CLSI[b] %S	%I	%R	EUCAST[b] %S	%I	%R
MRSA (1,139)								
Linezolid	1	1	100.0		0.0	100.0		0.0
Clindamycin	≤0.25	>2	62.2	0.2	37.6	62.2	0.1	37.8
Daptomycin	0.5	0.5	100.0			100.0		0.0
Erythromycin	>8	>8	35.2	4.0	60.8	35.6	1.3	63.0
Gentamicin	≤1	>8	75.1	0.4	24.5	74.6		25.4
Levofloxacin	>4	>4	35.6	1.4	62.9	35.6		64.4
Teicoplanin	≤0.5	1	100.0	0.0	0.0	96.7		3.3
Tetracycline	≤0.5	>8	79.0	0.8	20.2	78.1	0.4	21.5
Tigecycline	0.06	0.25				100.0		0.0
TMP-SMX	≤0.5	≤0.5	96.4		3.6	96.4	0.2	3.4
Vancomycin	1	1	100.0	0.0	0.0	100.0		0.0
MSSA								
Linezolid	1	1	100.0		0.0	100.0		0.0
Ceftriaxone	4	8	100.0[c]		0.0			
Clindamycin	≤0.25	≤0.25	97.7	0.1	2.2	97.6	0.1	2.3
Daptomycin	0.5	0.5	100.0			100.0		0.0
Erythromycin	0.25	>8	82.0	4.2	13.8	82.7	1.5	15.7
Gentamicin	≤1	≤1	96.2	0.3	3.5	95.8		4.2
Levofloxacin	0.25	0.25	96.7	0.3	3.0	96.7		3.3
Penicillin	1	>2	25.8		74.2	25.8		74.2
Piperacillin-tazobactam	1	2	100.0[c]		0.0	100.0		0.0
Teicoplanin	≤0.5	≤0.5	100.0	0.0	0.0	99.9		0.1
Tetracycline	≤0.5	≤0.5	94.8	0.5	4.7	94.3	0.1	5.6
Tigecycline	0.06	0.12				100.0		0.0
TMP-SMX	≤0.5	≤0.5	99.8		0.2	99.8	0.0	0.2
Vancomycin	0.5	1	100.0	0.0	0.0	100.0		0.0
CoNS (1,140)								
Linezolid	0.5	1	99.6		0.4	99.6		0.4
Ceftriaxone	>8	>8	32.7[c]		67.3			
Clindamycin	≤0.25	>2	73.4	1.2	25.4	72.4	1.1	26.6
Daptomycin	0.5	1	99.8			99.8		0.2
Erythromycin	>8	>8	40.4	1.8	57.9	40.4	0.7	58.9
Gentamicin	≤1	>8	60.5	5.3	34.2	56.7		43.3
Levofloxacin	0.5	>4	53.6	4.4	42.1	53.6		46.4
Oxacillin	>2	>2	32.7		67.3	34.8		65.2
Piperacillin-tazobactam	2	>16	32.7[c]		67.3	34.8		65.2
Teicoplanin	2	4	99.5	0.4	0.1	92.0		8.0
Tetracycline	≤0.5	>8	86.1	1.1	12.7	80.1	3.9	16.1
Tigecycline	0.06	0.25				100.0		0.0
TMP-SMX	≤0.5	>4	67.9		32.1	67.9	14.3	17.8
Vancomycin	1	2	100.0	0.0	0.0	100.0		0.0
E. faecalis (531)								
Linezolid	1	2	98.5	1.5	0.0	100.0		0.0
Ampicillin	1	2	100.0		0.0	99.8	0.2	0.0
Daptomycin	1	1	100.0					
Erythromycin	>16	>16	11.7	34.6	53.7			
Levofloxacin	1	>4	73.8	0.8	25.4	74.6		25.4
Piperacillin-tazobactam	4	8				99.8	0.2	0.0
Teicoplanin	≤0.5	≤0.5	99.6	0.0	0.4	99.6		0.4
Tigecycline	0.06	0.12				100.0	0.0	0.0
Vancomycin	1	2	99.6	0.0	0.4	99.6		0.4
