

In vitro activity of tedizolid (TR-700, formerly known as torezolid) against linezolid-resistant Gram-positive clinical isolates possessing the *cfr* methyltransferase gene and/or ribosomal gene mutations

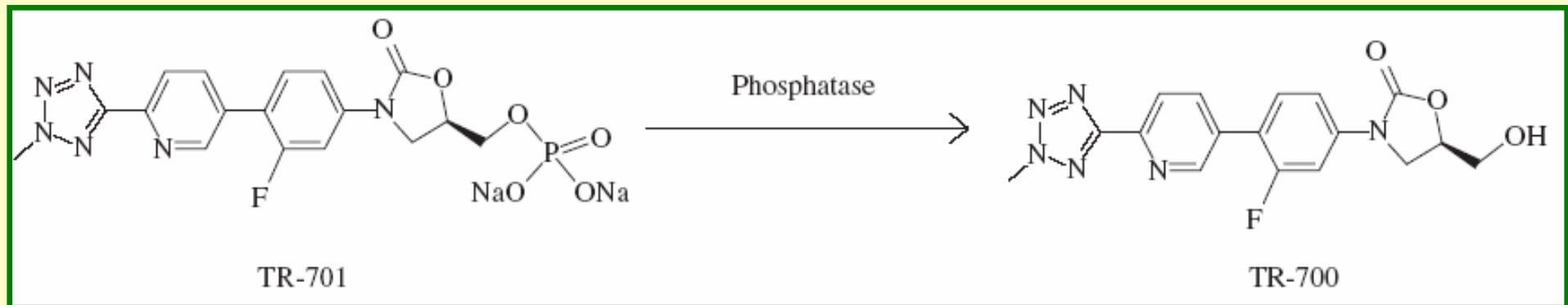
**CERCENADO E*, MARÍN M, GAMA B, IGLESIAS C, BOUZA E
Hospital General Universitario Gregorio Marañón. Madrid. SPAIN**

C2-945. 51th ICAAC. Chicago. September 2011



INTRODUCTION

- Tedizolid (TR-700) is the active moiety of tedizolid phosphate (TR-701), a new oral/ intravenous oxazolidinone prodrug with *in vitro* activity against Gram-positive microorganisms including staphylococci, enterococci, and streptococci.



- TR-700 is 4-fold more active than linezolid against linezolid-susceptible staphylococci and enterococci.
- Previous studies have demonstrated that TR-700 is also active against linezolid-resistant staphylococci (including *S. aureus*) and enterococci with different mechanisms of resistance to linezolid.

