Potency of Radezolid (RX-1741) and Torezolid (DA-7157) Tested Against a Collection of Linezolid-non-susceptible Strains with Genetically Defined Resistance Mechanisms

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ABSTRACT

Objectives: To provide a potency evaluation of the investigational oxazolidinone radezolid (RX-1741), against another investigational oxazolidinone, torezolid (DA-7157), and linezolid when tested by reference methods against a collection of linezolid-non-susceptible (NS) Gram-positive cocci having genetically defined mechanisms of oxazolidinone resistance.

Methods: A total of 90 linezolid-NS Gram-positive cocci, obtained through the SENTRY Antimicrobial Surveillance Program, were tested for susceptibility by CLSI broth microdilution methods (M07-A8 and M100-S21). Linezolid, radezolid and torezolid were manufactured by Rib-X Pharmaceuticals, Inc. Torezolid could not be tested at concentrations greater than 32 mg/L due to solubility interference. Strains were screened for mutations in the 23S rRNA, L3 and L4 encoding genes by PCR and DNA sequencing, and for the presence of the *cfr* gene.

Results: From this linezolid-NS strain collection, 40 coagulase-negative staphylococci (CoNS) isolates were shown to possess a broad variety of linezolid resistance mechanisms, which included 23S rRNA, L3 and L4 mutations and *cfr*, alone or in combination. The *S. aureus* strains tested were either 23S rRNA G2576T mutants or *cfr* positive. All *E. faecalis* and *E. faecium* isolates contained G2576T mutations, except for one strain that exhibited only a L4 F101L alteration. Data describing the antimicrobial activity of the three oxazolidinone compounds against these organisms are shown in Table 1.

Conclusions: Radezolid and torezolid both demonstrated enhanced activity against this collection of 90 linezolid-NS strains. Radezolid showed at least two-fold greater potency when compared directly to torezolid against most strains. These investigational oxazolidinones demonstrate encouraging *in vitro* activity against contemporary linezolid-NS Gram-positive pathogens.

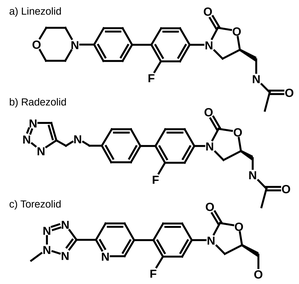
INTRODUCTION

Linezolid (Figure 1) is currently the only member of the oxazolidinone class of antimicrobial agents that has been approved for human use. Linezolid has demonstrated high clinical utility in a variety of human infections caused by Gram-positive cocci. Oxazolidinones inhibit protein synthesis by binding to a region in the peptidyl transferase center (PTC) of the 50S bacterial ribosome. Although rare (<1% in large surveillance studies), resistance to linezolid usually occurs by alterations to proteins (L3 or L4) or RNA (domain V of 23S rRNA) adjacent to the PTC, or by the expression of *cfr*, an acquired methyltransferase gene.

Radezolid (Figure 1, RX-1741) and torezolid (Figure 1, DA-7157) are investigational oxazolidinones in clinical development. Several studies to date have demonstrated that both of these antimicrobial agents retain some activity against several linezolid non-susceptible (NS) Gram-positive strains.

In this presentation, we perform a potency evaluation of radezolid, torezolid and linezolid when tested by reference methods against a collection of linezolid-NS Gram-positive cocci having genetically defined mechanisms of oxazolidinone resistance.

Figure 1. Chemical structures of oxazolidinones in this study



METHODS

Bacterial Strain Collection. Bacterial clinical isolates included in this investigation were obtained from the JMI Laboratories strain collection (number tested): *Enterococcus faecalis* (8), *Enterococcus faecium* (15), *Staphylococcus aureus* (27), and coagulase-negative staphylococci (CoNS; 40)

All isolates were identified to species level by at least two laboratories including a reference laboratory, JMI Laboratories (North Liberty, Iowa, USA).

Molecular Methods. Linezolid resistance mechanisms were characterized by molecular methods. All selected organisms were screened for mutations on 23S rRNA-, L3- and L4-encoding genes by PCR and nucleotide sequencing. In addition, the organisms were also screened for the *cfr* gene (Long et al., 2006; Mendes et al., 2008).

