

Streptococcus sanguinis Isolate Displaying a Phenotype with Cross-Resistance to Several rRNA-Targeting Agents

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This study describes a clinical case of a 71-year-old male with a history of ischemic cardiomyopathy after left ventricular assist device (LVAD) endocarditis caused by methicillin-resistant *Staphylococcus epidermidis* (MRSE) and a rare linezolid-resistant *Streptococcus sanguinis* strain (MIC, 32 µg/ml). The patient received courses of several antimicrobial agents, including linezolid for 79 days. The *S. sanguinis* strain had mutations in the 23S rRNA (T2211C, T2406C, G2576T, C2610T) and an amino acid substitution (N56D) in L22 and exhibited cross-resistance to ribosome-targeting agents.

Linezolid has been widely prescribed to treat serious infections caused by multidrug-resistant (MDR) Gram-positive pathogens since its clinical introduction as the first oxazolidinone in 2000 (1). Linezolid is currently approved by the U.S. Food and Drug Administration (FDA) for the treatment of complicated and uncomplicated skin and skin structure infections and nosocomial and community-acquired pneumonia caused by susceptible organisms (2). Linezolid also has an FDA indication for the treatment of vancomycin-resistant *Enterococcus faecium* (VRE) infections (including bacteremia) (2).

This oxazolidinone alters protein synthesis by interacting with the 50S ribosomal subunit, and recent data suggest that this drug binds to the A site of the peptidyl-transferase center (PTC) of bacterial ribosome, interfering with the positioning of aminoacyl-tRNA and resulting in protein synthesis inhibition (3). Although the prevalence of linezolid resistance among Gram-positive organisms remains low among surveillance clinical isolates (1), the resistance mechanisms detected have been comprised mostly of mutations in domain V of 23S rRNA, but alterations in the ribosomal proteins L3 and L4 have also been associated with decreased susceptibility (3, 4). Moreover, a more recent resistance mechanism, *cfr*, has been recognized. *cfr* encodes a methyltransferase that catalyzes the posttranscriptional methylation of nucleotide A2503 in the 23S rRNA, causing decreased susceptibility to phenicol, lincosamide, oxazolidinone, pleuromutilin, and streptogramin A (PhLOPS_A) compounds (4).

Overall, staphylococci and enterococci represent the vast majority of linezolid-nonsusceptible clinical pathogens reported (3). Although published reports have described the *in vitro* selection of laboratory strains of streptococci displaying decreased susceptibility to linezolid due to mutations in the PTC (5, 6), only two clinical cases of linezolid-resistant streptococcal isolates (*Streptococcus pneumoniae* and *Streptococcus oralis*) have been described to date (1, 7, 8). Those reports demonstrated that these clinical and laboratory isolates had mutations in the 23S rRNA or amino acid substitutions in L4. Our study reports a clinical case of bacteremia due to a linezolid-resistant *Streptococcus sanguinis* and methicillin-resistant *Staphylococcus epidermidis* (MRSE) and investigates in the former the resistance mechanisms associated with ribosome-targeting agents.

Linezolid-resistant *S. sanguinis* (453-31288R) was recov-

ered from blood cultures from a 71-year-old male who had a history of ischemic cardiomyopathy after placement of a left ventricular assist device (LVAD). The isolate was submitted to a central monitoring laboratory (JMI Laboratories, North Liberty, IA) as part of the 2011 SENTRY Antimicrobial Surveillance Program, according to defined protocols. Species identification was performed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany) and confirmed by 16S rRNA sequencing. Susceptibility testing was performed by broth microdilution methods, according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (M07-A9) (9). Validation of the MIC values was performed by concurrent testing of *S. pneumoniae* ATCC 49619 (10). MIC interpretations were based on the CLSI M100-S23 document (10) or the FDA package insert (tigecycline) (21).

