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TR-700, a Novel Oxazolidinone, Tested Against Linezolid-Resistant Gram-positive Species with Well-Characterized Resistance Mechanisms

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ABSTRACT

Background: TR-700 is the active component of orally administered prodrug TR-701. TR-700 has demonstrated potent activity against numerous Gram-positive species and in this study, a worldwide collection of linezolid-resistant (LZD-R) organisms was investigated.
Methods: 240 strains were susceptibility (S) tested by CLSI reference broth microdilution methods including 120 LZD-R and 120 controls, matched by species, geographic origin, site of infection and time. Species of LZD-S/LZD-R strains were: *E. faecalis* (16/16), *E. faecium* (EFM; 55/55), *S. aureus* (SA; 8/8), coagulase-negative staphylococci (CoNS; 8 spp., 40/40) and viridans group streptococci (VGS; 2 spp., 1/1). 23S rRNA target mutations or *cfr* genes were detected by PCR and sequencing.
Results: Among LZD-R strains, the R-mechanisms were G2576TT (109), *cfr* (4) and unknown (7), with strains originating from Europe, Far East, North and South America. Most strains were multidrug-R (MDR) and *cfr* isolates exhibited the R to phenicols, clindamycin, LZD, pleuromutilins and streptogramin B. TR-700 MIC values, regardless of species, were 4- to 32-fold greater than LZD-S isolates. TR-700 MIC results were ≤4, ≤8 or ≤16 µg/mL for 88, 96 and >99% of LZD-R strains, respectively. TR-700 $MIC_{50/90}$ results were lower for LZD-R enterococci (1/2 µg/mL) compared to staphylococci (4/16 µg/mL).

Organisms (no. tested)	Agent	No. occurrences at MIC (µg/mL): ≤0.5	1	2	4	8	16	≥32
SA (8)	TR-700	2	3	3	0	0	0	0
	LZD	0	0	0	0	4[a]	3	1
CoNS (40)	TR-700	0	2	10	15	8	4	1
	LZD	0	0	0	0	4[a]	13	23
EF, EFM and VGS (72)	TR-700	6	26	34	5	1	0	0
	LZD	0	0	0	14[b]	23[c]	30	5

a. Non-susceptible
b. Intermediate
c. Resistant

Conclusions: TR-700 exhibits enhanced activity against LZD-R and control wildtype strains compared to LZD. A significant number (nearly 90%) of LZD-R strains were inhibited by achievable levels (≤4 µg/mL) of TR-700. All strains with the emerging *cfr*-mediated R had TR-700 MICs at ≤8 µg/mL.

INTRODUCTION

Linezolid, the first approved antibacterial drug of the oxazolidinone class, has become an important agent for the treatment of serious infections caused by Gram-positive cocci. Since its regulatory approval in 2000, several species of Gram-positive pathogens have been identified with elevated MIC values (≥8 µg/mL) for linezolid. The mechanisms of resistance have generally been determined and found to be dominated by 23S rRNA target mutations, usually at four sites (G2576T, T2500A, G2505A, G2447T), some only occurring in laboratory-derived strains.

More recently resistance due to a *cfr* rRNA methyltransferase has been described in veterinary and human isolates of staphylococci. Four human staphylococcal clinical isolates were assessed during this study.

Susceptibility to linezolid, as defined by the US-FDA and the CLSI, are strains having a linezolid MIC of ≤4 µg/mL (staphylococci) or ≤2 µg/mL (streptococci and enterococci). Oxazolidinone agents are considered static and clinical utility has been determined by pharmacokinetic / pharmacodynamic (PK/PD) parameters balanced against a predictable toxicity profile. Therefore, advances in this class will require greater potencies against linezolid-non-susceptible Gram-positive pathogens (includes *S. aureus*, coagulase-negative staphylococci [CoNS] and *E. faecium* or *E. faecalis*), improved PK/PD target attainment and greater safety.