Susceptibility Test Methods. All isolates were tested for susceptibility to radezolid, torezolid (both manufactured by Rib-X Pharmaceuticals), linezolid, erythromycin, clindamycin and quinupristin/dalfopristin by reference broth microdilution methods according to the Clinical and Laboratory Standards Institute (CLSI; M07-A8, 2009) recommendations using fresh-frozen panels manufactured by JMI Laboratories. The quality assurance of the minimum inhibitory concentration (MIC) values was performed by concurrent testing of CLSI-recommended (M100-S21, 2011) control strains, including: *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212. Categorical interpretation of comparator MIC values was performed according to CLSI (M100-S21, 2011) criteria. MIC values for linezolid, radezolid, and torezolid against *S. aureus* ATCC 29213 were 2, 0.5, and 0.5 mg/L, respectively. MIC values for linezolid, radezolid, and torezolid against *E. faecalis* ATCC 29212 were 2, 0.25, and 0.5 mg/L, respectively.

RESULTS

A graphical summary of the activity of radezolid, torezolid and linezolid against all 90 strains by species/group is shown in Figure 2.

Against *S. aureus*

- Overall, radezolid ($MIC_{50/90}$, 1/4 mg/L) was two-fold more active than torezolid ($MIC_{50/90}$, 2/8 mg/L) against this *S. aureus* collection (Table 1).
- Radezolid and torezolid were eight- and four-fold more potent than linezolid ($MIC_{50/90}$, 8/16 mg/L; Table 1), respectively.
- *S. aureus* strains with elevated linezolid MIC values (≥8 mg/L) were either G2576T (nine strains) mutants or Cfr-producers (six strains, Table 2).
- Four out of six *cfr*-carrying *S. aureus* strains demonstrated torezolid MIC results two- to four-fold lower than radezolid (Table 2).

Against CoNS

- Overall, radezolid ($MIC_{50/90}$, 2/16 mg/L) was at least two-fold more active than torezolid ($MIC_{50/90}$, 4/>32 mg/L) when tested against CoNS, respectively (Table 1).
- Radezolid and torezolid were 16- and eight-fold more potent than linezolid ($MIC_{50/90}$, 32/>64 mg/L, Table 1), respectively.
- The CoNS isolates possessed a broader variety of linezolid resistance mechanisms, which included 23S rRNA, L3 and L4 mutations and Cfr production, alone or in combination (Table 3).
- *S. epidermidis* with G2447T or G2576T modifications usually showed MIC results for radezolid lower than torezolid (Table 3). A similar trend was noted for these compounds when tested against L3 and/or L4 mutants (strains 4596, 1590, 5174, 7715, 2466 and 8177).
- The *cfr*-carrying strain 4303, which also possessed L3 alterations, showed MIC values of 2, 2 and 64 mg/L for radezolid, torezolid and linezolid, respectively (Table 3). When this clinical isolate was subjected to plasmid curing, the resulting *cfr*-cured strain (8177) exhibited a similar MIC value (2 mg/L) for torezolid, while MIC results four-fold lower were noted for radezolid (0.5 mg/L). Linezolid MICs (8 mg/L) were eight-fold lower for the cured strain when compared to the index strain (4303).
- *S. epidermidis* with C2534T and T2504A alterations in 23S rRNA demonstrated elevated MIC results (≥32 mg/L) for radezolid, torezolid and linezolid (Table 3).
- Five out of ten *cfr*-carrying CoNS strains demonstrated radezolid MIC results two- to four-fold lower than torezolid (Table 3).

Against *E. faecium*

- Against *E. faecium,* radezolid ($MIC_{50/90}$, 0.25/0.5 mg/L) was eight- to 16-fold more active than torezolid ($MIC_{50/90}$, 2/8 mg/L). Linezolid $MIC_{50/90}$, was 4/16 mg/L (see Table 1).
- All *E. faecium* isolates included in this study harbored G2576T mutations (Table 4).