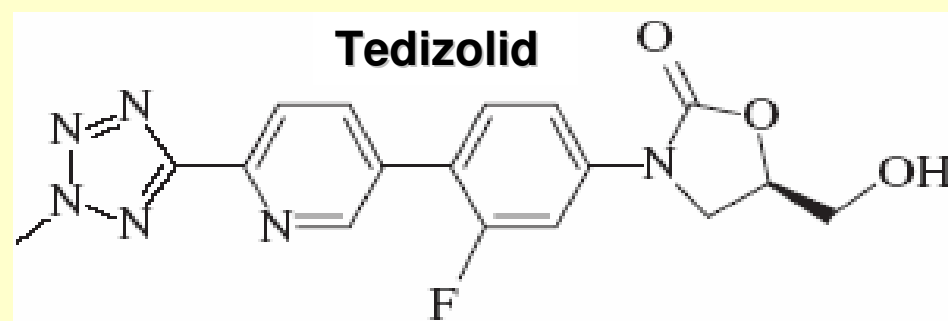
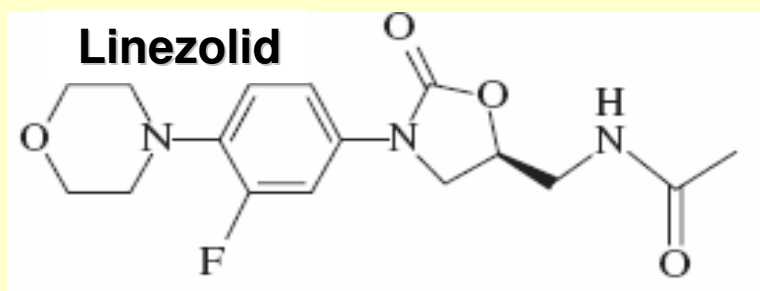
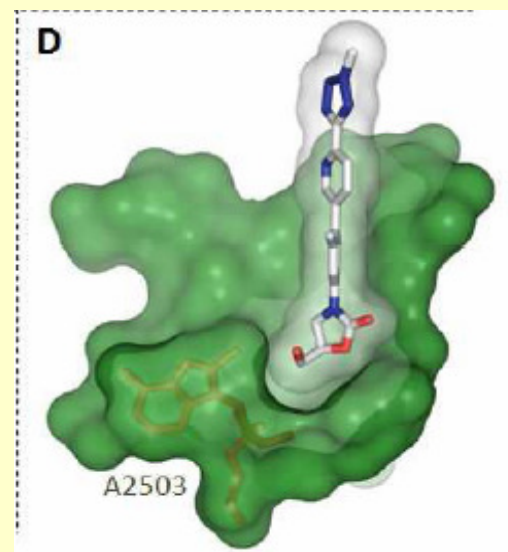
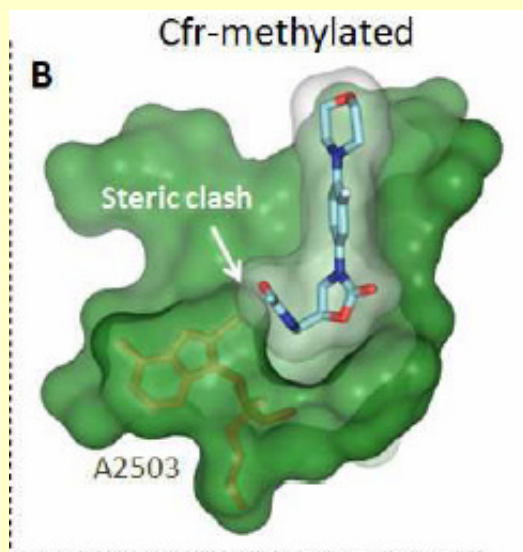
• Shaw KJ, et al; AAC 2008; 52:4442
• Livermore DM, et al; JAC 2009; 63:713

INTRODUCTION

- **Mechanisms of linezolid resistance:**
 - A) Mutations in 23S rRNA-encoding genes (*rrn*)**
 - In one or more copies (heterozygous/homozygous)
 - Mutations: G2576T, T2500A, others
 - Alteration of the compound's binding site
 - B) Mutations in *rplC*, *rplD* (ribosomal proteins L3, L4)**
 - C) Cfr methyltransferase (*cfr* gene)**
 - Modification of 23S rRNA at A2503
 - Plasmid-mediated
 - Resistance to phenicols, lincosamides, pleuromutilins, streptogramin A, 16-membered ring macrolides, and linezolid
- **Multiple mechanisms can occur within a single strain**

