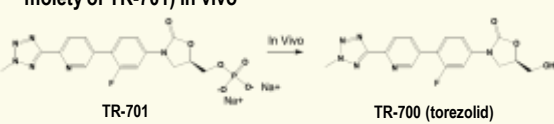
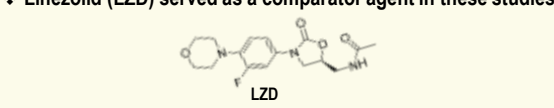


## INTRODUCTION AND PURPOSE

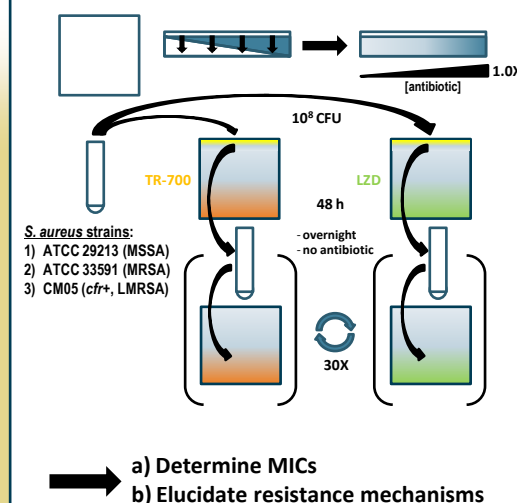
- ❖ TR-701 is a novel oxazolidinone prodrug that has completed Phase 2 clinical trials for complicated skin and soft tissue infections
  - ❖ TR-701 is rapidly converted to TR-700 (torezolid, the active moiety of TR-701) in vivo
- 
- ❖ Linezolid (LZD) served as a comparator agent in these studies
- 
- ❖ Objectives:
    - Characterize the potential for *S. aureus* to develop resistance over time to TR-700 and LZD through 30 cycles of serial passage
    - Elucidate underlying resistance mechanisms

## METHODS

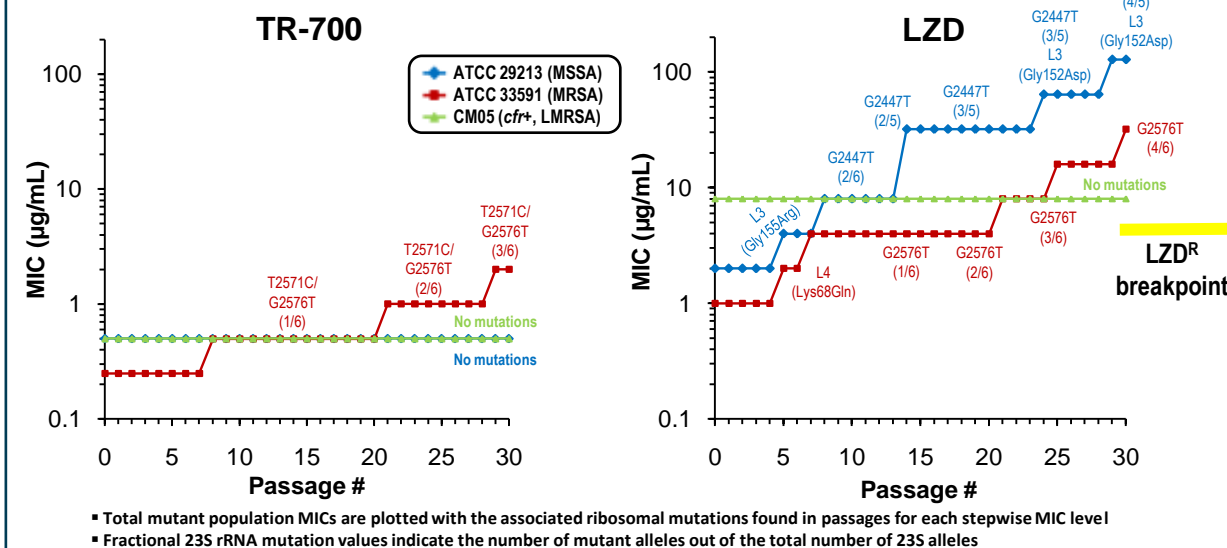
- ❖ *S. aureus* strains ATCC 29213 (MSSA), ATCC 33591 (MRSA), and CM05 [*cfr+*, LZD-resistant MRSA (LMRSA)] were cultured at 37°C on Mueller-Hinton II agar (MHA) or in liquid broth (MHB)
- ❖ Serial passage experiments were carried out by plating ~1 x 10<sup>8</sup> CFU on TR-700 and LZD antibiotic gradient plates
- ❖ After 48 h incubation, the leading edge of growth was subcultured into MHB without antibiotics and grown overnight for the subsequent passage plating
- ❖ MIC values were determined (CLSI) for total mutant populations in each passage and for individual colonies from representative passages
- ❖ PCR was used to amplify individual *rrn* alleles and *rpIC*, *rpID*, and *rpIV* genes encoding ribosomal proteins L3, L4, and L22, respectively
- ❖ The domain V region of each 23S rRNA gene and entire *rpIC*, *rpID*, and *rpIV* genes were sequenced to elucidate underlying resistance mechanisms

