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Detection of Linezolid-Resistant *Staphylococcus aureus* with 23S rRNA and Novel L4 Riboprotein Mutations in a Cystic Fibrosis Patient in Spain

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Linezolid-resistant *Staphylococcus aureus* (LRSA) emerged in cystic fibrosis (CF) patients in different countries several years ago (1), and we have also observed this problem in Spain. The most frequent mechanism of linezolid resistance in staphylococci is a G2576T point mutation within domain V of the 23S rRNA; mutations in ribosomal L3 and L4 of the peptidyltransferase center, besides the *cfr* gene codified in a plasmid, contribute to decreased susceptibility to linezolid (1, 2).

A 17-year-old female patient with CF was admitted to our hospital. Several microorganisms were isolated from this patient from 2010 to 2012, specifically, *Stenotrophomonas maltophilia*, *Escherichia coli*, and methicillin-resistant *Staphylococcus aureus* (MRSA), and she has been treated with different doses of antibiotics (levofloxacin, colistin [polymyxin E], and teicoplanin plus meropenem) and three courses of linezolid (600 mg/12 h/14 days), as recommended previously (3). Organisms in sputum cultures were identified by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Bruker) and 16S rRNA gene sequence analysis. Among the microorganisms characterized were three LR MRSA strains. Antibiotic susceptibilities were determined using the MicroScan WalkAway system (Siemens), Etest, and agar dilution according to CLSI guidelines (4) for ampicillin (MIC > 4 μg/ml), clindamycin (1 μg/ml), erythromycin (32 μg/ml), oxacillin (>16 μg/ml), teicoplanin (1 μg/ml), vancomycin (2 μg/ml), and linezolid (32 μg/ml). Genetic relatedness among the three *Staphylococcus aureus* strains was determined by a pulsed-field gel electrophoresis method, revealing that they were genetically similar, and spa typing (spa type t002, belonging to clonal group CC5, which is very usual in Spain) (5). The strain was positive for the *mec* gene.

We have studied the resistance mechanisms of LR MRSA; *cfr* is present according to Kehrenberg and Schwarz (6). As a positive control, we used linezolid-resistant *Staphylococcus epidermidis* from our collection. We amplified by PCR and sequenced domain V of the 23S rRNA using the primers described by Pillai and colleagues (7). The sequences were compared with the *Staphylococcus aureus* ATCC 12600 strain. We also amplified the *rplC*, *rplD*, and *rplV* genes, which codify L3, L4, and L22 riboproteins, with the primers described by Mendes and colleagues (8). Amplicons were sequenced and analyzed by using the DNAStar system, Madison, WI, USA. The L3 amino acid sequences were compared with that of linezolid-sensitive *Staphylococcus aureus* N315, and L4 and L22 were compared with those of *Staphylococcus aureus* MW2168 and *Staphylococcus aureus* N315.

The study showed that the three *Staphylococcus aureus* strains did not acquire the *cfr* gene; neither had *rplC* nor *rplV* mutations, but they had the 23S rRNA G2576T mutation (in some genes, because there was an incomplete digestion with the NheI restriction enzyme) and Gly69Ala and Thr70Pro substitutions in ribosomal protein L4 of the peptidyltransferase center.

To our knowledge, this is the first clinical case reported with the *rplD* gene (encoding the L4 riboprotein) mutations among the linezolid-resistant *Staphylococcus aureus* mechanisms (2, 9); however, in a previous work published when linezolid was not widely used (10), similar mutations related with the macrolide resistance were cited. The strains of our patient have also been resistant to macrolides. We need to develop new experiments to prove the contribution of L4 mutations.

The detection of these strains is a cause of concern, and it is necessary to maintain surveillance.

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Published ahead of print 4 March 2013

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doi:10.1128/AAC.00208-13

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