E. faecium (292)								
Linezolid	1	1	99.3	0.0	0.7	99.3		0.7
Ampicillin	>16	>16	8.2		91.8	7.2	1.0	91.8
Daptomycin	2	2	99.7					
Erythromycin	>16	>16	5.1	6.5	88.4			
Levofloxacin	>4	>4	5.1	4.1	90.8	9.2		90.8
Piperacillin-tazobactam	>16	>16				7.2	1.0	91.8
Teicoplanin	≤0.5	>16	75.7	9.6	14.7	74.0		26.0
Tigecycline	0.03	0.06				99.7	0.0	0.3
Vancomycin	0.5	>16	70.2	1.4	28.4	70.2		29.8
S. pneumoniae (1,136)								
Linezolid	1	2	100.0			100.0	0.0	0.0
Amoxicillin-clavulanic acid	≤0.03	2	90.8	3.5	5.6			
Ceftriaxone	0.03	1	83.6 92.9	9.2 5.2	7.1[d] 1.9[e]	83.6	14.4	1.9
Clindamycin	≤0.25	>2	77.6	0.2	22.2	77.8		22.2
Erythromycin	0.03	>32	67.3	0.3	32.5	67.3	0.3	32.5
Levofloxacin	1	2	98.1	0.4	1.6	98.1		1.9
Penicillin	0.03	2	65.9 65.9 91.0	18.5 7.9	15.6[f] 34.1[g] 1.1[h]	65.9 65.9	25.1	34.1[d] 9.0[e]
Piperacillin-tazobactam	≤0.06	4						
Tetracycline	≤0.25	>8	69.3	0.3	30.5	69.3	0.3	30.5
Tigecycline	0.03	0.06						
TMP-SMX	0.25	>4	68.8	11.4	19.7	76.3	4.0	19.7
Vancomycin	0.25	0.5	100.0			100.0		0.0
VGS (469)								
Linezolid	1	1	99.6					
Amoxicillin-clavulanic acid	0.06	2				81.0	12.2	6.8
Ceftriaxone	0.12	1	91.3	3.0	5.8	87.2		12.8
Clindamycin	≤0.25	>2	86.1	0.9	13.0	87.0		13.0
Daptomycin	0.25	0.5	100.0					
Erythromycin	0.03	>32	56.5	0.9	42.6			
Levofloxacin	1	2	94.2	1.1	4.7			
Penicillin	0.06	1	73.6	19.6	6.8	81.0	12.2	6.8
Piperacillin-tazobactam	0.25	4				81.0	12.2	6.8
Teicoplanin	0.12	0.25				100.0		0.0
Tetracycline	0.5	>8	65.7	2.3	32.0			
Tigecycline	0.03	0.06						
TMP-SMX	≤0.12	4						
Vancomycin	0.5	0.5	100.0			100.0		0.0
BHS (736)								
Linezolid	1	1	100.0			100.0	0.0	0.0
Amoxicillin-clavulanic acid	≤0.03	0.06	99.6			99.9		0.1
Ceftriaxone	0.03	0.06	100.0			99.9		0.1
Clindamycin	≤0.25	>2	87.9	0.7	11.4	88.6		11.4
Daptomycin	≤0.06	0.25	100.0			100.0		0.0
Erythromycin	0.03	>32	77.7	1.8	20.6	77.7	1.8	20.6
Levofloxacin	0.5	1	95.9	0.8	3.3	95.9		4.1
Penicillin	0.015	0.06	99.6			99.9		0.1
Piperacillin-tazobactam	0.12	0.25				99.9		0.1
Teicoplanin	0.25	0.25				100.0		0.0
Tetracycline	0.5	>8	52.0	2.7	45.3	51.3	0.7	48.0
Tigecycline	0.06	0.06				100.0	0.0	0.0
TMP-SMX	≤0.12	0.25						
Vancomycin	0.25	0.5	100.0			100.0		0.0

