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Streptococcus sanguinis Displaying a Cross Resistance Phenotype to Several Ribosomal RNA Targeting Agents, Including Linezolid

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SENTRY ANTIMICROBIAL SURVEILLANCE

Abstract

Background: Linezolid has been in clinical use for a decade and large surveillance studies have reported low and stable resistance rates among Gram-positive clinical pathogens. This study reports a clinical case of bacteremia due to a linezolid-resistant *S. sanguinis*.

Methods: Identification was performed by Vitek® 2 and confirmed by 16S rRNA sequencing. Susceptibility testing was performed by CLSI methods (M07-A9 and M100-S22). Strain was screened for *cfr* and mutations in the 23S rRNA-, L3-, L4- and L22-encoding genes. Clindamycin [*vga*(A/B/C and D) and *lsa*(A/B and C)] and tetracycline [*tet*(K/L/M and O)] genes were screened by PCR.

Results: A 71-year-old male had a history of ischemic cardiomyopathy post left ventricular assist device (LVAD) implantation. Other complications included hyperlipidemia and asthma. Patient was diagnosed (April, 2011) with LVAD endocarditis caused by methicillin-resistant *Staphylococcus epidermidis* (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-May, 2011). Patient was discharged and continued receiving linezolid (79 days). He was readmitted in July (2011) due to persistent MRSE bacteremia and therapy was switched to vancomycin (3 days) and linezolid (3 days). Patient was discharged with linezolid and doxycycline. Linezolid-resistant *S. sanguinis* was recovered from blood cultures in September and had elevated MICs for linezolid (32 μg/mL), chloramphenicol (32 μg/mL), clindamycin (2 μg/mL), tiamulin (32 μg/mL), tetracycline (>16 μg/mL), doxycycline (8 μg/mL), and quinupristin/dalfopristin (4 μg/mL). Linezolid-resistant *S. sanguinis* showed low MICs to all other agents, including erythromycin (≤0.12 μg/mL) and radezolid (1 μg/mL). T2211C, T2406C, G2576T and C2610T alterations were detected in 23S rRNA and a I59V mutation was noted in L22. Wildtype amino acid sequences were observed for L3 and L4. PCRs were negative for *cfr*, *vga* and *lsa* genes, and positive for *tet*(M). Linezolid-resistant *S. sanguinis* was cleared, but MRSE persisted.

Conclusions: This clinical case describes a patient with a chronic LVAD endocarditis, requiring multiple hospitalizations, and the extremely rare detection of a linezolid-resistant *S. sanguinis* strain causing bacteremia.

Introduction

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. Linezolid is currently approved by the 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. Linezolid also has an FDA indication for the treatment of vancomycin-resistant *Enterococcus faecium* (VRE) infections (including bacteremia).

This oxazolidinone alters protein synthesis via binding to the 50S ribosomal subunit with recent data suggesting that this drug binds to the A site of the peptidyl-transferase center (PTC) of bacterial ribosome interfering with the positioning of aminoacyl-tRNA, resulting in protein synthesis inhibition. Although the prevalence of linezolid resistance among Gram-positive organisms remains relatively low among surveillance clinical isolates, the resistance mechanisms have been extensively characterized. These mechanisms are mostly comprised of mutations in the domain V of 23S rRNA, and alterations in the ribosomal proteins L3, L4 and L22 have also been associated with decreased susceptibility. A more recent resistance mechanism, *cfr*, has been recognized. *cfr* encodes a methyltransferase that catalyzes the post-transcriptional methylation of nucleotide A2503 in the 23S rRNA causing decreased susceptibility to phenicol, lincosamide, oxazolidinone, pleuromutilin, and streptogramin A ($PhLOPS_A$) compounds.

Overall, staphylococci and enterococci represent the vast majority of linezolid non-susceptible clinical pathogens reported to date. Although, reports have described the *in vitro* selection of laboratory strains of streptococci displaying decreased susceptibility to linezolid due to mutations in the PTC, only one *Streptococcus pneumoniae* and one *Streptococcus oralis* clinical isolate resistant to linezolid have been reported to date. It was demonstrated that the pneumococcal strain had $_{65}WR_{66}$ and $_{68}KG_{69}$ deletions in L4, which increased the linezolid MIC result four-fold when compared with the isogenic parent strain. This study reports a clinical case of bacteremia due to a linezolid-resistant *Streptococcus sanguinis* and investigates associated resistance mechanisms.