This study determined the potency and spectrum of a new oxazolidinone TR-700, the active component of the prodrug TR-701. CLSI reference broth microdilution methods were used against a collection of linezolid-non-susceptible isolates having well-characterized resistance mechanisms. Comparator agents were concurrently tested as well as geographically matched control strains (linezolid-susceptible) of the same species occurring during the same sampling period.

METHODS

Organisms (240 clinical strains): Linezolid-resistant or -intermediate clinical strains, as determined by CLSI M100-S18 (2008) breakpoint criteria were utilized in this study. These strains have been characterized by molecular methods to determine the mechanism of resistance (113 total).

- *E. faecalis* (15; all G2576T mutants)
- *E. faecium* (54; all G2576T mutants)
- *S. aureus* (eight; six G2576T mutants and two with a *cfr* mechanism)
- *S. epidermidis* (24; 22 G2576T mutants and two with *cfr*)
- *S. capitis* (two G2576T mutants)
- *S. haemolyticus* (five G2576T mutants)
- *S. simulans* (two G2576T mutants)
- *S. xylosis* (two; both G2576T mutants)
- *Streptococcus oralis* (one G2576T mutant)

Linezolid-resistant or -intermediate phenotype strains without detectable mechanisms (seven total; mechanism under continued study) were also tested.

- *E. faecalis* (one)
- *E. faecium* (one)
- *S. epidermidis* (five)

In addition, matched linezolid-susceptible control strains (120 total) were used to compare oxazolidinone potencies.

- Strains were matched by species, time (same year of isolation) and institution.
- Secondary matching by site of infection and clinical service were also applied.

Antimicrobial agents (13 comparator agents): TR-700 and linezolid MICs were compared to 12 other agents including vancomycin and daptomycin (Table 1).

Susceptibility testing methods: CLSI M7-A7 (2006) broth microdilution method using cation-adjusted Mueller-Hinton broth was applied throughout. Additional medium supplements to 50 mg/L calcium for testing daptomycin and lysed horse blood (2-5%) were used for testing fastidious streptococci. The CLSI M2-A9 (2006) disk diffusion method with oxacillin and cefoxitin disks was used to confirm methicillin resistance patterns among the staphylococci. CLSI M100-S18 (2008) interpretive criteria and quality control ranges of comparison agents were applied.

Selection of resistant strains: From the MIC panel, the entire contents of the last well with growth was removed and placed into broth media. Tubes were placed in an ambient air incubator to allow growth to reach a 0.5 McFarland standard (1.5 to 3 hours). MIC panels were then inoculated using the appropriate volume and concentration, and repeated through 14 passaging days. The strains demonstrating emerging resistance were tested against selected antimicrobial agents from other classes upon completion of passaging in order to evaluate the emergence of cross-resistance. Reversion to susceptible was also assessed by three passages performed on drug-free media with final retesting by the broth microdilution method. All strains with oxazolidinone MIC values increasing by >four-fold were screened for resistance mechanisms.

Single-step mutation rate: Fresh colonies from an agar plate were emulsified in sterile broth or saline until a suspension equal to at least a 4 McFarland Standard (≥1 to 2 X 10^8 CFU/mL) was achieved. 1ml of the inoculum suspension was plated on appropriate agar plates containing 4X, 8X and 16X the TR-700 MIC. Serial dilutions of the inoculum suspension were plated on antimicrobial-free agar plates to quantitate the colony count (CFU/mL).

