Clonal Dissemination of Linezolid-Resistant *Staphylococcus haemolyticus* Exhibiting the G2576T Mutation in the 23S rRNA Gene in a Tertiary Care Hospital in Brazil

Linezolid, the first oxazolidinone class agent to be introduced clinically, has broad activity against many important multidrug-resistant Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Linezolid inhibits protein synthesis by interacting with 23S rRNA in the 50S ribosomal subunit, and it should interfere with the binding of aminoacyl-tRNA to the ribosomal A site in the bacterial ribosome (5). Although linezolid resistance is mediated by the cfr-encoded product (8) or by mutations in ribosomal proteins (6, 9), mutations in the central loop of domain V of 23S rRNA have been reported as the main mechanism associated with the expression of linezolid resistance among clinical staphylococcal strains (2, 3, 11).

In this study, we report the first clonal dissemination of linezolid-resistant *Staphylococcus haemolyticus* strains with the G2576T mutation in 23S rRNA in Brazil. From March 2008 to January 2010, nine *S. haemolyticus* strains exhibiting high-level resistance to linezolid (MICs of 32 to 128 μg/ml) were isolated from blood and catheter cultures from different inpatients in a tertiary care hospital. In this institution, linezolid was introduced in November 2000 and is currently the drug of choice for the treatment of VRE infections, which are highly prevalent. Identification testing was performed by the Vitek-2 system (bioMérieux, St. Louis, MO), and the susceptibility of bacterial strains was tested using disk diffusion and agar dilution methods according to the Clinical and Laboratory Standards Institute (CLSI) (1). Etests (AB Biodisk, Solna, Sweden) were used for oxacillin and vancomycin. The chromosomal DNA was obtained using the technique of phenol-chloroform extraction. The presence of the cfr gene and mutations in the domain V region of 23S rRNA and the rplC, rplD, and rplV genes were investigated as described previously (2, 4, 9, 10). The PCR products were sequenced and aligned with the corresponding nucleotide sequence from linezolid-susceptible *S. haemolyticus* strain JCSC1435 (GenBank accession no. AP006716.1). Pulsed-field gel electrophoresis (PFGE) typing was performed according to the method of Struelens et al. (12) with a modification. Bacterial DNA was digested with the restriction enzyme FastDigest Smal (Fermentas Life Sciences, Canada).

The G2576T mutation in the domain V region of 23S rRNA was identified in all strains except the linezolid-susceptible *S. haemolyticus* control strain, which was obtained from the same hospital. On the other hand, all strains showed the wild-type L3, L4, and L22 ribosomal proteins when compared to the linezolid-susceptible *S. haemolyticus* strain JCSC1435. Furthermore, the cfr gene was not identified in any isolate (Table 1).

Cases of linezolid resistance in *S. haemolyticus* are still extremely rare and associated with the G2576T mutation in domain V of 23S rRNA (7, 11, 13). In our institution, linezolid-resistant *S. haemolyticus* strains with the G2576T mutation have been disseminated since 2008 and represent 1% of all *S. haemolyticus* isolates obtained during the period of this study, whereas linezolid-resistant *Staphylococcus epidermidis* strains represent 0.04% of total isolates of this species (2). No linezolid-resistant *Staphylococcus aureus* strain has been identified so far. Because these strains were collected over a 2-year period, it is possible to infer that these strains belong to an endemic clone of *S. haemolyticus* that has probably spread in our institution and become linezolid resistant under selective pressure. Although linezolid is potentially active against *S. haemolyticus* strains, reports of clinical *S. haemolyticus* infections are extremely rare.

### TABLE 1 Demographic data and antimicrobial susceptibility profiles of the linezolid-resistant *S. haemolyticus* clinical strains exhibiting the G2576T mutation in the 23S rRNA gene

| S. haemolyticus strain | Patient ID | Date obtained (mo-yr) | Clinical sample | PFGE type | 23S rRNA mutation | MIC (μg/ml) | Resistance profile
|------------------------|------------|-----------------------|----------------|-----------|------------------|------------|--------------------------|

**TABLE 1** Demographic data and antimicrobial susceptibility profiles of the linezolid-resistant *S. haemolyticus* clinical strains exhibiting the G2576T mutation in the 23S rRNA gene

- **MIC (μg/ml):**
  - **L22:** 256 R R R R S R R S R R I S
  - **R:** 256 R R R R S R R S R R I S
  - **S:** 256 R R R R S R R S R R I S

**Resistance profile:**
- **L22:** 256 R R R R S R R S R R I S
- **R:** 256 R R R R S R R S R R I S
- **S:** 256 R R R R S R R S R R I S

**TABLE 1** Demographic data and antimicrobial susceptibility profiles of the linezolid-resistant *S. haemolyticus* clinical strains exhibiting the G2576T mutation in the 23S rRNA gene

**LETTER TO THE EDITOR**

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strains have become more frequent. Our results enhanced concern about the continued judicious use of linezolid for infections caused by this species.

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REFERENCES