The presence of *cfr* and mutations in the 23S rRNA and ribosomal proteins (L3, L4, and L22) was investigated by PCR and sequencing. Primers utilized were as follows: L3-F, ATGACCAAAGGAATCTTAGGG; L3-R, TTAGCACCTGGTACGTTACC; L4-F, AGCGATGCAGTATTTGGTATCG; L4-R, AAATTCATTATGCAAGAACC; L22-F, GAACTCAGCTGTAGCTAACGC; L22-R, TTCTGCAACAGCTACAGTGATG. The PCR conditions were as follows: initial denaturation at 95°C (5 min); 35 cycles of 94°C (30 s), 55°C (30 s), and 72°C (1 min); and final extension at 72°C (5 min). Amplicons were sequenced on both strands. 23S rRNA and ribosomal proteins obtained were compared to those from wild-type *S. sanguinis* ATCC 10556 using the Lasergene software package (DNASar, Madison, WI). This isolate was also screened for *vga*(ABCD), *lsa*(ABC), and tetracycline [*tet*(KLMO)] genes by PCR (11). The *S. sanguinis* strain was subjected to serial daily passage on drug-free blood agar plates for 60 days, followed by susceptibility testing. Growth curves were performed in duplicate for the index and wild-type ATCC 10556 strains, as previously described (12).

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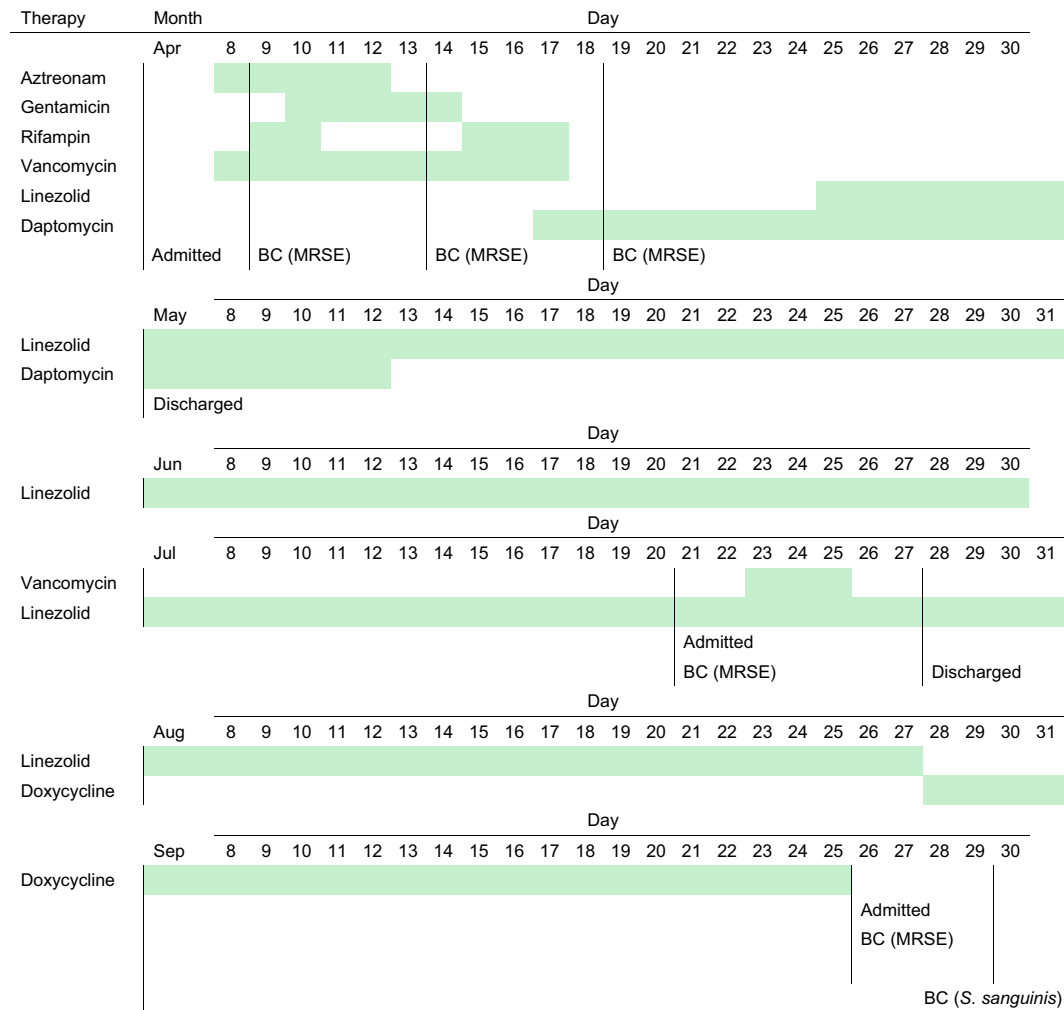


FIG 1 Summary of patient hospitalization history prior to the recovery of linezolid-resistant *S. sanguinis*, antimicrobial agents prescribed (green dates/lines), and culture dates of organisms recovered from blood cultures (BC). MRSE, methicillin-resistant *S. epidermidis*.