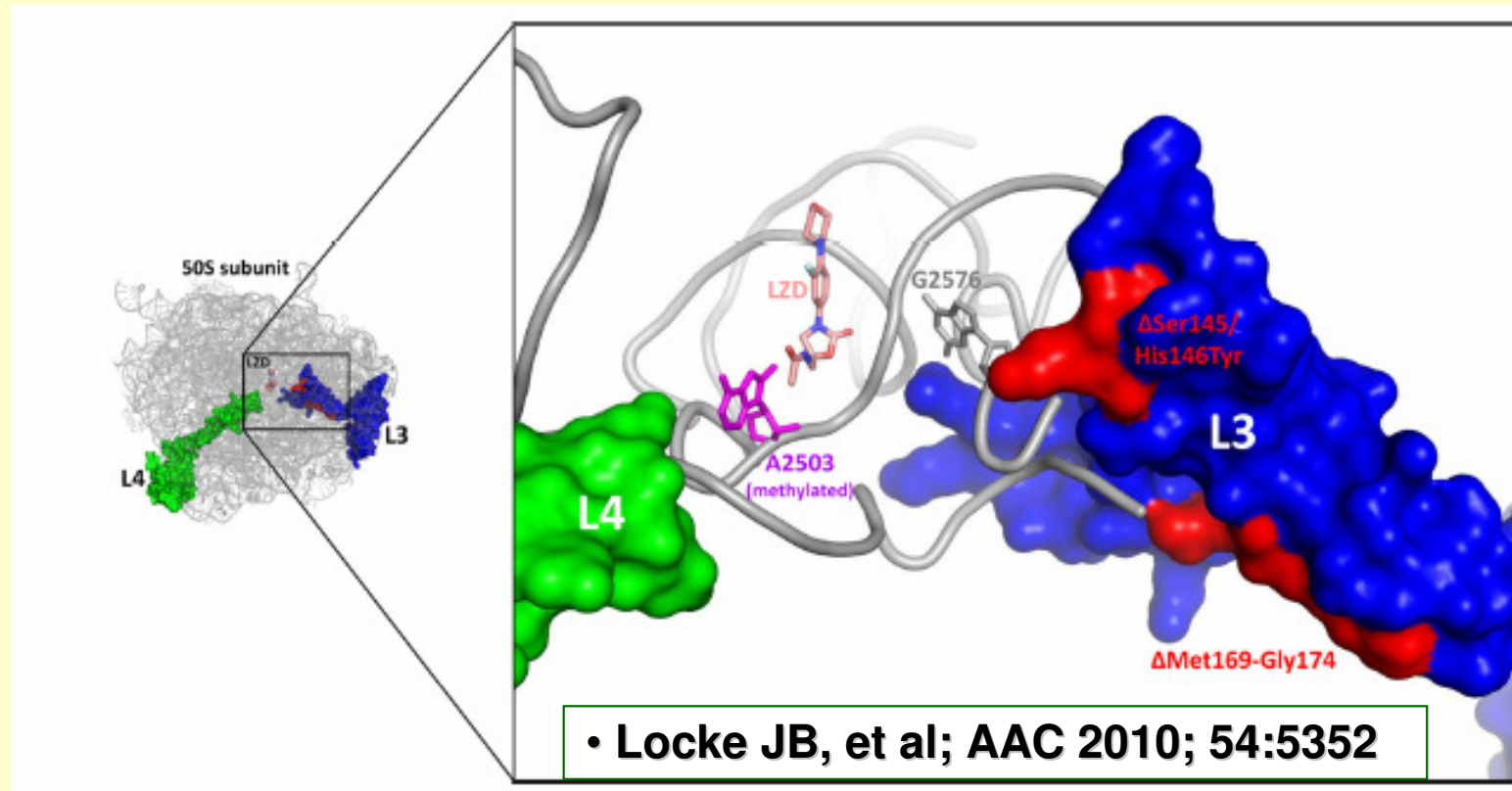
INTRODUCTION

- The increased potency of TR-700 (C-5 hydroxymethyl) over linezolid (C-5 acetamide) is due to steric clash by the acetamide with the methylated A2503 (*cfr*-positive isolates). In addition, TR-700 binding is less reliant on target residues associated with resistance to linezolid



OBJECTIVE

- We investigated the in vitro activity of tedizolid (TR-700) against a series of methicillin-resistant *Staphylococcus aureus* (MRSA), coagulase-negative staphylococci (CoNS) and enterococci with different mechanisms of resistance to linezolid



MATERIAL AND METHODS

- **Isolates:** 170 linezolid-resistant Gram-positive clinical isolates corresponding to 136 patients recovered at our institution (2009-2010) with characterized mechanisms of resistance to linezolid. (Cercenado et al. 2010; 20th ECCMID A-869; and 50th ICAAC C2-1490)

- Total 170 isolates:

MRSA (n=21); *S. epidermidis* (n=128); *E. faecium* (n=13); *E. faecalis*: (n=8)

- **Characterization of linezolid resistance mechanisms:**

Performed by PCR and sequencing as previously described:

- mutations in the 23S rRNA ribosomal subunit (G2576T and others)
- mutations in the genes encoding L3 (*rpIC*), and L4 (*rpID*) (*S. aureus*)
- detection of the *cfm*-methylase gene

- Hong T, et al; JCM 2007; 45: 3227
- Kehrenberg C, et al; AAC 2006; 50:1156
- Locke JB, et al; AAC 2009; 53:5265

