Clonal dissemination of linezolid-resistant *Staphylococcus haemolyticus* exhibiting
the G2576T mutation in the 23S rRNA gene in a tertiary care hospital, Brazil

Running title: “G2576T mutation in linezolid-resistant *S. haemolyticus*”

Keywords: linezolid, ribosomal mutation, coagulase-negative staphylococci.

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 (1, 3, 11).

In this study, we report the first clonal dissemination of linezolid-resistant *S. haemolyticus* strains with G2576T in 23S rRNA in Brazil. From March 2008 to January 2010, nine *S. haemolyticus* strains exhibiting high-level resistance to linezolid (MICs of 32-128 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 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 Laboratory Standards Institute (CLSI) (2). 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 \textit{cfr} gene and mutations in the domain V region of 23S rRNA and the \textit{rplC}, \textit{rplD} and \textit{rplV} genes were investigated as described previously (1, 4, 9, 10). The PCR products were sequenced and aligned with the corresponding nucleotide sequence from linezolid-susceptible \textit{S. haemolyticus} strain JCSC1435 (GenBank accession no. AP006716.1). PFGE typing was performed according to Struelens \textit{et al} (12) with a modification. Bacterial DNA was digested with the restriction enzyme FastDigest® Smal (Fermentas Life Sciences, Canada).

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

Cases of linezolid resistance in \textit{S. haemolyticus} are still extremely rare and associated to the mutation G2576T in domain V of 23S rRNA (7, 11, 13). In our institution, linezolid-resistant \textit{S. haemolyticus} strains with G2576T have been disseminated from 2008 and represent 1% of all \textit{S. haemolyticus} isolated during the period of this study whereas linezolid-resistant \textit{S. epidermidis} strains represent 0.04% of total isolates of this species (1). No linezolid-resistant \textit{S. 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 \textit{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* 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**


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

<table>
<thead>
<tr>
<th>Strain</th>
<th>ICU</th>
<th>Culture</th>
<th>Clinical sample</th>
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<th>23S rRNA</th>
<th>MIC (µg/ml)</th>
<th>Resistance profile</th>
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<td>-</td>
<td>2</td>
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</table>

ICU, intensive care unit; LZD, linezolid (30 mg); VAN, vancomycin; OXA, oxacillin; CIP, ciprofloxacin (5 µg); CEF, cefalotin (30 µg); PEN, penicillin (10 U); SAM, ampicillin/sulbactam (10/10 mg); TEK, teicoplanin (30 mg); ERY, erythromycin (15 mg); AMK, amikacin (30 mg); GEN, gentamicin (10 mg); CHL, chloramphenicol (30 mg); SXT, trimethoprim/sulfamethoxazole (1.25/23.75 mg); CLI, clindamycin (2 mg); TET, tetracycline (10 mg). The strain Lin<sup>+</sup> corresponds to a linezolid-susceptible control strain recovered from a clinical sample in an inpatient from the same hospital.
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