RESULTS

- Table 1 summarizes MIC results for TR-700 and comparator agents tested against 69 enterococcal and 39 staphylococcal isolates having a G2576T mutation of the 23S rRNA target. The highest MIC values were 16 and >32 µg/mL for TR-700 and linezolid, respectively. TR-700 was four- to eight-fold superior, inhibiting 88.4% of *Enterococcus* strains at ≤4 µg/mL.
- The *cfr*-positive strains (four isolates; *S. aureus* [two] and *S. epidermidis* [two]) results showed linezolid MIC values ranging from 8 to >32 µg/mL, and TR-700 results were generally several-fold lower (MIC range, 0.5-8 µg/mL; Table 1)
- Seven strains having linezolid MIC results reproducibly at ≥8 µg/mL, but without a detectable resistance mechanism, are tabulated in Table 1. TR-700 was 16-fold more active than linezolid.
- Only two viridans group *streptococcal* species (*S. mitis* [susceptible control] and *S. oralis*) were tested. The *S. oralis* isolate was non-susceptible to linezolid and TR-700 was at least four-fold more potent than linezolid (data not shown).
- Table 2 compares the TR-700 activity to that of vancomycin against all 240 strains. Against linezolid-susceptible isolates, TR-700 was generally more potent than vancomycin, however against some linezolid-non-susceptible strains, vancomycin remained more effective in vitro (examples: CoNS and viridans group streptococci).
- TR-700 MIC distributions (see Table 3) for two organism groups (enterococci/streptococci and staphylococci) are compared to those of linezolid when testing only the linezolid-non-susceptible isolates (120 strains). The differences in the median MIC values favored TR-700 by four-fold. Only one enterococcus and 13 staphylococci among these 120 strains (11.7%) would be considered TR-700-non-susceptible, if the same susceptibility breakpoints as linezolid were applied to this investigational oxazolidinone.
- TR-700 results among the compared strains, showed no passaging series MIC increases. In contrast, the three tested strains exhibited a two-fold increase in the control linezolid MIC results (data not shown). The tests with the non-compared strains indicated a consistent two-fold TR-700 MIC elevation for three organisms (MSSA, VanA *E. faecalis*, *emtA S. pyogenes*) and no change in the TR-700 MIC for *S. aureus* USA100 and USA400 clonal representatives. Strains with elevated MIC values at the end of the experiment (all remained in susceptible MIC ranges ≤4 µg/mL for linezolid; ≤0.5 µg/mL for TR-700), had MIC values that returned to baseline.

Table 1. TR-700 antimicrobial activity compared to other antimicrobials when tested against linezolid-non-susceptible strains with defined mechanisms of resistance.

