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Re-Emergence of Linezolid Resistance in Clonally Distinct *Staphylococcus aureus* Strains Causing Pulmonary Infection in a Cystic Fibrosis Patient: Initial Case Studies from Latin America

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ABSTRACT

Background:
To continue the characterization of acquired linezolid (LZD) resistance (R), a very uncommon phenomenon among Gram-positive clinical isolates even in geographic locations of high LZD use. We previously reported the first LZD-R *S. aureus* strain from Brazil which was isolated from a cystic fibrosis (CF) patient (year 2002 strain JMI-700; LZD MIC, >8 µg/ml). We now report the occurrence of a second LZD-R *S. aureus* from the same patient after 3 years.

Methods:
Strain 57-233X was isolated in January 2005 from the sputum of a 12 y-o female as part of the Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) program. The patient was receiving LZD periodically since December 2000 for the treatment of pulmonary infections and had the index LZD-R *S. aureus* strain isolated in July 2002. Strain 57-233X was tested for susceptibility (S) against LZD and >30 antimicrobials by CLSI broth microdilution methods. Both strains were epidemiologically typed by automated ribotyping and PFGE. The presence of the G2576U mutation in domain V of the 23S rRNA was evaluated by PCR and restriction endonuclease digestion of the PCR fragments.

Results:
Strain 57-233X had a LZD MIC at > 8 µg/ml and was R to oxacillin, ciprofloxacin, clindamycin, trimethoprim/sulfamethoxazole, gentamicin and rifampin, but S to vancomycin, teicoplanin, quinupristin/dalfopristin, daptomycin (MIC, 0.25 µg/ml), dalbavancin (≤ 0.03 µg/ml) and tigecycline (MIC, 0.06 µg/ml). LZD-R was confirmed by Etest (32 µg/ml) and disk diffusion (14 mm) methods. The molecular types of the two isolates were markedly different indicating the emergence of distinct LZD-R *S. aureus* strains in the same patient. The G2576T mutation was observed in both strains.

Conclusions:
LZD-R emerged in two distinct clones of *S. aureus* from the respiratory tract of a CF patient over a period of 30 months. CF patients receiving LZD frequently may represent an important reservoir for LZD-R *S. aureus* and should be monitored carefully.

INTRODUCTION

Linezolid is the first member of the oxazolidinone class of antimicrobials approved for clinical use in 2000. Linezolid is active against the vast majority of clinically important Gram-positive cocci, including multi-drug resistant *Staphylococcus* spp., *Enterococcus* spp., and streptococci; and acquired resistance has remained rare due to its unique mechanism of action.

The compound inhibits the formation of the initiation complex formed with mRNA, fMet-tRNA and the 30S ribosomal subunit. Mutations in the central loop of domain V of 23S rRNA have been associated with resistance to linezolid in several species.

Linezolid has been used extensively since 2000 to treat pulmonary infections caused by methicillin-resistant *S. aureus* (MRSA) in cystic fibrosis patients attending the Cystic Fibrosis Service of the Hospital de Clínicas de Porto Alegre (HCPA), a tertiary university hospital located in the city of Porto Alegre, Brazil. We previously reported the first linezolid-resistant *S. aureus* strain from Brazil isolated in 2002 from a cystic fibrosis patient (strain JMI-700; MIC, >8 µg/ml). In the present study, we report the occurrence of a second linezolid-resistant *S. aureus* strain from the same patient, occurring three years later.

MATERIALS AND METHODS

Case history:
Strain 57-233X was isolated in January 2005 from the sputum of a 12 year old female as part of the Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) program. The patient had been receiving linezolid periodically since December 2000 for the treatment of pulmonary infections and had the index linezolid-resistant *S. aureus* strain isolated in July, 2002 (JMI-700).

Susceptibility testing:
Strain 57-233X was tested for susceptibility against linezolid and >30 antimicrobials using CLSI broth microdilution methods (CLSI M7-A7 and M100-S16, 2006). Linezolid resistance was confirmed by Etest (AB BIODISK, Solna, Sweden), as well as disk diffusion methods.

Ribotyping:
Strain 57-233X and the previous linezolid-resistant strain from this patient (JMI-700) were subjected to ribotyping using the Riboprinter Microbial Characterization System (Qualicon Inc., Wilmington, DE) as per manufacturers' recommendations. Ribotype was compared to that of the Brazilian MRSA clone.

Detection of G2576U mutation in V domain of 23S rRNA:
Presence of G2576U mutation in the V domain of 23S rRNA responsible for linezolid resistance was detected using PCR amplification of the region and identification of a NheI restriction site (GCTAGC) within the amplicon.

RESULTS

- Strain 57-233X showed high-level resistance to linezolid with a MIC of 32 µg/ml. The susceptibility pattern of strain 57-233X, as well as the previous linezolid-resistant strain isolated from this patient, are shown in Table 1.

Table 1. Antimicrobial susceptibilites of the two linezolid-resistant strains isolated from the patient admitted in the Brazilian hospital.

	MIC in µg/ml	
Antimicrobial	57-233X	JMI-700
Linezolid	32	16
Oxacillin	>2	>256
Levofloxacin	4	4
Clindamycin	>2	>8
Doxycycline	≤2	>4
Chloramphenicol	16	>16
Trimethoprim/sulfamethoxazole	>2	>32
Gentamicin	>8	>8
Rifampin	>2	>2
Vancomycin	0.5	2
Teicoplanin	≤2	2
Quinupristin/dalfopristin	0.5	1
Daptomycin	0.25	1
Tigecycline	0.06	-
Dalbavancin	≤0.03	0.12

- PCR amplified fragment of the V domain of 23S rRNA gene exhibited a restriction site for NheI enzyme, confirming the G2576T mutation in both strains (Figure 1).

- Strain 57-233X showed a ribotype distinct from that of the previous linezolid-resistant strain JMI-700 (Figure 2).

Figure 1. Photo of PCR electrophoresis gel showing the detection of G2576T mutation on 23 rRNA gene

Legend: λ, lambda ladder; columns 1 and 4, negative controls (linezolid-susceptible strains); column 2, strain 57-233X; column 3, strain JMI-700; column 5, *S. aureus* positive control strain.

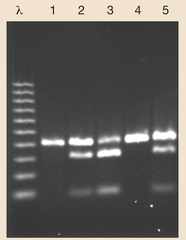
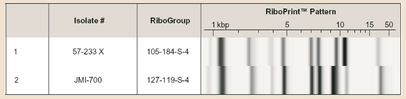


Figure 2. Ribotype of both linezolid-resistant strains from the cystic fibrosis patient.



CONCLUSIONS

- Linezolid resistance emerged in two distinct strains of *S. aureus* from the respiratory tract of a cystic fibrosis patient over a period of 30 months.

- Cystic fibrosis patients receiving linezolid periodically may represent an important reservoir for linezolid-resistant *S. aureus* and should be monitored carefully.

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