## RESULTS

### I. Gradient plate serial passage



### II. Serial passage MICs and associated 23S rRNA and ribosomal protein mutations

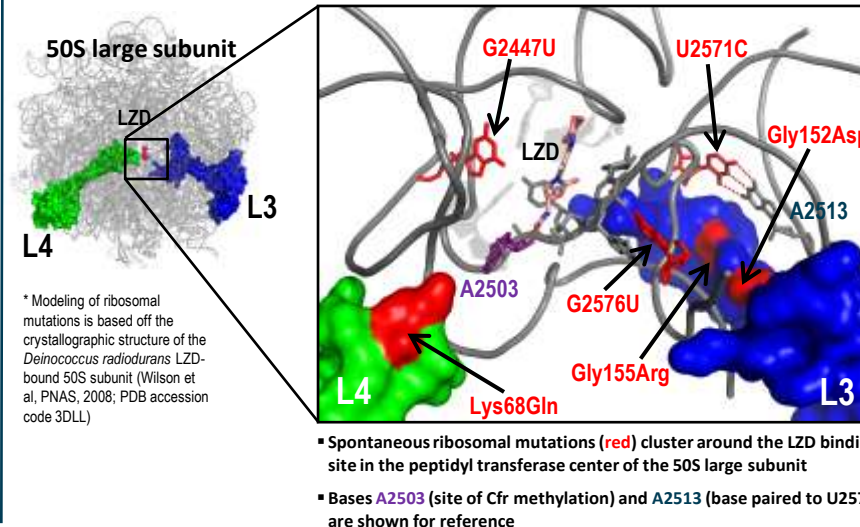


### III. Characteristics of wild-type and isogenic serial passage mutant strains

strain	selection	23S rRNA mutations			ribosomal protein mutations				MIC (µg/mL)			
		rDNA	gene #(s)	prop.	rpIC	L3	rpID	L4	TR-700	LZD	TIA	VAN
ATCC 29213	wild-type	-	-	-	-	-	-	-	0.5	2	1	1
	LZD	G2447T	3, 4	2/6	-	-	-	-	1	8	2	1
	LZD	G2447T	3, 4	2/5	-	-	-	-	2	16	4	1
	LZD	G2447T	3, 4, 5	3/5	-	-	-	-	4	32	8	1
	LZD	G2447T	3, 4, 5	3/5	G455A	Gly152Asp	-	-	8	128	32	1
	LZD	G2447T	1, 3, 4, 5	4/5	G455A	Gly152Asp	-	-	8	128	64	1
ATCC 33591	wild-type	-	-	-	-	-	-	-	0.25	1	0.5	1
	LZD	G2576T	3	1/6	-	-	-	-	0.5	4	2	1
	LZD	G2576T	3, 4	2/6	-	-	-	-	0.5	4	2	1
	LZD	G2576T	3, 4, 5	3/6	-	-	-	-	1	16	2	1
	LZD	G2576T	2, 3, 4, 5	4/6	-	-	-	-	2	32	4	1
	TR-700	T2571C/G2576T	4	1/6	-	-	-	-	0.5	2	0.5	1
CM05	wild-type	-	-	-	-	-	-	-	0.5	8	nd	1
	LZD	-	-	-	-	-	A202C	Lys68Gln	0.5	2	1	1
	TR-700	T2571C/G2576T	2, 4, 5	3/6	-	-	-	-	2	16	1	2

- The genetic basis of 23S rRNA mutations (rDNA), the specific mutant 23S alleles (gene #s), and the proportion (prop.) of mutant alleles over the total number of 23S alleles are given
- Riboprotein L3 and L4 mutations are reported for the genes (*rpIC*, *rpID*) and proteins (L3, L4), respectively
- MICs were performed for all strains against TR-700, linezolid (LZD), tiamulin (TIA), and vancomycin (VAN)

### IV. Structural modeling of serial passage ribosomal mutations



## RESULTS

- ❖ All mutations were located in close proximity to the oxazolidinone binding site in the peptidyl transferase center
- ❖ Cross resistance between TR-700 and LZD was observed for all mutants
- ❖ **TR-700 serial passage**
  - ATCC 29213 & CM05 (*cfr+*): no mutations or MIC increases
  - ATCC 33591: 8-fold MIC increase (0.25 to 2 µg/mL) associated with increasing copy # of a novel, coupled T2571C/G2576T 23S rRNA mutation
- ❖ **LZD serial passage**
  - ATCC 29213: 64-fold MIC increase (2 to 128 µg/mL) associated with an independent Gly155Arg L3 mutation, increasing copy # of the independent 23S rRNA mutation G2447T, loss of a 23S rRNA allele, and coupled G2447T and Gly152Asp mutations
  - ATCC 33591: 32-fold MIC increase (1 to 32 µg/mL) associated with a Lys68Gln mutation in ribosomal protein L4 and increasing copy # of the 23S rRNA mutation G2576T
  - CM05 (*cfr+*): no mutations or MIC increases

## CONCLUSIONS

- ❖ Novel mutations in 23S rRNA and ribosomal proteins L3 and L4 were identified
- ❖ TR-700 maintained a 4-16-fold potency advantage over LZD for all mutants
- ❖ Relative to LZD, resistance to TR-700 was difficult to generate and required multiple mutations to achieve an MIC of 2 µg/mL, the highest level observed through selection with TR-700

## ACKNOWLEDGEMENTS

- ❖ We thank Dr. John Quinn for the *cfr+* CM05 strain