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LETTER TO THE EDITOR

Linezolid Resistance in Brazilian *Staphylococcus hominis* Strains Is Associated with L3 and 23S rRNA Ribosomal Mutations

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Among different clinical staphylococcal strains, linezolid resistance has mainly been mediated by mutations in the central loop of domain V of 23S rRNA, with the change of G-to-T at position 2576 (G2576T mutation) being the most prevalent. However, linezolid resistance associated with mutations in ribosomal proteins has recently been described, and the precise role of these mutations is under investigation. Many L3 mutations have been associated with resistance to linezolid in *Staphylococcus aureus*, *Staphylococcus cohnii*, and *Staphylococcus epidermidis* (1), but to the best of our knowledge, they have not been reported in *Staphylococcus hominis* so far. We report both 23S rRNA and L3 mutations in linezolid-resistant *S. hominis* strains circulating in an intensive care unit (ICU) of a tertiary care hospital located in Brazil.

From August 2008 to September 2011, three *S. hominis* strains exhibiting high-level resistance to linezolid (MIC values of 32 to 64 μg/ml) were isolated from blood and catheter cultures from different inpatients in an intensive care unit. These strains represented 1.2% of all *S. hominis* strains isolated in this period at this institution. All the patients were treated with linezolid (Table 1). The identification of bacterial strains was performed with the Vitek-2 system (bioMérieux, St. Louis, MO) and according to the protocol of Hirotaki et al. (2). Antimicrobial susceptibility was tested using the disk diffusion and broth dilution methods of the CLSI (3). The presence of the *cfr* gene and mutations in the domain V region of 23S rRNA, *rplC*, and *rplV* genes were investigated by PCR as described previously (4), except that the *rplD* gene was amplified using the forward primer for *rplD* and the reverse primer for *rplC*. PCR products were sequenced and aligned with the corresponding nucleotide sequence from linezolid-susceptible *S. hominis* strain SK119 (NCBI accession no. 629742). Additionally, a linezolid-susceptible *S. hominis* strain (6/831) isolated from a patient hospitalized in the same hospital was also tested for comparison purposes. The domain V fragment was digested with the NheI restriction enzyme, and determination of the clonal relatedness of strains was carried out by pulsed-field gel electrophoresis (PFGE) using the restriction enzyme SmaI.

All isolates were clonally related and multidrug resistant but remained susceptible to vancomycin, teicoplanin, and tetracycline. Two strains, with a linezolid MIC of 64 μg/ml, were found to have G2576T in 23S rRNA and mutations Gly139Arg/Met156Thr in the L3 protein, whereas one strain, with a MIC of 32 μg/ml, had Phe147Ile in the L3 protein, in addition to G2576T. The incomplete digestion of domain V with NheI suggested the presence of fragments with both the G2576T mutant and wild-type sequences in all linezolid-resistant *S. hominis* strains. Mutations in the proteins L4 and L22, as well as the presence of the *cfr* gene, were not identified in any isolates.

Previous studies have already reported Phe147Ile, Gly139Arg, and Met156Thr in the L3 protein of other species (5, 6). The linezolid MIC of the *S. hominis* strain 35/1228 was similar to those reported by Kosowska-Shick et al. (5) for two *S. epidermidis* strains that also had Phe147Ile simultaneously with G2576T in 23S rRNA (wild-type L4 and *cfr* negative). The combination of Gly139Arg and Met156Thr substitutions in L3 has not been reported previously, especially accompanied by the G2576T mutation, which was present in multiple copies of the 23S rRNA gene of the *S. hominis* strains evaluated in this study. However, these mutations were not able to confer high levels of linezolid resistance in *S. hominis*. A linezolid-resistant *S. aureus* strain (wild-type L4 and L22 proteins and *cfr* negative), which also had G2576T and only Gly139Arg in L3, displayed a higher linezolid MIC (>256 μg/ml) (6) than the *S. hominis* strains of this study (64 μg/ml) with both the Gly139Arg and Met156Thr mutations.

The identification of these same mutations in the L3 protein of the linezolid-resistant *S. hominis* strains strengthens the role of these sites

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TABLE 1 Demographic data and antimicrobial susceptibility profiles of the linezolid-resistant *S. hominis* clinical strains

Strain	ICU[a]	Culture date (mo/yr)	Culture day/duration of treatment (days)	Clinical sample	*cfr* gene	Mutation(s)		Resistance profile[b]											
								MIC (μg/ml)											
						23S rRNA	L3 protein	LZD	VAN	OXA	CIP	TEC	SXT	AMK	GEN	ERY	CLI	TET	CHL
S. hom..6/831[c]	Yes	09/10		Blood culture	Absent	None (wt[d])	None (wt)	1	<4	>256	R	S	S	S	R	R	R	S	R
S. hom..35/1228	Yes	08/10	42/68	Catheter tip	Absent	G2576T	Phe147Ile	32	<4	>256	I	S	R	I	R	R	R	S	R
S. hom..41/1283	Yes	04/11	38/30	Blood culture	Absent	G2576T	Gly139Arg/Met156Thr	64	<4	>256	R	S	R	R	R	R	R	S	R
S. hom..43/257	Yes	09/11	39/41	Catheter tip	Absent	G2576T	Gly139Arg/Met156Thr	64	<4	>256	I	S	R	R	R	R	R	S	R

[a] ICU, intensive care unit.

[b] LZD, linezolid; VAN, vancomycin; OXA, oxacillin; CIP, ciprofloxacin (5 μg); TEC, teicoplanin (30 mg); SXT, trimethoprim-sulfamethoxazole (1.25/23.75 mg); AMK, amikacin (30 mg); GEN, gentamicin (10 mg); ERY, erythromycin (15 mg); CLI, clindamycin (2 mg); TET, tetracycline (30 mg); CHL, chloramphenicol (30 mg); R, resistant; S, susceptible; I, intermediate.

[c] Strain *S. hom*..6/831 corresponds to a linezolid-susceptible strain isolated from a patient hospitalized in the same hospital.

[d] wt, wild type.

in the acquisition of linezolid resistance in *Staphylococcus* spp., although the presence of G2576T in the 23S rRNA gene makes it difficult to determine the exact role of L3 mutations in conferring the elevated linezolid MIC values shown by these clinical strains.

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