Against *E. faecalis*

- The MIC_{50} value of 0.25 mg/L for radezolid was eight- and 32-fold lower than torezolid (MIC_{50}, 2 mg/L) and linezolid (MIC_{50}, 8 mg/L), respectively, when tested against this collection of eight *E. faecalis* strains (Table 1).

Table 2. Resistance mechanisms and MIC values for radezolid, torezolid and linezolid when tested against linezolid-non-susceptible *S. aureus*

		Resistance mechanisms[a]				Antimicrobial agents (mg/L)[b]						
Organism	Isolate	*cfr*	23S	L3	L4	RZD	TZD	LZD	LEV	ERY	CLI	Q/D
S. aureus	1633	–	G2576T	WT	WT	0.5	2	8	0.12	64	0.25	0.25
S. aureus	12591	–	G2576T	WT	WT	1	1	8	16	4	0.25	1
S. aureus	99	–	G2576T	WT	WT	1	2	8	4	>128	>64	1
S. aureus	3342	–	G2576T	WT	WT	1	2	8	0.12	32	0.25	0.5
S. aureus	4303	–	G2576T	WT	WT	1	8	16	8	>128	0.5	0.5
S. aureus	699	–	G2576T	WT	WT	2	4	8	8	>128	>64	1
S. aureus	700	–	G2576T	WT	WT	2	4	16	4	>128	>64	1
S. aureus	269	–	G2576T	WT	WT	4	4	16	32	0.5	0.5	0.5
S. aureus	261	–	G2576T	WT	WT	4	>32	32	16	0.5	0.5	0.5
S. aureus	1848	+	WT	WT	WT	0.5	0.5	8	0.12	16	>64	2
S. aureus	8615[c]	+	WT	WT	WT	0.5	1	8	0.25	0.25	>64	2
S. aureus	737	+	WT	WT	WT	1	0.5	8	64	>128	>64	8
S. aureus	272	+	WT	WT	WT	2	1	8	>64	>128	>64	2
S. aureus	1687	+	WT	WT	WT	2	0.5	8	64	>128	>64	2
S. aureus	6952	+	WT	WT	WT	4	1	16	32	0.12	16	4

a. WT, wildtype.
b. RZD, radezolid; TZD, torezolid; LZD, linezolid; LEV, levofloxacin; ERY, erythromycin; CLI, clindamycin; Q/D, quinupristin/dalfopristin.
c. *S. aureus* RN4220 where a *cfr*-carrying plasmid was introduced by electroporation.

- All linezolid-NS *E. faecalis* possessed G2576T mutations, except for one strain that showed only a L4 F101L alteration (Table 5).
- Overall, radezolid displayed MIC values two- to eight-fold lower than torezolid when tested against linezolid-NS *E. faecalis,* except against isolate 6480, where a ≥32-fold difference was noted (Table 5).

Table 4. Resistance mechanisms and MIC values for radezolid, torezolid and linezolid when tested against linezolid-non-susceptible *E. faecium*