Mechanism/Organism group (no.tested)/ Antimicrobial agent	MIC (µg/mL) 50%	90%	Range	% by category[a] Susceptible/Resistant
G2576T				
Enterococcus spp. (69)[b]				
TR-700	2	2	0.5-8	-/-
Linezolid	8	16	4-32	0.0 / 81.2
Ciprofloxacin	>4	>4	1->4	1.4 / 98.6
Daptomycin	1	2	0.12-4	100.0 / -
Erythromycin	>2	>2	≤0.25->2	10.1 / 34.8
Quinupristin/dalfopristin	1	>2	≤0.25-8	75.4 / 23.2
Teicoplanin	>16	>16	≤0.12->16	34.8 / 62.3
Tetracycline	≤2	>8	≤2->8	59.4 / 40.6
Tigecycline	0.06	0.25	≤0.03-0.5	98.6 / -
Vancomycin	>16	>16	0.5->16	27.5 / 69.6
Staphylococci (39)[c]				
TR-700	4	8	0.5-16	-/-
Linezolid	16	>32	8->32	0.0 / -
Ciprofloxacin	>4	>4	0.25->4	2.6 / 97.4
Clindamycin	1	>2	≤0.25->2	20.5 / 23.1
Daptomycin	0.25	0.5	0.25-1	100.0 / -
Erythromycin	>2	>2	≤0.25->2	23.1 / 7.9
Gentamicin	>8	>8	≤2->8	30.8 / 56.4
Oxacillin	>2	>2	≤0.25->2	5.1 / 94.9
Quinupristin/dalfopristin	0.5	1	≤0.25-2	92.3 / 0.0
Teicoplanin	4	8	0.5-16	92.3 / 0.0
Tetracycline	≤2	>8	≤2->8	74.4 / 20.5
Trimethoprim/sulfamethoxazole	>2	>2	≤0.5->2	33.3 / 66.7
Vancomycin	2	2	0.5-2	100.0 / 0.0
cfr gene (4)[d]				
TR-700	1	-	0.5-8	-/-
Linezolid	32	-	8->32	0.0 / -
Ciprofloxacin	>4	-	>4	0.0 / 100.0
Clindamycin	>4	-	>4	0.0 / 100.0
Daptomycin	≤0.25	-	≤0.25	100.0 / -
Erythromycin	≤0.25	-	≤0.25->2	50.0 / 50.0
Gentamicin	>8	-	≤2->8	25.0 / 75.0
Oxacillin	>2	-	>2	0.0 / 100.0
Quinupristin/dalfopristin	>2	-	1->2	25.0 / 75.0
Teicoplanin	≤2	-	≤2	100.0 / 0.0
Tetracycline	≤2	-	≤2->8	75.0 / 25.0
Trimethoprim/sulfamethoxazole	>2	-	≤0.5->2	25.0 / 75.0
Vancomycin	1	-	1-2	100.0 / 0.0
Unknown (7)[e]				
TR-700	2	-	1->32	-/-
Linezolid	32	-	8->32	0.0 / 85.7
Ciprofloxacin	>4	-	1->4	14.3 / 85.7
Daptomycin	0.25	-	≤0.03-0.5	100.0 / -
Erythromycin	≤0.25	-	≤0.25->2	57.1 / 42.9
Quinupristin/dalfopristin	0.5	-	≤0.25-8	85.7 / 14.3
Tetracycline	≤2	-	≤2->8	85.7 / 14.3
Tigecycline	0.06	-	0.06-0.25	100.0 / -
Vancomycin	2	-	1->16	85.7 / 14.3

a. CLSI (2008) interpretive criteria for all agents except tigecycline where USA-FDA breakpoints were applied. - = no criteria are published.
b. Includes *E. faecalis* (15) and *E. faecium* (54).
c. Includes *S. aureus* (six), *S. epidermidis* (22), *S. capitis* (two), *S. haemolyticus* (five), *S. simulans* (two) and *S. xylosis* (two).
d. Includes *S. aureus* (two) and *S. epidermidis* (two).
e. Includes *E. faecalis* (one), *E. faecium* (one) and *S. epidermidis* (five).

Table 2. Activity of TR-700 and vancomycin tested against enterococci (142 strains), S. aureus (16 strains), coagulase-negative staphylococci (CoNS; 80) and viridans group streptococci (two strains).

Organism/resistance pattern (no. tested)	MIC (µg/mL) 50%	90%	Range	Resistance mechanism (no.) G2576T	cfr
E. faecium (110)					
Linezolid-susceptible (55)				0	0
TR-700	0.25	0.5	0.12-0.5	-	-
Vancomycin	>16	>16	0.5->16	-	-
Linezolid-intermediate (10)				10	0
TR-700	1	1	0.5-1	-	-
Vancomycin	>16	>16	1->16	-	-
Linezolid-resistant (45)				44	0
TR-700	2	2	1-4	-	-
Vancomycin	>16	>16	0.5->16	-	-
E. faecalis (32)					
Linezolid-susceptible (16)				0	0
TR-700	0.5	0.5	0.12-1	-	-
Vancomycin	2	4	1->16	-	-
Linezolid-intermediate (4)				3	0
TR-700	1	-	0.5-2	-	-
Vancomycin	2	-	1->16	-	-
Linezolid-resistant (12)				12	0
TR-700	2	4	0.5-8	-	-
Vancomycin	1	>16	1->16	-	-
S. aureus (16)					
Linezolid-susceptible (8)				0	0
TR-700	0.25	-	0.12-0.5	-	-
Vancomycin	1	-	1	-	-
Linezolid-non-susceptible (8)				6	2
TR-700	1	-	0.5-2	-	-
Vancomycin	1	-	0.5-1	-	-
CoNS (80)					
Linezolid-susceptible (40)[a]				0	0
TR-700	0.12	0.25	0.12-0.25	-	-
Vancomycin	2	2	1-4	-	-
Linezolid-non-susceptible (40)[b]				33	2
TR-700	4	16	1->32	-	-
Vancomycin	2	2	1-2	-	-
Viridans-group streptococci (2)					
Linezolid-susceptible (1)[c]				0	0
TR-700	0.12	-	0.12	-	-
Vancomycin	0.5	-	0.5	-	-
Linezolid-non-susceptible (1)[d]				1	0
TR-700	2	-	2	-	-
Vancomycin	0.5	-	0.5	-	-