MATERIAL AND METHODS

- **Antimicrobials:** TR-700 (tedizolid) was provided by Trius Therapeutics, Inc., San Diego, CA, USA, and linezolid was obtained from Sigma-Aldrich Corp., St. Louis, MO.
- **Susceptibility testing:** MICs were determined using the broth microdilution method (CLSI guidelines) in Mueller-Hinton II broth (Becton Dickinson, Sparks, MD).
- Concentrations of TR-700 (tedizolid) and linezolid ranged from ≤ 0.06 to 128 mg/L
- **Control strains:** *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212

RESULTS

- **Isolates and distribution of linezolid resistance mechanisms:**

Total: 170 isolates

MRSA (n=21); *S. epidermidis* (n=128); *E. faecium* (n=13); *E. faecalis*: (n=8)

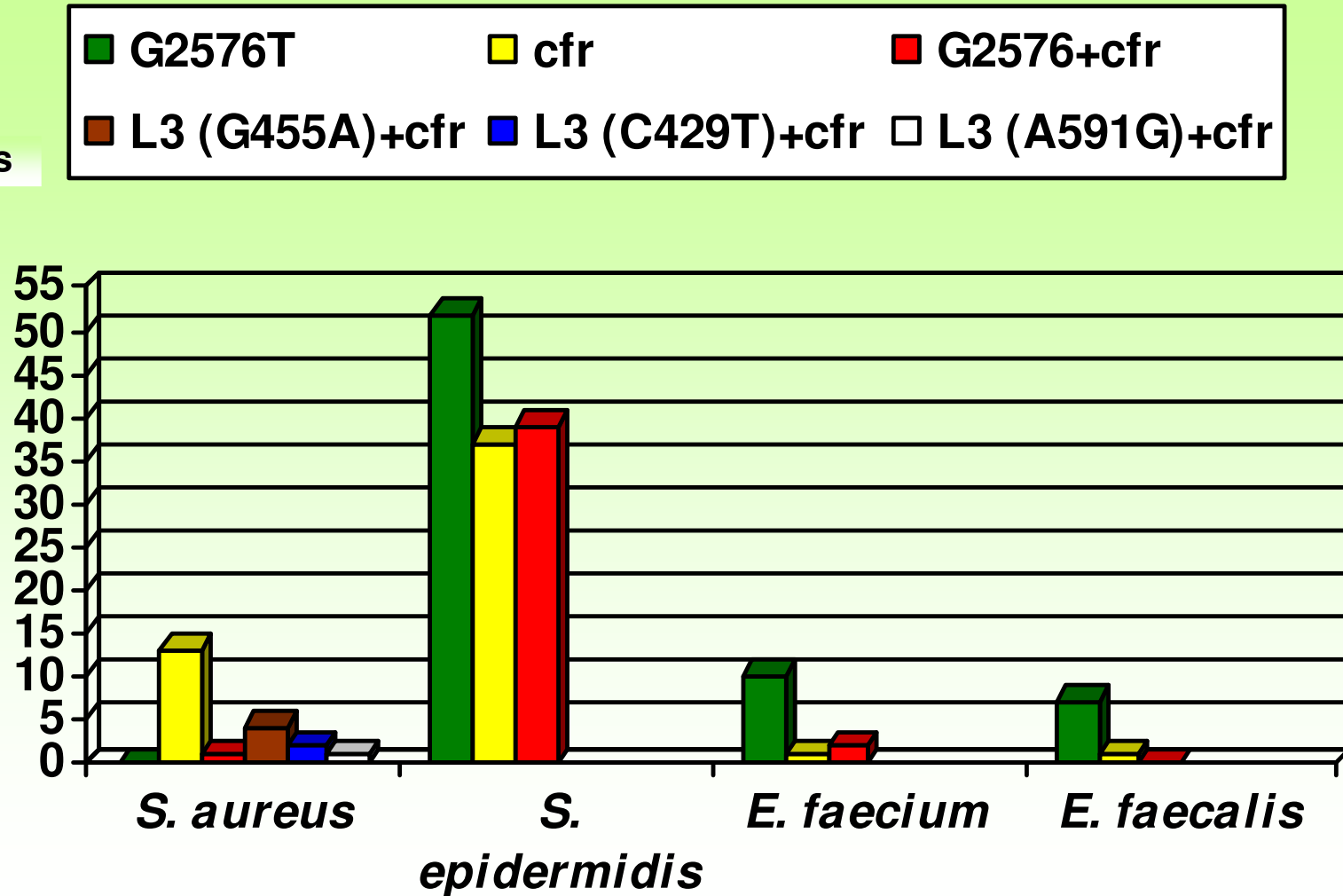
Mechanisms of linezolid resistance (all isolates)	No. Isolates (%)
G2576T	69 (40.6)
<i>cfr</i>	59 (34.7)
G2576T+ <i>cfr</i>	42 (24.7)