		Resistance mechanisms[a]				Antimicrobial agents (mg/L)[b]						
Organism	Isolate	*cfr*	23S	L3	L4	RZD	TZD	LZD	LEV	ERY	CLI	Q/D
E. faecium	2132	–	G2576T	WT	WT	0.25	1	4	64	>128	>64	1
E. faecium	36	–	G2576T	WT	WT	0.25	1	4	>64	>128	>64	0.5
E. faecium	1710	–	G2576T	WT	WT	0.25	1	4	64	>128	>64	0.5
E. faecium	5353	–	G2576T	WT	WT	0.25	1	4	>64	>128	>64	0.5
E. faecium	939	–	G2576T	WT	WT	0.25	1	4	64	>128	>64	0.5
E. faecium	2539	–	G2576T	WT	WT	0.25	1	4	>64	>128	>64	1
E. faecium	5036	–	G2576T	WT	WT	0.25	2	4	>64	>128	>64	1
E. faecium	6994	–	G2576T	WT	WT	0.25	2	4	64	>128	>64	0.5
E. faecium	2997	–	G2576T	WT	WT	0.25	2	8	32	64	>64	0.5
E. faecium	12093	–	G2576T	WT	WT	0.25	2	8	64	>128	>64	1
E. faecium	652	–	G2576T	WT	WT	0.25	2	4	>64	>128	>64	0.5
E. faecium	8199	–	G2576T	WT	WT	0.25	2	8	>64	1	0.06	0.25
E. faecium	8205	–	G2576T	WT	WT	0.25	2	8	64	>128	>64	4
E. faecium	4184	–	G2576T	WT	WT	0.25	2	4	64	>128	>64	0.5
E. faecium	559	–	G2576T	WT	WT	0.25	2	8	>64	>128	>64	0.5
E. faecium	6237	–	G2576T	WT	WT	0.25	4	16	32	>128	>64	1
E. faecium	535	–	G2576T	WT	WT	0.5	1	4	64	>128	>64	0.5
E. faecium	2129	–	G2576T	WT	WT	0.5	1	4	64	>128	>64	1
E. faecium	414	–	G2576T	WT	WT	0.5	2	4	>64	>128	>64	1
E. faecium	243	–	G2576T	WT	WT	0.5	2	4	32	>128	>64	2
E. faecium	2069	–	G2576T	WT	WT	0.5	2	8	64	>128	>64	2
E. faecium	6803	–	G2576T	WT	WT	0.5	4	16	64	>128	>64	1
E. faecium	14306	–	G2576T	WT	WT	0.5	8	16	64	>128	>64	1
E. faecium	1896	–	G2576T	WT	WT	0.5	8	32	>64	0.12	0.5	0.5
E. faecium	5534	–	G2576T	WT	WT	0.5	8	32	64	0.25	0.25	0.5
E. faecium	381	–	G2576T	WT	WT	0.5	4	8	64	>128	>64	1
E. faecium	6128	–	G2576T	WT	WT	1	4	16	>64	0.12	0.12	0.5

a. WT, wildtype.
b. RZD, radezolid; TZD, torezolid; LZD, linezolid; LEV, levofloxacin; ERY, erythromycin; CLI, clindamycin; Q/D, quinupristin/dalfopristin.

Table 3. Resistance mechanisms and MIC values for radezolid, torezolid and linezolid when tested against linezolid-non-susceptible CoNS