In April 2011, a 71-year-old male patient was diagnosed with LVAD endocarditis caused by MRSE, and aztreonam (5-day therapy), gentamicin (5 days), rifampin (5 days), and vancomycin (10 days) were prescribed, followed by daptomycin (25 days) and linezolid (8 days) (April to May 2011) (Fig. 1). The patient was discharged and continued receiving linezolid (79 days). He was readmitted in July 2011 due to persistent MRSE bacteremia, and vancomycin therapy (3 days) was introduced, while linezolid (3 days) remained. The patient was discharged on linezolid and then switched to doxycycline. The patient was readmitted in September 2011, and blood cultures yielded MRSE, followed by linezolid-resistant *S. sanguinis* (MIC, 32 µg/ml) (Fig. 1 and Table 1). *S. sanguinis* was cleared, but the MRSE infection persisted, with another positive blood culture in December 2011, despite several (five) changes of peripherally inserted central venous catheters (PICCs) between April and December 2011. In March and May 2012, *Pseudomonas aeruginosa* was recovered from blood cultures, which cleared later. The patient was discharged but expired in May from further complications.

Linezolid-resistant *S. sanguinis* also exhibited elevated MIC

results for chloramphenicol, clindamycin, tiamulin, tetracycline, doxycycline, and quinupristin-dalfopristin (Table 1). When tested against other ribosome-targeting agents, the *S. sanguinis* strain showed susceptible and/or low MIC values for erythromycin and tigecycline. Mutations were observed within the 23S rRNA at positions T2211, T2406, G2576, and C2610 (*Escherichia coli* numbering of rRNA), and there was an N56D amino acid substitution in L22. Wild-type amino acid sequences were observed for L3 and L4 (Table 1). The *S. sanguinis* strain was PCR negative for *cfr*, *vga*, and *lsa* genes and positive for *tet(M)*. After 60 days of passage in nonselective medium, passage-derived *S. sanguinis* strains demonstrated antimicrobial susceptibility profiles equivalent to that of the parent strain (Table 1). The *S. sanguinis* mutant strain demonstrated a lag phase longer than the control strain, and both growth curves had distinct log and stationary phases (Fig. 2). In addition, 31228R showed a generation time of approximately 30 min, which was 2-fold longer than that of ATCC 10556 (i.e., ~15 min).

Among the 23S rRNA mutations observed, G2576T and C2610T are within the PTC and are known to affect the

TABLE 1 Antimicrobial susceptibility profile and molecular findings for the linezolid-resistant *S. sanguinis* strain (453-31288R) investigated in this study

Parameter	Value/result	
	453-31288R	453-31288R ₆₀ ^b
Antimicrobial agent MIC ($\mu\text{g/ml}$) [susceptibility category] ^a		
Linezolid	32 [R]	64 [R]
Chloramphenicol	32 [R]	32 [R]
Clindamycin	2 [R]	1 [R]
Virginiamycin	2	2
Quinupristin-dalfopristin	4 [R]	4 [R]
Retapamulin	2	2
Tiamulin	32	32
Tigecycline	≤ 0.03 [S]	≤ 0.03 [S]
Tetracycline	> 16 [R]	> 16 [R]
Doxycycline	8	
Vancomycin	0.5 [S]	
Levofloxacin	0.5 [S]	
Erythromycin	≤ 0.12 [S]	
Penicillin	≤ 0.06 [S]	
Molecular finding		
<i>cfr</i>	Negative	
23S rRNA	T2211C, T2406C, G2576T, and C2610T	
L3	Wild type	
L4	Wild type	
L22	N56D	

^a MIC interpretive criteria as published by CLSI M100-S23 (10), when available. The tigecycline susceptible breakpoint for *Streptococcus* spp. other than *S. pneumoniae* (≤ 0.25 $\mu\text{g/ml}$) approved by the Food and Drug Administration was applied (21). S, susceptible; I, intermediate; R, resistant.