[a] MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; CoNS, coagulase-negative staphylococci; VGS, viridans group streptococci; BHS, β-hemolytic streptococci; TMP-SMX, trimethoprim-sulfamethoxazole
[b] Criteria as published by CLSI (2017) and EUCAST (2017)
[c] Susceptibility based on oxacillin results
[d] Using meningitis breakpoints
[e] Using non-meningitis breakpoints
[f] Using oral breakpoints
[g] Using parenteral, meningitis breakpoints
[h] Using parenteral, non-meningitis breakpoints

Oxazolidinone resistance mechanisms and epidemiology

- One *S. aureus* from Panama City (linezolid MIC, 4 µg/mL) carried *cfr*, while 4 *Staphylococcus epidermidis* (linezolid MIC, 16-32 µg/mL) from Italy (3) and Germany (1) carried 23S rRNA alterations with or without mutation in the L3 and/or L4 (Table 3)
- Eight *E. faecalis* (Guatemala City [4], Durango [2], Taipei [1], and Paris [1]) had linezolid MIC values of 4 µg/mL and all harbored *optrA* (Table 3)
- Two clonally related *E. faecium* (Rome) were linezolid-resistant (MIC, 8 µg/mL) and had 23S rRNA mutations (Table 3)
- One *S. gallolyticus* (linezolid MIC, 4 µg/mL) and 1 *S. mitis* (linezolid MIC, 16 µg/mL) displaying elevated MIC results for linezolid had *optrA* and 23S rRNA mutations, respectively (Table 3)
- Clonally disseminated isolates with decreased susceptibility to linezolid have been observed in Guatemala City (*E. faecalis*), Rome (*E. faecium*), and Milan (*S. epidermidis*) (Table 3)

Table 3 Isolates with elevated or resistant-level linezolid MIC values (≥4 µg/mL) in the 2016 ZAAPS Program

Collection#	Organism	City	Country	Linezolid MIC (µg/mL)	Linezolid Resistance mechanism(s)	PFGE	MLST
983392	*S. aureus*	Panama City	Panama	4	*cfr*		ST72
973450	*E. faecalis*	Paris	France	4	*optrA*		ST775
956335	*E. faecalis*	Guatemala City	Guatemala	4	*optrA*	EF361A	ST256
956343	*E. faecalis*	Guatemala City	Guatemala	4	*optrA*	EF361A	ST256
956349	*E. faecalis*	Guatemala City	Guatemala	4	*optrA*	EF361A	ST256
956359	*E. faecalis*	Guatemala City	Guatemala	4	*optrA*	EF361A	ST256
986223	*E. faecalis*	Durango	Mexico	4	*optrA*	EF126A	ST480
986247	*E. faecalis*	Durango	Mexico	4	*optrA*	EF126B	ST480
981649	*E. faecalis*	Taipei	Taiwan	4	*optrA*		ST766
954245	*E. faecium*	Roma	Italy	8	23S rRNA (G2576T)	EFM86A[a]	ST117
954473	*E. faecium*	Roma	Italy	8	23S rRNA (G2576T)	EFM86A[a]	ST117
948652	*S. epidermidis*	Kiel	Germany	16	23S rRNA (G2576T), L3(G137S, F147Y, M156T, H146P), L4 (71G72ins)		ST2
978113	*S. epidermidis*	Florence	Italy	32	23S rRNA (G2576T), L3 (M156T), L4 (71G72ins)		ST2
939504	*S. epidermidis*	Genova	Italy	16	23S rRNA (G2576T)		ST2
956526	*S. epidermidis*	Milan	Italy	16	23S rRNA (G2576T), L3 (M156T)	SEPI377A[b]	ST2
959706	*S. gallolyticus*	Bangkok	Thailand	4	*optrA*		ND
939344	*S. mitis* group	Ljubljana	Slovenia	16	23S rRNA (G2576T)		ND

[a] Isolates with an EFM86A PFGE profile were also observed in 2002, 2006, 2008, and 2009 in this site
[b] A *S. epidermidis* with an SEPI377A PFGE profile was also detected in 2015 in this site

Conclusions

- Linezolid remained active against 99.8% of isolates belonging to a global (except US) collection included in the 2016 ZAAPS Program
- Alterations in the 23S rRNA and/or L3/L4 proteins remain the main oxazolidinone resistance mechanisms in *E. faecium* and CoNS clinical isolates
- *optrA* emerged as the sole oxazolidinone resistance mechanism in *E. faecalis* and it was also detected in a *S. gallolyticus* isolate
- Surveillance and infection control will be important strategies to detect and contain the plasmid-mediated *optrA* resistance gene from disseminating

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