a. Includes *S. aureus/a* (one), *S. capitis* (one), *S.epidermidis* (26), *S. haemolyticus* (six), *S. hominis* (two) and CoNS-NOS (four).
b. Includes *S. capitis* (two), *S. epidermidis* (29), *S. haemolyticus* (five), *S. simulans* (two), and *S. xylosis* (two).
c. *S. mitis*.
d. *S. oralis*

- Table 4 illustrates the rare occurrence of single-step TR-700 target mutations. At screening concentrations of 4X, 8X, and 16X TR-700 MICs, no mutant strains were detected at the inoculum densities of 1.1 to 2.2 x 10^9 CFU/mL.

Table 3. TR-700 and linezolid MIC values for oxazolidinone-non-susceptible strains of Gram-positive cocci (120 strains).

Organism/agent (no. tested)	No. occurrences at each MIC (µg/mL): 0.25	0.5	1	2	4	8	16	32	>32
Enterococci and streptococci (72)									
Linezolid	0	0	0	0	14[a]	22[b,c]	30	5	0
TR-700	0	6	26	34	5	1	0	0	0
Staphylococci (48)									
Linezolid	0	0	0	0	0	8[a]	16	15	9
TR-700	0	2[a]	5[a]	13[a]	15	8	4	0	1

a. Intermediate MIC for linezolid when testing enterococci.
b. Non-susceptible or resistant MIC value for staphylococci and enterococci, respectively.
c. Underlined value is the median MIC.
d. *S. aureus* TR-700 MIC range was at 0.5-2 µg/mL, MICS0 at 1 µg/mL, versus 4 µg/mL for CoNS (see Table 2).

Table 4. TR-700 single-step mutation study results.

Isolate	Inoculum concentration	At TR700 concentration of: 4X MIC	8X MIC	16X MIC
S. aureus ATCC 29213	2.2E+09	ND[a]	NG	NG
S. aureus USA 300-0114	2.2E+09	NG	NG	NG
E. faecalis ATCC 29212	1.1E+09	NG	NG	NG

a. ND = no growth.

CONCLUSIONS

- Reference CLSI broth microdilution methods were used to evaluate TR-700, the active component of the prodrug TR-701, when tested against linezolid-refractory Gram-positive cocci (120 isolates), and an equal number of wildtype strains that were matched for species, geographic location, isolation time interval and infection type (see Tables).
- TR-700 was observed to be four- to 32-fold more active than linezolid, but potency varied by species and documented resistance mechanism.
- Many (nearly 90%) linezolid-non-susceptible isolates had TR-700 MIC results at ≤4 µg/mL.
- The occurrence of MIC elevation after 14 passage days for TR-700 was less than linezolid by direct comparison experiments.
- Single-step mutational rates for TR-700 were extremely low at <1.1 x 10^{-9}, and emergence of TR-700 resistance on chemotherapy would be considered to be a low risk based on these results.
- Continued investigation of this novel oxazolidinone is warranted.

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