Methods

Bacterial strain. Linezolid-resistant *S. sanguinis* was recovered from blood cultures of a 71-year-old male, who had a history of ischemic cardiomyopathy post placement of a left ventricular assist device (LVAD). The isolate was submitted to a central monitoring laboratory (JMI Laboratories, North Liberty, Iowa) as part of the 2011 SENTRY Antimicrobial Surveillance Program, according to defined protocols. Species identification was performed by the Vitek® 2 System (bioMérieux; Hazelwood, Missouri) and confirmed by 16S rRNA sequencing.

Antimicrobial susceptibility testing. Susceptibility testing was performed by broth microdilution methods, according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (M07-A9, 2012). Validation of the MIC values was performed by concurrent testing of CLSI-recommended quality control reference strains (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619). MIC interpretations were based on the CLSI M100-S22 document, when available. In addition, the inoculum density was monitored by colony counts to assure an adequate number of cells for each testing event.

Screening for linezolid and other resistance mechanisms, and drug-free passaging. *S. sanguinis* was screened for the presence of *cfr*, and mutations in the 23S rRNA and ribosomal proteins (L3, L4 and L22) by PCR and sequencing. Amplicons were sequenced on both strands. Ribosomal proteins obtained were compared to those from wildtype *S. sanguinis* ATCC 10556 using the Lasergene® software package (DNAStar; Madison, Wisconsin). This isolate was also screened for *vga*(A/B/C and D), *lsa*(A/B and C) and tetracycline [*tet*(K/L/M and O)] genes by PCR. In addition, the *S. sanguinis* strain was subjected to serial daily passage in drug-free blood agar plates for 20 days, followed by susceptibility testing.

Results

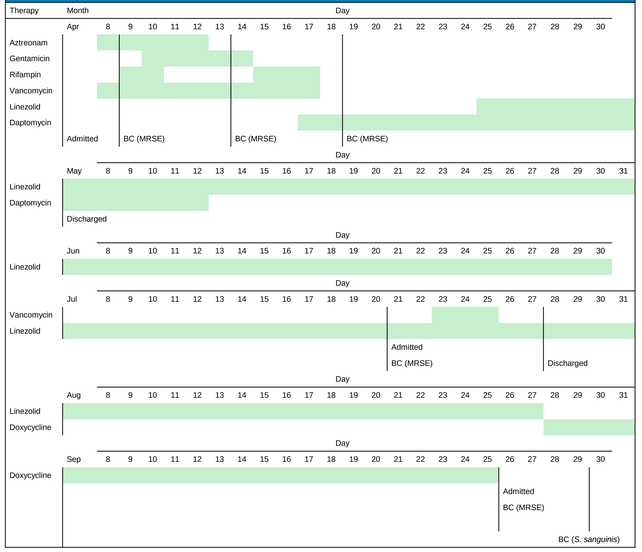
- On April 2011, patient was diagnosed with LVAD endocarditis caused by methicillin-resistant *Staphylococcus epidermidis* (MRSE) and aztreonam (5-day therapy), gentamicin (5 days), rifampin (5 days) and vancomycin (10 days) were prescribed, followed by daptomycin (25 days) and linezolid (8 days, April-May, 2011; **Figure 1**).
- Patient was discharged and continued receiving linezolid (79 days). He was readmitted in July (2011) due to persistent MRSE bacteremia and therapy was switched to vancomycin (3 days) and linezolid (3 days). Patient was discharged on linezolid and doxycycline therapy (**Figure 1**).
- Linezolid-resistant *S. sanguinis* was recovered from blood cultures in September (2011). This isolates had elevated MIC results for linezolid (32 μg/mL), chloramphenicol (32 μg/mL), clindamycin (2 μg/mL), tiamulin (32 μg/mL), tetracycline (>16 μg/mL), doxycycline (8 μg/mL), and quinupristin/dalfopristin (4 μg/mL; **Table 1**).
- Among other ribosomal targeting antimicrobial agents, the *S. sanguinis* strain was susceptible and/or showed low MIC values for erythromycin (≤0.12 μg/mL) and tigecycline (≤0.03 μg/mL), as well as the new oxazolidinone radezolid (1 μg/mL; **Table 1**).
- Mutations were observed within the 23S rRNA protein at positions T2211, T2406, G2576 and C2610 (*Escherichia coli* numbering of rRNA), as well as a I59V alteration was noted in L22. Wildtype amino acid sequences were observed for L3 and L4. The *S. sanguinis* index strain was PCR-negative for *cfr*, *vga* and *lsa* genes, and positive for *tet*(M).
- After 10 days of passage in non-selective medium, passage-derived *S. sanguinis* strains demonstrated similar antimicrobial susceptibility profile when compared to parent strain. The linezolid-resistant *S. sanguinis* was cleared, but the MRSE infection persisted.