		Resistance mechanisms[a]				Antimicrobial agents (mg/L)[b]						
Organism	Isolate	*cfr*	23S	L3	L4	RZD	TZD	LZD	LEV	ERY	CLI	Q/D
S. epidermidis	512	–	G2447T	WT	WT	1	4	32	64	0.12	0.5	0.5
S. epidermidis	805	–	G2447T	WT	WT	1	4	32	16	≤0.06	0.25	0.5
S. epidermidis	7625	–	G2447T	WT	WT	2	4	>64	32	2	0.5	0.25
S. epidermidis	2268	–	G2447T	WT	WT	2	16	>64	32	2	0.5	0.5
S. epidermidis	3207	–	G2447T	WT	WT	64	>32	>64	32	≤0.06	2	1
S. epidermidis	2286	–	G2576T	H146R/M156T	71G72 ins	4	>32	>64	>64	8	2	0.5
S. epidermidis	3650	–	G2576T	WT	WT	0.5	2	8	8	>128	>64	1
S. epidermidis	1708	–	G2576T	WT	WT	0.5	2	8	8	0.12	0.25	0.12
S. epidermidis	11107	–	G2576T	WT	WT	0.5	4	16	64	0.25	0.5	0.25
S. hominis	10728	–	G2576T	WT	WT	0.5	4	8	8	>128	>64	1
S. epidermidis	1645	–	G2576T	WT	WT	1	4	16	16	0.25	0.5	0.25
S. capitis	856	–	G2576T	WT	WT	1	4	16	4	32	0.5	0.5
S. epidermidis	13064	–	G2576T	WT	WT	1	4	16	>64	0.25	0.5	0.25
S. epidermidis	1440	–	G2576T	WT	WT	1	8	32	>64	0.12	0.5	0.25
S. epidermidis	5401	–	G2576T	WT	WT	2	>32	64	>64	4	1	0.5
S. haemolyticus	2061	–	G2576T	WT	WT	2	>32	32	32	0.5	2	0.5
S. haemolyticus	2322	–	G2576T	WT	WT	4	8	32	16	64	1	1
S. hominis	2847	–	G2576T	WT	WT	4	8	16	2	0.5	0.5	0.5
S. haemolyticus	1923	–	G2576T	WT	WT	4	32	32	16	64	1	0.5
S. epidermidis	3417	–	G2576T	WT	WT	4	>32	>64	8	4	1	0.25
S. epidermidis	7521	–	G2576T	WT	WT	4	>32	>64	>64	8	2	0.5
S. epidermidis	8676	–	G2576T	WT	WT	4	>32	>64	>64	4	1	0.5
S. epidermidis	5289	–	G2576T	WT	WT	4	>32	>64	64	128	1	0.5
S. epidermidis	4596	–	WT	H146Q	N158S	1	8	32	8	2	0.25	0.5
S. epidermidis	1590	–	WT	H146Q	N158S	2	4	32	>64	2	0.25	0.5
S. epidermidis	5174	–	WT	L101V/F147L/A157R	N158S	0.5	2	8	8	64	>64	2
S. epidermidis	7715	–	WT	L101V/V154L/A157R	N158S	2	4	16	>64	2	0.12	0.25
S. epidermidis	2466	–	WT	L101V/V154L/A157R	P171S	2	2	8	>64	>128	0.12	0.25
S. epidermidis	8177[c]	–	WT	L101V/F147L/A157R	N158S	0.5	2	8	16	>128	>64	0.5
S. epidermidis	4303	+	WT	L101V/F147L/A157R	N158S	2	2	64	16	64	>64	8
S. epidermidis	5738	+	WT	L101V/S158Y/D159Y	WT	2	8	>64	8	≤0.06	>64	1
S. epidermidis	3058	+	WT	NT	NT	2	2	32	8	≤0.06	16	2
S. epidermidis	4045	+	WT	NT	NT	0.5	0.5	16	16	>128	>64	2
S. capitis	4593	+	WT	WT	WT	0.5	0.5	8	0.25	0.12	16	1
S. epidermidis	12898	+	WT	D159Y/S158Y	WT	0.5	2	16	8	>128	>64	1
S. cohnii	10842	+	WT	D159Y/S158F	A133T/N20S/V155I	1	2	16	8	>128	>64	8
S. epidermidis	2104	+	WT	NT	NT	16	8	>64	>64	4	>64	2
S. epidermidis	2174	+	WT	A157R/H146Q/	71G72ins/N158S	32	>32	>64	>64	8	>64	2
S. epidermidis	3147	+	C2534T	H146Q/V154L/A157R	N158S	32	>32	>64	>64	2	>64	2
S. epidermidis	3242	+	T2504A	WT	WT	64	>32	>64	32	≤0.06	4	1

a. WT, wildtype; NT, not tested; L3 L101V and L4 N158S mutations are known to not confer decreased susceptibility to linezolid.
b. RZD, radezolid; TZD, torezolid; LZD, linezolid; LEV, levofloxacin; ERY, erythromycin; CLI, clindamycin; Q/D, quinupristin/dalfopristin.
c. *cfr*-cured *S. epidermidis* 4303.

Table 1. MIC (mg/L) results obtained for radezolid, torezolid and linezolid when tested against 90 linezolid-non-susceptible strains

	Radezolid		Torezolid		Linezolid		Erythromycin			Clindamycin			Quinupristin/dalfopristin		
Organism (n)	$MIC_{50/90}$	Range	$MIC_{50/90}$	Range	$MIC_{50/90}$	Range	$MIC_{50/90}$	Range	%R[a]	$MIC_{50/90}$	Range	%R	$MIC_{50/90}$	Range	%R
S. aureus (15)	1/4	0.5 - 4	2/8	0.5->32	8/16	8 - 32	64/>128	0.12->128	66.7	>64/>64	0.25->64	60.0	1/4	0.25-8	13.4
CoNS (40)	2/16	0.5 - 64	4/>32	0.5->32	32/>64	8 - >64	2/>128	≤0.06 - >128	40.0	1/>64	0.12->64	37.5	0.5/2	0.12-8	5.0
E. faecium (27)	0.25/0.5	0.25 - 1	2/8	1-8	4/16	4 - 32	>128/>128	0.12->128	85.2	>64/>64	0.06->64	NA[b]	0.5/2	0.25-4	3.7
E. faecalis (8)	0.25/-[c]	0.25 - 2	2/-	0.5->32	8/-	4 - 32	128/-	8 - >128	100.0	>64/-	>64	NA	8/-	8 - >16	100.0