^b MIC results and interpretations obtained for some antimicrobial agents tested against the *S. sanguinis* strain 31288R₆₀ after 60 subcultures on drug-free blood agar plates.

linezolid MIC values and to cause cross-resistance to other ribosome-targeting agents, such as clindamycin, chloramphenicol, streptogramin A, and pleuromutilins (13–16). However, strain 31288R was susceptible to erythromycin, which corroborates the fact that this drug interacts with the 50S rRNA exit tunnel wall, and binding is mostly affected by mutations at 23S rRNA residues 2058 and 2059 (17). Moreover, amino acid substitutions in L22 can result in macrolide resistance, but the ribosomal protein alteration observed here seems to have little effect on erythromycin (18).

It is well known that mutations in the 23S rRNA may affect protein synthesis and confer lethal phenotypes; otherwise, they may confer a high bacterial fitness cost (16). Although the resistance phenotype displayed by strain 31228R seemed to be stable based on the passaging experiment, the ribosomal mutations and/or the L22 amino acid alteration seemed to adversely affect the growth rate (fitness) of *S. sanguinis*. However, it is important to mention the limitations of the growth rate experiments, since strains with different backgrounds were utilized and the results observed may have been originated due to factors other than the ribosomal modifications detected. In summary, further experiments are necessary to understand the impact of each 23S rRNA and the L22 amino acid substitution on the susceptibility profile and fitness cost. In addition, any modifications observed in the 23S rRNA may also act as compensatory mutations, as well as the L22 amino acid substitution, since alterations in this protein have not been associated with linezolid resistance due to a more distal position from the PTC (6, 19, 20). Lastly, the combination of ribosomal modifications observed in this strain appears to affect binding of several ribosome-targeting agents and produce a resistance profile similar to that of Cfr (PhLOPS_A) (4).

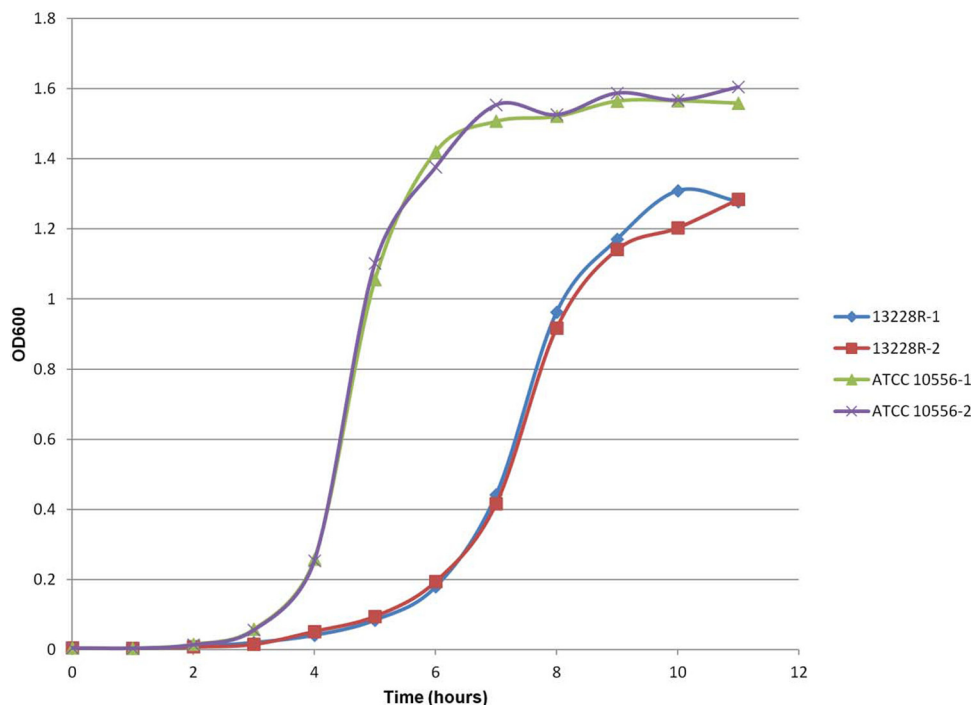


FIG 2 Growth pattern experiments performed for *S. sanguinis* 453-31288R and control strain ATCC 10556. The optical density at 600 nm (OD₆₀₀) values of two cultures for each strain are shown for each time point.

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