Table 1. Antimicrobial susceptibility profile and molecular findings for the linezolid-resistant *S. sanguinis* investigated in this study.

Parameters	MIC (μg/mL) [susceptibility category][a]	Parameters	MIC (μg/mL) [susceptibility category][a]
Antimicrobial agent		Antimicrobial agent	
Linezolid	32 [R]	Tigecycline	≤0.03 [S]
Radezolid	1	Tetracycline	>16 [R]
Chloramphenicol	32 [R]	Doxycycline	8
Clindamycin	2 [R]	Vancomycin	0.5 [S]
Virginiamycin	2	Levofloxacin	0.5 [S]
Quinupristin/dalfopristin	4 [R]	Erythromycin	≤0.12 [S]
Retapamulin	2	Penicillin	≤0.06 [S]
Tiamulin	32		
Molecular findings			
cfr	Negative		
23S rRNA	T2211C, T2406C, G2576T and C2610T		
L3	WT		
L4	WT		
L22	I59V		

a. MIC interpretive criteria as published by CLSI M100-S22 (2012), when available. Tigecycline susceptible breakpoint for *Streptococcus* spp. other than *S. pneumoniae* (≤0.25 μg/mL) approved by the Food and Drug Administration was applied (Tygacil Product Package Insert, 2005). S, susceptible; I, intermediate; and R, resistant; WT, wildtype.

Figure 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.*



Conclusions

- This clinical case describes a patient with a chronic LVAD endocarditis, requiring multiple hospitalizations, and the extremely rare detection of a linezolid-resistant *S. sanguinis* strain causing bacteremia.
- The *S. sanguinis* strain demonstrated several mutations in the 23S rRNA, among which G2576T and C2610T are within the PTC and known to increase linezolid MIC values and to cause cross resistance to other ribosomal targeting agents, such as clindamycin, chloramphenicol, streptogramin A and pleuromutilins.
- Alterations in C2610 seem to have minimal effects on the erythromycin binding, which establishes interactions with the 23S rRNA at residues 2057, 2611 and 2058. Therefore, the 23S rRNA mutations observed caused a resistance phenotype (except for tetracycline) similar to that of *cfr* ($PhLOPS_A$).
- Additional 23S rRNA modifications, such as T2211C and T2406C, were located more distal and further investigations are needed to determine their influences on binding of ribosomal targeting agents.

References

1. Clinical and Laboratory Standards Institute (2012). *M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: ninth edition*. Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute (2012). *M100-S22. Performance standards for antimicrobial susceptibility testing: 22nd informational supplement*. Wayne, PA: CLSI.
3. Diaz L, Kiratisin P, Mendes R, Panesso D, Singh KV, Arias CA (2012). Transferable plasmid-mediated resistance to linezolid due to *cfr* in a human clinical isolate of *Enterococcus faecalis*. *Antimicrob Agents Chemother* 56: 3917-3922.
4. 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.
5. Mendes RE, Deshpande LM, Farrell DJ, Spanu T, Fadda G, Jones RN (2010). Assessment of linezolid resistance mechanisms among *Staphylococcus epidermidis* causing bacteraemia in Rome, Italy. *J Antimicrob Chemother* 65: 2329-2335.
6. Mendes RE, Smith TC, Deshpande L, Diekema DJ, Sader HS, Jones RN (2011). Plasmid-borne *vga(A)*-encoding gene in methicillin-resistant *Staphylococcus aureus* ST398 recovered from swine and a swine farmer in the United States. *Diagn Microbiol Infect Dis* 71: 177-180.
7. Wolter N, Smith AM, Farrell DJ, Schaffner W, Moore M, Whitney CG, Jorgensen JH, Klugman KP (2005). Novel mechanism of resistance to oxazolidinones, macrolides, and chloramphenicol in ribosomal protein L4 of the pneumococcus. *Antimicrob Agents Chemother* 49: 3554-3557.