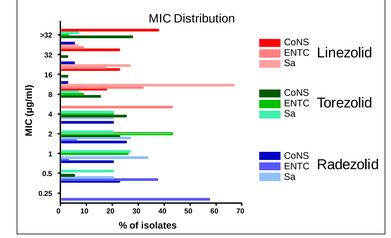
a. Criteria as published by CLSI (2011).
b. NA, indicates that breakpoint is not available.
c. Insufficient strains (<10) to determine MIC_{50}.

Table 5. Resistance mechanisms and MIC values for radezolid, torezolid and linezolid when tested against linezolid-non-susceptible *E. faecalis*

		Resistance mechanisms[a]				Antimicrobial agents (mg/L)[b]						
Organism	Isolate	*cfr*	23S	L3	L4	RZD	TZD	LZD	LEV	ERY	CLI	Q/D
E. faecalis	1628	–	G2576T	WT	WT	0.25	2	16	16	32	>64	8
E. faecalis	534	–	G2576T	WT	WT	0.25	2	8	1	>128	>64	8
E. faecalis	6943	–	G2576T	WT	WT	0.25	0.5	8	32	128	>64	8
E. faecalis	2759	–	G2576T	WT	WT	0.5	4	8	32	>128	>64	8
E. faecalis	44	–	G2576T	WT	WT	0.5	4	8	64	16	>64	8
E. faecalis	1142	–	G2576T	WT	WT	0.5	4	16	32	8	>64	8
E. faecalis	6480	–	G2576T	WT	WT	2	>32	32	64	>128	>64	>16
E. faecalis	4071	–	WT	WT	F101L	0.25	2	4	2	>128	>64	8

a. WT, wildtype.
b. RZD, radezolid; TZD, torezolid; LZD, linezolid; LEV, levofloxacin; ERY, erythromycin; CLI, clindamycin; Q/D, quinupristin/dalfopristin.

Figure 2. Activity of the three oxazolidinones against 90 linezolid non-susceptible strains by species/group



CONCLUSIONS

- Radezolid and torezolid demonstrated enhanced activity against this collection of 90 linezolid-NS strains. Radezolid showed at least two-fold greater potency when compared directly to torezolid against most strains.
- The activities of these two new investigational oxazolidinones against contemporary linezolid-NS Gram-positive pathogens are encouraging and support further clinical development of these compounds.

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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® Annual Appraisal of Potency and Spectrum program. *Diagn Microbiol Infect Dis.* 68:459-67.
2. Clinical and Laboratory Standards Institute (2009) *M07-A8, Methods of dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-seventh edition*. Wayne, PA: CLSI.
3. Clinical and Laboratory Standards Institute (2011). *M100-S21. Performance standards for antimicrobial susceptibility testing: 21st informational supplement*. Wayne, PA: CLSI.
4. Livermore DM, Mushtaq S, Warner M, Woodford N (2009). Activity of oxazolidinone TR-700 against linezolid-susceptible and –resistant staphylococci and enterococci. *J. Antimicrob. Chemother*. 63:713-75.
5. Locke JB, Finn J, Hilgers M, et al. (2010). Structure-activity relationships of diverse oxazolidinones for linezolid-resistant *Staphylococcus aureus* strains possessing the *cfr* methyltransferase gene or ribosomal mutations. *Antimicrob. Agents Chemother*. 54: 5337-5343.
6. Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B (2006). The *cfr* rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. *Antimicrob. Agents Chemother*. 50: 2500-2505.
7. Mendes RE, Deshpande LM, Castanheira M, DiPersio J, Saubolle MA, Jones RN (2008). First report of *cfr*-mediated resistance to linezolid in human staphylococcal clinical isolates recovered in the United States. *Antimicrob. Agents Chemother*. 52: 2244-2246.