Mechanisms of linezolid resistance (MRSA)	No. Isolates (%)
<i>cfr</i>	13 (61.9)
<i>cfr</i> + G2576T	1
<i>cfr</i> + L3 G455A	4
<i>cfr</i> + L3 C429T	2
<i>cfr</i> + L3 A591G	1

RESULTS

Isolates and distribution of linezolid resistance mechanisms

No. isolates



- None of the MRSA isolates showed mutations in the gene encoding ribosomal protein L4

RESULTS

In vitro activity of TR-700 (tedizolid) and linezolid (LZD) against LZD-resistant strains

MICs (mg/L)

Organism (No. tested)	Antimicrobial agent	Range	50%	90%
Total (170)	TR-700	≤0.06-8	1	2
	LZD	4- >128	32	>128
MRSA (21)	TR-700	≤0.06-0.5	0.12	0.5
	LZD	4-32	8	16
<i>S. epidermidis</i> (128)	TR-700	≤0.06-8	1	4
	LZD	8- >128	32	>128
<i>E. faecium</i> (13)	TR-700	0.12-2	1	1
	LZD	8- 16	8	16
<i>E. faecalis</i> (8)	TR-700	0.12-2	NA	NA
	LZD	8- 32	NA	NA

RESULTS

In vitro activity of TR-700 (tedizolid) and linezolid (LZD) according to the different mechanism/s of resistance

MICs (mg/L)

Mechanism of resistance (No. isolates)	Antimicrobial agent	Range
G2576T (69 isolates)	TR-700 LZD	0.12-8 8- >128
<i>cfr</i> (59 isolates)	TR-700 LZD	≤0.06-2 4->128
G2576T+ <i>cfr</i> (42 isolates)	TR-700 LZD	0.12-8 8- >128

- LZD MIC ranges were the same in strains with different mechanisms of resistance (range 4->128 mg/L)
- TR-700 (tedizolid) MICs were not impacted in strains possessing the Cfr mechanism alone (range ≤0.06-2 mg/L)

RESULTS

In vitro activity of TR-700 (tedizolid) and linezolid (LZD) against LZD-resistant MRSA with different mechanisms of resistance
MICs (mg/L)

Mechanisms of resistance (No. isolates)	Antimicrobial agent	Range
<i>cfr</i> (13)	TR-700 LZD	≤ 0.06 -0.5 4-16
<i>cfr</i> + G2576T (1)	TR-700 LZD	0.25 8
<i>cfr</i> + L3 G455A (4)	TR-700 LZD	0.12-0.5 8-32
<i>cfr</i> + L3 C429T (2)	TR-700 LZD	0.12-0.5 8
<i>cfr</i> + L3 A591G (1)	TR-700 LZD	0.12 8

- TR-700 (tedizolid) MICs were not impacted in strains with L3 mutations co-occurring with the *cfr* mechanism (range ≤ 0.06 -0.5 mg/L)
- LZD MICs ranged from 4 to 32 mg/L

CONCLUSIONS

- **TR-700 (tedizolid) maintained a ≥ 4 -fold potency advantage over linezolid against all Gram-positive clinical isolates tested (including MRSA) with different mechanisms of resistance to linezolid.**
- **TR-700 (tedizolid) retained its activity against *cfr*-positive isolates.**
- **The presence of L3 mutations co-occurring with the *cfr* mechanism did not significantly affect TR-700 (tedizolid) MICs against linezolid-resistant MRSA.**